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Electrical and Magnetic properties Comparison between LCMO and LSMO system

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Abstract

Electrical and magnetic properties of La_{0.67}Ca_{0.33}MnO₃ and La_{0.67}Sr_{0.33}MnO₃ systems were studied via solid-state reaction method. The studies have been carried out by powder X-ray diffraction analysis (XRD), resistance techniques by using D.C four point probe and magnetic susceptibility by using A.C susceptometer. These materials are extensively studied also by the substitution of rare-earth compound to understand the nature of transport phenomena in each system. From X-ray diffraction (XRD) patterns, the samples of LCMO and LSMO systems show single phase perovskite structure and our powder samples crystallize in a orthorhombic and rhombohedral structures respectively. The electrical transport studies exhibit metal to insulator transition (MIT) at low susceptibility studies exhibit ferromagnetic temperature, ACparamagnetic transition with an antiferromagnetic ordering at low temperature.

مقارنة الخصائص الكهربية والمغناطيسية بين أنظمة LSMO و LCMO

في الآونة الأخيرة ظهر اهتمام كبير في دراسة نظام $A=(Sr,\ Ca)$ $(La_{1-x}A_xMnO_3)$ في الآونة الأخيرة ظهر اهتمام كبير في دراسة نظام من خواص تطبيقية وعلمية هامة في مختلف نواحي الحياة. لذا محاولات لتطوير وتحسين خواص هذه المواد لما لها من خواص تطبيقية وعلمية هامة في مختلف نواحي الحياة. لذا

فقد وقع الاختيار على (LSMO, LCMO) للدراسة في محاولة لتقديم مواد ذات تطبيقات كهربية عالية الكفاءة وذلك بمدف دراسة خواصها الكهربية والمغناطيسية لإمكانية استخدامها في تطبيقات تكنولوجية كثيرة كأجهزة الاحساس والاستشعار وزيادة حساسية تخزين البيانات.

ففي هذا العمل تمت دراسة بعض الخصائص الكهرومغناطيسية والتركيبية لأنظمة (La_{0.67}Ca_{0.33}MnO₃) و La_{0.67}Ca_{0.33}MnO₃) بطريقة تفاعل الحالة الصلبة عند درجات حرارية مختلفة وباستخدام طريقتي التلدين والتلبيد . وقد فحصت العينات باستخدام حيود الأشعة السينية (XRD) ، كما اشتملت على فحوصات كهربائية لقياس المقاومية الكهربائية باستخدام تقنيات تحقيق الأربع نقاط وفحوصات مغناطيسية لإيجاد ودراسة خصائص التأثيرية المغناطيسية وكذلك قياس الانفاذية المغناطيسية باستخدام (A.C susceptometer) (وايضا يتم دراسة هذه المواد على نطاق واسع بالاستعاضة عن مجموعة المركبات الأرضية النادرة وذلك لفهم طبيعة الخواص في كل نظام). ومن خلال أنماط حيود الأشعة السينية (XRD) ، اظهرت عينات LCMO والمحالة في الصورة البلورية الأحادية الطور ذو الشكل معيني قائم (الاورثورومبيك) ومنشور سداسي منتظم (روهومبوهيدرا) على التوالي وانما تعتمد في الحقيقة عل درجة الحرارة والتركيز في هذا الجانب. اما الخواص الكهربائية فأظهرت الانتقال من المعدنية الى العازل (MIT) عند درجة الحرارة المنخفضة، ودلت دراسة قابلية و درجة الحرارة المنخفضة. كما أوضحت هذه الدراسات ان درجة الحرارة الحرجة في هذه المركبات تكون حساسة في درجة الحرارة المنخفضة. كما أوضحت هذه الدراسات ان درجة الحرارة الحرجة في هذه المركبات تكون حساسة جدا تبعا لظروف تحضير العينة ونقاوتها.

هذه الخصائص، جنبا إلى جنب مع الاستقطاب الدوراني 100%، يجعل LSMO مادة مثيرة للاهتمام لتطبيقها في الأجهزة من النوع الإلكتروني الدوراني. ومن الأمثلة على ذلك نفق التقاطعات المغناطيسية والأجهزة الكهربائية والمغناطيسية وعلاوة على ذلك، يتم استخدامه كالموصلات الفائقة لتحقيق الاستقطاب الدوراني. بينما الاهتمام التقني ل LCMO للتطبيقات المختملة على سبيل المثال , المحسسات المغناطيسية, قراءة رؤوس مولدات المقاومة المغناطيسية للذاكرة العشوائية وكذلك تخزين البيانات.

Keyword: Electrical, magnetic and colossal magnetoresistance

Introduction

The interest in perovskite manganites with the form, La_{1-x}A_xMnO₃ (A = divalent alkaline earth metal, i.e. Ca⁺², Sr⁺², B⁺² and Pb⁺²) in recent years was due to their observed colossal magnetoresistance (CMR) effects near the ferromagnetic-paramagnetic transition temperature T_C in this material and the potential applications in magnetoresistive transduser and magnetic sensors [1]. These systems have technological importance such as in sensor application and increasing data storage by increasing in sensitivity of hard disk drive read heads [2]. The phenomenon of CMR effects, explained by Zener through the double exchange mechanism, occurs in the phase transition from a paramagnetic insulator at high temperature to the ferromagnetic metal at low temperature T_p [3]. Above the metal-insulator transition temperature T_p , the sample shows activated behavior $(d\rho/dT < 0)$ or behave like a semiconductor material. The LaMnO₃ is a perovskite-type oxide and it's antiferromagnetic insulator due to the superexchange antiferromagnetic coupling interaction between the Mn³⁺ and Mn⁴⁺ ions.LSMO is metallic ferromagnetic manganite with Curi temperature of \approx 360 K [4,5]. La_{1-x}Sr_xMnO₃ is considered as an alloy of LaMnO₃ with a(La) atom substituted by a(Sr) atom. It shows colossal MR and exhibits halfmetallic properties for x=0.3 [6]. At x=0.3, it has been shown that its spin polarization is 95% at 4K and ~ 90% at 100K [7,8]. As early ,provided evidence La_{1-x}Ca_xMnO₃ is an HM [9]. The manganites doped with calcium and strontium [10-13] received much attention due to the high Tc and small variance of the B site ionic radii. Recent studies on these materials revealed that CMR phenomenon is attributed not only to the double exchange (DE) mechanism but also to the interactions such as electron-phonon coupling, electronmagnon interaction, and the complicated band structure. In the present paper, we have studied the electrical and magnetic properties of

probe and to compare between $La_{0.67}Ca_{0.33}MnO_3$ and $La_{0.67}Sr_{0.33}MnO_3$ systems.

Experiment

The manganites samples of $La_{0.67}Ca_{0.33}MnO_3$ and $La_{0.67}Sr_{0.33}MnO_3$ were prepared via the conventional solid-state reaction method. A well-mixed stoichiometric mixture of La_2O_3 , $CaCO_3$, $MnCO_3$ and La_2O_3 , $SrCO_3$, MnO_2 with purities higher than 99.9% were mixed with acetone, ball milled for 6 h and oven dried at 110 °C overnight. The dried powder was heated at 900 °C in air for 12 h with rate 3°C/min to produce a highly reactive powder. After calcination, the black powdery mixture was grounded and sieved in order to ensure good homogeneity, then pressed into pellets using 1.2g powder by hydraulic press at (3Tcm⁻²) and sintered at 1300 °C for 24 hours with rate 2°C/min, followed by furnace cooling at room temperature.

Characterization of the samples were done by Philip x-ray diffractometer (XRD) with a rotating anode at room temperature with $CuK\alpha$ radiation using Phillips (PW1830) in order to see the structure of the samples with λ =1.54056 °A. The resistance was measured by the conventional four-probe technique in the temperature range of 20 to 300 K. Ac magnetic susceptibility χ measurements for two systems as a function of temperature in a magnetic field of H=10 Oe were measured by a Lakeshore AC(Model 7000) susceptometer with temperature range of 30-300 K and a frequency of 125 Hz.

Results and Discussion

1- Structure properties:

X-ray diffraction spectrum (XRD) 2 Θ pattern ranged from 20° to 80° is obtained with the scattering vector perpendicular to the plane of the samples are asshown in Figure 1. The Miller indexed XRD peaks correspond to an orthorhombic structure for La_{0.67}Ca_{0.33}MnO₃ [14] and to rhombohedral structure for La_{0.67}Sr_{0.33}MnO₃. No clear evidence of reflection peaks is

observed in the XRD spectrum. Lattice parameters and unit cell volume for two systems were calculated and listed in table 1. The unit cell volume for LCMO sample is bigger than that of LSMO.

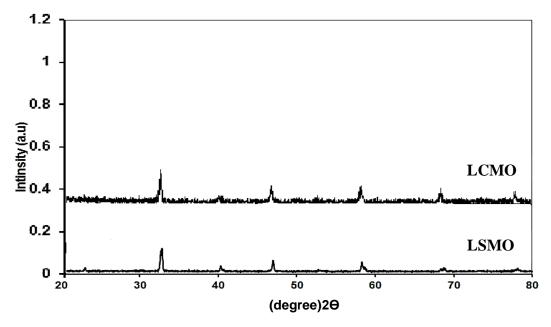


Figure 1: XRD spectrum for LCMO and LSMO sample.

Table 1: Lattice parameters and unit cell volume for LCMO and LSMO sample

System	Lattice parameters (Å)	Volume (Å ³)
La _{0.67} Ca _{0.33} MnO ₃	a = 5.474 b = 7.702 c = 5.448	229.69
$La_{0.67}Sr_{0.33}MnO_3$	a=b=c=5.471	116.66

2- **Electrical properties**:

Figure 2 shows the temperature dependence of the resistance under zero magnetic field. The measurement was done by using the standard four-probe method with a constant current 1mA.

The insulator-metal transition occurs at (246K) with metallic behavior at lower temperature side for LCMO sample as shown in figure (2a) [14]. Whereas the samples of LSMO is metallic throughout the measured temperature region and it's a round 360K (Tc is not indicated because the measuring system is incapable to measure up to 300 K) as shown in figure (2b).

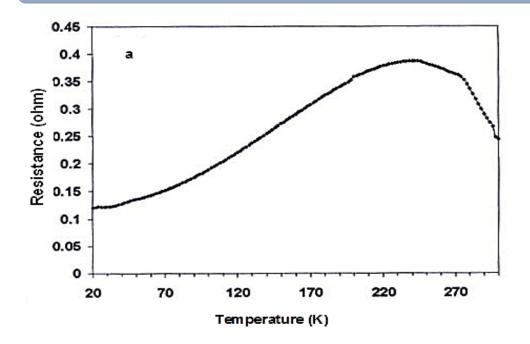


Figure 2a: Temperature dependence of resistance for LCMO sample.

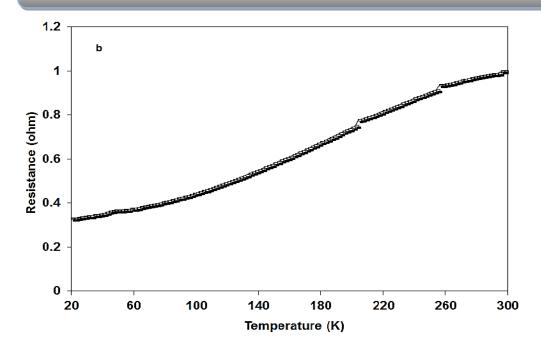


Figure 2b: Temperature dependence of resistance for LSMOsample.

3- Magnetic properties:

The temperature dependence of the ac magnetic susceptibility, χ ' at the magnetic field of 10 Oe of the samples are as shown in Figure 3. Both samples display classical phase transition from paramagnetic to ferromagnetic state at Cuire temperature, Tc. The LCMO sample display lower Tc at around 245K as shown infigure (3c) [14]. Whereas, The sample of LSMO exhibits the high Tc at 365 K, but here Tc is not indicated because the measuring system is incapable to measure up to 300 K only as shown in figure (3d). As the magnetic field increases from 0.1 Oe to 10 Oe the LSMO sample shows increase of Ac susceptibility value. However, as the temperature increases the Ac susceptibility increases.

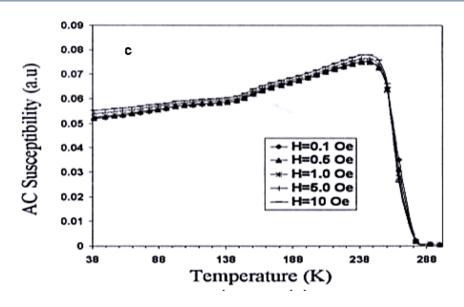


Figure 3c: Temperature dependence of ac-Susceptibility for LCMO sample.

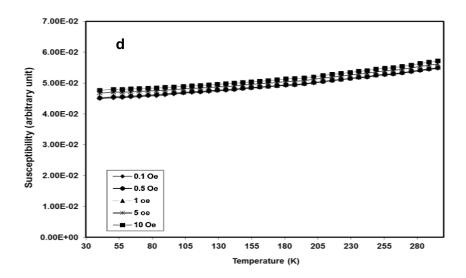


Figure 3d: Temperature dependence of ac-Susceptibility for LSMO sample.

Conclusion

The electrical and magnetic properties of LCMO and LSMO samples were investigated. Both the metal insulator and ferromagnetic transition temperatures were found. An insulator-metal (I-M) transition is observed at 246K for the LCMO sample, while a broad MIT is observed for LSMO sample and it shows a high phase transition temperature at around 360 K.Parent compound (LCMO) shows a paramagnetic (PM) to ferromagnetic (FM) transition with Curie temperature ($T_C \approx 245$ K) near to that peak in electrical resistivity (≈246 K), and the Curie temperature T_C for LSMO sample is ≈ 365 K. The perovskite structure manganites, specifically LCMO and LSMO exhibits colossal magnetoresistance behavior which is of interest to magnetic information storage applications and LSMO has received much attention due to its largest single electron bandwidth and the highest Curie temperature. These properties, together with the 100% spin polarization, make LSMO an interesting material for application in spintronic devices. Examples are magnetic tunnel junctions magnetoelectric devices .Furthermore, LSMO is also used to investigate other materials, e.g. by spin-injection into cuprite superconductors to probe spin polarization. While, the CMR effect for LCMO is of great technological interest. Potential applications are for instance magnetic sensors, magnetoresistive read heads, random access memory and data storage.

References

- 1. Xiong, C., Hu, H., Xiong, Y., Zhang, Z., Pi, H., Wu, X., Li, L., Wei, F. & Zheng, C. "Electrical properties and enhanced room temperature magnetoresistance in (La_{0.7}Ca_{0.2}Sr_{0.1}MnO₃)_{1-x/Pdx} composites. Journal of Alloys and Compounds 479: 357-362 (2009).
- 2. N.Kobayashi, M. Isa, T. Nishizaki, M. Fujiwara, "Electrical and Magnetic properties of La_{1-x}Ca_xMnO₃ and Pr_{1-x}Ca_xMnO₃ thin films J. of magnetism and magnetic material 177-181(1998).

- 3. Turilli, G. and Licci, F., Relationship between Spin Order and Transport and Magnetotransport Properties in La_{0.67}Ca_{0.33}Mn_{1-x}Al_xO₃ Compounds, Phys. Rev. B: Condens. Matter, 54, 18, 13052–13057 (1996).
- 4. A. Urushibara, Y. Moritomo, T. Arima, A. Asamitsu, G. Kido, and Y. Tokura, "Insulator-metal transition and giant magnetoresistance in La_{1-x}Sr_xMnO₃". Phys. Rev. B 51, 14103 (1995).
- 5. M. Imada, A. Fujimori, and Y. Tokura, "Metal-insulator transitions". Rev. Mod. Phys. 70, 1039 (1998).
- 6. J.-H. Park, E. Vescovo, H.-J. Kim, C. Kwon, R. Ramesh & T. Venkatesan." Direct evidence for a half-metallic ferromagnet". Nature 392,794,(1998).
- 7. M. Bowen, M. Bibes, A. Barthélémy, J.-P. Contour, A. Anane, Y. Lemaître and A. Fert." Nearly total spin polarization in La_{2/3}Sr_{1/3}MnO₃ from tunneling experiments". Appl. Phys. Lett., 82, 233, (2003).
- 8. M Bowen, A Barthélémy, M Bibes, E Jacquet, J P Contour, A Fert, D Wortmann and S Blügel." Half-metallicity proven using fully spin-polarized tunneling". J. of Phys..Condensed Matter, .17, 41,(2005).
- 9. J. Y. T. Wei, N.-C. Yeh, and R. P. Vasquez." Tunneling Evidence of Half-Metallic Ferromagnetism in La_{0.7}Ca_{0.3}MnO₃".Phys. Rev. Lett.79,5150, (1997).
- 10. Karmakar, S., Taran, S., Chaudhuri, B.K., Sakata, H., Sun, C.P., Huang, C.L. & Yang, H.D. "Study of grain boundary contribution and enhancement ofmagnetoreisistance in La_{0.67}Ca_{0.33}MnO₃/V₂O₅ composites". J. Phys. D: Appl. Phys. 38: 3757-3763(2005).
- 11. Ravi, V., Kulkarni, S.D., Samuel, V., Kale, S.N., Mona, J., Rajgopal, R., Daundkar, A., Lahoti, P. S. & Joshee, R. S. "Synthesis of La_{0.7}Sr_{0.3} MnO₃ at 800 °C using citrate gel method. Ceramics International 33: 1129-1132(2007).
- 12. Roul, B.K., Sahu, D.R., Mohanty, S. & Pradhan, A.K. "Effect of high temperature sintering schedule for enhanced CMR properties of La0.67Ca0.33MnO3 close to room temperature. Materials Chemistry and Physics 67: 267-271(2001).

- 13. Venkataiah, G., Prasad, V. & Venugopal Reddy, P. "Influence of A-site cation mismatch on structural, magnetic and electrical properties of lanthanum manganites. Journal of Alloys and Compounds 429: 1-9(2007).
- 14. Hazar A.Salama, S. A. Halim, W.M.D.W. Yusof, Imad Hamadneh, Noorhana Yahya, Zaidan Abdul Wahab, E. B. Saion and Z. Gebrel. "A comparative study on the structural, magnetic and electrical properties of the $La_{0.7}Ca_{0.3}MnO_3$ synthesize via co-precipitation method and solid-state reaction". Solid State Science and Technology, 12, 1, 98-103, (2004).

List of Abbreviations and Symbols

(الرموز والمصطلحات)

Symbol/Term	Designation/Explanation	
Sr	Strontium	
Ca	Calcium	
Mn	Magnesiur	n
La	Latium	
0	Oxygen	
C	Carbon	
MR	Magnetoresistance	
Cuka	The wavelength of the	
	Kα1 line of copper	
CMR	Colossal magnetoresistance	
Oe	Oersted	
MIT	Metal to insulator transition	
HM	Half Metal	
K	Kelvin	
Тр	Phase	transition
temperature		

Tc	Curie temperature	
R	Resistance	
AC	Alternating Current	
XRD	X-ray diffractometer	
ρ	Resistivity	
a.u	Arbitrary unit	
DC	Direct current	
χ	Susceptibility	
°C	Degree Celsius	
h	Hour	
Å	Angstrom	
Hz	Frequency unit	
FM	Ferromagnetic	
PM	Paramagnetic	
C	Curie constant for material	
λ	Wavelength	
θ	Angle of diffraction	
a, b, c	Lattice parameters	

The agricultural activities in Libya during the second Era of Ottmanians

(1835-1911AD)

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Abstract

The second era of Ottmanian (1835-1911AD) had witnessed a stable economic development and growth due to internal and external policy and because of the reforms achieved by the Ottmanian Empire in all its states.

Agriculture is considered a significant formation in the economical growth due to its direct relation to population's income and effect with people's needs in any country in the world because agriculture is the main source of food to the people.

The agricultural and animal products have played an essential role in the economy of Tripoli at that time, the Ottoman authority was responsible on supervision of farms while the workforce was local people of Tripoli. This situation had led to improve the economical condition of the state and its people, also it was the main source for income to the Ottoman government.

1. Introduction

The research discusses the agricultural activities in Libya during the second Ottomanian era in the state of west Tripoli. The era of Ottomanian in west Tripoli has witnessed a significant development and growth, and the history of that time shows many historical information about Libya. The study will discuss the economical activities of the state of west Tripoli such as agricultural activities and agricultural products because agriculture was

very important to the people at that time until now. The study will also discuss the livestock in west Tripoli because of its importance to people's living and was considered as one of the main sources of income.

This paper divided into introduction and many sections. The first section discusses the agricultural activities. The second section discusses the livestock. At the end, the study concluded with significant results related to this topic.

1.1 The Agricultural Activity

Agriculture is a typical pattern in the economic activity in general because it is related to the needs of people in any country of the world, and more importantly, agriculture in west Tripoli had provided food for the population of the state. The agricultural activities in west Tripoli was considered the main productive sources and ranked top in the state economy because of the wide area of Libya along the northern coast from east to west of Libya which is 1900km long on the Mediterranean sea (Mustafa Hamid, 1999, p.276)

Agriculture had provided a stable life for more than 95% of the population at that time, it was an important source of income for the Ottoman state. (Fadia Abdul Aziz. 2006, p.45). Despite the fact that agriculture was the main pillar of the Libyan's life but it was primitive and not developed, and also depended on traditional techniques. In addition to that, agriculture was suffering from crises and many problems, including:

- 1. Lack and shortage of water resources.
- 2. Climatic fluctuations which was affecting crops.
- 3. Failure to fight and control animal and pests.
- 4. The shortage of labor force.

- 5. The difficulty of transporting crops.
- 6. Farmer's dependence on old agricultural tools used for harvesting and composting.
 - 7. The neglect from local authorities to improve agricultural activity.
- 8. The state authorities had imposed local taxes on farmers. (Francesco Koro, 1984, p.92)

The drought and few rainfalls had affected the whole country and there was almost periodically a clear impact on the economy of the state. Every ten years there had been four good years for production of good crops, and four years of medium production. The remaining two years' production was slightly few (Ahmed Sidki, 1971, p.238). The areas which was suitable for farming and producing good crops but it was limited only in the region of west Tripoli area and in particular Al Jafara region, Mislata coast, Tripoli oases, and the mountains and valleys of Fazzan. With regards to Cyrenaica, it was including Al Marj plain, Green Mountain, and desert oases; the other part of the state's land consist of desert and rocks or sand dunes. (Anthony: Ikea, 1975, p.99)

At the beginning of the second Ottomani era (1250AH/1835AD), the Ottomani governors had turned to pay attention to agricultural activities, and this resource has become a major contribution to the economy of the state in achieving simple economic growth.

At that time, the local newspapers had worked on alerting and bringing attention to the importance of agricultural activity. For example; Al Raqeeb newspaper in its issue dated July 27, 1327AH/1909AD, had stated the requirement to renew the agricultural works and using new machines to ease agricultural works and to help the labor working in the

farms at that time, the newspaper had highlighted the need for professional working using these machines because the land of the state was the best, the newspaper had advised the specialists in agricultural projects to adopt modern methods. (Al Raqeeb newspaper, 1329AH), whilst the newspaper of Tripoli had described the extract of water from wells using animals as a slow task and not efficient, and according to that using this way to extract water would not develop the agriculture in the state, and therefore the newspaper advised the necessity to replace it, and demanded the government to allocate special funds for this purpose, (Tripoli Newspaper: 1327-1329AH).

Abu Kasha had wrote "no harvesting, no farming, with extreme poverty increasing, what a big disaster to the people of the state, it should for government agents who had deposited into the sponsorship to ensure interest of the country and to think in the future with fears and dangers and threatened to take off security and stability of the state. (Fadia Abdul Aziz, 2006AD. p. 89)

After that the Ottmanian governors had been alerted to the huge problems associated with agriculture activity, and then Al-Wali (governor) Ali Ridha Basha (1282/1287AH/1866/1870AD) had issued orders to improve some of the agricultural areas and develop the production in these areas through all possible means to encourage the farmers to work in farms (Niqola Ziadah, 1966, p.55). Then he had established the fund for public benefits of agricultural credit in year 1285AH, 1869AD (paper number 129, management affairs file). During the same time Al-Wali (governor) had submitted a report to (the high door) explaining the importance of agriculture to the economy of the state as well as highlighted the obstacles they were facing and the required means to develop agriculture in the state. According to that, he had suggested a proposal to drill wells in many part of the state. His project had received an acceptance from the Ottomanian administration center but the project was not executed and produced the

desired goals because Al-Wali (governor) had resigned and did not continue his job.

Ahmed Rustum Basha¹ (1896/1882AD/1313/1299HD) then had worked to encourage the work in unused lands and develop agriculture in these lands, therefore he had sold the lands that was belonging to the state in the eastern areas of Tripoli with a condition to invest these lands. To achieve his objectives he had imported some new plants such as potatoes and berry trees (Al Mousely Shihab Yasin, 2006, p.71). At that time the government of the state had worked on plantation of Olive trees and understanding how to instill these trees and taking care of them. According to that a resolution by the management council of the state was issues (paper no 270, file of agriculture, Industry and Minerals). The resolution indicated rules to punish the failure farmers or employees, as well as managers and supervisors who did not follow the terms and instructions indicated in the resolution. In addition to that to punish the people who had caused damage to olive trees through random grazing (paper no 1080, file of agriculture, Industry and Minerals). This was according to the report of Al-Wali (governor) Ahmed Basha to the high door dated 8th of Shawal 1308HD 16 May/1891AD on the occasion of the tenth anniversary of his mandate. In 1325AH/ 1907AD a special directorate for agriculture under the supervision of agricultural experts staff was established in order to provide agricultural guidance for farmers. The Mabel-Lomis Todo has referred that some European authors like James Hamilton had spoke about soil fertility in the state and how few water and a little work would bring a big harvest. Mabel-Lomis Todo explained how water drift paths of rich

¹.Ahmed Rustum Basha. Was born in Istanbul in 1825 AD, and studied at Athens, Greece, and spoke a number of languages Alaworbeh, and held several management positions and political in

the Ottoman Empire, then appointed ruler of the mandate of Tripoli in 1882 and continued in power until the year 1896. He died in the year 1897. Source Ahmad Sidqi Dajani .1971. Libya, such as the Italian occupation. 1edition. RR 64.136

Muslims and Jews reflect a real beauty where fruit trees and flowers were grown. (Mobil Lomsi, 1968, p. 101-104)

Al Hashashi (Tunisian travelers)² showed that agriculture in Tripoli as seen in year 1312HD/1895AD was divided into two divisions in this country. The first division of those, people who were qualified for doing farm works and serving the land very well. The 2nd division was Arab people who were living far from the state and were not qualified to work in the farms and were not serving the land properly even their lands was suitable for agriculture, those people of the 2nd division were more interested in doing trading deals instead of working in agriculture.

The process of encouraging farmers to harvest the lands included all regions of the land, in a letter sent by the manager of Sirt region to Al-Wali (governor) Mustafa Nouri Basha indicated his intention to encourage the local people to tilled lands and planting wheat and barley fields (1270HD/1854AD) (Mohammed Abdul Majid 2004AD, p.82)

Ottmanian authorities has used a new system to organize real-estates and to register them as well as special lands belong to the population and government inside the state. On 18th of Shaaban 1274AH/ April 2, 1858AD, the Ottmanian law related to lands had stated the presence of what was called "Daftar Khana" job in the administration of real estate registration for the purpose of ownership and registering of lands, it was called "Daftar Hakani".

² .Mohammed bin Othman Al Hashashi. Is a historian and traveler Tunisian born 26 Ramadan 1271 AH = 12 of July 1855 AD, and visited the mandate Tripoli in 1896 and clerks (evacuation anguish for Tripoli) and book (Date Zitouna Mosque) and died on Tuesday, 3 de argument 1330 e _1911 m. Source Al Hashashi: Mohammed,Benotman -1965, trip Al Hashashi to Libya. provide and achieve Ali Mustafa Misrati. rr 23.24

The process of land registering and indicating the names of land owners was known as "Al Tabo" ³ and the land owner would receive a royal certificate which was known as Koshan Tabo. This law was implemented in the state until 1291HD, 1874AD and had been applied in the region of Tripoli and surrounding areas, like the western mountain and some coastal cities (ahmed Sudki Al Dajani, 1971AD, p.112)

To support the process of land registration, the establishment of the first agricultural bank of the Ottomanian government during the late period of their rule was done in 1328 AH / 1910 AD. The capital of the bank was funded from institutions similar to its work in the Ottomanian Empire. The city of Tripoli had paid an interest estimated about 4% on the capital and the most important activities and facilities of the bank such as credit based on mortgage of immovable property. And the banks had imposed certain conditions in the process of granting loans to the citizens such as the land registration and legal editor. (Naji Mahmoud, 1995. p. 94)

In return, the policemen had been assigned for many economic tasks, for example, guarding crops and fruit trees in agricultural areas and protect them from spoil and thefts, alert their owners to pay more attention and care and nurturing as well as punishing owners who was neglecting or cause damage to the crops and fruit trees. The policemen were responsible to send the heads of administrative units monthly reports on the status of those plants and their conditions. (Mabrouka Omar, police in the state of Tripoli during the Ottoman era 2, 1835/1911. 2007, p 138)

1.2 Agricultural products

³. Tabou, the origin is Bricks it means following or dependency. Launches on Sindh submitted by the State is delegated to dispose of the land has held ownership. Recognition by extension the state, Hussein Mujibur Egyptian, 2004, Lexicon of the Ottoman Empire, i 1, the House of Culture for publication, Cairo. P 84

The most important agricultural products was varied according to the sestem of irrigation. Agriculture basically in west Tripoli is wintry and in the summer .The wintry agriculture depends on rainfalls for planting many several crops such as barley and wheat, which was considered the main feeding for the population, and the barley was the main feeding for the horses which was the means of transportation for the Ottmanian soldiers. The planting of barely was taking the priority in grain production. Libyan grain was demanded by the European countries especially England for making Beer and Alcohol. The state was exporting huge quantities to England at that time, whereas wheat was exported to European counties (Fatehiyah Ali Abdullah, 2006, p.46)

In 1327AH/1909AD, the planted area with wheat in Tripoli was about 35.7 thousand hectares and barley was 7.8 thousand hectares. In the context of the Ottoman state's interest in agriculture in the state of Tripoli, one of the documents indicated that in year 1321AH/1903AD, they had brought 40 sacks of wheat seeds and distributed to the Libyans farmers to take advantage of fertilized land and production of this type of wheat which was highly demanded in Turkey at that time. The estimation of total area allocated to cereal cultivation in the state of Tripoli was about 990 thousand hectares with 78 hectares allocated to the cultivation of fodder for animals. (Mohamed Mabrouk, d. T., p.120)

The English consular Jago had referred in his report that the exported quantities of wheat and barley from the west Tripoli during the period 1295-1278AH/1878-1862AD reached a total amount of 18,78,000 Lira and with a total amount 5,067,000 Lira of wheat and barley. The imported wheat was 7,952,000 Lira and wheat 10,422,000, with a net difference for the trading budget equal to 1,478,00 Lira, which led to an increase in the production (English consular report in west Tripoli, Franshisko Koro 1984AD, p.93). Even though, the agricultural crops were affected by rainfall from year to year. This impact of rainfall was clear in the exported quantities of barley and wheat to England from years 1324-1328AH/1906-

1910AD. The instability in the quantity of agricultural crop in the year 1324 AH /1906AD reached an amount to 380,300 quintals and fell down in year 1328AH /1910AD to 227,100 quintals. Other products of west Tripoli region was dates which was exported on an average of 120-130 thousand lira. The kind of dates was not excellent and not at the same quality level as in Tunisia and Egypt. It should be noted that the dates of Fezzan was one of the best dates in Libya. The estimated exports of dates from 1316-1319AH/1899-1902AD as follows:

In 1316AH /1899AD, the exported dates was 120,000 francs, in 1317AH/1900AD the exported dates was 145,000 francs in 1318AH/1901AD was 50,000. During the year 1319AH/1902AD the exported dates was 120,000 Francs (Fatehia Ali Abdullah, 2006, p. 48).

The palm tree has played an important role in the lives of the Libyan people because its relationship with daily feeding. Therefore, the Libyan people had got great benefits from the palm trees because palm trees was the only food for them and for their animals. They made roofs for homes and hats from palm stumps, they also made brooms from palm leaves (Yassin Shihab, 2006, p. 102)

The state of Tripoli was known for olive planting since ancient times. Especially during the times of Carthaginians and Romans, olive trees spread across the state especially in Misalata region, Tarhunah, Nafusa Mountains, Gado, Rahibat and Gharyan, which was famous for the quality of olive, (Nikolai Iblich, 1988, p. 29).

The production of west Tripoli in the year 1328AH, 1910AD was about 60 thousand quintals of olives. The oils and derivatives of olives were exported through the port of Tripoli to Europe. Also olive was exported from old Sabratha, as indicted in the historical monuments with the existence of barmaid of oil until the present time, which was made since the Roman era (Mustafa Hamed, 1999AD, p.281)

The governing authorities were always encouraging the people through periodic publications and instructions about the best ways of planting olive trees, despite the lack of capability. (Document No. 28. Dated 1900)

The authorities worked through official newspapers to raise the level of awareness and interest about the olive tree and converted the vast unplanted areas to planted areas with olive trees. The authorities also had focused on the implementation of the program of taxation and its formation of committees inventory of land and agricultural property including olive trees.

In 1302AH/1884AD a decision was made from the state commissioned officer who went to Mislata city and meet the members of the city council at that time, he had selected local officers form the council, and then established a committee included writer and accountant to count the number of olive trees in the city because of the economic importance to the state (Ghaith Abdullah, 2010, p. 98-99)

One of the fruit trees we have found was citrus, which were grown in orchards and farms in Tripoli and Al-Zawyah. Most of the production of citrus was orange and lemon. Also other trees such as grape, figs, peaches and apricot, apples, that was exported to Malta , Tunisia, England and Germany at that time. The estimated export value was about 60 thousand Lira per year.

We can identify the types of trees and their number from the following table (Naji Mohammed, Nouri Mohammed, p.33,1973AD)

Type	Number of trees	Type	Number of trees
Orange	39.000	Pomegranate	750
Grapes	40.000	Quince	300
Limon	19.000	Almond	800
Pears	300	Apples	400
Figs	800	Total number: 101	,350

Tobacco: The plantation of Tobacco and working with it in the state was locaed in coastal cities in Libya and some cities in the south of Libya like Sukna and was familiar of trading in Tobacco. After the process of harvesting, the agricultural production was delivered to the government office. Tobacco was classified into two types, and these two types were planted in Fezzan and Tripoli region. Tobacco of Tripoli was planted in the western region like Al Zawiyah and Gharyan city, which was close to the state center like AL Jumah market and Tajora'a with an estimated area 300 hectares, but it was a low quality class, whereas the Tobacco of Fezzan was planted in the southern region of the state or what is known Fezzan or Sabha in the current time, and this Tobacco was the best class, and it was produced double times of Tobacco of Tripoli per one hectares, also it was cultivated and harvested in June. The single hectares produce 3-4 tons (Adel Mohammed Abdul Aziz, 2000, p.340) and this agriculture needs for plenty of water, which was one of the main obstacles to develop due to lack of water resources (Kakia Antoni Jousef, 1975, p.174)

The government authorities have worked to monopolize the plantation of Tobacco and had made agreements with foreign companies in order to be responsible for plantation of Tobacco. In year 1301HD/1884AD, the state government had made an agreement with a French company which was known AL Reji which had granted a resolution to authorize the company to start the plantation of Tobacco as well as manufacturing. This company had allocated areas for planting Tobacco in Tripoli and in the surrounding areas, and the city of Al Zawiah and Gharyan as well (Adel Abdul Aziz, 2000,

p.340) therefore we can say that the activities of Jewish people was limited even though they were doing trading in the agricultural products of the state, but the Jewish people were not interested to work in this type of business because at that time they were transferring their business from agriculture to trading and industrial crafts, and it was known that Jewish people did not own lands for agriculture purpose.

1.3 Livestock

The livestock has been associated with the life of Bedouin people and rural areas either for their food or non-food products. People everywhere since the ancient history had used animals for many purposes in the fields such as plowing, irrigation and provided natural fertilizer for the land. Animal had represented the sole mean of transportation at that time in the state of Tripoli, whether for individuals or for transportation of goods, whereas camels was playing a major role in the transport and trading on the convoy route. On camels' back, the man cross the deserts and travelled across the whole desert areas. (Ben Moussa Taiseer, 1988. p. 131)

The spread of many types of animals in the state according to the appropriate areas for animal's life, and there were many animals in different regions of the state. The livestock was one of the main sources of income to the state and had contributed to develop the economies of the state. The livestock included; sheep, goats, cows, camels, horses, mules, and donkeys. (Mustafa Hamed: 1999,p. 281, Kakia Anthony Jouseph 1975, p.126).

The rainfall affected the livestock from year to year, in the year where there was increasing rate of rainfall, the agricultural and animal products were overflowed, and the opposite occurred during drought years, for example; in years 1326AH/1908AD and 1328AH/1910AD, the drought period was very hard on agriculture in the state and worsened their economic conditions. (Fatehia Ali Abdullah, 2006, p.51).

Cooper (the English) had reported in his testimony for livestock an existence of 350,000 camels and 30,000 horses in the state. Kakia Anthony had also referred to the existence of cattle, sheep and goats, and he stated that the cows in Libyan were giving a little quantities of milk and was used in the manufacturing of oil exported to Turkey and other foreign countries, and this was because the people was preferring goat milk.

Livestock had contributed in some years in supporting the state's economy, and had played an important role in the export volume of the state. In 1324AH/1906AD, the state of Tripoli had exported about 500,000 heads of cattle and during 1326AH/1908AD, and according to a report from the Italian consulate in Benghazi at that time, a delivery of 700 heads of small bulls from Barca to Malta, and at the same time the state had exported about 340,000 heads of sheep and goats to Egypt.

Wool was delivered monthly to France and Italy as a good quality product, but the price of exported wool was not expensive because the wool was full with sands and dirt and not clean. Other exported products was bones and goat hair and dander from camel leather, also there was few small factories established for tanning. (Francesco Kourou, 1984. p. 96)

And according to official and non-official sources, the estimated number of goats and sheep was two million heads, and in one of the statistical documents for the year 1323AH/1905AD that shows what belonging to citizens in a number of areas of the sheep and the goats, the numbers according to the following table. (Ben Moussa Taiseer, 1988, p.134)

Region	Number of Sheep	Number of Goats
14.154	13.906	Al Azizyah
4.391	2.923	Tajoraa
27.550	30.977	Al Ruqat
4.962	5.732	Al Alawnah
145	336	Al Sahel
367	373	Al Manshiah

The report of Salnamah of year 1200/1285AH, 1786/1869AD, was not an accurate statistics and numbers of animals to the state as outlined in the following table (Yassin Shihab, 2006, p.106)

Туре	Number of Animals	Туре	Number of Animals
Sheep	1.287.000	Camels	35.000
Goats	700.000	Horses	14.000
Cows	32.000	Mules	300
Calves Cows	10.000	Donkeys	Undefined number
Bulls	25.000	All types of Poultry	5,000,300

The livestock was an important source for the local economy, and was not only because of the animals themselves but because of their products such as wool, leather, dairy, and camel dander, and these products and animals were exported to the neighboring countries and the countries of the Mediterranean Sea. According to that the Jewish people had been professional in grazing and animal husbandry, and this because the countries of north Africa was famous in sheep production due to population nature and geographic factors (Hirschberg.A.1975 p262), and since then livestock was of great importance to the economy of Maghreb countries, and because the majority of the population was Bedouin at that time, therefore many of the industries were connected to animals and their

production. Jewish was famous for making cheese from milk and selling them. In summary the agriculture, grazing, animal husbandry was not mainly the main profession of immigrant Jewish people (Bashir Abdul Rahaman, 2001, p.91)

The Jewish companies had played a vital role in importing and exporting of livestock. The report of Italian consular showed that the animals were exported from west Tripoli to Malta because the type of meat of cows in Tripoli was very good and strongly demanded.

Due to the lack of milk, the process of fattening was achieved in Malta; the sheep were excellent kind and have tails which was weighing up of 5-6 kg. The exported cattle annually was 7800 head which was worthing to 624,000 francs, as for the sheep, only 2880 head worth amount 34,560 francs. The price per sheep was 8-12 francs. The export through Carmili Follett "English" and Carmili to Tkabardo "Italian" and Shalom Qbeson "Ottmanian"

Jewish people also worked in exporting animal bones through a Jewish company called Berjis De Silva from Portugal, the company was exporting annually around 100 tons with an estimated amount reached to 8000 francs at that time, and the company we receiving the bones from the centers for slaughtering animals at a price 8 Franc per kg and then delivered to Genoa, Italy. The horns and hooves of animals were exported to Marseille annually, the estimated quantity was 10,000-12,000 kg, with a total amount equal to 1500 Franc from the same Jewish company.

The work of both Jacob Arbib "English" and Joseph Ksar "English" and Shalom Qeson "Ottmanian " and Shalom Habib " Ottmanian " and Haim Hassan "Italian" on exporting the skins of cows, goats and sheep

The number of goat skin that were shipped annually was about 208,000 dozen colorful and dried, and the total amount was reaching around one million francs exported to United States through Marseille.

The number of sheep skins that were shipped annually was about 20,000 kg, and the total amount was around 12,000 Francs exported to Naples, and Hamburg (Al Ahwal, Khalifa Mohammed. 1985. p. 341-342)

1.4The results

The regulations and reforms that had been made by the Ottmanian authorities and in particular what were associated with applying the law and rules that regulates the economy of the Ottmanian state that include the western region of Tripoli had achieved contrast results in some times due to foreign interfere as well as the natural factors.

The study showed that Jewish people had dealt with the economic activities they had practiced at that time through handcraft, industrial and commercial professions. The volume of economic activities of Jewish people had been expanded at the end of the 19th century because of the foreign interference and giving the Jewish people foreign protection that allowed them to work freely without obstacles from the state authorities and government bodies at that time.

In addition to that the foreign privileges in all Ottmanian states had gave the Jewish people complete freedom and essential role in building economical and political relationships between the state and European countries, they had acquired commercial agencies and exporting rights to the European nations.

The study showed that agricultural activities in the state of west Tripoli was very active, and it had provided a good income for the population at that time despite that agriculture was primitive and depended on traditional old means and ways.

In addition to that the study emphasized on the role of climate in the agricultural environment such as rainfall and shortage in water resources.

The study highlight that the governors of the Ottmanian state were mainly interested in agricultural activities and encourages the local people and farmers for plantation and taking care of fruit trees, as in year 1869AD the funds of public benefits had been established in order to provide loans for the farmers and agricultural projects

The policemen were giving several tasks such as protection of agricultural crops and monitor people who damage the trees and crops and punish them.

The common agricultural crops in the state were; (barley, wheat, olive and citrus trees, tobacco). The Jewish people had small contributions in agricultural activities, because they had owned small agricultural areas suitable for plantations.

The role of Jewish people in agriculture was limited even though they were doing trading deals in agricultural products of the state, but the Jewish people were not mainly interested to work in agriculture because at that time they were transferring their business from agriculture to trading and industrial crafts, and it was known about Jewish people they did not own lands for agriculture purpose.

The study found that Jewish companies had played an important role in exporting and importing of livestock products represented in; (leather, bones and sheep wool) were export from Tripoli to Malta, Italy, Marseille, England and other European countries, and because these products are essential for factories in European countries as a raw material as well as livestock had contributed to the economy of the state for many years and was one of the main factors for economic growth in the state at that time.

References

- 1. Abdo: Mabrouka age (2007), Police in the state of Tripoli during the second Ottoman 1835. 1911. Unpublished Master's Thesis, University of the seventh of April, Libya.
- 2. Abou AL the Kasaba: No. 13 on 1328AH: Tripoli, the Historical Archives.
- 3. Ahmad Sidqi Dajani (1971). Libya and the Italian occupation. 1st edition. House Ferjani Tripoli Libya
- 4. Al Araby Ghaith Abdullah (2010), Mislata in the second Ottoman 1835 m -1911 m study in the political, administrative, economic and cultural, edition 1, publications of the National Center for Mahvutat and historical studies, Tripoli, Libya.
- 5. Al Mahdawi Mohamed Mabrouk, d. T., Population geographic Libya, established the People for publication and distribution, advertising, Libya.
- 6. Al Mousely Yassin Shihab (2006), Economic conditions in the state of Tripoli and Provincial Benghazi 183,(1911), 1st edition. Libya.
- 7. Alhacaúhi Mohammed bin Othman (1965), trip Alhacaúhi to Libya. Provide and achieve Ali Mustafa Misrati.
- 8. Anthony Kakia (1975). Libya during the Ottoman occupation II 1835 1911 m. edition 1. House Ferjani Tripoli Libya
- 9. Bashir Abdel Rahman (2001), Jews in the Arab Maghreb 642-1070m, 1st edition 1, appointed for Islamic Studies and Research.
- 10. Benmoussa facilitate (1988), Libyan Arab society in the second Ottoman, Libya Arab Book Publishers, Tunisia. Libya.
- 11. British consul in Tripoli report west, center of Libyan Jihads for Historical Studies, Tripoli, Libya.
- 12. Brocan: Nikolai Iblich (1988). Libya History in the nineteenth century until 1919, translated by Imad Eddin Ghanem, the Libyan Jihad Center for Historical Studies, Tripoli, Libya. Construction Press, Damascus.

- 13. Document No. 1080, The file of agriculture, industry and minerals. Libyan Jihad Center for Historical Studies, Tripoli, Libya.
- 14. Document No. 129, The file of Administrative Affairs. The house of historical manuscripts, Tripoli, Libya.
- 15. Document No. 13. The Economic file documents. Libyan Jihad Center for Historical Studies, Tripoli.
- 16. Document No. 270, The file of agriculture, industry and minerals. Libyan Jihad Center for Historical Studies, Tripoli, Libya.
- 17. Document No. 28 on the instructions for planting olive trees. Dated 1900, the agriculture file, the house of historic manuscripts, Tripoli, Libya.
- 18. Egyptian Hussein Mujib (2004), Lexicon of the Ottoman state, 1st edition, Culture Publishing House, Cairo.
- 19. Francesco Kourou, 1984, Libya during the second Ottoman, translation Khalifa Mohammed Al Tlesi, edition 2, Libya, Tripoli.
- 20. Ghariani. Adel Mohammed Abdul Aziz (2000), Mandate of Tripoli during the second Ottoman 1835-1911, with particular emphasis on the study of patterns of economic and social life in the city Sukna. Ph.D. thesis. Malaysian National University of Bangui.
- 21. Hubail. Mohammed Abdul Majeed 2004, Tripoli 1795-1858m study of patterns and economic conditions. Unpublished Master's Thesis, University Alzaria. Libya.
- 22. Ibrahim Fadia Abdel Aziz (2006). Federal Turks position of the Italian invasion of Libya. edition 1. Publications Libyan Jihad Center for Historical Studies, Tripoli, Libya.
- 23. Lyon: John Francest (1976). Tripoli to Fezzan Mustafa quality. Arab Book Publishers, Libya. Toon.
- 24. Marian: Fathia Ali Abdullah, in (2006), the plant allies as an economic resource for the State of Tripoli West (1868 1911), 1st edition, Libyan Jihad Center for Historical Studies, Tripoli, Libya.
- 25. Mobil to Omci (1968), the secrets of Tripoli, library Ferjani, Tripoli. Libya.

- 26. Naji Mahmoud (1995), Tripoli, Libya, Tripoli, The house of Ferjani.
- 27. Naji Mohammed Nuri Mohammed (1973), Tripoli, Translation of Ikmal Mohammed Ihsan, Library House thought, Libya.
- 28. Nicholas increase (1966), Libya in early modern times, Cairo Institute Arab Research and Studies.
- 29. Rahouma. Mustafa Hamed (1999), Economic life in Tripoli patterns of economic activity and trade prospects with home and abroad, Libyan Jihad Center for Historical Studies.
- 30. Sergeant Gazette No. 21, the first year in Tripoli 1329AH. Tripoli, the Historical Archives
- 31. Tripoli newspaper, Volume 13, Issue 226. 18 April 1377, 10 November 1957.
- 32. Tripoli newspaper: Year 4 No. 1358. Date March 26, 1329AH 14 March 1327. Tripoli. Historical Archives.

Why Many People Wish To Use Public Transport System: A Case Study in Tripoli-Libya

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Abstract

In many cities today, owning a car has become an important and dominant mode of transport. Increasing dominance of own cars as a mode of transport is due to its inherent advantages associated with its usage. In Tripoli, Libya due to rapid increase in car usage associated with poor public transport system has resulted in an increase of traffic congestion, accidents and lack of parking spaces. This study aims to identify the factors preventing owning cars from shifting to public transport system (PT). And to develop model shift from owning cars to public transport in order to formulate policies to achieve this. A policy to enhance public transport system usage and control car ownership simultaneously has become necessary for solving traffic congestion problems. A questionnaire survey was carried out on users of transport in Tripoli such as own car, taxis, microbuses and coaches drivers (n = 900). The probability of car drivers shifting to public transport was examined based on scenario of several options such as reduction in public transport travel time and travel cost. Reduction in total travel time and cost for public transport mode emerged as the most important elements in attracting car users towards using public transport system. Logistic regression technique has been used to analyze the factors that impact users to switch their trips to public transportation alternatives. Statistical Package for Social Science (SPSS) version 19 and Excel 2007 software were used to analyze the questionnaire in this study.

Key words: public transport, car ownership, logistic regression, mode choice and traffic congestion.

Introduction

Tripoli is the largest Libyan city and port, and it is the country's capital. It is the meeting place for the People's Congress representing the government; it is also known in Arabic as Tarabalus Al-Gharb, or Tripoli of the West. The population of Tripoli is about 1,682,000 people with area of 400 square kilometers and population density 2207.32 people / sq km [8]. Figure 1 Shows the location of Tripoli where the survey was carried out.



Fig. 1 Tripoli location and study area

Source: Google Earth 2009

The survey was carried in Tripoli due to the high number of car ownership and poor public transport system services together with high traffic congestion at the selected area. This paper is a part of the study that

focused on model shift initiatives. These initiatives focused on shifting own car users to safer modes of public transport in order to increase road safety and enhance road environment. Many cities have attempted to restrict the use of own cars in favour of public transport, such as policies exist in Egypt [11], Dubai [4], Saudi Arabia [2], Lebanon [7], England [5], Romania [9], Malaysia [1], Asian countries [6], Dublin [13] and France [5]. The attempts have been by changing the public perception to it.

Own car is one of the important modes of personal transport in Libyan cities, mainly because it is cheap and more reliable than the current transport modes available namely taxi, coaches and microbuses. Every Libyan adult person almost owns one or more cars. They can travel from place to place in safe way. Also, it is provided with comfortable mechanisms such as a cover to protect him from the heat and rain. The other transport type available in Libya is Taxi. Taxi is a very reasonable and easily available mode of transport. Statistics of the secretariat of the Libyan justice in seventies to end of 2009 illustrated that 80% of Tripoli residents used their own cars to go to work, study, shopping and else. The number of vehicles in Libya was steadily increased in 1970s and early 1980s. By 1985 there were 313,000 automobiles and trucks in the country, as well as about 70,000 different types of buses. At the end of 2008, vehicles has increased approximately to 2,052,679 vehicles [12].

Study Problem

Understanding travel time, cost and the reasons for choosing one transport mode over another is an essential issue. However, travel time is more complex. For each trip, commuters have the choice between different modes of transportation. Each mode has specific characteristics, such as advantages and disadvantages associated with travel time and travel cost. Commuters in Tripoli used public transport namely microbuses, private taxies and coaches and own cars to their works, study and shopping activities. Public transport can be owned and operated by individuals or

private companies. The uncontrolled usage of these transport modes has caused traffic congestion problems which has increased travel time, road accidents and air pollution to the city environment [3]. Nevertheless, own cars users have become more popular and dominant than other modes of transport in Tripoli city due to their availability, flexibility and convenient for travel when required. Own cars also represent high status, comfort and safety. Due to the complex scenarios happening here, a study has been carried out to understand the traffic congestion and try to establish suitable models system to reasonably described travellers attitude and perception in Tripoli city. Figure 2 shows framework factors that has caused traffic congestion in Tripoli city.

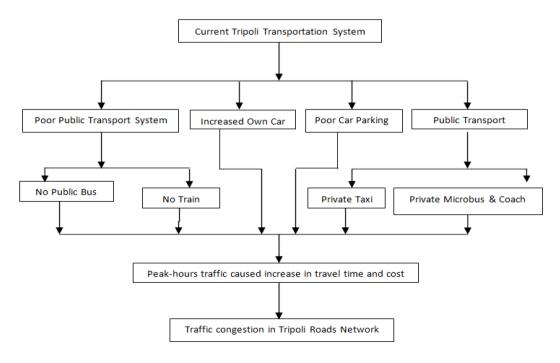


Figure 2 Tripoli traffic congestion factors framework

Methodology

Primary data are generated by researcher who is responsible for the design of the study and the collection, analysis and reporting of the data. These data are used to answer specific research questions [10]. The data collection was done through a field study i.e. observation and survey. The survey was done using questionnaires to get relevant data. The respondents for this survey are the own car users at study areas who use his / her own car to make his / her trips to work, study and shopping. The questionnaires were distributed to the private vehicle users who do not use other modes of were transportation. The respondents selected randomly. questionnaires were formed to ensure user's comprehension and they are in the form of open and close ended questions. The questionnaires were printed by two languages, Arabic and English language, to provide easier understanding and answering for some respondents. There are three sections in this survey. Section A is about respondent's personal information that will help the researcher to get the respondent's basic information for this research. Section B is a section that requires respondents to fill in information about their trip characteristics and purpose such as work, study and shopping trips. Section C is a section that requires respondents to highlight their preference in mode switching in the study area. This questionnaire has 51 questions which covered question three sections as explained above, frequency using a private vehicle for work, study and shopping purposes, problems when using private vehicles and travel time and cost. These questions were formed based on the research questions and the hypothesis. This survey had been conducted in areas under Tripoli authority which do not have public transportation system services, inadequate public transport services and shortage in parking facilities. In total, there are four zones which do not have public transportation services. This survey was done on work days (Saturday through Thursday)

A total of 900 questionnaires were collected in 5 months from (25 July to 23 December 2015). There are several questions that correspond with respondents' views, recommendation and opinions. These questions provide the opportunity for the respondents to give their opinion. The questions are formulated in such a way that could help the respondent answer the questionnaire easily and quickly. Respondents were selected randomly from residential areas which does not have public transportation system services. The selected respondents are based on public transportation vehicles and own car users who use their vehicles as their mode of transportation to go to their trips. The following section will elaborate how this selection is made. Statistical Package for Social Science (SPSS) and Excel software were used for analysis the questionnaire and logistic regression method was used in this study.

Results

Alternative Mode of Transport for Car Users

The study made an attempt to determine whether car users had access to other modes of transport. Results from the study indicated that about 88% of car users had an access to the public transport mode, while 12% had no access to alternative modes. The question was written as to explain if the government provides a good level of service by establishing separate lanes of public buses and providing car parking areas in main public bus stations, where these services will encourage the respondents' shift into PT as can be seen in Figure 3. Figure 3 shows that 88% of respondents liked to use PT if the government improved and initiated good PT services, also providing the bus lane and car park facilities and suitable parking cost per hour and just 12% disliked using PT and preferred to use private car.



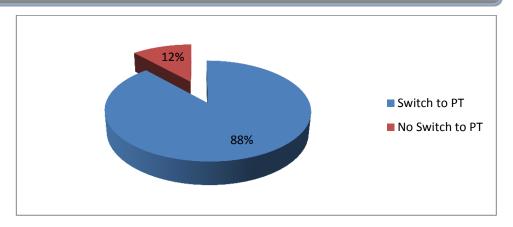


Figure 3 Improvement of the PT services can encourage people to shift

Factors Contributing to Car Popularity

The study describes specific questions in order to explain factors that contributed to car use as opposed to bus and train use. The key factors addressed were: 1) Available, 2) Reliable, 3) Comfortable, `4) Satisfactory, 5) No expensive, 6) Safe and 7) Prestige. Table 1 shows that private cars are more desired over all other available modes in Tripoli. The 39.5% or 296 respondents said the car was always available, 14.7% or 110 respondents pointed out that it was reliable, 23.2% or 174 respondents said that it was comfortable, 5.3% or 40 respondents stated that it was satisfactory, 4.4% or 33 respondents mentioned that it was not expensive, 12.9% or 97 respondents mentioned that it was safe and 0% had seen having the car as prestigious.

Table 1 Reasons support to use your own car

No	Statement	Percent
1	It is available	39.5
2	It is reliable	14.7
3	It is comfortable	23.2
4	It is satisfactory	5.3
5	It is no expensive	4.4
6	It is safe	12.9
7	It is prestige	0

Reasons Convince to Switch from Using Own Car to Public Transport

Considering the reasons to a modeshift from car travel to public transport, it is necessary to understand the factors which support the great majority of active car users t use public transport (bus and train) as a regular means of transport. Table 2 presents the major reasons identified when survey respondents were asked to name or select from a given list, the factors which would influence their decision to use public transport. Table 2 shows the factor of most significance encouraging car users to use the public transport was that the "High traffic congestion and delay". This statement received an average rating of 1) 30.3 % if the PT service is available, 2) 6.3 % if the PT fare is cheap, 3) 20.1 % if the PT service is fast, 4) 29.7 % if the PT covered all desirable routes, and 5) 13.6 % if the PT vehicles are clean and comfortable.

Table 2 Reasons can convince to switch from using own cars to public transport

No	Statement	Percent
1	If the service is available	30.3
2	If the fare is cheap	6.3
3	If the service is fast	20.1
4	If the desirable routes are covered by public transport	29.7
5	If the vehicles are clean and comfortable	13.6

Deterrents to Mode Shift from Car to Public Transport

Considering the deterrents to a modeshift from car travel to public transport, it is necessary to understand the factors which deter the great majority of active car users from using public transport (bus and train) as a regular means of transport. Table 3 presented the major deterrents identified when survey respondents were asked to name or select from a given list, the factors which would influence their decision not to use Public transport(PT). Table 3 shows the factor of most significance discouraging car users from using the public transport was that the 1) 31.2 % PT do not covered all desirable routs, 2) 26.4 % PT vehicles are too crowded, 3) 21.7 % in frequented PT, 4) 10.9 % PT unreliable services, and 5) 9.7 % PT uncomfortable.

Table 3 Factors can discourage you from riding public transport instead of your car

No	Statement	Percent
1	Vehicles are too crowded	26.4
2	Unreliable services	10.9
3	Uncomfortable	9.7
4	In frequented public transport	21.7
5	Desirable routes not covered by public transport	31.2

Improving Travel Time for Public Transport

The main factors affect decision making in choosing travel mode from, in and to Tripoli city are travel time and travel cost. Travel time is considered an important reason for mode choice. Using public transport services is perceived as a waste of time by almost all own car users. The data were represented in the form of cumulative format in the third column in Table 4. The main factors affecting travel mode from/to Tripoli city are travel time and travel cost which had been supported by the findings in this study. Given its significant impact, travel time is a salient consideration criterion in travel mode shift. The mode shift probabilities were categorized by various categories (percentages) of travel time reduction, simplified in Figure 4. According to Table 4 and Figure 4 the mode shift probabilities ranged from 92.8% probability of private car use with public transport reduction travel time by 10% to 1% probability of own car use with a travel

time reduction of 90%. At the same reduction time level (10%), the probabilities of public transport user increased from 7.2% to 99% of probability when the reduction level of travel time per trip reached 90%. A 50:50 split (or own car to public transport ratio 1:1) may be achieved if travel time of public transport was to reduce approximately 50% per trip. For instance, if 70% of respondents were to use public transports, the travelling time has to be reduced by approximately 85%.

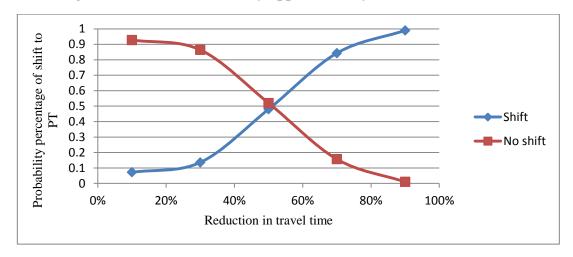


Figure 4 Shifting to public transport if the travel time improved

The study further reveals that the main factor influencing decision making in choosing travel mode in Tripoli city is the reduction of travel time. Table 4 shows reduction Of travel time to work and study by public transport with respect to survey results and probability of prediction (P) values. The P value is derived from Equation 1 which involve constant (D) and coefficient (α) values to verify the logistic prediction model used in this study.

$$P = \frac{1}{1 + De^{\alpha(x)}}$$
 (1)

Where P = Probability prediction of shift to PT

D = constant

 α = coefficient of x

x = level of travel time reduction

e = the base of natural logarithms (approximately 2.718)

Table 4 Survey results and data calibration

Category	Reduction Travel Time	Cumulative survey result (P)	1-P	1-P/P	Ln (1-P/P)
1	10%	0.072	0.928	12.88889	2.556366
2	30%	0.136	0.864	6.352941	1.848918
3	50%	0.48	0.52	1.083333	0.080043
4	70%	0.843	0.157	0.18624	-1.68072
5	90%	0.99	0.01	0.010101	-4.59512

Simple linear regression analysis was then conducted by using Microsoft Excel to obtain the intercept constant and the α value. The results in Table 4 above reflect the process of calibration which then introduced into Excel to estimate the alpha (α) and intercept values in ANOVA which is described on Table 5. Based on the ANOVA table, some important factors reflect the study significance which is the values of R^2 , intercept

coefficient and the important factor was alpha value which is used in the equation 1 above to verify the used model. p values of 0.00395 as well as 0.00643 showed that the coefficient (-1.7833) and intercept (4.99168) were significant at 0.05 significance level. With R² value of 0.956148 shows that the variable, namely travel time reduction was able to explain 95.61% of variation in the dependent variable, Ln (1-p/p). The probability function for transport shift is written in Equation 2.

Table 5 The ANOVA table result

		X Variable
	Intercept	1
Coefficients	4.99168	-1.7833
Standard Error	0.73128	0.22049
t Stat	6.82593	-8.0877
P-value	0.00643	0.00395
Lower 95%	2.66441	-2.485
Upper 95%	7.31895	-1.0816
Lower 95.0%	2.66441	-2.485
Upper 95.0%	7.31895	-1.0816

LN D = 4.99168, $\alpha = -1.7833$

D = 147.1835

 $R^2 = 0.956148$

Where R^2 approaches one value that indicates the model's strong correlation power. Thus, the result of the prediction models can be shown in Table 6 and Figure 5

$$P = 1/(1 + 147.1835e^{-1.7833(x)})$$
 (2)

Table 6 shows the respective survey results and model prediction results pertinent to the travel time reduction. Respective results can be substituted into the calibrated logit model to validate its functionality. For instance, at 50% level of reduction travel time, the predicted cumulative p = 0.588581, which has maximum 10.9% (0.588581-0.48) probability different from the data collected from the survey. Furthermore, Figure 5 shows the plots for both survey and prediction results. From the graph, the steep slope was discovered from 30% to 70% travel time reduction, indicates that high probability of shifting into public transport occurred at time reduction level range 50%-70%. Based on prediction model, the probability to shift at 50% is 0.39 (0.588581-0.19354) while probability to shift at 70% is 0.31 (0.894859-0.588581). These findings have implied that the majority of commuter users desired the travel time to be reduced by 50% to 70% if they were to take public transport.

Table 6 Survey results and logit model results

Category	Travel time reduction	Survey result (P)Cumulative	Result from logit model (P)
1	10%	0.072	0.03885
2	30%	0.136	0.193854
3	50%	0.48	0.588581
4	70%	0.843	0.894859
5	90%	0.99	0.980633

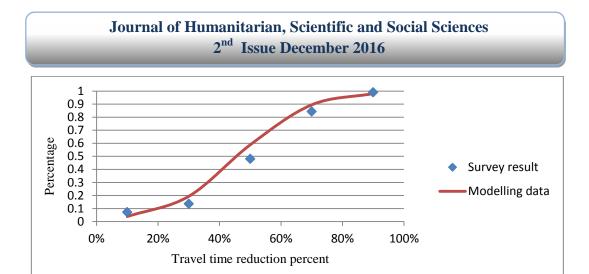


Figure 5 Improving travel time category for public transport shift

Figure 5 shows the high correlation between survey results and Logit model, where both of the survey results and the modelled ones were increased with the reduction of travel time.

Improving Travel Cost for Public Transport

The data are represented in the form of cumulative format in the third column in Table 7 Improving the travel cost for public transport by reducing it may motivate the public transport use. The model shift probabilities were categorized by various categories of travel cost reduction (%) as shown in Figure 6. Mode shift probabilities ranged from 91.7% probability of own car use with public transport reduction travel cost (10%) to 1% probability of own car use with a reduction of travel cost 90%. In other words, public transport users' probability increased from 0.083 with 10% public transport travel cost reduction to 0.99 of probability when 90% reduction in travel cost per trip applied. A 1:1 split may be achieved when travel cost reduction succeeded around 45% per trip by public transport (intersection between two lines).



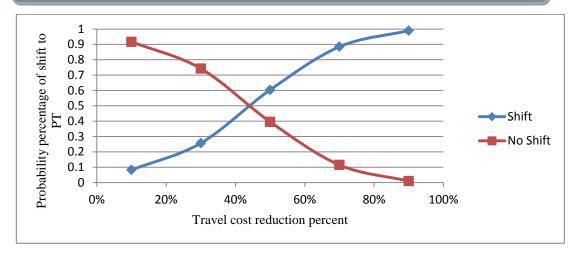


Figure 6 Shifting to public transport if reduction in travel cost

The results as shown in Table 7 below indicate some important statistics which reflect the model significance, such as R², T-test, intercept, and coefficient (alpha value). The intercept as well as alpha values were involved in the equation below to verify the used model see Equation 3.

$$P = \frac{1}{1 + De^{\alpha(x)}}$$
Where,
$$P = \text{probability prediction of shift to PT}$$

$$D = \text{constant}$$

$$\alpha = \text{coefficient of x}$$

$$x = \text{level of travel cost reduction}$$

$$e = \text{the base of natural logarithms (approximately 2.718)}$$

Table 7 Survey results and data calibration

Category	Reduce travel Cost	Survey result (P)Cumulative	1-P	1-P/P	Ln (1-P/P)
1	10%	0.083	0.917	11.04819	2.402267
2	30%	0.257	0.743	2.891051	1.06162
3	50%	0.604	0.396	0.655629	-0.42216
4	70%	0.885	0.115	0.129944	-2.04066
5	90%	0.99	0.01	0.010101	-4.59512

Simple linear regression analysis was then conducted by using Microsoft Excel to obtain the intercept constant and the α value. The results in the above Table 7 reflect the process of calibration which then introduced into Excel to estimate the alpha (α) and intercept values in ANOVA which is described in Table8. Based on the ANOVA table, some important factors that reflect the study significance are the values of R square, intercept coefficient and the important factor was alpha value which is used in the equation 3 above to verify the used model. p values of 0.00105 as well as 0.00219 showed that the coefficient (-1.7097) and intercept (4.4103) were significant at 0.05 significance level. With R² value of 0.981822 showed that the variable, namely travel cost reduction was able to explain 98.18% of the variation in the dependent variable, Ln (1-p/p). The probability function for transport shift is written in Equation 4.

Table 8 The ANOVA table result

	Intercept	X Variable 1
Coefficients	4.410305	-1.7097
Standard Error	0.44547	0.13431
t Stat	9.90044	-12.7293
P-value	0.00219	0.00105
Lower 95%	2.99264	-2.13715
Upper 95%	5.82797	-1.2823
Lower 95.0%	2.99264	-2.13715
Upper 95.0%	5.82797	-1.2823

Thus

 $LN\ D=4.410305$

 $\alpha_{=}$ -1.7097

D = 82.29456

 $R^2 = 0.981822$

Where R^2 approaches one value indicating the model's strong correlation power

Thus, the result of the prediction models can be shown in Table 9 and Figure 7

$$P = 1/(1 + 82.29456e^{-1.7097(x)})$$
 (4)

Table 9 shows the survey result together with model estimated data pertinent to the traveling cost reduction (%). The largest prediction gap was detected at 50% of travel cost reduction. Cumulative p value of 0.672345, which had maximum 6.8% scores was different from the survey data. Figure 7shows the graph plotted with the proportion (probability) of shift versus travel cost reduction (public transport) for both the survey and model. Based on the graph, it is noted that dramatic increment occurred at 30% to 50% travel cost reduction (model estimated) which suggests that the majority of users will most likely shift to public transport at 50% of cost reduction with probability of 60%.

Table 9 Survey results and logit model results

Category	Improving Travel cost	Cumulative Survey result (P)	result from logit model
1	10%	0.083	0.062938
2	30%	0.257	0.270735
3	50%	0.604	0.672345
4	70%	0.885	0.918976
5	90%	0.99	0.984299

The high correlation between survey results and logit model is clarified in Figure 7 where both of the survey results and the modelled one was increased with the reduction of travel cost.

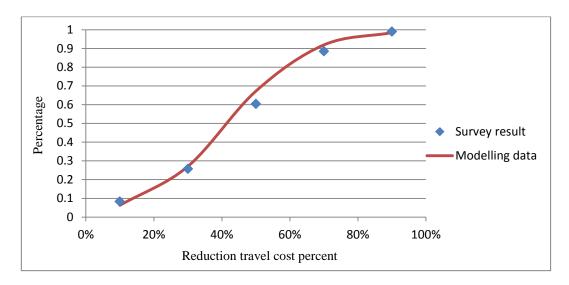


Figure 7 Improving travel cost for public transport

Own Car and Public Transport Mode in Present Time

Majority of Tripoli residents are suffering from the daily congestion in most city streets especially at peak period of working days (Saturday through Thursday). Table 10 shows in Tripoli 494 out of 600 respodents or 82.3 % of the respondents prefer to use public transport to avoid traffic congestion.

Table 10 Would like to use public transport or prefer your own car (n 600)

Statement	Frequency	Percent
Yes, use public transport	494	82.3
No, prefer use own car	106	17.7

Buses is the Best Public Transport Mode use in Tripoli

Table 11 illustrates 33.3% of the respondents prefer to use buses in city streets while, 28% like to use light rail transit, 24.5% for train and low percent about 14.2% prefer to use public taxi.

Table 11 The best public transport mode use in Tripoli (n 600)

Statement	Frequenc y	Percent
Buses	200	33.3
Light Rail Transit	168	28.0
Under Ground Train	l 147	24.5
Public Taxi	85	14.2

Discussion

Traffic congestion is not a new problem for the central city; it has spread to cover suburban areas. The study in Tripoli city has revealed that own car and public transport vehicles are becoming popular among urban

travellers which has caused acute traffic congestions and spread out widely, especially along the roads to Tripoli city centre. This study has revealed that positive and negative operations are needed to encourage commuters to shift from own car and private transport to public transport. The positive operation would be to reduce travel time, cost and improve service (more frequent and more on-time trips), and the negative operation such as to increase the parking fees and reduce the parking spaces. The collected data were obtained from our survey which was subjected to the logit model prior to the calibration process. D and α value were extracted to be used in our model equation. Then the validation process took place to fit our results into the model. From the ANOVA results, the value of R square was within the normal range. Based on the given results, our model was approximately significant with the P value of < 0.05. Results of the study have shown that more time will be used to travel by own car and private transport for all trips. These scenarios have encouraged the commuters to shift to the public transport (PT) system.

Conclusion

Transportation sector is an important thing in human daily life to make their daily trips easier. But, the total of private vehicle on road was increased and cause many problems such as traffic congestion, air pollution and else [8]. Private vehicle users in Tripoli are suffering from transportation travel time, congestion and lack parking if compared to public transportation users in some big cities such as Cairo, Dubai, London, Paris and else. Increasing of travel time causes several problems to all vehicles users like increasing daily traffic congestion. Changing to public transport system alternatives namely buses, train and LRT could reduce the traffic congestion. A study has been done at Tripoli areas among private vehicle namely own car, taxi, coaches and microbuses users, who live at areas with acute poor public transportation system services in the city. Logistic Regression Model has been used to analyze the factor that will be

influencing users to switch their travel behaviour to public transport system alternatives. Poor public transportation system services especially public buses at certain places in Tripoli city is the reason why travellers prefer to use own cars. In fact, there are several areas that do not have public transportation services in Tripoli. This situation forces residents in these areas to use own car vehicles to make their daily trips. If public transportation services provide good and adequate, the trip maker might shift their trip behaviour from travelling by own cars to public transportation systems. This will save their expenditure on transportation and reduce traffic congestion. The improvement and enhancement of travel time and cost will encourage switching to use public transport mode in providing the best services and suitable travel facilities. The current mode split is 80% use private car and 20% who use the public transport namely taxi, coaches and microbuses. If improving the public transport travel time by 70%, this will encourage the 81.2%. And by 70% improve of travel cost, will encourage the 86.2%, to switch from using own cars to use public transport system services. The need of realignment the Public Transport system namely Buses, Light Rail Transit and Underground train is very important to releaf the traffic congestion problems in Tripoli roads network and streets.

References

- [1] Abdalla N, Kamba, Riza Atig O,K Rahmat & Amiruddin Ismail. 2007. Why Do People use Their Cars: A Case Study In Malaysia. Journal of Social Sciences 3(3):117-122.
- [2] Abdul Jalil. Statistical Analysis of the Number of Traffic Accident in Saudi Arabia paper Presented to symposium Studies Traffic in Big Cities. Security Academy of Naif 1401.
- [3] Colonel Fathi Bashir Alrabotti: Traffic Lights, part two 2007 (Tripoli).

- [4] Ghaya Adam Almannaei, Dr Arif Mehmood: Analyzing the impact of Traffic Congestion Solution on Road Safety. A case of Emirates and Sheik Zayed Road in Dubai (2005)
- [5] Harrison, S, Henderson, G, Humphreys, E & Smyth, A 1998. Quality bus corridors and green routes: can achieve a public perception of permanence of bus services? In Proceedings of the PTRC 26th European Transport Conference: Public Transport and Operation, 14-18 September 1998, Loughborough University, UK
- [6] Land transport Authority LTA, 1996. A world class land transport system' white paper presented to Parliament, 2 January 1996
- [7] Lebanon state of the Environment report, Ministry of Environment / LEDO, Chapter 5 pages from 65 83 (2001)
- [8] Libya country Profile Published 13 November 2007, last update 21 September 2008
- [9] Marshall,S & McLellan, A. 1998. Reducing the need to travel: a European guide to good practice in proceedings of PTRC 26th European transport Conference: policy, planning and sustainability :14-18 Sept. Loughborough University, UK
- [10] Regional workshop on urban transport in the Mediterranean Region, Blaikie 2003, Janury 22&23,2008 –Skhirat- Morocco.
- [11] Traffic Analysis on Main Corridors in Mansoura City, Dakahlia Governieate, Egypt. Dr. Mahmoud El-Shourbagy, Associate Professor, King Saud University, Civil Eng. Department, college of Eng (2003).
- [12] Traffic office and licensing of Tripoli (data as (2007-2008))
- [13] Transportation 21: Future for Dublin A policy Paper by the Dublin Chamber of Commerce 10th January 2005.

GENETIC MARKERS FOR HEALTHY MILK PRODUCTION

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summary

The development of DNA-based technologies, within the concept of selection marker gene, allows animals to evaluate the allelic variants of genes that are directly or indirectly related to production traits. Development and function of mammary is controlled mainly by growth hormone and prolactin, which are secreted by the anterior pituitary. The article presents the results of studies of the effect gene polymorphism pituitary transcription factor (POU1F1), kappa-casein gene (k-CSN) and prolactin gene (PRL) on milk production of cows of Frisian breed. Analysis of the genotypes was performed by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). It contains information describing how to conduct research and reagent kits for each of the genes studied. POU1F1 gene controls the transcription of genes PRL, TSH and GH, as well as the functioning of the breast. Mutations kappa-casein gene (k-CSN) affect the protein content in milk, cheese yield and cheese as well as on the coagulation properties of milk. All this makes the consideration of these three genes (POU1F1, k-CSN, PRL) as a promising candidate genes dairy cattle productivity. As a result of studies

have shown the presence of two populations of allelic variants of each gene and all three variants of genotypes. As a result of molecular genetic studies have investigated the genetic structure of Frisian breed and established three genotypes AA, AB and BB. The population was in Hardy-Weinberg equilibrium.

Animals genotype BB (POU1F1) characterized by the best milk yield per lactation (500.5 kg at (P <0,01) and 835.6 kg (P <0,001) compared with genotypes AB and BB, respectively), and genotype AB (POU1F1) was significantly associated with higher protein content. Genotype AB gene k-CSN provides higher performance milking (at 572.7 kg (P<0,05) compared with the AA genotype), whereas the effect of polymorphism of the gene for the protein and fat content in milk has not been established . PRL gene revealed that the animals of AA genotype have better milk yield per lactation. Superiority over similar genotype AG amounted to 388.2 kg and above analogy with the GG genotype at 564.1 kg (P<0,05).

Keywords: gene, polymorphism, productivity, POU1F1, k-CSN, PRLR, Frisian breed.

Introduction

In the current economic environment and foreign policy, it has increased substantially the relevance of the problem of increasing the productivity of farm animal genetic resources of domestic breeds[14]. One of the problems of domestic dairy cattle is the transition to the use of foreign tribal resources, in particular Frisian. Both genetic and environmental factors are known to influence production traits in cattle. Selection of animals with higher production or better reproductive performance is of great significance to breeders and consumers[2]. Current technologies enable scientists to improve on the accuracy and efficiency of traditional selection methods by applying genetic markers trough marker-assisted

selection. Therefore, genetic polymorphisms that are significantly associated with certain traits of interest are very useful [8]. Polymorphism detection in genes related to production traits and the identification of the allele which results in a phenotype of interest can allow for marker assisted selection (MAS) [12][17]. Genes affecting polygenic traits characterizing production performance are difficult to identify. In our conditions, such animals, giving 10 thousand Kg of milk per lactation, has a low reproductive capacity and propensity to various diseases, which significantly reduces the terms of economic use of high yielding cows and, consequently, the efficiency of production. Thus, it is advisable to increase the genetic potential of local breeds differing productive longevity of the use of modern breeding techniques. High efficiency for solving this problem having methods of DNA marker selection aimed at identifying genotypes of different genes markers of efficiency and selection of animals with desirable genotypes for further reproduction. Today, the interest of pituitary transcription factor genes (POU1F1), kappa-casein (k-CSN) and prolactin (PRL), as promising genetic markers productive qualities of cattle [7]. The aim of this work was to study the effect of gene polymorphism POU1F1, k-CSN and PRL on the performance of dairy efficiency of cows of Frisian breed.

Materials and methods

The subject of this research was the cows of Frisian breed of "ALHERA" region. Data on milk production obtained from the card directly on the farm. Genomic DNA of cows (n = 136) were isolated from 200µl of whole blood using a reagent kit Diatom DNA . Analysis was performed by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Restriction of the amplified gene fragments POU1F1 carried out with the restriction enzyme HinfI, k-CSN- HindIII, PRL – RsaI . The size of restriction products were evaluated by electrophoresis on a 2% Agarose gel. Statistical analysis was performed by standard methods.

Kit for DNA Extraction Diatom DNA Prep

The kit is intended to be used for easy and effective extraction of DNA from biological samples, based on selective sorption of DNA on the surface of silica-gel granules in the presence of a high concentration of chaotropic agent. The extracted DNA is used for further molecular biological reactions without additional purification. The kit is optimized for DNA extraction from the volume of $100\mu l$ (up to $20\mu g$) from 100 samples.

The kit contains:

Lysing reagent – 60 ml;

10×Salt buffer for washing − 10 ml;

Glass beads, sorbing agent -2 ml;

Reagent for elution, ExtraGene – 10 ml

Product specifications:

IEXITACIION MEINOO	Sorption in a volume on the silica-gel granules
Purity of the extracted DNA	OD 260/280 – 1,6-2,0
Time consumption for DNA extraction	< 40 min
Yield of DNA	3-5 μg from 100 μl of blood

RFLP analysis—DNA sequence polymorphisms display different migration profiles from wild-type fragment patterns when DNA is digested with restriction fragments and separated by size using electrophoresis.

Results and Discussion

POU1F1 is a candidate gene for regulating the growth and development of cattle and other mammals, controls transcription of a gene of prolactin (PRL), thyrotropin (TSH) and growth hormone (GH). It is also required for effective functioning of breast cancer and increases milk production. According to the research of Drozdov E.V., mutation POU1F1, followed by disruption of the structure of its product we can have a significant impact on the expression of the genes controlled by them, and thus change the phenotypic expression of traits of milk production in cattle. [2] The gene POU1F1 in cattle localized on chromosome 1q21-22, located between TGLA57 and RM95 (A \rightarrow G, AC_000158.1, 35008949..35024718), consists of six exons and five introns derived protein comprises 129 amino acids[12].

The researchers has been proven to influence kappa-casein gene (k-CSN) on milk productivity of cattle and technological properties of milk. According to Vladimir Kryukov in several populations has 6 different alleles of the gene. Genetic variants A and B alleles are most common. Many researchers have attributed to the presence of alleles in a higher protein content in milk, a higher yield of cheese and cheese, as well as the best coagulation properties of milk [3]. It is found that high-quality hard cheese can only be produced by a milk obtained from cows having genotype BB gene k-CSN. In many countries, to increase the frequency of the desired allele B gene k-CSN in programs of artificial insemination using semen of bulls with genotype BB and AB [10]. Locus k- CSN relates to synteny group U15 and stored in the chromosome 6. Variant A comprises amino acids threonine (Thr) and aspartate (Asp) at positions 136 and 148, respectively. Variant B Thr-136 is replaced by isoleucine (Iso), a Asp-148 to alanine (Ala). These differences are the result of point mutations in k-CSN [2].

Prolactin (PRL, lactogenic hormone mammotropin (luteotrophic hormone), growth hormone (GH), and chorionic somato mammotrophin (placental lactogen, PL) are a family of protein hormones with significant

sequence homology. Their molecules in various species account for 190-199 amino acid residues. Homology of amino acid sequences of human GH and PRL – 35%. All three hormones share common antigenic determinants and have a similar effect: have growth-stimulating and lactogenic activity [10].

PRL is involved in the initiation and maintenance of lactation in mammals and was originally identified as lactotropic hormone secreted by the pituitary gland. To date, we accumulated a lot of data about a variety of physiological functions of prolactin, including osmoregulation, reproduction, behavioral responses. Prolactin is synthesized in various tissues, including immune and endothelial cells, neurons and others. Moreover, part of prolactin in immune response regulation has led to the concept of a dual functional role of prolactin – as hormones and cytokines. Many biological functions, such as gene expression caseins are made through the prolactin receptor [8].

In cattle PRL gene is localized in the q21 region of chromosome 23 and is closely linked with the genes of class I and class II major histocompatibility complex [4]. The analysis studied the genetic structure of cows of breed Frisian genes POU1F1, k-CSN and PRL, set the desired impact and identified genotypes on these genes. In the studied sample of cows of Frisian breed gene POU1F1established the presence of the three genotypes AA, AB and BB at a frequency of 18.8; and 43.7%, respectively (Table 1). The highest frequency is determined for allele B(0.62) and genotype BB(43.7%).

In carrying out similar studies Drozdov EV et al. in the cattle herds of the Bryansk region found the prevalence of allele B and genotype BB POU1F1 gene in all studied populations [2], which is consistent with our research. The maximum prevalence of the genotype frequencies observed in animals BB black-motley breed: group of cows (81.0%), and the calves of black-motley breed of polymorphism in this gene was not observed. The highest frequency of A allele was identified in cows ayshirskoy breed. The

European and North American rocks observed in the prevalence of allele, in most cases is very significant [13]. It was also noted that cattle Iran is bucking the trend of the prevalence rate of A-allele. In groups of cows of black-motley breed in allele frequency in the range of 0.64 (Belarus) to 0.88 (Russia, Bryansk).

In the study of the distribution of frequencies of alleles and genotypes of the gene k-CSN in the study population were identified three possible genotypes AA, AB, BB. The lowest frequency was determined for the BB genotype, which occurred at a frequency of 6.3%. The frequency of A allele was 0.75, allele B – 0.25. The highest frequency in a population characterized by a homozygous genotype AA (56.3%). In studies of Goncharenko G. in the gene k-CSN for cows of red steppe breed established frequency of allele A 0.65, allele B–0.34, AA genotype – 40.8%, AB–48.9%, BB– 10.3 % [1]. Similar results were obtained Selionovoy MI (-0 Allele A, 62; B allele –0.38 genotypes AA–44% AB–36% BB–20%) [9], LN Chizhova (Alleles A–0.74 and C–0.26; genotypes AA–58.5; AB–32.1 and BB–9.4%) [11].

Thus, our data are consistent with studies of several domestic authors in terms of the low incidence of homozygotes BB and significantly more prevalent allele, compared with allele B. On the other hand, our results, as well as data Selionovoy MI and Chizhova LN showed superiority over the frequency of homozygotes AA AB heterozygotes [9], while studies Goncharenko GM et al. found a slight superiority over the heterozygote AB homozygous AA [1]. Our studies of the genetic structure of Frisian breed cows according to PRL gene polymorphism showed the presence of genotypes AA, AG and GG with a frequency of 6.3; 31.3; 62.5%, respectively. The highest frequency was allele G (0,78) and the genotype GG (0,62). Studies Alfonso E. et al. PRL gene in the Americanstyle Swedish cows in front showed the prevalence in the population allele (0.87). The frequencies of genotypes AA, AG and GG were 78.4; 17.2; and 4.4%, respectively [12]. Research results Tyulkina SV et al. [10] from

purebred and crossbred Holstein bulls of PRL gene locus showed that 53 out of 70 bulls (75.7%) had the AA genotype, 16 (22.9%) - AB and 1 (1, 4%) - BB. The frequency of A allele was 0.87, and the allele B - 0.13. The frequency of allele A PRL gene in cattle herds of black-motley, Jersey, Kholmogory, Yaroslavl, Simmental breeds was 0,31-0,85. A higher frequency of allele A PRL gene was observed among cows of black-motley breed of 0.85, with the lowest frequency of allele A was the animals Jersey Cattle–0.31 [10].

Studies M. Ozdemir on different breeds of cattle in different directions productivity also showed different frequencies of genotypes and alleles of the gene PRL [16].

Table1: The distribution of the frequencies and alleles to the DNA-markers of the Frisian cows

DNA-marker	Allel frequency		Genotype frequency, %		
	Α	В	AA	AB	ВВ
POU1F1/Hinfl	0,38	0,62	18,8	37,5	43,7
k-CSN/HindIII	0,75	0,25	56,3	37,4	6,3
PRL/Rsal	Α	G	AA	AG	GG
	0,22	0,78	6,3	31,3	62,4

The data (Table 2) showed the influence of gene polymorphisms POU1F1, k-CSN and PRL on productive qualities of cows. In the study of gene POU1F1, animals with BB genotype have better milk yield per lactation and surpasses analogues with genotypes AB in 500,5 (P <0,01) and AA to 835.6 kg (P <0,001). Fat content in the milk of cows of different genotypes was similar, but the concentration of the protein in milk was higher in cows with heterozygous genotype AB, which surpasses analogues AA genotype at 0,05% (P <0,01). Drozdov E.V. et al obtained opposite results where individuals of genotype AA gene POU1F1 nadoyu

at the average for a month was significantly superior to animals with other genotypes [2]. In the group of cows with Aagenotype individuals present with efficiency of more than 550kg of milk per month. Regarding indicators of body fat percentage in the milk there was no statistically significant differences between individuals with different genotypes.

In the study there was a significant influence of gene polymorphism k-CSN on milk production of cows. The presence of AB genotype is associated with a better milk yield per lactation. Thus, the milk yield per lactation, cows with genotype AB exceeds analogues with genotype AA in 572.7 kg (P < 0.05). At the same time, the relationship of genotypes with a mass fraction of fat is not included in our study. Cows with BB genotype were excluded from the analysis, because of sample (n = 1) does not provide reliable results, nonetheless; genotype AB animals carrying the desired allele intended to increase the mass proportion of protein is 0.09%. In the future when dealing with the breed you need to increase the proportion of animals with a desired genotype BB in the population, in order to increase milk production of cows of Frisian breed.

Table2:Milk productivity of cows of different genotypes POU1F1, k-CSN and PRL

Genotype of gene	Milk yield for 305 days of lactation, kg	Mj, %	MPS, %			
POU1F1/Hinfl						
AA	4959,6 ± 112,8	3,88±0,04	3,08±0,02			
AB	5294,7±104,6**	3,92±0,01	3,13±0,003**			
BB	5795,2±137,8***	3,88±0,03	3,12±0,007			
k-CSN/HindIII						
AA	5259,1±187,2	3,89±0,02	3,09±0,01			
AB	5831,8±175,2*	3,89±0,01	3,18±0,06			
BB	-	-				
PRL/Rsal						
AA	5815,5±201,0*	3,90±0,07	3,15±0,02			
AG	5427,3±227,0	3,95±0,01	3,12±0,01			
GG	5251,4±196,5	3,88±0,03	3,10±0,06			

^{*}P<0,05, **P<0,01, ***P<0,001

In studies of Selionovoy M.I., cows of Frisian breed with genotype AB gene k-CSN udoyu superior to their counterparts with genotype AA at 154.1 kg. In these studies, the highest productivity was observed in genotype BB that we have not studied. As in our case, the effect of the gene k-CSN with indicators of fat content in milk is not established [9]. At the same time, the results of research and other Goncharenko. Significant effect of the gene k-CSN on the performance and yield of milk fat from cows of Frisian breed were found [1]. Thus, the structure of the allelic gene may contribute significantly to polygenic traits of milk production, at least in the Frisian breed . This B-allele associated with increased

productivity, can be seen as relatively rare. In the analyzed group of cows is absent and there is a high probability of extinction in this sector in the coming generations. PRL gene revealed that the animals AA genotype have better milk yield per lactation and surpasses analogues genotype AG for 388.2kg and 564.1 in the genotype GGkg (P<0,05). Effect of PRL gene genotypes on the amount of fat and protein in the milk of the study population, has not been established, but there is a positive trend in animal genotype AA, which are relatively unique GG genotype had better scores on the protein mass fraction of 0.05%. Research results Alfonso E. et al. [12] gene PRL showed that animals with AA genotype had a higher milk production during lactation than animals with genotype AG and GG (p <0.05), consistent with our results. Studies of S.N. Dong for a large population of local Holstein cows also showed that cows with AA genotype of the gene PRL was higher milk protein content than cows with genotypes AG and GG [14].

Conclusion

The pituitary-specific positive transcription factor (*POU1F1*) gene has been the subject of many recent studies because of its important roles in growth and development of mammals.

As a result of studies, it found the effect of genotypes of genes POU1F1, k-CSN and PRL in the milk production of cows of Friesian breed. For cows of Friesian breed desirable genotypes established POU1F1 / BB, k-CSN / AB and PRL / AA consolidation that in the study population will increase the milk yield per lactation to 835,6 (P <0,001), 572,7 (P <0,05) and 564.1 kg (P<0,05), respectively. No significant differences in the mass fraction of fat in milk has been established. The largest mass fraction of protein in milk was observed in animal genotype AB / POU1F1, which surpasses analogues on the 0,05% (P <0,01). These results confirm the possibility of using these DNA-

markers as additional criteria for the selection of animals for increasing milk productivity. The animals breeding selection assisted with genetic markers can increase the production traits or optimize reproduction performance in dairy cattle. The results from many associations analysis of this effect shows potential positive effect of B allele occurrence on growth and negatively on milk production performance. Preferably average values for milk, growth and reproduction parameters.

These studies are part of a comprehensive study of domestic cattle breeds, which aims to study the genetic basis of the variability of milk production in cattle by examining the polymorphism of a number of key genes involved in the development of breast cancer and her lactation capacity.

References

- 1. Goncharenko G.M. Comparative evaluation efficiency milk Simmental and Red Steppe breeds considering genotypes k-casein // Bulletin of the Altai State Agrarian University. 2013. № 12(110).
- 2. Drozdov E.V., Zayakin V.V. IJ Allelic polymorphism of Pit-1 in the cattle herds of the Bryansk region and its relationship with milk production // Bulletin of Samara Scientific Center of the Russian Academy of Sciences. 2011. T.13. №5 (3). Pp235-239.
- 3. Kryukov V. DNA diagnostics in breeding cattle // Herald OrelGAU. 2012. № 1(12).
- 4. Leonova M.A. Prospective marker genes of agricultural animals // Young scientist 2013. №12(59).
- 5. Leonov M.A. Gene polymorphism POU1F1 <u>cows of red steppe breed</u> // Agrarian He- rald Urals. 2014. № 12 (130). S.23-25.
- 6. Leonov M.A. Features of the chemical composition of the milk of different species of farm animals // Valeology. 2014. №4.
- 7. Leonova M.A. Intensification in livestock breeding process using PCR // Young scien- tist. -2014. № 11. S.172-175.
- 8. Leonova M.A. The role of prolactin gene and its receptor formation

- characteristics of agricultural animals // Genetics and breeding of animals4/2014.
- 9. Selionova M.I. The milk yield and the level of the natural resistance of cows of different genotypes of kappa-casein gene // Herald of the Stavropol region. 2011. №1(1).
- 10. Tyulkin S.V. Polymorphisms in genes of growth hormone, prolactin, leptin, thyroglobu- lin bulls // Journal of Genetics and Breeding. 2012. Vol 16. №4/2.
- 11. Chizhov L.N. Genotyping results of dairy cattle on kappa-casein gene // Collection of scientific works. Stavropol. 2013. V.2. Vol.6.
- 12. Alfonso E., Rojas R., Herrera G., Lemus C. et al. Polymorphism of the prolactin gene (PRL) and its relationship with milk production in American Swiss cattle. African Jour- nal of Biotechnology. 2012. Vol. 11 (29). pp.7338-7343.
- 13.Beigi Nassiri MT, Biranvand Z., Hartatik T., Fayazil J., Tavakoli S. The Study of PIT1 Gene Polymorphism in the Najdi Cattle Using PCR-RFLP // Method. J. Anim. Vet. Adv. 2012. 9 (15):2001-2003.
- 14. Dong CH, Song XM, Zhang L., Jiang JFJiang. New insights into the prolactin-RsaI (PRL-RsaI) locus in Chinese Holstein cows and its effect on milk performance traits. Genetic and Molecular Research. 2013. 12 (4):5766-5773.
- 15. Misriantia, R., Sumantria C., Farajallahb A. Polymorphism Identification of Pit1 Gene in Indonesian Buffaloes (Bubalus-bubalis) and Holstein-Friesian Cows. Media Peterna- kan. 2010. 33 (3):131-136.
- 16. Ozdemir M. Determination of Pit-1 / Hinf1 Polymorphism in Holstein and Native Ear cattle raised as genetic resource in Turkey. J. Anim. and Plant Sciences. 2012. 22 (1): 25-28.
 - 17. ZHAO, Q. DAVIS, M. E. HINES, H. C. 2004. Associations of polymorphisms in the Pit-1 gene with growth and carcass traits in Angus beef cattle. In *Journal of Animal Science*, vol. 82, 2004, p.2229-2233.

Involvement of HIF-1 &HIF-2 in the hypoxic induction of VEGF and MMP-7 genes

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Abstract

Aim: To describe the use of siRNA technology to inhibit HIF- 1α and HIF- 2α expression in primary macrophages cultured *in vitro*, to determine whether pro- MMP-7 expression regulated by these two HIFs. In these experiments, the well described HIF-target, VEGF, is used as a positive control whenever possible.

Methods: Monocytes were isolated from buffy coats & grown for seven days to be differentiated into MDM, then HIF-1 α , HIF-2 α expressions were inhibited by transfection with siRNA oligonucleotide, finally the expressions of VEGF & MMP-7 were examined.

Results: Complete inhibition of pro-MMP-7 & VEGF release by MDM showing that their hypoxic regulation is entirely dependent of HIF-1 and HIF-2.

Conclusion: These findings indicate that both genes identified could be important for the survival and functioning of macrophages in hypoxic diseased tissues. Moreover, these data emphasize that the role of HIF- 2α in

addition to HIF-1 α needs to be considered when developing agents that target the HIF pathway.

Introduction

The presence of multiple areas of hypoxia (low oxygen tension) is a hallmark feature of human and experimental tumors. Monocytes are continually recruited into tumors, where they differentiate into tumorassociated macrophages (TAM) and gather in hypoxic and/or necrotic areas. A number of recent studies have shown that macrophages respond to the levels of hypoxia found in tumors by up-regulating such transcription factors as hypoxia-inducible factors 1 and 2, which then activate the expression of a broad array of mitogenic, pro-invasive, pro-angiogenic and pro-metastatic genes. We showed the mRNA for the gene encoding the pro-tumour enzyme, matrix metalloproteinease-7 (MMP-7 or matrilysin), to be upregulated by human macrophages exposed to hypoxia *in vitro* (Burke *et al.*, 2003). Here, we extend this finding to show that these cells also upregulate their release of Pro-MMP-7 protein when exposed to hypoxia and that this is a HIF-1 & HIF-2 dependent phenomenon.

Introduction

The presence of multiple areas of hypoxia is a characteristic feature of malignant tumours resulting from local imbalance between the supply of oxygen by blood vessels and its consumption by the surrounding tumour mass (Vaupel *et al.*, 2001).

Many of the adaptations to hypoxia are mediated by the activation of specific genes through the action of hypoxia inducible transcription factors (HIF-1 and HIF-2) (Wiesener *et al.*, 1998). These proteins are heterodimers consisting of two different α units and a common constitutive β subunit, both basic helix loop helix (BHLH)-PER ARNT SIM (PAS) domain protein (Semenza 2002). Under normoxic conditions the α subunit is hydroxylated

at specific proline residues resulting in ubiquitination through the interaction with von Hippel-Lindau protein (pVHL) and proteosomal degradation. This process inhibited under hypoxia, as a result HIFa accumulates in the nucleus and bind to short DNA sequences called hypoxia-response elements (HREs) '5-RACGTG-3` near or in oxygensensitive genes, stimulating gene expressions which have important roles in tumour biology such as angiogenesis, glucose/energy metabolism, cellular growth, metastasis and apoptosis (Maxwell *et al.*, 1997; Semenza *et al.*, 2000; Zarember *et al.*, 2005).

Tumour associated macrophages (TAMs) are present in large numbers in most types of human tumour. Indeed they can constitute up to 80% of the total tumour mass in breast carcinomas (Kelly *et al.*, 1988). They originate mainly from the circulating monocytes rather than tissue macrophages (Yamashiro *et al.*, 1994). In breast carcinomas, macrophages are found mainly in the poorly vascularized, hypoxic regions (Leek *et al.*, 1996). Under hypoxic conditions, it is thought that they alter their gene expression and adapt their metabolic activity to anaerobic glycolysis, with increased production of lactate and pyruvate, and metabolic acidosis (Lewis *et al.*, 1999). TAMs seem to have a complex role in tumour growth as they can exert both anti-tumour and pro-tumour effects through a variety of cytokines and enzymes (Bingle *et al.*, 2002).

TAMs respond to hypoxia by expressing pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF) (Lewis *et al.*, 2000). Moreover, macrophages exposed to hypoxia in *vitro* up-regulate their release of VEGF (Harmey *et al.*, 1998). Macrophages also known to release PR-39 amino acid peptide, that inhibits the degradation of HIF-1α by neighbouring cells and stimulate their expression of VEGF (Lie *et al.*, 2000). Once macrophages reach hypoxic regions in the tumour they promote tumour progression by releasing factors which stimulate tumour angiogenesis and reduced patient survival in various forms of cancers such as breast cancer (Leek *et al.*, 1996) and pulmonary adenocarcinoma

(Takanami *et al.*, 1999). Macrophages express abundant HIF-1 α in hypoxic areas of various tumour types (Burke *et al.*, 2002).

MMP-7 (matrilysin) is one of the smallest MMPs, consisting of two domains, a pro-domain and a catalytic domain (wossner *et al.*, 1998). MMP-7 is secreted as a 28 kDa proenzyme. Activation of the proenzyme involves a proteolytic removal of the N-terminal pro-region containing the cysteine switch motif conserved in MMPs. The resulting mature and active enzyme (19 kDa) consists of a catalytic domain with a zinc binding motif (Van Wart, *et al.*, 1990).

MMP-7 is expressed in epithelial cells of normal and diseased tissues (Wilson et al., 1996). The protein localizes in normal tissues to secretory and ductal epithelium in the endometrium, in various exocrine glands and macrophages (Busiek et al., 1992; Rodgers et al., 1994). MMP-7 up regulated by cytokines in inflammation result in increased level of TNF from macrophages to remodel the regenerated tissue (Gearing et al., 1994) It is also expressed in a variety of tumours ranging from breast, colon, prostate, stomach, skin and soft tissue tumours (Hashimoto et al., 1998; Shapiro et al., 1999; Deny et al., 2004; Yamamoto et al., 2004) and contributes to proliferation, angiogenesis and metastasis. Knockout mice lacking the gene have suppressed intestinal tumourigenesis (Wilson et al., 1997; Jiang et al., 2005). On the other hand, Patterson et al., 1997 has demonstrated that two member of human matrix metalloproteinase MMP-7 and MMP-9 hydrolyse human plasminogen to generate angiostatin fragment. Therefore, MMP-7 and MMP-9 may regulate new blood vessel formation through angiostatin which is a specific inhibitor of angiogenesis.

MMP-7 mRNA has been shown to be up regulated in hypoxic macrophages and that the promoter of MMP-7 is hypoxia- inducible in macrophages, and element from this promoter may prove useful in the

future development of hypoxia inducible therapeutic constructs optimized for use in macrophages.(Burke *et al.*, 2003).

The aim of this study is to detect the MMP-7 and VEGF protein in hypoxic macrophages and use the siRNA technology to knockout HIF-1 α & HIF-2 α and see if these two genes are HIF-1 & HIF-2 dependent.

Materials and Methods

Isolation and culture of primary human monocyte-derived macrophages

Human monocytes were isolated from leukocyte enriched buffy coats obtained from healthy blood donors (National Blood Service, Sheffield, UK). Blood was diluted 1:1 with HBSS (without calcium or magnesium), layered on Ficol-Paque Plus (Amersham Biosciences, UK) and centrifuged for 40 min at 400 g. The mononuclear cell-rich band was removed, washed twice with HBSS and resuspended in Iscove's Modified Dulbecco's Media (IMDM) supplemented with 2% human ABC serum, L-Glutamine, penicillin (100)IU/ml), and $(100\mu g/ml)$ (All from Sigma, Poole, UK). 8×10^7 mononuclear cells were seeded into 10 cm tissue culture plates (Iwaki, town, Japan) and cultured for 1 h after which non-adherent cells were removed by washing and the culture medium replenished. Adherent monocytes were cultured for 7 days, to allow differentiation into monocyte-derived macrophages. (Zuckerman et al., 1979). Purity and differentiation status of cells was checked using CD68 immunocytochemistry and carboxypeptidase M in flow cytometry. (Rehli, et al., 1995)

Hypoxic culture and protein extraction

Monocyte-derived macrophages were subjected to 0.1% (hypoxia) or 20.9% (normoxia) O_2 in 5% CO_2 humidified multi-gas incubators (Heto, Camberly, UK) for 1,6,16, and 24 h. Incubator oxygen levels were confirmed at the end of all experiments using mobile oxygen analyzers

(Analox Sensor Technology, Cleveland, UK). Culture medium depths of less than 2 mm were used throughout this study to ensure rapid removal of oxygen from the culture media during hypoxic experiments.

Preparation of MDM cell extracts and immunoblotting

Following hypoxic or normoxic incubation MDM were washed in PBS and total cell extracts obtained by lysing cells in lysis buffer (50 mM tris HCL pH 8.0, 150 mM NaCl, 1% triton X-100 and 1 protease inhibitor tablet (Roche, Mannheim, Germany). Cell lysates were incubated on ice for 40 minutes, sheered by repeated passage through a 25-gauge needle and then centrifuged at 400 g for 10 minutes at 4°C to remove cell debris. Nuclear and cytoplasmic MDM extracts was prepared using CelLytic Nuclear extraction (Sigma, Poole, UK). All extracts were stored at -20°C until immunoblot analysis. Protein concentration of cell extracts was estimated using QuantiPro BCA reagent (Sigma, Poole, UK). Samples for immunoblotting were prepared by heating to 100°C for 5 minutes in reducing loading buffer. 60µgsample was run on a 10% SDS-PAGE gels and after separation the proteins transferred onto polyvinylidene difluoride membranes (Amersham Biosciences, Little Chalfont, UK). The membrane were incubated for 16 hours at 4°C in blocking buffer (5% skimmed milk powder in TBST – 10mM Tris, 180mM NaCl, 0.05% Tween-20, pH 8) then probed with anti-HIF-1 ☐ (BD.Biosciences, San Jose, CA)or anti-MMP7 Systems, Abingdon, UK) antibody diluted to 1:250;1:100 (R&D concentration respectively in blocking buffer and incubated for 2 hatroom temperature. After washing with TBST the membranes were then exposed to anti-mouse secondary antibody conjugated with horseradish peroxidase (Dako Ltd, Copehnagen, Denmark) at a 1:2500 dilution in blocking buffer for 1 hour at room temperature. The secondary antibody was detected using enhanced chemiluminescence reagent plus (Amersham, Little Chalfont, UK). To confirm equal loading, membranes were stripped with stripping buffer (100 mM beta mercaptoethanol, 2% SDS, 62.5mM Tris HCl pH 6.7)

at 50° C for 30 minutes and re-blotted with a mouse monoclonal antibody to \Box -actin (Sigma, Poole, UK) at 1:15,000in blocking buffer. Protein expression was quantified by densitometry using Quantity One software (BioRad, Hercules, CA).

Transient transrection of cells with siRNA duplexes

The siRNA oligonucleotides used in this study were purchased from Eurogentec (Seraing, Belgium). The HIF-1α siRNA duplex targets nucleotides 1521-1541 of the HIF-1α mRNA sequence (NM001430) and is comprised of sense 5'-CUGAUGACCAGCA-ACUUGAdTdT-3 antisense 5'-UCAAGUUGCUGGUCAUCAGdTdT-3. The inverted HIF-1α control duplex did not target any gene and comprised of sense 5'-AGUUCAACGACC-AGUAGUCdTdT-3 5'and antisense GACUACUGGUCGUU-GAdTdT-3. Duplexes were prepared by mixing 50µM concentration of antisense and sense oligonucleotides with annealing buffer (30mM HEPES pH 7.0, 100mM potassium acetate, and 2mM magnesium acetate) heat denaturing for 1 min at 85°C and annealing at 37°C for 1 hour. Duplex formation was confirmed by electrophoresis through 5% low melting temperature agarose (NuSieve GTC; FMC Bioproducts, Rockland, ME)

siRNA treatment of human monocyte-derived macrophages

Monocyte-derived Macrophages were cultured for 5days in 6 well plates (initial seeding density was 20 x 10⁶ mononuclear cells/well). 5μg of 20 nM siRNA duplex was diluted in IMDM containing 2% AB serum, 2mM L-Glutamine, penicillin (100 IU/ml), and streptomycin (100μg/ml) to give a final volume of 100μl, then mixed by vortexing for 10 seconds. For complex formation 15μl of RNAiFect transfection reagent (Qiagen, Crawley, UK) was added to the diluted siRNA and mixed by vortexing for 10 seconds and then incubated for 15 minutes at room temperature. Adherent macrophages were washed with PBS and 1900μl of IMDM

medium added to the cells. 2000µl of the complexes were added drop wise onto the cells and the plate gently agitated before incubating 37°C. Following 24 h incubation macrophages were washed and incubated for an additional 24 h before beginning the hypoxic experiments.

Measurement of VEGF and MMP-7

Tissue culture supernatants of monocyte-derived macrophages exposed to normoxia and hypoxia were collected, centrifuged for 10 minutes at 400 x g to remove cell debris and then concentrated 5 x using vivaspin Millipore columns. Secreted MMP-7 and VEGF were measured in the concentrated supernatant using the respective Quantikine ELISA kit (R&D Systems, Abingdon, UK).

Statistical analysis:

A parametric t-test was used to evaluate significance between experimental groups. *p*

values exceeding 0.05 were considered not significant.

Results

Immunoblots for detection of HIF-1α in human MDM

A HIF-1 α protein was detected in macrophages in total cell extract by using Western blotting. The protein was up-regulated at 1 and 6 hours reaching high level at 16 hours up to five folds and then start to decline at 24 hours of hypoxia (0.1% O_2) with no HIF-1 α expression at 20.9% O_2 (Figure 1).

By looking at the cytoplasmic extract there is no HIF- 1α expression detected unlike the nuclear extract where HIF- 1α is up regulated after 16hours of hypoxia up to 7 folds and increase further when the cells treated

with LPS. There is small amount of HIF- 1α protein detected in the hypoxic cytoplasmic fraction after LPS treatment (Figure 2).

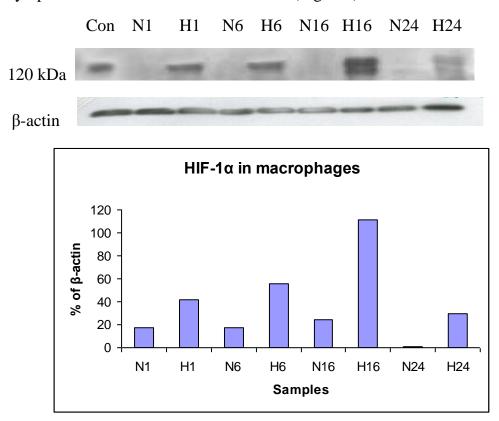


Figure1: HIF-1α expression in human MDM. Hypoxic induction of HIF-1α macrophage showing bands (at approximately120 kD) in total cell extracts following exposure to normoxia (N) (20.9% O_2) or hypoxia (H) (0.1% O_2) for 1, 6, 16 and 24 hours of hypoxia with the densitometry. The membrane was reblotted with the β-actin antibody to ensure equal loading.

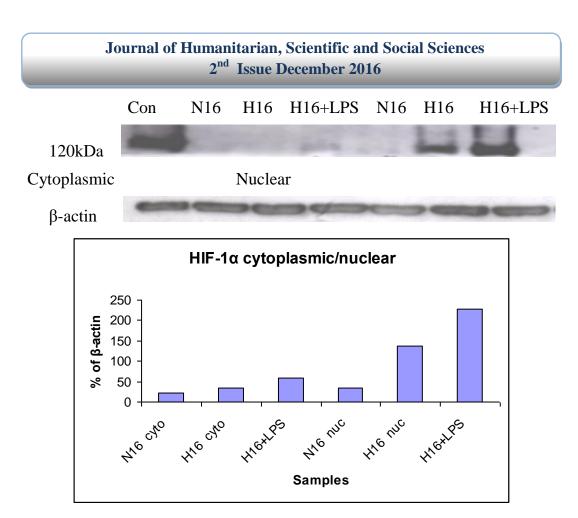
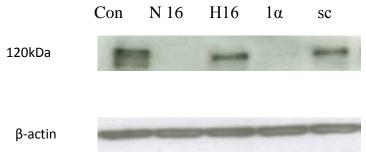


Figure 2: HIF-1α expression under the effect of LPS in the cytoplasmic and nuclear extracts in MDM. HIF-1α protein was detected mainly in the nuclear extract and increase further when the cell treated with LPS after 16 hour hypoxia (0.1% O_2). There was no HIF-1α expression in the cytoplasmic extract with the densitometry. The membrane was reblotted with the β-actin antibody to ensure equal loading.

Transient transfection of HIF-1α in human MDM

MDM were transfected with $5\mu g$ of 20 nM siRNA duplex and then the protein was checked through Western blotting after 48 hours transfection. HIF- 1α protein was reduced in hypoxic macrophages treated with siRNA. There was no HIF- 1α inhibition in the cells treated with the same concentration of the scrambled peptide (control) (Figure 3). The cells were viable after the siRNA transfection by using promidium iodide stain (PI) (data not shown).



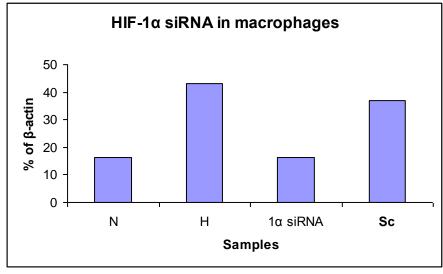
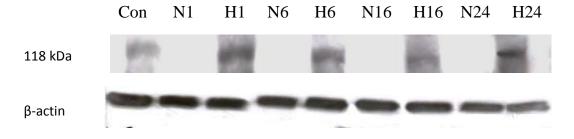


Figure 3: Transient transfection of HIF- 1α in MDM. Cells treated with $5\mu g$ siRNA for 48 hours shows complete inhibition of the HIF- 1α protein in the hypoxic macrophages as detected by Western blotting. There was no effect on HIF- 1α expression in the cells treated with the scrambled peptide (control). The β -actin protein was used as a loading control.

Immunoblots for detection of HIF-2α in human MDM

A HIF-2 α protein was tested in macrophages in total cell extract by using Western blotting. HIF-2 α protein was not detected under normoxic conditions (20.9%) at 1, 6, 16 and 24 hours. However, macrophages were found to upregulate HIF-2 α protein when subjected to hypoxia (0.1% O₂). In contrast to HIF-1 α , maximal levels of HIF-2 α were seen after 1 hour of hypoxia up to 6 folds. Thereafter, protein levels gradually declined. HIF-2 α protein was stable at 24 hours, unlike HIF-1 α , which had started to decline at this point (Figure 4).

As with HIF-1 α , HIF-2 α is translocated to the nucleus under hypoxic conditions HIF-2 α was found not to accumulate in either the cytoplasmic or nuclear fractions of MDM in normoxia. However, after 16 hours exposure to hypoxia, HIF-2 α protein was detected in the nuclear fraction up to 8 folds (Figure 5).



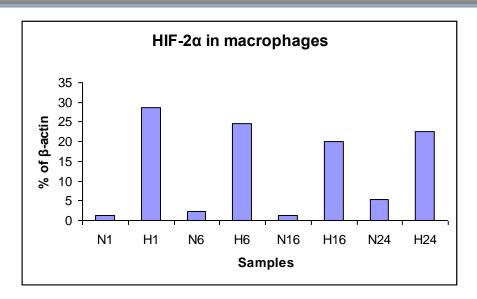
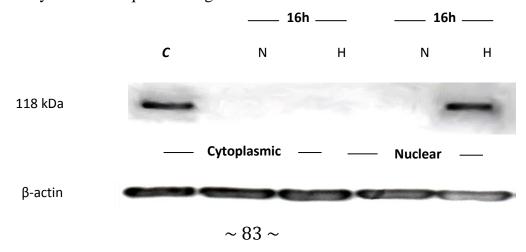


Figure 4. Effect of hypoxia on HIF-2α accumulation in human monocyte-derived macrophages *in vitro*. Immunoreactive HIF-2α protein bands (at 118 kD) were seen in total cell extracts (A) following exposure of cells to hypoxia ('H'; $0.1\% O_2$) for 1, 6 or 16 & 24 h. No bands were visible in extracts after cells experienced normoxia for the same periods of time ('N'; $20.9\% O_2$). The membrane was stripped and re-blotted with a β-actin antibody to ensure equal loading of all lanes.



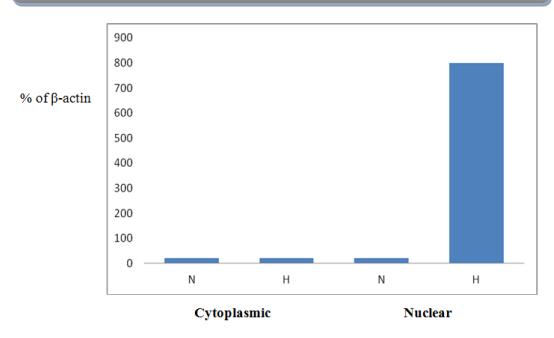


Figure 5. Immunoreactive HIF- 2α protein bands were seen in nuclear but not cytoplasmic cell extracts following exposure of cells to hypoxia for 16h. The control is human breast carcinoma cells (T47D), exposed to hypoxia for 16h.

Transient transfection of HIF-2α in human MDM

Macrophages were transfected with HIF- 2α siRNA at concentration of 250 nM. HIF- 2α protein expression was inhibited in these cells after 16 hours hypoxia. There was no effect on HIF- 2α expression after transfection with the scrambled oligonucleotide siRNA probe (Figure 6).

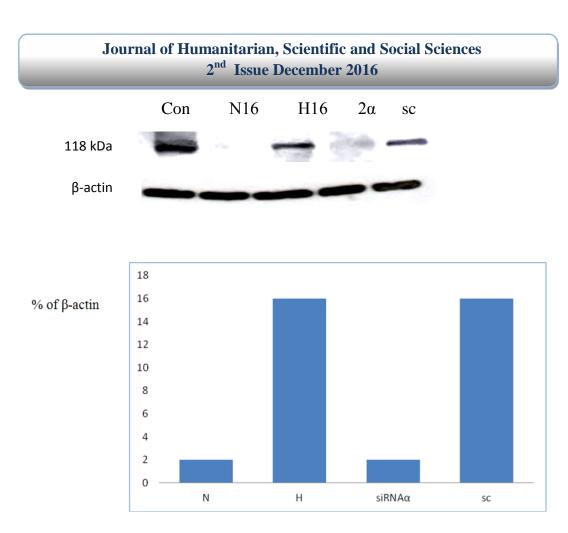


Figure 6: Transient transfection of HIF- 2α in MDM. Cells treated with $5\mu g$ siRNA for 48 hours shows complete inhibition of the HIF- 2α protein in the hypoxic macrophages as detected by Western blotting. There was no effect on HIF- 1α expression in the cells treated with the scrambled peptide (control). The β -actin protein was used as a loading control.

VEGF expression in HIF-1α knockout macrophages

Media was collected after the siRNA transfection and concentrated before the ELISA experiment. VEGF protein was decreased to the normoxic level

in the HIF-1 α knockout cells. This result suggests an important role for HIF-1 α in the expression of VEGF protein under hypoxia (Figure 7).

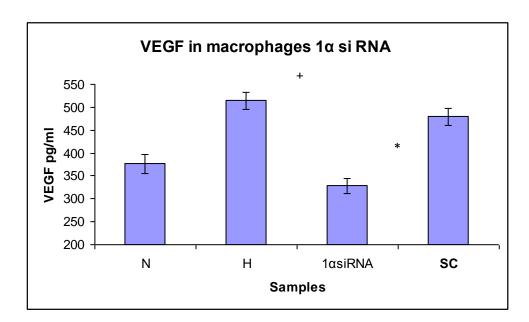


Figure 7: ELISA for VEGF expression in HIF-1 α knockout in macrophages. VEGF protein was detected at the normoxic level in the absence of HIF-1 α . + P<0.001 w.r.t. MDM cultured under normoxic conditions.* P<0.001w.r.t scrambled siRNA-treated MDM cultured under hypoxic conditions.

Hypoxic induction of pro-MMP-7 release by human macrophages: role of HIFs:

Pro-MMP-7 release was significantly (P < 0.001) upregulated by hypoxic macrophages compared to macrophages cultured in normoxic conditions. Treatment of macrophages with HIF-1 α siRNA & HIF-2 α siRNA significantly (P < 0.001) reduced the levels of hypoxia-induced pro-MMP-7

released when these macrophages were exposed to hypoxia for 16 hours. The levels of pro-MMP-7 found in HIF-1 α siRNA & HIF-2 α siRNA-treated macrophages were comparable to those found in the supernatants of macrophages cultured in normoxic conditions. Transfection of macrophages with the scrambled oligonucleotide siRNA probe had no effect on levels of pro-MMP-7 found in macrophage culture supernatants and these were similar to those found in macrophages subjected to hypoxia without siRNA treatment (Figure 8).

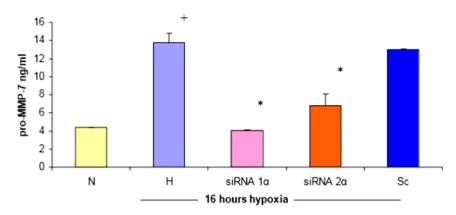


Figure 11: Inhibition of HIF- 1α & HIF- 2α ablates hypoxia-induced release of pro-MMP-7 by macrophages. The media from macrophages was collected and assayed for pro-MMP-7 expression. N = normoxia, H = hypoxia, siRNA 1α = hypoxic macrophages treated with siRNA for HIF- 1α , siRNA 2α = hypoxic macrophages treated with siRNA for HIF- 2α , Sc = hypoxic macrophages treated with a scrambled siRNA control. The level of pro-MMP-7 protein was reduced in both HIF- 1α & HIF- 2α -deficient macrophages compared with controls. A representative ELISA is shown of three separate experiments. + P<0.001 w.r.t. MDM cultured under normoxic conditions. * P<0.001 w.r.t scrambled siRNA-treated MDM cultured under hypoxic conditions.

Figure 8. The level of pro-MMP-7 protein was reduced in both HIF- 1α & HIF- 2α -deficient macrophages compared with controls. + P<0.001 w.r.t. MDM cultured under normoxic conditions. * P<0.001 w.r.t scrambled siRNA-treated MDM cultured under hypoxic conditions.

Discussion

In this study MMP-7 was shown to be up regulated in hypoxic macrophages at 16 hours of hypoxia but not at 1 and 6 hours using Western blotting for both whole cell extract and the media. This result was confirmed by zymography and ELISA. Induction of MMP-7 protein expression by hypoxia explain the high levels of this enzyme expressed by macrophages in human atherosclerotic lesions (Halpert *et al.*, 1996)) as these sites are known to be ischemic (Bjornheden *et al.*, 1999). The fact that MMP-7 levels are high at sites of plaque rupture suggests that it may be an important feature in the progression of certain cardiovascular diseases (Shah 1998). The hypoxic up regulated gene identified (MMP-7) could be important for the survival and functioning of macrophages in hypoxic diseased tissues and their promoters could prove useful in macrophage delivery gene therapy.

MMP-7 expression has been linked to resistance to doxorubicin chemotherapy. Owing to the ability of MMP-7 to cleave Fas ligand from the surface of the tumour cells, blocking the action of the drug (Mitsiades *et al.*, 2001). TAMs express high level of MMP-7 in hypoxic areas of human breast carcinoma (Burke *et al.*, 2003) suggesting that hypoxic induction of this protease in macrophage may contribute to the resistance of hypoxic tumour cells to treatment with doxorubicin.

In this study we used siRNA that specifically target degradation of mRNAs encoding HIF-1 α . After treatment with siRNA, the expression of HIF-1 α protein was greatly reduced under hypoxic conditions in the HIF-1 α knockout cells. The siRNA oligonucleotides used was specific to HIF-1 α protein tested. Inverted siRNA control had no effect on the expression of HIF-1 α protein.

Two hypoxia inducible genes (VEGF and MMP-7) (Harmey *et al.*, 1998; Burke *et al.*, 2003)) were tested in macrophages. Both VEGF and MMP-7 protein were decreased to the normoxic level in macrophages HIF-1 α knockout. This result indicates that both VEGF and MMP-7 expression is HIF-1 α dependent in primary macrophages. Human MMP-7 promoter contain a sequence closely resembling a HRE at position -617 to -590, containing a consensus HIF binding site (HBS; 5 –ACGTG $\dot{}$ 3) (Burke *et al.*, 2003 133). Although the hypoxic regulation of MMP-7 and VEGF appears to involve HIF-1 in human MDM, we have yet to ascertain whether this is a direct or an indirect effect in the transcription of these two genes. In addition HIF-2 α knockout worth to be tested in macrophages to check if it has any effect in expression of these genes in macrophages. As the activity of individual HREs varies markedly between different cell types. Therefore, the most appropriate HRE for use in macrophage based gene therapies would be derived from a gene highly up regulated in hypoxic macrophages.

References

- 1. Bingle, L., Brown, N. J., & Lewis, C. E. 2002, "The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies", *J Pathol*, vol. 196, no. 3, pp. 254-265.
- 2. Bjornheden, T., Levin, M., Evaldsson, M., & Wiklund, O. 1999, "Evidence of hypoxic areas within the arterial wall in vivo", *Arterioscler.Thromb.Vasc.Biol*, vol. 19, no. 4, pp. 870-876.
- 3. Burke, B., Giannoudis, A., Corke, K. P., Gill, D., Wells, M., Ziegler, H., & Lewis, C. E. 2003, "Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy", *American Journal of Pathology*, vol. 163, no. 4, pp. 1233-1243.

- 4. Burke, B., Tang, N., Corke, K. P., Tazzyman, D., Ameri, K., Wells, M., & wis, C. E. 2002, "Expression of HIF-I alpha by human macrophages: implications for the use of macrophages in hypoxiaregulated cancer gene therapy", *Journal of Pathology*, vol. 196, no. 2, pp. 204-212.
- Busiek, D. F., Ross, F. P., McDonnell, S., Murphy, G., Matrisian, L. M., & Welgus, H. G. 1992, "The matrix metalloprotease matrilysin (PUMP) is expressed in developing human mononuclear phagocytes", *J Biol Chem*, vol. 267, no. 13, pp. 9087-9092.
- 6. Denys, H., De Wever, O., Nusgens, B., Kong, Y., Sciot, R., Le, A. T., Van Dam, K., Jadidizadeh, A., Tejpar, S., Mareel, M., Alman, B., & Cassiman, J. J. 2004, "Invasion and MMP expression profile in desmoid tumours", *Br J Cancer*, vol. 90,no. 7, pp. 1443-1449.
- 7. Gearing, A. J., Beckett, P., Christodoulou, M., Churchill, M., Clements, J., Davidson, A. H., Drummond, A. H., Galloway, W. A., Gilbert, R., Gordon, J. L. 1994, "Processing of tumour necrosis factor-alpha precursor by metalloproteinases", *Nature*, vol. 370, no. 6490, pp. 555-557.
- 8. Halpert, I., Sires, U. I., Roby, J. D., Potter-Perigo, S., Wight, T. N., Shapiro, S. D., Welgus, H. G., Wickline, S. A., & Parks, W. C. 1996, "Matrilysin is expressed by lipid-laden macrophages at sites of potential rupture in atherosclerotic lesions and localizes to areas of versican deposition, a proteoglycan substrate for the enzyme", *Proc Natl Acad Sci U S A*, vol. 93, no. 18, pp. 9748-9753.
- 9. Harmey, J. H., Dimitriadis, E., Kay, E., Redmond, H. P., & Bouchier-Hayes, D. 1998, "Regulation of macrophage production of vascular endothelial growth factor (VEGF) by hypoxia and

- transforming growth factor beta-1", *Ann.Surg Oncol*, vol. 5, no. 3, pp. 271-278.
- 10. Hashimoto, K., Kihira, Y., Matuo, Y., & Usui, T. 1998, "Expression of matrix metalloproteinase-7 and tissue inhibitor of metalloproteinase-1 in human prostate", *J. Urol.*, vol. 160,no. 5, pp. 1872-1876.
- 11. Jiang, W. G., Davies, G., Martin, T. A., Parr, C., Watkins, G., Mason, M. D., Mokbel, K., & Mansel, R. E. 2005, "Targeting matrilysin and its impact on tumor growth in vivo: the potential implications in breast cancer therapy", *Clin.Cancer Res.*, vol. 11,no. 16, pp. 6012-6019.
- 12. Kelly, P. M., Davison, R. S., Bliss, E., & McGee, J. O. 1988, "Macrophages in human breast disease: a quantitative immunohistochemical study", *Br J Cancer*, vol. 57, no. 2, pp. 174-177.
- 13. Leek, R. D., Lewis, C. E., Whitehouse, R., Greenall, M., Clarke, J., & Harris, A. L. 1996, "Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma", *Cancer Res*, vol. 56, no. 20, pp. 4625-4629.
- 14. Lewis, J. S., Landers, R. J., Underwood, J. C., Harris, A. L., & Lewis, C. E. 2000, "Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas", *J Pathol*, vol. 192, no. 2, pp. 150-158.
- 15. Lewis, J. S., Lee, J. A., Underwood, J. C., Harris, A. L., & Lewis, C. E. 1999, "Macrophage responses to hypoxia: relevance to disease mechanisms", *Journal of Leukocyte Biology*, vol. 66, no. 6, pp. 889-900.

- 16. Li, J., Post, M., Volk, R., Gao, Y., Li, M., Metais, C., Sato, K., Tsai, J., Aird, W., Rosenberg, R. D., Hampton, T. G., Li, J. Y., Sellke, F., Carmeliet, P., & Simons, M. 2000, "PR39, a peptide regulator of angiogenesis", *Nature Medicine*, vol. 6,no. 1, pp. 49-55.
- 17. Maxwell, P. H., Dachs, G. U., Gleadle, J. M., Nicholls, L. G., Harris, A. L., Stratford, I. J., Hankinson, O., Pugh, C. W., & Ratcliffe, P. J. 1997, "Hypoxia-inducible factor-1 modulates gene expression in solid tumors and influences both angiogenesis and tumor growth", *Proc Natl Acad Sci U S A*, vol. 94, no. 15, pp. 8104-8109.
- 18. Mitsiades, N., Yu, W. H., Poulaki, V., Tsokos, M., & Stamenkovic, I. 2001, "Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity", *Cancer Res*, vol. 61, no. 2, pp. 577-581.
- 19. Patterson, B. C. & Sang, Q. A. 1997, "Angiostatin-converting Enzyme Activities of Human Matrilysin (MMP-7) and Gelatinase B/Type IV Collagenase (MMP-9)", *Journal of Biological Chemistry*, vol. 272, no. 46, pp. 28823-28825.
- 20. Rehli, M., Krause, S. W., Kreutz, M., & Andreesen, R. 1995, "Carboxypeptidase M Is Identical to the MAX.1 Antigen and Its Expression Is Associated with Monocyte to Macrophage Differentiation", *Journal of Biological Chemistry*, vol. 270,no. 26, pp. 15644-15649.
- 21. Rodgers, W. H., Matrisian, L. M., Giudice, L. C., Dsupin, B., Cannon, P., Svitek, C., Gorstein, F., & Osteen, K. G. 1994, "Patterns of matrix metalloproteinase expression in cycling endometrium imply differential functions and regulation by steroid hormones", *J Clin Invest*, vol. 94, no. 3, pp. 946-953.

- 22. Semenza, G. L. 2000, "HIF-1 and human disease: one highly involved factor", *Genes Dev*, vol. 14, no. 16, pp. 1983-1991.
- 23. Semenza, G. L. 2002, "HIF-1 and tumor progression: pathophysiology and therapeutics", *Trends Mol Med*, vol. 8, no. 4 Suppl, p. S62-S67.
- 24. Shah, P. K. 1998, "Role of inflammation and metalloproteinases in plaque disruption and thrombosis", *Vasc.Med*, vol. 3, no. 3, pp. 199-206.
- 25. Shapiro, S. D. 1999, "Diverse roles of macrophage matrix metalloproteinases in tissue destruction and tumor growth", *Thromb.Haemost.*, vol. 82,no. 2, pp. 846-849.
- 26. Takanami, I., Takeuchi, K., & Kodaira, S. 1999, "Tumor-associated macrophage infiltration in pulmonary adenocarcinoma: association with angiogenesis and poor prognosis", *Oncology*, vol. 57,no. 2, pp. 138-142.
- 27. Van Wart, H. E. & Birkedal-Hansen, H. 1990, "The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family", *Proc Natl Acad Sci U S A*, vol. 87, no. 14, pp. 5578-5582.
- 28. Vaupel, P., Thews, O., & Hoeckel, M. 2001, "Treatment resistance of solid tumors: role of hypoxia and anemia", *Med Oncol*, vol. 18, no. 4, pp. 243-259.
- 29. Wiesener, M. S., Turley, H., Allen, W. E., Willam, C., Eckardt, K. U., Talks, K. L., Wood, S. M., Gatter, K. C., Harris, A. L., Pugh, C. W., Ratcliffe, P. J., & Maxwell, P. H. 1998, "Induction of endothelial PAS domain protein-1 by hypoxia: characterization and

- comparison with hypoxia-inducible factor-1alpha", *Blood*, vol. 92, no. 7, pp. 2260-2268.
- 30. Wilson, C. L. & Matrisian, L. M. 1996, "Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions", *Int J Biochem Cell Biol*, vol. 28, no. 2, pp. 123-136.
- 31. Wilson, C. L., Heppner, K. J., Labosky, P. A., Hogan, B. L., & Matrisian, L. M. 1997, "Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin", *Proc Natl Acad Sci U S A*, vol. 94, no. 4, pp. 1402-1407.
- 32. Wilson, C. L., Heppner, K. J., Labosky, P. A., Hogan, B. L., & Matrisian, L. M. 1997, "Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin", *Proc Natl Acad Sci U S A*, vol. 94,no. 4, pp. 1402-1407.
- 33. Woessner, J. F., Jr. 1998, "Role of matrix proteases in processing enamel proteins", *Connect.Tissue Res*, vol. 39, no. 1-3, pp. 69-73.
- 34. Yamamoto, H., Horiuchi, S., Adachi, Y., Taniguchi, H., Nosho, K., Min, Y., & Imai, K. 2004, "Expression of ets-related transcriptional factor E1AF is associated with tumor progression and over-expression of matrilysin in human gastric cancer", *Carcinogenesis*, vol. 25,no. 3, pp. 325-332.
- 35. Yamashiro, S., Takeya, M., Nishi, T., Kuratsu, J., Yoshimura, T., Ushio, Y., & Takahashi, K. 1994, "Tumor-derived monocyte chemoattractant protein-1 induces intratumoral infiltration of monocyte-derived macrophage subpopulation in transplanted rat tumors", *American Journal of Pathology*, vol. 145, no. 4, pp. 856-867.

- 36. Zarember, K. A. & Malech, H. L. 2005, "HIF-1alpha: a master regulator of innate host defenses?", *J Clin Invest*, vol. 115, no. 7, pp. 1702-1704.
- 37. Zuckerman, S. H., Ackerman, S. K., & Douglas, S. D. 1979, "Longterm human peripheral blood monocyte cultures: establishment, metabolism and morphology of primary human monocytemacrophage cell cultures", *Immunology*, vol. 38,no. 2, pp. 401-411.

ANALGESIC EFFECTS OF LIBYAN FRESH POMEGRANATE FRUIT OF PUNICA GRANATUM

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Context: The severe side effects of analgesic drugs require the search of new analgesics from natural products and nutritional resources known as nutraceutical.

Objective: This work aimed to evaluate the analgesic effect of fresh pomegranate *Punica granatum* fruit juice (fpj) been consumed by Libyans for a century. In this study we investigated the fresh fruit juice given to mice for three subsequent days versus the effect of (fpj) given instantly and those given the ethanol extract of pomegranate

Materials and Methods: Antinociceptive activity of fresh pomegranate fruit juice (fpj) was examined using two models of pain. In the writhing test the (fpj) was administered for three subsequent days where no food was given,the (fpj) was also given instantly i.p.(0.15ml/kg) and orally p.o.(0.15ml/kg) for hot tail one group of animals were prefed (fpj) the ethanol pomegranate extract was administered by intraperitoneal route in

doses of (100,150 and 200 mg/kg) for writhing , hot tail flick test and compared to aspirin 100 mg/kg in all tests

Results:

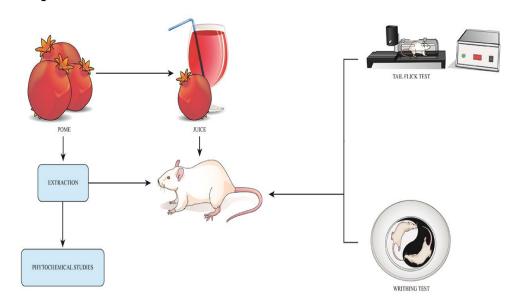
In the writhing test for mice the index of pain inhibition (IPI) was 47.8% for mice prefed with (fpj) and 37% for ethanol pomegranate extract (200mg/kg i.p.) and 59% for aspirin whereas, the groups treated instantly (0.15ml/10g) i.p.and orally failed to inhibit acetic acid induced writhings.

In the hot-tail flick test fresh pomegranate fruit juice (fpj) antinociceptive effect was evident within 15min the maximum possible analgesia (MPA) was 18% and remained elevated throughout the observation period ethanol pomegranate extract (200mg/kg) showed significant analgesia reaching its peak at 60 min MPA was 24.1% as compared to aspirin 37.5%

Conclusion: The results demonstrated that consuming pomegranate fresh fruit leads to significant analgesic activity which may be both peripheral and central.

Keywords: Antinocieptive, pomegranate fruit juice, maximum possible analgesia, *Punicagranatum*

Graphical abstract



Introduction

The pomegranate (Punica granatum L.Punicaceae) ,is one of the oldest known fruit species. It has a long history of herbal use dating back more than 3000 years. In Libya pomegranate is considered as a health giving fruit and home remedy and is consumed widely as a fruit juice, pomegranate fruit peels are used for gastric ulcers, visceral pain, and as a natural dye. It grows in the middle east extending throughout the Mediterranean, eastward to China and India, and on to the American Southwest, California and Mexico in the New world. The pomegranate plant is either a small tree or a large shrub, its fruit often considered to be a large berry (Levin,1994). Pomegranate is used traditionally for treatment of many illnesses these include ulcers diarrehea, aphthe and diabetes mellitus (Lansky and Newman,2007). It has been reported to possess anti-inflammatory effect (Lansky and Newman,2007;Shukla et al 2008;

Quachrif et al, 2012),antioxidant effect(Mertens-Talcott et al., 2006) anticancer and antiatherosclerotic effect (Perze-Vicente et al., 2002).

The major class of phytochemical present in pomegranate is the polyphenols and includes flavoinoids, condensed tannins and hydrolysable tannins(Gil et al., 2000). In addition pomegranate fruit husk is rich in ellagitannins and gallotannins (Mayer, 1977). Pomegranate juice is an important source of anthrocyanins and phenolic (puicalin, pedunculagin, puicalagin and ellagic acid) which give the fruit and aril its red colour (Gil et al,2000; Kulkarnit Aradhya,2005). It has been reported that pomegranate juice has an antioxidant capacity three times of that known beverages such as red wine and green tea, presumably due to the presence of hydrolysable tannins in the rind along with anthrocyanins and ellagic acid derivatives (Gil et al., 2000). In a comparative study, anthocyanins from pomegranate fruit were also shown to possess higher antioxidant activity than vitamin E(alpha tochopherol), ascorbic acid and B carotene (Seeram and Nair, 2002).

NSAIDs remain among the most widely used drugs for management of pain and inflammation despite their serious side effects. This highlights a need for safe and alternative treatments. The prevention and alternative treatments could come from nutrition. By nature nutrition is better positioned to have long term rather than short term health effect (Ameye and Chee, 2006).

NSAIDs inhibit both COX1which has a protective effect and COX2 enzyme which is responsible for inflammation and pain which lead to discovery of selective COX2 inhibitors which are known to be very expensive (Peterson and Cryer ,1999). This gave a rise to research for natural COX2 inhibitors with no side effects and less expenses. Pomegranate is rich in flavonoids and phenolics which are plant based chemicals that hold great promise as COX2 inhibitors. Despite the wide consumption of pomegranate fruit and juice in Libya no scientific studies

have been done regarding the Libyan variety of *Punica granatum*. In this study we tested the fruit juice and fruit extract which are consumed by Libyans in order to verify their ethno pharmacological use and to join other colleagues in the search of natural analgesics.

Materials and methods

Plant material

Fresh pomegranate were collected from an orchard in the region of Tajoura,in the fall of 2010

Animals

For the writhing test and hot tail flick test albino mice (20-30g) of both sexes were used. The animals were supplied by the animal house of faculty of medicine University of Tripoli. Two groups of mice was fed pomegranate fruit juice for 3 subsequent days and no other food was given.

The experiments were performed according to the guidelines set by the National institute of health regarding the treatment of experimental animals

Preparation of juice

The fresh pomegranate fruits were washed and manually peeled without separating the seeds. Juice was obtained using commercial blender filtered and diluted with distilled water.

Extraction method

Fresh fruits were cleaned freeze-dried and grounded into fine powder using electric blender. The powder was dried in an oven at 40 °C for 24hrs then the fine powder was sieved through 24 mesh .The fine powdered sample was extracted with 250ml 80%ethanol in water at room temperature

for 24hr in a shaking water bath. The extract was filtered by a Millipore filter with a 0.45um nylon membrane under vaccum at 25°C

Phytochemical analysis

Preliminary phytochemical analysis of pomegranate extract implicated determination of the following compounds alkaloids, flavoinoids, tannins, anthocyanes, sterols, terpenes and saponins (Trease and Evans 1989).

Acetic acid writhing test

Writhing was induced by the intra peritoneal injection of 0.6% acetic acid at a dose of 0.1ml/kg (Collier et al, 1968). Five groups of 6 mice were used. Saline, ethanol pomegranate extract 100,150and 200mg/kg, aspirin 100mg/kg were given to animals 30min before the injection of acetic acid and the number of writhes were counted for a period of 15min

The index of pain inhibition was calculated as follows:

$$IPI = \left(\frac{X0 - Xi}{X0}\right) \times 100$$

X0 is the number of writhes observed in control group. Xi the number of writhes in the tested groups

Hot tail flick test

Albino mice weighing between 25-30g were fasted for 24hrs with water given ad libitium maintained at room temperature and were divided into five groups of six mice. Mice were treated with normal saline 0.15ml/10g, aspirin100mg/kg and ethanol pomegranate extract(Epe)100,150 and 200mg/kg. One or two cm of the tail was immersed in warm water kept constant at 50°C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as the mean of the next two readings. The

latent period of tail flick response was taken as the index of antinociception and was determined before and at 15,30,45 and 60 min after the administration of drugs. The maximum reaction time was fixed at 15 sec (Sewell and Spencer ,1976).

The maximum possible analgesia was calculated as (MPA)

MPA=<u>Test reaction time</u>-<u>Saline reaction time</u>

15-Saline reaction time

Results

Acetic acid -induced writhing test

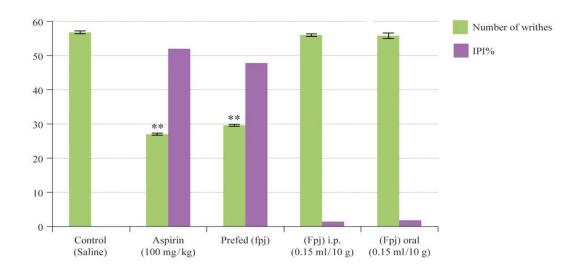
The results in Table 1 showed that pomegranate fruit juice given for a period of three subsequent days reduced the number of writhes induced by acetic acid IPI was 47.8%

Whereas, pomegranate fruit juice given instantly i.p or orally failed to produce significant effect. Aspirin (100mg/kg,i.p.) exhibited 52% of pain inhibition.

Table 1: The antinociceptive effect of administration of *P. granatum* juice and aspirin on acetic –acid induced visceral pain in mice.

Treatment	Number of writhes	IPI%
Control (Saline)	56.8 ± 0.4	0
Aspirin (100 mg/kg)	27 ± 0.3***	52%
Prefed (fpj)	29.6 ± 0.3***	47.8%
(Fpj)i.p. (0.15 ml/10 g)	56.0 ± 0.4	1.4%
(Fpj)oral (0.15 ml/10 g)	55.8 ± 0.8	1.8%

Values are represented as the mean \pm SEM (n=6).Differences between groups were statistically analysed by ANOVA followed by t-test ***p<0.001vs control (saline).Fpj: pomegranate fruit juice; IPI index of pain inhibition



In Table 2 the ethanol pomegranate extract reduced writhes in a dose dependent manner IPI was 37% for (200mg/kgi.p.) and 28% for (150mg/kg.i.p.) and 21% for (100mg/kg i.p.) aspirin 100mg/kg exhibited 59% of pain inhibition

Table 2: The antinociceptive effect (i.p.) of P.*granatum* extracts and aspirin on acetic acid induced visceral pain in mice.

Treatment	Number of writhes	IPI%
Control (Saline)	55.8 ± 0.6	0%
Aspirin (100 mg/kg)	22.8 ± 0.4***	59%
Epe extract (100 mg/kg)	44.3 ± 0.7***	21%
Epe extract (150 mg/kg)	40 ± 0.6***	28%
Epe extract (200 mg/kg)	35 ± 0.4***	37%

Values are represented as the mean $\pm SEM(n=6)$.Differences between groups were statistically analysed by ANOVA followed by t test.***p<0.001vs control (saline).Epe: ethanol extract of pomegranate; IPI:index of pain inhibition

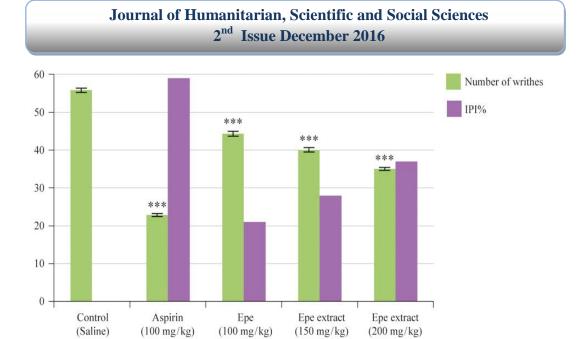


Table 3 :The effect of administration of *P.granatum* extract, juice and aspirin(100mg/kg) on hot tail tail flick test

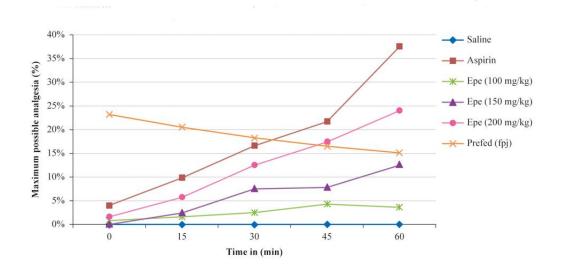
Treatment	0 min	15 min	30 min	45 min	60 min
Saline	2.5 ± 0.0	2.8 ± 0.03	3 ± 0.02	3.5 ± 0.05	3.8 ± 0.06
Aspirin (100 mg/kg)	3 ± 0.02	4 ± 0.04***	5 ± 0.04***	6 ± 0.04***	8 ± 0.03***
Epe (100 mg/kg)	2.6 ± 0.03	3 ± 0.02	3.3 ± 0.04*	4 ± 0.03**	4.2 ± 0.03
Epe (150 mg/kg)	2.5 ± 0.04	$3.1 \pm 0.04*$	3.9 ± 0.05***	4.4 ± 0.06**	5.2 ± 0.04***
Epe (200 mg/kg)	2.7 ± 0.02	3.5 ± 0.04***	4.5 ± 0.04***	5.5 ± 0.06***	6.5 ± 0.04***
Prefed (fpj)	4.7 ± 0.03	5 ± 0.06***	5.2 ± 0.08	5.4 ± 0.06***	5.5 ± 0.06***

Values are represented as mean+SEM(n=6).Differences between groups were statistically analysed byANOVA followed by t test *p<0.05,*p<0.01,***p<0.001vs control(saline)

Epe:Ethanol extract of pomegranate IPI:index of pain inhibition

Table 4: The percentage of maximum possible analgesia (MPA%) caused by *P.granatum* extract (100,150,200mg/kg) *P.granatum* juice and aspirin100mg/kg

Treatment	0 min	15 min	30 min	45 min	60 min
Saline	0%	0%	0%	0%	0%
Aspirin	4%	9.8%	16.6%	21.7%	37.5%
Epe (100 mg/kg)	0.8%	1.6%	2.5%	4.3%	3.6%
Epe (150 mg/kg)	0%	2.4%	7.5%	7.8%	12.5%
Epe (200 mg/kg)	1.6%	5.7%	12.5%	17.4%	24%
Prefed (fpj)	18%	18%	18.3%	16.5%	15.1%



Discussion

The aim of this study was to evaluate the analgesic effects of *P.granatum* fruit juice and ethanolic extract which is commonly used by Libyan traditional medicine and to try to find alternative for analgesics which are widely used and known for their common side effects. We also focused on making a preliminary comparison between the juice and fruit extract.

To investigate the analgesic effect of pomegranate fruit juice and ethanolic extract three different models were conducted. The peripheral analgesic effect of the extract was tested by using the writhing test. This test is widely accepted as a model for visceral pain (Kozak et al 1998;Vogel andVogel1997). Writhing induced by chemical substances eg acetic acid injected i.p. are due to sensitization of nociceptors by prostaglandins and this test is useful for the evaluation of mild analgesic nonsteroidal anti-inflammatory compounds which act peripherally.

The group of mice given pomegranate ethanolic extract produced a significant dose related effect in writhing test, the prefed group with pomegranate fruit juice also produced a significant effect whereas those given (fpj) instantly i.p or p.o.failed to produce response.

This could be to the fact that biologically active dietary constituent has only limited effects on its relevant target and significant differences are only reached over time through a cumulative effect where daily benefits add up day after day (Ameye and Chee ,2006).

Another explanation is that the effect given by pomegranate fruit juice may be due to one or more metabolites. A study by (Seeram et al., 2006) confirmed that after pomegranate juice injection ellagic acid metabolites which were not present in juice consumed as dimethylellagic acid glucoronide were detected in plasma and urine while urolithins formed

by intestinal bacteria were detected in urine samples and urolithin metabolites excreted in urine can persist for 48 hours thereby suggesting an explanation of long term administration.

In another study three pomegranate juice metabolites were detected in plasma urolithinA, urolithinB and a third unidentified metabolite ,maximum excretion rate occurred 3-4days after juice ingestion (Cerda et al., 2004). The same author also reported that ellagitannins bioaccumulate in small animals (Cerda et al., 2003).

The inhibition of writhing in mice by the extract suggest a peripheral mechanism of action possibly mediated by inhibition of PGE among several possibilities. Pomegranate extract revealed the presence of phytochemicals including flavoinoids which have a role in analgesic activity and work by targeting prostaglandins and inhibiting prostaglandin synthetase and tannins. Flavoinoids are also known to suppress COX2 transcription (Oleary et al., 2004). Cold pressed pomegranate seed oil has been shown to inhibit both cyclooxygenase and lipooxygenase enzymes invitro (Schubert et al., 1999).

Tail flick test is used to determine both centrally acting analgesics (Rambadrane et al., 1989) like morphine (Domer,1990) and peripherally acting analgesics like NSAIDs which inhibit cyclooxygenase in peripheral tissues ,thereby interfering with the mechanism of transduction of primary afferent nociceptors(Fields,1987). In this study aspirin 100mg/kg given intraperitonealy produced significant antinociception in both tests which agrees with (Miranda et al., 2001)who reported that NSAIDs elicited antinociception in writhing test and hot tail flick test.

The pomegranate ethanolic extract produced significant dose related analgesia with the hot tail flick test, at the dose of 100 mg/kg the onset of action started at 30 min whereas at dose150,200 mg/kg the onset was at 15 min which reached its peak at 60 min similar to aspirin This suggests that

it is possible that the extract produces analgesia by both a central and peripheral component on the other hand, the juice showed significant analgesia at 15min MPA18% which then declined to MPA15.1% at 60 min this either could be due to low concentration of bioavailable compounds such as ellagic acid or it may be due to a conditioning effect that develops due to repeated testing (Adovokat and McInnis 1992)Pomegranate extracts 100,150 and 200mg/kg showed significant suppression on pain induced by the radiant heat applied to the plantar surface of the heel of the right hind paw injected with carrageenan and left hind paw injected with saline. These results indicate that pomegranate action was via peripheral(right paw) and via central nervous system mechanisms (left paw)

The dose 200mg/kg of pomegranate produced an analgesic effect comparable to aspirin 100mg/kg in both right and left hind paw. This confirms our previous finding that pomegranate extract acts by peripheral and central mechanism of action.

While several researches have reported the health promoting effects of pomegranate juice (eg.,protection against cancer,diabetes, cardiovascular disease, inflammation, dental conditions erectile functions,bacterial functions antibiotic resistance, UV-induced skin damage, infant brain ischemia, male infertility, Alzeheimers disease and arthritis (Lansky and Newman,2007;Aviram et al2000;Aviram et al 2004;Jurenka,2008; Baso and Penugonda 2009) no studies on pomegranate juice analgesic effect have been reported. The most striking observation in this study is the ability of pomegranate juice to possess an analgesic effect, it has been reported that regular consumption of pomegranate juice provide significant amounts of water soluble hydrolysable ellagitannins (Gil et al 2000,Seeram et al 2004). Ellagitannins are hydrolysable tannins releasing ellagic acid on hydrolysis it was also reported that after a single administration of pomegranate juice 250 ml urolithins A and urolithin B are formed and conjugated in the liver prior excrection in the urine over12-56 hrs (Heber 2011). Furthermore a

study by Adams et al. (2006) reported that pomegranate juice inhibits COX2 enzyme. This evidence gives rise to use pomegranate as an alternative to NSAIDs

Conclusion

Based on the results of this study, it can be concluded that pomegranate fruit juice has an analgesic effect which may be peripheral and pomegranate ethanol extract has both central and peripheral analgesic effects. These findings may justify the use of this plant in medicine to manage pain.

References

- 1.Adams LS, Seeram NP, Aggarwal BB, Takada Y, Sand D, Heber D. (2006). Pomegranate juice, total pomegranate ellagitannins and punicalagin suppress inflammatory cell signalling in colon cancer cells. *J Agric Food Chem* 54:980–5
- 2.Advokat C, McInnis C. (1992). Environmental modulation of behavioral tolerance in spinal rats *Brain ResMay* 22:581(1)46-52
- 3.Ameye LG and Chee WSS. (2006). Osteoarthritis and Nurtrition .From nutraceuticals to functional foods a systemic review of the scientific evidence 8:4 1-229
- 4.Aviram,M.;Dorenffeld,L.;Rosenblat,M.;Volkova,N.;Kaplan,M.;Coleman, R.;Hayek,T.;Presser,D.;Fuhurman,B.(2000).Pomegrante juice consumption reduces oxidative stress,atherogeic modifications to LDL,and platelet aggregation:Studies in humans and in atherosclerotic apolipoprotein Edeficeient mice *American Journal of Clinical Nutrition* 71,1062-1076

- 5.Aviram,M.;Rosenblat,M.;Gaitini,D.;Nitecki,S.;Hoffman,A.;Dorenfield,L., Volkova,N.;Presser,D.;Attias,J.;Liker,H.;Hayek,T. (2004). Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common cold carotid intima-media thickness,bloodpressure and LDLoxidation .*Clinical Nutrition* 23,423-433
- 6.Basu, A.; Penugonada, K. Pomegranate juice Aheart-healthy fruit juice. Nutrition Review (2009) 67,49-56
- 7.Cerda B,Ceron JJ,Tomas-Barberan FA,Espin JC.(2004). Repeated oral administration of high doses of pomegranate ellagitannin punical agin to rats for 37days is not toxic *JAgric FoodChem* 51:3493-501
- 8.Cerda B, LlorachR, CeronJJ, EspinJC, Tomas-Barberan FA. (2003). Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol of pomegranate juice *Eur JNutr* 2003;42:18-28
- 9.CollierHO,Dinneen LC, Johnson CA,ScheiderC.(1968). The abdominal constriction response and its suppresssion by analgesic drugs in the mouse BrJPharmacolChemother, 32,295-310
- 10.Domer,F.(1990) Characterization of the analgesic activity of ketorolac in mice *Europ JPharmacol*177:127-137
- 11.Gil M I,TomasBarberanFA,Hess-PierceBetal .(2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing *JAgricFoodChem* 48:4581-4589
- 12.Heber, D. (2006). Preface to N.P. Seeram, R.N. Schulman & D. Heber, Pomegranates : ancient roots to modern medicine. New York: CRC Press.

- 13. Jurenka, J. Therapeutic applications of pomegranate (Punica granatum L.: A review Alternative Medicine Review 2008, 13, 128-144s
- 14.Kozak,W;Archuleta,I;Mayfield,K.P;KozakA;Rudolph,Kand KlugerMJ.(1998).Inhibitors of the alternative pathways of arachidonate metabolism differentially affect fever in mice.*AmJPhysiol*.275:1031-1040
- 15.Kulkarni AP,Aradhya SM.(2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development *Food Chemistry* 93:319-324
- 16.Lansky EP, Newman RA. (2007) Punicagranatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J Ethnopharmacol* jan 19,109(2):177-206
- 17.Levin ,GM.(1994) Pomegranate (*Punicagranatum*) plant genetic resources in Turkmenistan Plant genetic resources Newsletter 97,31
- 18.Mayer, W., Gurner, A., Andra, K. Punicalagin und punicalin zwel gerbstoffe aus den schalen der granatapfel Liebigs *Ann. Chem* 1977, 1976-1986
- 19.Mertens-Talcott SU,Jilma-StohlawetZP,Rios Jet al. Absorption ,metabolism and antioxidant effects of pomegranate (Punicagranatum L.)polyphenols after ingestion of standardized extract in healthy human volunteers.(2006). *J Agric Food Chem* 54:8956-8961
- 20.Miranda HF,LopezJ,SierraltaF,CorreaA andPinardi.(2001). NSAIDs antinociception measured in a chemical and a thermal assay in mice *Pain ResManage* 6(4):190-196

21.OLeary KA, dePascual-

TereasaS,NeedsPW,BaoYP,OBrienNM,WilliamsonG Effect of flavonoids and vitamineE on cyclooxygenase-2(C0X-2)transcription Mutat Res ;2004:13;551(1-2):245-54

- 22.PetersonW.L.,CyrerB.COX,sparing NSAIDs is the enthusiasm justified?JAMA 1999;282(20)1961-3
- 23.Perez-Vicente, A., Gil-Izquierdo, A., Garcia-Viguera C. (2002). Invitro gastrointestinal study of pomegranate juice phenolic compounds, anthocyanins and Vitamine C *Journal of Agricuktural and food chemistry* 50,2308-2312
- 24.QuachrifA,Khalkitt,ChaibS,MuntassirM.AbufatimaR,FaroukL,Benharraf A,ChaitA. (2012).Comparitive study of the anti-inflammatory and antinociceptive effects of two varieties of Punicagranatum. *PharmBiolApr* 50(4)429-38
- 25.RambadraneK,BansinathM,Turndorf H,PuigMM. (1989). Tail immersion test for the evaluation of a nociceptive reaction in mice methodological considerations *JPharmacol Methods* 21(1):21-31
- 26.Seeram NP,HenningsSM,ZangY,SuchardM,LiZ,Herber D. (2006). Pomegranate juice ellagitannins metabolites are present in human plasma and some can persist in urine up to 48 hrs *J Nutr* 136 2481-2485
- 27.Sewell,R.D.E.andP.S.J.Spencer (1976). Antinociceptive activity of narcotic agonist and partial analgesics and other agents in the tail immersion test in mice and rats . *Neuropharmacol* 15,23-29
- 28. Schubert, S.Y., Lansky, E.P., & Newman, I. (1999). Antioxidant and eicosanoid enzyme

inhibition properties of pomegranate seed oil and fermented juice flavonoids. J

Ethnopharmacol 66:11-17

29.Shukla M,GuptaK,RasheedZ,Khan,KAand HaqqiTM. (2008).Bioavailable constituents metabolites of pomegranate (Punicagranatum L)preferentially inhibit COX2 activity exvivo andIL-6 beta induced PGE2 production in human chondrocytes invitro, *Journal of inflammation*5;9

30.Trease ,GE&EvansMC,Textbook of pharmacognosy ,13thedn(BailiereTindall,London,Toronto,Tokyo)1989

31.Vogel,H.&Vogel,W.H.1997.Drug discovery &Evaluation in Pharmacoogical Assay,Berlin&Springer,pp402-407

Semantic Repetition in Translating between Arabic and English

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Abstract

Languages are important medium for communication between nations. They are naturally distinctive. Arabic and English are among these languages which belong to two distant language families. Arabic is a Semitic language whereas, English is Anglo – Saxon. Accordingly, they are different. Several linguistic features pose variable problems when translating between these two languages. Semantic repetition in Arabic is among the problems which encounter professional as well as trainee translators when translating from Arabic into English. Despite the attempts by many translation researchers to deal with the problems encountered translators when translating, only a few investigated the problem of semantic repetition in Arabic This paper is an attempt to shed light on this translation problem using examples to illustrate it and explore the possible techniques suggested by translation scholars to tackle this and help to produce satisfactory translation to the target language reader of English. The discussions in this paper, could provide a point of reference for many translation researches throughout the Arab world.

Introduction

Texts as a form of communication are used by people to express their feelings, exchange ideas and communicate their mutual concerns. Therefore, the structure of a text is extremely important. Rowland and

Avery (2001. 111) emphasize that "Careful structuring is essential with any piece of writing, of whatever length or subject matter and whomever it is written, whether you are writing a novel, a letter to a friend. Or a recipe, clear structuring is the key of effective communication". They also suggest a number of considerations that influence the structuring of any material for an article, one of them and perhaps the most important is that any material must be clear, readable and comprehensible.

As far as semantic repetition is concerned, and according to Kaplan (1966) writings written by Arab students are characterized by repetion, elaborate parallelism rather than the linear arrangement. Moreover, Karama and Hajaj (1989: 185) on the other hand, claim that over assertion and exaggeration are among the characteristics ascribed to Arabic rhetoric.

Therefore, repetition is a unique phenomen in Arabic language. Arabs frequently make use of semantic repetion which is not normally used by English. To illustrate this, let us consider the following examples; the noun heart for instance, is monosemous in English, it describes one single word whereas, in Arabic, it is synonymous. It describes two words. It can be translated into Arabic either as: الدفن or الدفن This also applied to the English word rain which may be translated into Arabic as: عنت مطر The unawareness of such semantic distinction in both languages no doubt can be problematic to student of translation and trainee translators.

What is semantic repetition?

Repetion is the style that can be dominant in any text. Some key words are frequently repeated to emphasize aspects that central to the overall message of a certain text. But what is meant by semantic repetion?

Semantic repetion is the "repetion of synonym or near synonym" (cf. Dickens and Watson 1999:541). But what is meant by synonym and near synonym in language? Before discussing the types of semantic repletion

and in order to understand the definition semantic repetition, it is better to begin with the recognition of the definition of the synonym and near synonym which they exist in the definition of the semantic repetition.

What is a synonym and near synonym?

The Longman English defines a synonym as" a word with the same meaning or nearly the same

meaning as another word in the same language" A slight difference in the meaning of words in either language can result in inappropriate selection of the right equivalent synonym.

Thornburg (2002:9) defines synonyms as: "words that share a similar meaning". Thus: old, ancient, antique, aged, elderly are all synonyms in that they all share the common meaning of not young/new. He also argues that synonyms are similar, but not seldom the same. Even in the case between words which are interchangeably sued, such as Taxi and Cab, or aubergine and egg- plant, one will be preferred over the in certain contexts and by particular speakers(ibid) Although synonyms have the a useful function in languages, they on the other hand, provide a real challenge to both student of translation and trainee translator.

Similarly, Griffiths (2006:26) concludes that synonymy as 'Synonymy is equivalence of sense'. The nouns mother, mom, and mum are synonymous. When a single word in a sentence is replaced by a synonym – a word equivalent in sense – then the literal meaning of the sentence is not changed: My mother's / mom's / mum's family was Christie.

Types of synonyms

According to Ghazala (1995: 91) synonyms are divided into two types:

- 1. absolute synonym; words which are perfectly identical in meaning, i.e. words such as big, large, and huge.
- 2. near (or close) synonym; words which are similar to one another in meaning, i.e.

Angry

غاضب

Discomforted

ممتعض

Annoyed

متضايق

Disturbed

منزعج

It is worth mentioning in this context, that the later type of synonyms is commonplace in any language, written or spoken.

Types of semantic repetition

According to Dickens (2005:59) semantic repetition is of two basic kinds:

1. where the two words or phrases used have closely – related distinguishable meanings; an example of this is the Arabic nouns الاستقصاء which can be translated into English as:

Investigation and analysis

Another example is the Arabic nouns, التقويم والقياس which can be translated into English as:

Evaluation and measurement

Although the Arabic nouns used in the example above have different meanings but at the same time they are closely -- related to each other.

2. where the words or phrases are fully synonymous or. At least in the context in which they are being used, there's no clear difference in the meaning. As an example of this are the Arabic doublet adjectives in the phrase:

Literally translated into English by Dickens (2005:59) as:

In a continuing continuous manner

Another example of this type of semantic repetition is the Arabic doublet verbs:

It can be translated into English as:

Clarify and explain

Semantic repetition can also syndetic and may involve the use of connective (\mathfrak{g}) or - in the case of adjectives in particular, but also occasionally in the case of using nouns and verb - it may a syndetic that may occur without the use of a connective. To illustrate this, the use of the Arabic adjectives:

It can be translated into English as:

Savage and barbaric

Further example of syndetic repetition is the use of Arabic adjectives:

فاتنات جميلات

It can be translated into English as:

Pretty attractive

Having discussed definitions and types of semantic repetition a key question needs to be answered which is what translation techniques can be used to translate semantic repetition when translating between English and Arabic?

Dickens (2005:59-61) suggests a number of translation techniques that can be used during the translation of semantic repetition from English into Arabic. They are as follow:

1. Merging the semantic repetition of the Arabic doublet in the sentence.

The two Arabic words are combined in one English word. This translation technique is likely to be an appropriate strategy when there is no clear difference in the meaning between the two Arabic words. Thus, the semantic repetition of the Arabic words صارمة وقاسية

phrase اجراءات صارمة وقاسية .The two Arabic Adjectives صارمة وقاسية merged into one English Adjective severe to repeat one single concept.

Further example of using this technique in translating semantic repetition into English, is the Arabic nouns تحدیث وعصرنة merged into one English noun modernize.

2. Employing grammatical transposition.

This translation technique is used when the semantic repetition clearly has different meanings i.e. the Arabic nouns القيم الاخلاقية in this case the Arabic

noun doublet has been transposed by an – adjective – noun phrase: *moral* values.

3. Semantic distancing.

Another technique for translating semantic repetition into English is called semantic distancing. In this translation technique, two words give two sets of meaning. It also involves relaying both elements of the Arabic doublet by different words in English whose meanings more distant than their Arabic counterparts. (Dickens 2005:60). The Arabic doublet "يدهش ويذهل "for instance, is a good example of this type of repetition. The phrase منظرها يدهشه ويذهله has been translated into English by St Jones 1999:5) as:

Her appearance had both astonished and alarmed him

Itis worth mentioning here that the semantic differences in the English words astonish and alarm is greater than the Arabic doublet يدهش ويذهل.

As far as sematic repetition is concerned it also possible to combine semantic repetition with grammatical transposition. A good example of this perhaps the extract taken from the Lebanese politician attitude towards his party when he said:

This was translated into English by Jones (1999: 7) as:

I remain committed more than ever to the project of unification and reform

In this example, the Arabic adjectives(Active participle) مستمر ومتمسك has been transposed to a verb - adjective(past participle) English doublet remain committed in the sense of the Arabic doublet have been distanced more in the English translation.

4. Maintaining the same form of repetition

The final technique can be used in translating Arabic semantic repetition into English is to maintain the same form of repetition – the repetition in the English version carries the same character and force as Arabic does. The following example illustrates this:

It can be translated into English as:

This civilized and high class behavior

It is obvious in the example above that the repetition in the English translation carries the same emphatic – and emotive force as it does in Arabic.

Conclusion

To conclude, a structure of a text is extremely important. To guarantee good communication, texts need to be well-structured. The contrastive knowledge and the awareness of Linguistic differences between languages specially the one concerned with rhetoric in general, and style in particular, is a vital factor and plays an important role in the production of acceptable target language texts. Due to the sharp linguistic differences between Arabic and English, Semantic repetition in Arabic for stance, represents certain problems when translating between Arabic and English and vice versa. Therefore, semantic repetition in Arabic requires special attention to overcome this difficulty in translation.

Despite the various issues of translation between Arabic and English, little has been devoted to the translation problems in translating between Arabic and English. Furthermore, limited attention and effort has been given to addressing the problem of semantic repetition when translating between Arabic and English. As stated in the introduction, the main aim of this paper has been to examine the semantic repetition when translating

between Arabic and English. To achieve this aim, we have discussed this translation problem that encounters both student of translation as well as trainee translators when translating between Arabic and English. Taking the differences between Arabic and English into consideration, and because bilingual dictionaries (Arabic / English) are not always appropriate for handling this problem, the paper also introduced number of techniques suggested by some translation scholars to tackle this translation problem. Finally, this study also shows that more research need to be conducted especially those with cultural nature – so that any other cultural problems that this paper did not revealed can be uncovered.

References

- 1. Avery. and Roland. D. (2001) 'Styles of journalism'. In Stott. and Avery.(ed)Writing with Style. London, UK: Longman Group Limited, pp. 105-126.
- 2. Dickens, J. and Watson, J.C.E (1999) Standard Arabic Course. Cambridge: Cambridge University Press.
- 3. Jones, L. (1999) Translation of الحاج رئيسا للكتائب بفارق سبع اصوات newspaper 2. March. 1999). BA Translation Project: University of Durham
- 4. James. D. Hervey. and Higgins. I(2005) Thinking Arabic Translation: Course in Translation Method: Arabic to English .London Routledge.
- 5. Kaplan, R.B. (1966) Cultural thought patterns in Intercultural education in Language Leaning, XVI (1-2). Pp 1-20.
- 6. Karama. N. and Hajaj. A. (1989) Errors in English among Arabic Speakers: Analysis and Remedy. London: Longman.
- 7. Ghazala. H. (1995) Translation as Problems and Solutions: A course book for Students and trainee translators.
- 8. Scott Thornbury (2002) How to teach vocabulary. Series editor: Jeremey Harmer. Pearson Longman.

A NEW CMOS DESIGN AND ANALYSIS OF CURRENT CONVEYOR SECOND GENERATION (CCII)

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Abstract

This paper describes the current conveyors used as a basic building block in a variety of electronic circuit in instrumentation and communication systems. Today these systems are replacing the conventional Op-amp in so many applications such as active filters, analog signal processing. Current conveyors are unity gain active building block having high linearity, wide dynamic range and provide higher gain bandwidth

The proposed current conveyors are simulated using TSMC 0.18 μ m CMOS technology on Advanced Design System and the results are also tabulated for comparison. The main features of these current conveyors are low voltage, less power, high slew rate and wide bandwidth for voltage transfer (V_y to V_x) and current transfer (I_x to I_z) which make them suitable for high frequency and low power applications.

Keywords

Bulk-Driven transistors, Current Conveyor of Second Generation CCII, CMOS integrated circuit, PSPICE simulation.

1. INTRODUCTION

One of the most basic building blocks in the area of current-mode analogue signal processing is the current conveyor (CC). The principle of the current conveyor of the first generation was published in 1968 by K. C. Smith and A. S. Sedra [1]. CCI was then replaced by a more versatile second-generation device in 1970 [2], the CCII. Current conveyor designshave mainly been with BJTs due to their high transconductance values compared to their CMOS counterparts. They are used as current-feedback operational amplifiers like the MAX477 high-speed amplifier and the MAX4112 low-power amplifier, which both feature current feedback rather than the conventional voltage feedback used by standard operational amplifiers. Current conveyors are used in high-frequency applications where the conventional operational amplifiers cannot be used, because the conventional designs are limited by their gain-bandwidth product.

The current mode circuits such as Current conveyors (CCs) have received considerable attention and emerged as an alternate building block to the Op-Amp (voltage mode circuit) in the field of analog signal processing [3] due to its potential performance feature. In CCs, the use of current rather than voltage as the active parameter can result in a higher usable gain, accuracy and bandwidth due to reduced voltage excursion at sensitive nodes [4]. The current conveyors are not only useful for current processing, but also offer certain important advantages in voltage processing circuits. The nonlinear circuits and dynamics [5] can easily be developed using CCs.

With the reduction in the supply voltage and device threshold voltage of CMOS technology, the performance of CMOS voltage mode circuits has greatly affected which results in a reduced dynamic range, an increased propagation delay and reduced noise margins. The CCs have simple structure, wide bandwidth and capability to operate at low voltage. It also offer unity current gain, unity voltage gain, higher linearity, wider dynamic range and better high frequency performance.

2. THE CURRENT CONVEYOR CC

The current conveyor is functionally flexible and versatile in nature as it has precise unity voltage gain between X and Y; unity current gain between Z and X as shown in Fig. 1, rather than the high ill-defined open loop gain of Op-Amps. Because of this fact, CCII is generally used without feedback in amplifier applications [6, 7].

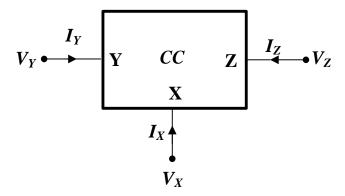


Fig. 1: Building block of Current conveyor

The build block of current conveyor and its generalised characteristics equation are represented by the following hybrid matrix.

$$\begin{bmatrix} I_Y \\ V_X \\ I_Z \end{bmatrix} = \begin{bmatrix} 0 & a & 0 \\ b & 0 & 0 \\ 0 & c & 0 \end{bmatrix} \begin{bmatrix} V_Y \\ I_X \\ V_Z \end{bmatrix} \tag{1}$$

The current conveyor is a grounded three-port network represented by the black box (Fig 1) with the three ports denoted by X, Y, and Z. Its terminal characteristics can be represented best by a hybrid matrix giving the outputs of the three ports in terms of their corresponding inputs [8].

3. CURRENT CONVEYOR SECOND GENERATION CCII

The second-generation current conveyor (CCII) is used as a basic building block in many current-mode analog circuits. It offers high input impedance at voltage input port Y, which is preferable in order to avoid loading effect. So, second generation current conveyor is developed to overcome the problem loading effect of CCI. The CCII is considered as a basic building block in analog circuit design because all the analog applications can be developed by making suitable connections of one or more CCIIs with passive and active components.

The second-generation current conveyor is a grounded three-terminal (X, Y and Z) device as shown in Fig. 2 (a), and the equivalent circuit of the ideal CCII is shown in Fig. 2 (b).

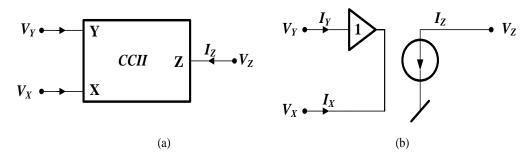


Fig. 2: (a) The CCII symbol, (b) ideal equivalent circuit.

The characteristics of ideal CCII are represented by the following hybrid matrix

$$\begin{bmatrix} I_Y \\ V_X \\ I_Z \end{bmatrix} = \begin{bmatrix} 0 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & +1 & 0 \end{bmatrix} \begin{bmatrix} V_Y \\ I_X \\ V_Z \end{bmatrix}$$
 (2)

An ideal CCII has the following characteristics:

- Infinite input impedance at terminal Y ($R_Y = \infty$ and $I_Y = 0$)
- Zero input impedance at terminal $X (R_X = 0)$
- Accurate voltage copy from terminal Y to X $(V_X = V_Y)$
- Accurate current copy from terminal X to Z with infinite output impedance at Z ($I_Z = I_X$ and $R_Z = \infty$)

4. OPERATIONS USING THE IDEAL CCII

• Amplifiers using CCII

The CCII can easily be used to form the current output amplifiers and voltage-output amplifier as shown in Fig. 3.The voltage- and current- gains are as follows:

$$\frac{I_{out}}{I_{in}} = \frac{R_1}{R_2} \tag{3}$$

$$\frac{V_{out}}{V_{in}} = \frac{R_2}{R_1}$$
 (4)

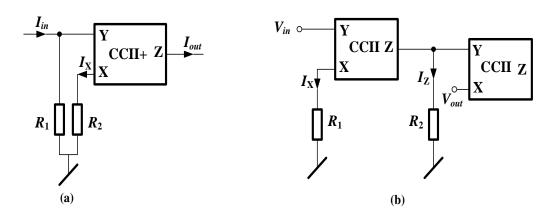


Fig. 3: (a) CCII-based current amplifier, (b) CCII-based voltage amplifier.

• Integrators using CCII

In Fig. 4, simple current- and voltage- integrators are presented.

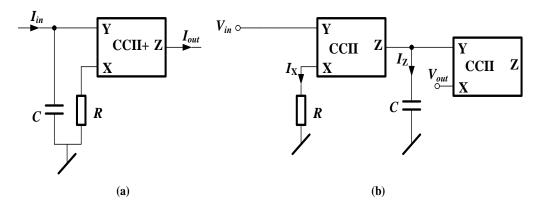


Fig. 4: (a) CCII-based current integrator, (b) CCII-based voltage integrator.

The output signals are as follows:

$$I_{out} = \frac{I_{in}}{sCR} \tag{5}$$

$$V_{out} = \frac{V_{in}}{sCR} \tag{6}$$

Adders using CCII

In Fig. 5, CCII-based current adder and CCII-based voltage adder are reported, with the following equations:

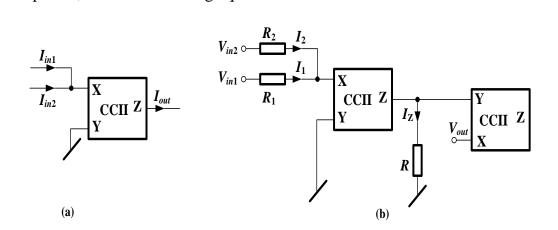


Fig. 5: (a) CCII-based current adder, (b) CCII-based voltage adder.

$$I_{out} = -(I_{in1} + I_{in2})$$
 (7)

$$V_{out} = -\frac{R}{R_1} V_{in1} - \frac{R}{R_2} V_{ir}$$
 (8)

• Differentiators using CCII

Current- and voltage-mode versions are shown in Fig. 6. The output signals are as follows:

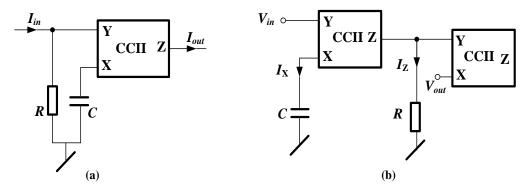


Fig. 6: (a) CCII-based current differentiator, (b) CCII-based voltage differentiator.

5. PROPOSED CMOS CURRENT CONVEYOR SECOND GENERATION

A new connection of Bulk-driven OTA is used to realize the CCII. In the OTA-based approach, presented in Fig.7, Bulk-driven OTA is used to implement the unity gain buffer between the Y and X inputs [9]. The X input current I_x is sensed by duplicating buffers, output transistors M_6 and M_7 using transistors M_8 and M_9 , and extracting the X current from them as I_z . Since transistors M_8 and M_9 have the same size and gate-source voltage as the output stage transistors M_6 and M_7 , the current I_z should be a copy of the current flowing through M_6 and M_7 which is I_x . Transistors M_{10} - M_{15} are used to generate I_z . Since no additional transistors need to be inserted between the OTA and rails, the approach will not increase the minimum operating voltage over that of the operational core. In addition the voltage follower is based on an OTA, thus it will maintain all the benefits and also the disadvantages of such a circuit i.e. a good voltage follower at the cost of lower bandwidth [10].

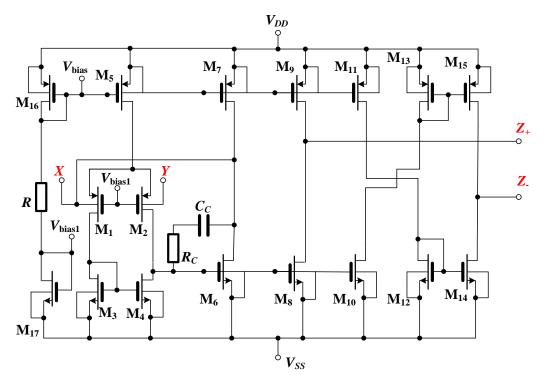


Fig. 7: Bulk-driven CCII± based on Bulk-driven OTA.

The aspect ratios of each of the transistors used the CCII in Fig. 7 are listed in Table1.

$$V_{DD}\&V_{SS} = \pm 0.6 \text{V}, R = 5\text{k}\Omega, R_C = 4.7\text{k}\Omega, C_C = 0.5\text{pF}$$

Transistor	Length (µm)	Width (µm)
M_1,M_2	2	30
M ₃ ,M ₄	2	4
M ₅ ,M ₁₆	3	20
M ₆ , M ₈ , M ₁₀ ,M ₁₂ , M ₁₄	2	16
M ₇ , M ₉ , M ₁₁ ,M ₁₃ , M ₁₅	3	40
M ₁₇	3	10

Table 1. Aspect ratios of the transistors used in the CCII in Fig. 7.

6. SIMULATION RESULTS

The simulated frequency responses of current gains I_{z+}/I_x , I_z/I_x are given in Fig. 8. The cut off frequencies for the gains are 20 MHz and 52 MHz, respectively.

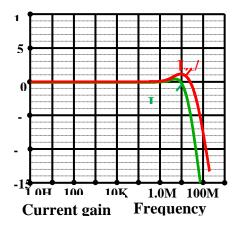
In Fig. 9, the input voltage buffer behaviour is shown. A DC sweep simulation has been performed, to check the range in which the voltage on X node is equal to the voltage applied to Y node.

The current linearity between X and Y terminal of the bulk-driven current conveyor (CCII±) from Fig. 7, is demonstrated in Fig. 10. Note that for input currents I_x and I_z , the boundary of linear operation is $ca\pm 16\mu A$.

The corresponding small-signal current gains are as follows: I_{z+}/I_x , $I_z/I_x = 1$, and the corresponding voltage gain $V_x/V_y = 0.97$.

The small-signal low frequency resistance of the X terminal R_x is equal to 166Ω as shown in Fig. 11. The small-signal resistance of the Y terminal R_Y is equal to $50G\Omega$.

The small-low frequency signal resistances of the Z_+ , Z_- outputs terminals are equal to $560k\Omega$, and $554k\Omega$, respectively. Simulation results of the CCII± are summarized in Table 2.



600m V_Y[V 400m 0m 0m 0m 0m V_V[V

Fig. 8: Frequency variation of the current gains I_{z+}/I_x , I_z/I_x in dB of the CCII in Fig. 7.

Fig. 9: Voltage follower between X and Y of the CCII in Fig. 7.

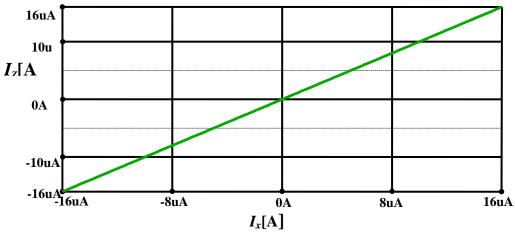
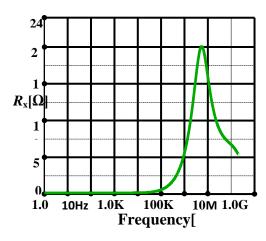


Fig. 10: Current linearity between X and Y of the CCII in Fig. 7



600K
R_{Z+-}
400K

200

1.0H 100H 10KH 1.0MH100MH
Frequency

Fig. 11: The X node input resistance $r_{in,x}$ of the CCII in Fig. 7.

Fig. 12: The Z node output resistance $r_{in,Z}$ of the CCII in Fig. 7.

Characteristics	Simulation Result
Power consumption	119 μW

3-dB bandwidth I_{Z+}/I_X	20 MHz	
3-dB bandwidth $I_{\rm Z}/I_{\rm X}$	52 MHz	
DC voltage range	-400, 600 mV	
DC current range	±16 μA	
Current gain $I_{\rm Z}/I_{\rm X}$	1	
Voltage gain $V_{\rm X}/V_{\rm Y}$	0.97	
Node X parasitic DC resistance	166 Ω	
Node Y parasitic DC resistance	50 GΩ	
Node Z+ parasitic DC resistance	560 kΩ	
Node Z- parasitic DC resistance	554 kΩ	
Measurement condition: $V_{DD} = 0.6V$, $V_{SS} = -0.6V$		

Tab. 2: Simulation results of the Bulk-driven CCII.

7. CONCLUSION

In this paper Bulk-driven CCII based on operational Transconductance Amplifier OTA is simulated using TSMC 0.18um CMOS technology with 0.6V power supply. Differential pair partially improves for power dissipation and terminal impedances but bandwidth reduces a when scaled down from 0.35um to 0.18um. CCII can be used as a voltage buffer and current buffer.

REFERENCES

- [1] SMITH, K.C., SEDRA, A. The current conveyor: a new circuit building block. *IEEE Proc. CAS*, 1968, vol. 56, no. 3, pp. 1368-1369.
- [2] CHUN-MINGCHANG. Multifunction biquadratic filters using current conveyors, *IEEE Transaction on Circuits and Systems-II, Analog and Digital signal processing*, vol. 44, no. 11, pp.956-958, 1997.

- [3] R V Yenkar, R S Pande and S S Limaye. _The Survey of Historical Technical Development In Current Conveyors And Their Applications'. *IJCA Proceedings on National Conference on Innovative Paradigms in Engineering and Technology* (NCIPET 2012) ncipet (4):17-23, March 2012.
- [4] B. Wilson, Recent developments in current conveyors and current-mode circuits', *IEE Proceedings*, 1990, 137(2) pp. 63-77
- [5] Liu, Tsao, J. Wu and Tsay, Nonlinear circuit applications with current Conveyors', *IEEE Proc.* 140(1), 1993, pp. 1-6.
- [6] Sedra A, Robert G, Gohn F S: The current conveyor: history and progress, *IEEEInternational Symp. on Circuits and Systems*, Portland, USA, 1989,3, pp. 1567-1570.
- [7] Grigorescu L, Amplifier built with current conveyors', *Rom. Journ. Phys.*, 2008, 53(1-2) pp. 109-113.
- [8] Senani R, Novel application of generalized current conveyor', *ElectronicsLetter*, 1984, 20(4), pp. 169-170.
- [9] KHATEB, F., BIOLEK, D., NOVACEK, K. On the design of low-voltage low-power bulk-driven CMOS current conveyors.

[10] Shaktour, M. Design of low power low voltage bulk driven operational transconductance amplifier, *Poznan University of Technology Academic Journals*, Poland, 2014, 19, pp. 63-70.

Accelerating the Modified Picard Iteration By Using Green's Function Approach

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Abstract

We consider the Picard's iteration method as a technique for solving initial value problems of the first and second order linear differential equations. The basic idea is the use of Green's function to collect some of the terms in a perfect differential term, and then use the decomposition techniques. For the second order differential equations, we transform the equation to a system of two first order equations and in addition we use the Gauss Seidel technique. The algorithm of the proposed method is discussed. Comparisons with the classical Picard method and modified Picard have illustrated the rapid convergence of the proposed method. Numerical examples have illustrated that the technique obtains the theoretical fixed point quicker than that obtained with other techniques including the modified Picard.

الملخص العربي:

في العقد الأخير من القرن الماضي و إلى الآن لا يزال الاهتمام بنظرية الوجود و الوحدانية مستمراً و ذلك للوصول لحل تقريبي لأي معادلة تفاضلية بشروط ابتدائية، و كانت الطرق التقريبية المتبعة سابقاً متعبة و مملة و تتطلب مجهوداً في العمليات الحسابية.

و لهذا قمت بالبحث عن طريقة أخرى للتخلص من بعض العيوب لتلك الطرق و تعطى نتائج جيدة، فكانت طريقة بيكار د التكرارية و طريقة بيكار د المعدلة هما الأنسب حيث قمت بتعديلها بواسطة معادلة جرين التكاملية و ذلك لزيادة سرعة التقارب والوصول إلى الحل المضبوط لأي مسألة قيمة ابتدائية.

Keywords: Picard iteration, Green's function, Differential equations, Gauss-Seidel method.

1. Introduction

The authors introduced many studies to find solution for differential equation from through using several methods and they said that say the solution must be a unique, among all the available methods, the Picard's method, in [4,7] the authors applied Contraction Mapping Principle which used to prove Picard Theorem "Existence and Uniqueness theorem " which play a vital role in the theory of differential equation, the idea of approach is very simple; the ODE of the first order will be converted to an integral equation, which defines a mapping T, and a conditions of the theorem will imply that T is a contraction which implies that T has a fixed point. There many theorems on the existence of a unique solution of the differential equations under certain condition, and also many approximate techniques for solving systems of ordinary differential equation have been developed.

The Picard iteration is important to construct the existence and uniqueness of solutions of first order of differential equations and apposition to systems of first order of differential equations. In [1], El-Arabawy interested in symbolic computations in treating initial and boundary value problems. In [7] Yilldiz studied nonlinear boundary value problem where they construct an operator equation and show that the special approximations for the operator equation get better convergence speed. Also investigated nonlinear BVP by used the successive approximation method.

The Gauss-Seidel method is a technique for solving systems of linear algebraic equations which was used in researching of Youssef [8] which was concerned with the study of optimizing for Picard iteration method. In [3] Ha studied the Green's function to find numerical solutions of second-order linear and nonlinear differential equations with various boundary conditions. And discussed and analyzed numerical solutions which are

obtained by the Green's function and shooting method and comparison between them.

The objective of this paper is to use the Green function for the first order to find solution of initial value problems for first-order systems of ordinary differential equations.

2. Green Functions for First and Second Order Equations

The Green's function is type of function used to solve the non-homogenous differential equation subject to initial and boundary conditions, a homogeneous linear ODE has trivial solution only, but has nontrivial solution when the initial conditions are not zero, a Green's function G(x,s) of linear operator L, at a point s is any solution of the following problem:

$$LG(x,s) = \delta(x-s) \tag{1}$$

where δ is the Dirac delta function, this method can be solve differential equations of the form;

$$Ly(x) = f(x) \tag{2}$$

If kernel of L is nontrivial, then the Green's function is not unique. If we multiply the equation (1) for the Green's function by f(x), then by integration we obtain:

$$\int LG(x,s)f(s)ds = \int \delta(x-s)f(s)ds = f(x)$$

Case (I): Consider the first order non-homogeneous equation

$$L[y] = f(x)$$
 for $x > a$ (3)

Where $L = \frac{d}{dx} + p(x)$ Subject to initial condition $u(a) = u_0$

The Green function G(x,s) is defined as the solution to $L[G(x,s)] = \delta(x-s)$ subject to G(a,s) = 0

We can represent the solution to the inhomogeneous problem in Eq. (3) as an integral involving the Green function,

$$y(x) = y_h + \int_a^{\infty} (0) f(s) ds$$
 (4)

Where G(x,s) is continuous in x and s. For $x \neq s$, LG(x,s) = 0, the integral also satisfies the initial condition.

$$\int_{a}^{\infty} G(x,s)f(s)ds = \int_{a}^{\infty} (0)f(s)ds = 0$$

We integrate the differential equation on the interval (s^-, s^+) to determine this jump.

$$G'+p(x)G=\delta(x-s)$$

$$G(s^+, s) - G(s^-, s) + \int_{s^-}^{s^+} p(x)G(x, s)ds = 1$$

$$G(s^+,s)-G(s^-,s)=1$$

The homogeneous solution of the differential equation is

$$y_h = y_0 e^{-\int p(\tau)d\tau}$$

Since the Green function satisfies the homogeneous equation for $x \neq s$, it will be a constant times this homogeneous solution for x < s and x > s.

$$G(x,s) = \begin{cases} c_1 e^{-\int p(\tau)d\tau}, & a < x < s \\ c_2 e^{-\int p(\tau)d\tau}, & s < x \end{cases}$$

In order to satisfy the homogeneous initial condition G(a, s) = 0, the Green function must vanish on the interval (a, s).

$$G(x,s) = \begin{cases} 0, & a < x < s \\ e^{-\int_{s}^{x} p(\tau)d\tau}, & s < x \end{cases}$$

Then the eq. (4) will be come

$$y(x) = y_0 e^{-\int_a^x p(\tau)d\tau} + \int_a^\infty e^{-\int_s^x p(\tau)d\tau} f(s)ds(5)$$

Consequently, we can rewrite an eq. (5) as the form [2, 9]

$$y(x) = y_0 G(a, x) + \int_a^\infty G(x, s) f(s) ds$$

Where
$$G(a,x) = e^{-\int_a^x p(\tau)d\tau}$$

Clearly the Green function is of little value in solving the inhomogeneous differential equation in eq. (3), as we can solve that problem directly.

Case (II): We consider the second order non-homogeneous equation

$$L[y] = f(x) \text{ for } a \le x \le b \tag{6}$$

Subject to a homogeneous boundary conditions, y(a) = y(b) = 0.

Where
$$L = \frac{d^2}{dx^2} + p(x)\frac{d}{dx} + q(x)$$
 and where $p(x), q(x)$ and $f(x)$ are

continuous functions on interval [a,b].

The Green function G(x, s) is defined as the solution to

$$L[G(x,s)] = \delta(x-s)$$
 subject to $G(a,s) = 0$

The solution of the non-homogeneous problem in eq. (6) as an integral involving the Green function.

$$y(x) = \int_{a}^{b} G(x, s) f(s) ds \tag{7}$$

Let y_1 and y_2 be two linearly independent solutions to the homogeneous equation L[y] = 0 Since the Green function satisfies the homogeneous equation for $x \neq s$, it will be a linear combination of the homogeneous solutions.

$$G(x,s) = \begin{cases} c_1 y_1 + c_2 y_2, & x < s \\ d_1 y_1 + d_2 y_2, & x > s \end{cases}$$

Since G(x,s) is continuous and G'(x,s) has only ajump discontinuity, which determined by:

$$\int_{s^{-}}^{s^{+}} [G''(x,s) + p(x)G'(x,s) + q(x)G(x,s)] dx = \int_{s^{-}}^{s^{+}} \delta(x-s) dx$$
$$G'(s^{+},s) - G'(s^{-},s) = 1$$

In [3] studied examples of boundary value problem to find solutions by using Green's function, and in [7] Green's function used in successive approximation equations.

If eq. (6) has initial conditions $y(a) = \alpha_1$, $y'(a) = \alpha_2$, in this case, the solution of eq. (6) is $y = y_h + y_p$.

where

$$y_p = \int_a^b G(x, s) f(s) ds$$

and

$$y_h$$
"+ $p(x)y_h$ '+ $q(x)y_h = 0$, $y_h(a) = \alpha_1, y_h$ ' $(a) = \alpha_2$

where y_p is solution of the nonhomogeneous equation $Ly_p = f$, which satisfies homogeneous boundary conditions $y_p(a) = y_p(b) = 0$ and y_h is solution of the homogeneous equation [5,6,9].

3. Materials and Methods

The objective of this work is the use of the Green's function integral approach for the first order equations to accelerate the convergence of Picard iteration method. As well as decompose the system corresponding to the linear second order initial value problems into two parts and use the Green's function integral for one part and use the Gauss seidel approach described in [8].

We consider the second order of linear differential equation (6) with the initial conditions $y(a) = \alpha_1$, $y'(a) = \alpha_2$, and reducing the eq.(6) to a system of the first order differential equations which takes the form

$$y_1 = y_2$$
 $y_1(a) = \alpha_1$ (8)

$$y_2 = f(x) - p(x)y_2 - q(x)y_1$$
 , $y_2(a) = \alpha_2$

Consequently; we use Green's function G(x,s) to find solution for y_2 , so it can be write solution of y_2 as the form

$$y_2 = y_2(a)e^{-\int_a^x p(\tau)d\tau} + \int_a^x e^{-\int_s^x p(\tau)d\tau} (f(s) - q(s)y_1)ds$$

where
$$G(x,s) = e^{-\int_{s}^{x} p(\tau)d\tau}$$
.

Hence, the system of equations (8) can be written as the form

$$y_{1,n} = y_{1,0} + \int_{a}^{x} y_{2,n-1} ds$$
 $n = 1, 2, ...$ (9)

$$y_{2,n} = y_{2,0}e^{-\int_a^x p(\tau)d\tau} + \int_a^x e^{-\int_s^x p(\tau)d\tau} (f(s) - q(s)y_{1,n-1})ds$$

According to the [7], we will decrease from the steps of the previous iteration by using the Gauss-Seidel method for linear system subsequently the iteration will become the form

$$y_{1,n} = y_{1,0} + \int_{a}^{x} y_{2,n-1} ds, \qquad n = 1, 2, \dots$$

$$y_{2,n} = y_{2,0} e^{-\int_{a}^{x} p(\tau) d\tau} + \int_{a}^{x} e^{-\int_{s}^{x} p(\tau) d\tau} (f(s) - q(s)y_{1,n}) ds$$
(10)

Since the Picard method for eq. (8) is converges, also this method is converges and will do on increasing of convergence. we will introduced the different cases of differential equations to show the comparison between Picard method and the proposed method.

Firstly, we will consider the linear equation with constant coefficients

$$y"+\alpha y'+\beta y = f(x) \tag{11}$$

with the initial conditions $y(a) = \alpha_1$, $y'(a) = \alpha_2$, where the right hand side f(x) of equation takes the different formulas.

Secondly, The Euler Cauchy equations are differential equations of the form

$$\alpha x^2 y'' + \beta x y' + \gamma y = f(x) \tag{12}$$

with given constants α , β , γ and unknown y(x).

4. Main results

In this section, we will mention different types of examples of differential equations, the first type examples are linear with constant coefficients and second type with variable coefficients and the third type are examples of nonlinear differential equations with constant and variable coefficients.

Example 1. Consider the initial value problem

$$y'' + 4y' + 4y = e^{-2x}$$
, $0 \le x \le 1$ $y(0) = 1$, $y'(0) = -1$

The nonhomogeneous term is a part of the complementary function of the differential equation, the exact solution is: $y = (1 + x + \frac{x^2}{2})e^{-2x}$

The corresponding system takes the form:

$$y_1' = y_2$$
 $y_1(0) = 1$ (13)
 $y_2' = e^{-2x} - 4y_2 - 4y_1$ $y_2(0) = -1$

Accordingly, the classical Picard iteration method takes the form

$$y_{1,n} = 1 + \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, ...$$

$$y_{2,n} = -1 + \int_0^x (e^{-2x} - 4y_{2,n-1} - 4y_{1,n-1}) ds$$
(14)

Hence, the corresponding modified Picard iteration with Green's function integral is

$$y_{1,n} = y_{1,0} + \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, \dots$$

$$y_{2,n} = y_{2,0} G(x,0) + \int_0^x G(x,s) (e^{-2x} - 4y_{1,n-1}) ds$$
where:
$$G(x,s) = \begin{cases} 0, & x < s \\ e^{4(s-x)}, & s < x \end{cases}, G(x,0) = e^{-4x}$$

Consequently, the corresponding Picard iteration modified by Gauss Seidel with Green's function integral is

$$y_{1,n} = 1 + \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, ...$$

$$y_{2,n} = -e^{-4x} + \int_0^x e^{4(s-x)} (e^{-2x} - 4y_{1,n}) ds$$
(16)

Then we obtain the solution, and in the following table (1) we give the comparison between the Picard's solution and solution of the system of equations by using Green's function for first order.

T _v	2	Exact	Picard	Picard with	Picard G-S.
1	'i	у	y_7	$Gr. F y_7$	with $Gr. y_7$

0.0	1	1	1	1
0.1	0.904697	0.904697	0.904697	0.904697
0.2	0.81779	0.81779	0.81779	0.81779
0.3	0.738152	0.738152	0.738152	0.738152
0.4	0.665007	0.665012	0.665007	0.665007
0.5	0.597804	0.597835	0.597807	0.597804
0.6	0.536126	0.53626	0.536137	0.536126
0.7	0.479631	0.48009	0.479665	0.479631
0.8	0.428021	0.429353	0.428108	0.428021
0.9	0.381014	0.384422	0.381212	0.381014
1.0	0.338338	0.346225	0.338746	0.338338

Table1, the comparison between exact solution and the numerical approximations

Example 2. Consider the initial value problem

$$y'' + y' - 2y = 2x + (x^2 - 1)e^x$$
, $0 \le x \le 1$ $y(0) = 0$, $y'(0) = 1$

The nonhomogeneous term contains a part of the complementary function of the differential equation multiplied by a second degree polynomial plus another simple function, the exact solution is:

$$y = -\frac{1}{2} - x + \frac{88}{81}e^x - \frac{95}{162}e^{-2x} + \frac{(3x^3 - 3x^2 - 7x)}{27}e^x$$

The corresponding system takes the form:

$$y_1 = y_2$$
 $y_1(0) = 0$ (17)
 $y_2 = 2x + (x^2 - 1)e^x - y_2 + 2y_1$ $y_2(0) = 1$

Accordingly, the classical Picard iteration method takes the form

$$y_{1,n} = \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, \dots$$

$$y_{2,n} = 1 + \int_0^x (2s + (s^2 - 1)e^s - y_{2,n-1} + 2y_{1,n-1}) ds$$

$$\sim 148 \sim$$
(18)

Hence, the corresponding modified Picard iteration with Green's function is

$$y_{1,n} = \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, \dots$$

$$y_{2,n} = G(x,0) + \int_0^x G(x,s)(2x + (x^2 - 1)e^x + 2y_{1,n-1}) ds$$
Where: $G(x,s) = \begin{cases} 0, & x < s \\ e^{(s-x)}, & s < x \end{cases}$, $G(x,0) = e^{-x}$

Consequently, the corresponding Picard iteration modified by Gauss Seidel with Green's function integral is

$$y_{1,n} = \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, ...$$

$$y_{2,n} = e^{-x} + \int_0^x e^{(s-x)} (2s + (s^2 - 1)e^s + 2y_{1,n}) ds$$
(20)

In table (2) we give the comparison between the Picard's solution and the solution by using the Green's function integral of the first order.

ν.	Exact	Picard	Picard with	Picard G-S.	Absolute Error
x_i	У	y_7	$Gr. F y_7$	with $Gr. y_7$	Hosoinie Error
0.0	0	0.	.0	0.	0.
0.1	0.0908019	0.0908019	0.0908019	0.0908019	0.000000
0.2	0.166192	0.166192	0.166192	0.166192	0.000000
0.3	0.230241	0.230241	0.230241	0.230241	0.000000
0.4	0.286632	0.286634	0.286632	0.286632	0.000000
0.5	0.33885	0.33886	0.338851	0.338849	0.000001
0.6	0.390365	0.390411	0.39037	0.390365	0.000000
0.7	0.444821	0.444975	0.444836	0.44482	0.000001
0.8	0.50623	0.506666	0.506271	0.506226	0.000004
0.9	0.579183	0.580274	0.579282	0.579173	0.000001
1.0	0.669092	0.671566	0.669307	0.669067	0.000025

Table 2, the comparison between exact solution and the numerical approximations

Example 3 Consider the initial value problem

$$y''-3y'+2y = \sin x$$
, $0 \le x \le 1$ $y(0) = 1$, $y'(0) = 2$

The corresponding system of the IVP takes the form

$$y_1' = y_2$$
 $y_1(0) = 1$ (21)
 $y_2' = \sin x + 3y_2 - 2y_1$ $y_2(0) = 2$

Accordingly, the classical Picard iteration method takes the form

$$y_{1,n} = 1 + \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, ...$$

$$y_{2,n} = 2 + \int_0^x (\sin s + 3y_{2,n-1} - 2y_{1,n-1}) ds$$
(22)

From the system (21), we obtain the Green's function for first order DE y_2 , and defined by

$$G(x,s) = \begin{cases} 0, & x < s \\ e^{3(x-s)}, & s < x \end{cases}, G(x,0) = e^{3x}$$

then the solution to the equationy₂ represented by

$$y_2 = 2G(x,0) + \int_0^x G(x,s)(\sin s - 2y_1)ds$$
 (23)

The analogous modified Picard iteration with Green's function integral eq. (21) is approximated as the formula

$$y_{1,n} = 1 + \int_0^x y_{2,n-1} ds,$$
 $n = 1, 2, ...$ (24)

$$y_{2,n} = 2e^{3x} + \int_0^x e^{3(x-s)} (\sin s - 2y_{1,n-1}) ds$$

And so, the Picard iteration method modified by Gauss Seidel with Green's function integral takes the form

$$y_{1,n} = 1 + \int_0^x y_{2,n-1} ds, \qquad n = 1, 2, ...$$

$$y_{2,n} = 2e^{3x} + \int_0^x e^{3(x-s)} (\sin s - 2y_{1,n}) ds$$
(25)

We shall display results of the comparison between the Picard iteration as given by the procedure (22), the Picard method modified with Green's function as given by the procedure (24), and the modified Picard by Gauss Seidel method with Green's function as given by the procedure (25), which are indicated in the following tables (3-4) on the interval [0,1]. Also, the comparisons for all procedures with exact solution are shown in Figure 1.

ν.	Exact	Picard	Picard with	Picard G-S.	Absolute Error
x_i	y	$y_{1,7}$	<i>Gr. Fy</i> _{1,7}	with $Gr. F. y_{1,7}$	Hosoinie Error
0.1	1.22158	1.22158	1.22158	1.22158	0.000000
0.2	1.49338	1.49338	1.49338	1.49338	0.000000
0.3	1.82777	1.82777	1.82777	1.82777	0.000000
0.4	2.24	2.23999	2.24	2.24	0.000000
0.5	2.74879	2.74877	2.7488	2.74879	0.000000
0.6	3.37715	3.37702	3.37718	3.37715	0.000000
0.7	4.15324	4.15279	4.15335	4.15324	0.000000
0.8	5.11162	5.11027	5.112	5.11162	0.000000
0.9	6.29459	6.29103	6.29573	6.29459	0.000000
1.0	7.75396	7.74543	7.75702	7.75396	0.000000

Table 3, the comparison between exact solution and

the numerical approximations

From table (3), we find that the procedure (25) give results similar to the exact solution after only seven steps, and the convergence is faster than other procedures to the exact solution.

x_i	Exact	Picard	Picard with	Picard G-S.
λ_i	у	$y_{1,12}$	$Gr. Fy_{1,12}$	with $Gr.y_{1,12}$
0.1	1.22158	1.22158	1.22158	1.22158
0.2	1.49338	1.49338	1.49338	1.49338
0.3	1.82777	1.82777	1.82777	1.82777
0.4	2.24	2.24	2.24	2.24
0.5	2.74879	2.74879	2.74879	2.74879
0.6	3.37715	3.37715	3.37715	3.37715
0.7	4.15324	4.15324	4.15324	4.15324
0.8	5.11162	5.11162	5.11162	5.11162
0.9	6.29459	6.29459	6.29459	6.29459
1.0	7.75396	7.75396	7.75396	7.75396

Table 4, the comparison between exact solution and the numerical approximations

Table (4) demonstrates that the comparison between all procedures, where the approximate solutions which we obtained from Picard iteration and the Picard iteration modified with Green's function are arrive to fixed point after five steps from seventh step, while the Picard iteration modified by Gauss Seidel method with Green's function is still save the fixed point after the twelfth step, also will do on increasing of convergence, and is give the accurate results.

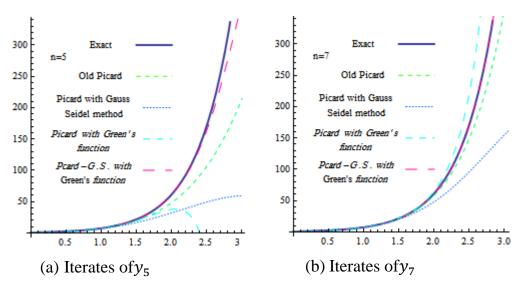


Figure 1 Comparison between exact solution and the numerical approximations

Example 4. Consider the Euler equation with initial conditions

$$x^{2}y'' + 4xy' + 2y = x^{2}$$
, $1 \le x \le 2$ $y(1) = 0$, $y'(1) = 1$

The exact solution for the differential eq. will be given $y = \frac{2}{3x} - \frac{3}{4x^2} + \frac{1}{12}x^2$

We rewrite differential equation as the form $y'' + \frac{4}{x}y' + \frac{2}{x^2}y = 1$, and then use the reduction technique to the system of first order differential equations

$$y_{1} = y_{2} y_{1}(1) = 0$$

$$y_{2} = 1 - \frac{4}{x}y_{2} + \frac{2}{x^{2}}y_{1} , y_{2}(1) = 1$$
(26)

So the Green's function is defined by

$$G(x,s) = \begin{cases} 0, & x < s \\ \frac{s^4}{x^4}, & s < x \end{cases}, G(x,1) = \frac{1}{x^4}$$

Therefore, the classical Picard iteration method takes the form

$$y_{1,n} = \int_{1}^{x} y_{2,n-1} ds,$$
 $n = 1, 2, ...$ (27)

$$y_{2,n} = 1 + \int_{1}^{x} (1 - \frac{4}{x} y_{2,n-1} - \frac{2}{x^2} y_{1,n-1}) ds$$

Accordingly, the corresponding modified Picard iteration with Green's function integral of (26) takes the form

$$y_{1,n} = \int_{1}^{x} y_{2,n-1} ds, \qquad n = 1, 2, \dots$$

$$y_{2,n} = \frac{1}{x^{4}} + \int_{1}^{x} \frac{s^{4}}{x^{4}} (1 - \frac{2}{x^{2}} y_{1,n-1}) ds$$
(28)

and the corresponding Picard iteration modified by Gauss Seidel with Green's function takes the form

$$y_{1,n} = \int_{1}^{x} y_{2,n-1} ds, \qquad n = 1, 2, ...$$

$$y_{2,n} = \frac{1}{x^{4}} + \int_{1}^{x} \frac{s^{4}}{x^{4}} (1 - \frac{2}{x^{2}} y_{1,n}) ds$$
(29)

We introduce the comparison between the Picard iteration, the Picard method modified with Green's function as given by the procedure (28), and the Picard iteration modified by Gauss Seidel with Green's function as given by the procedure (29), which are illustrated in the following tables (5-6) on the interval [1,2]. Also, the comparisons for all procedures with exact solution are shown in Figure 2.

ν.	Exact	Picard	Picard with	Picard G-S.
x_i	У	$y_{1,6}$	<i>Gr. F.</i> $y_{1,6}$	with Gr. F.
				$y_{1,6}$
1.0	0	0	0	0
1.1	0.0870592	0.0870592	0.0870592	0.0870592
1.2	0.154722	0.15472	0.154722	0.154722
1.3	0.209867	0.20984	0.209867	0.209867
1.4	0.256871	0.256714	0.256871	0.256871
1.5	0.298611	0.298009	0.298613	0.298611
1.6	0.337031	0.335276	0.337036	0.337031
1.7	0.373475	0.36923	0.373484	0.373475
1.8	0.408889	0.399917	0.408907	0.408889
1.9	0.443954	0.426825	0.443986	0.443954
2.0	0.479167	0.448953	0.479217	0.479167

Table (5), the comparison between exact solution and the numerical approximations

From table (5), we find that the procedure (29) give results analogous to the exact solution after only six steps, and the convergence is faster than other procedures to the exact solution.

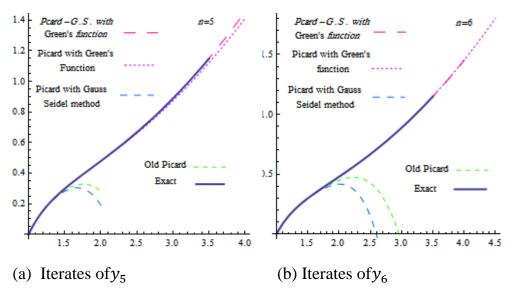


Figure 2 Comparison between exact solution and the numerical approximations

x_i	Exact	Picard	Picard with	Picard G-S.
	У	$y_{1,14}$	<i>Gr. F.</i> y _{1,9}	with $Gr. F. y_{1,6}$
0.0	0	0	0	0
0.1	0.0870592	0.087058	0.0870592	0.0870592
0.2	0.154722	0.154721	0.154722	0.154722
0.3	0.209867	0.209867	0.209867	0.209867
0.4	0.256871	0.256871	0.256871	0.256871
0.5	0.298611	0.29861	0.298611	0.298611
0.6	0.337031	0.33703	0.337031	0.337031
0.7	0.373475	0.373474	0.373475	0.373475
0.8	0.408889	0.408889	0.408889	0.408889
0.9	0.443954	0.443954	0.443954	0.443954
1.0	0.479167	0.479167	0.479167	0.479167

Table 6, the comparison between exact solution and

the numerical approximations

Table (6) is summarize the comparison between all procedures, where the approximate solutions which we obtained from Picard iteration and the Picard iteration modified with Green's function are arrive to fixed point after 8 and 3 steps from sixth step, respectively, while the Picard iteration modified by Gauss Seidel method with Green's function is still save the fixed point, also will do on increasing of convergence, and is give the accurate results.

It can be transformed equation $x^2y''+4xy'+2y=x^2$ into differential equations with constant coefficients, and then to find the numerical solutions from the successive approximations, consequently; let

$$x = e^t \implies \ln x = t$$
, so

$$\frac{1}{x} = \frac{dt}{dx}$$
, $\frac{dy}{dx} = \frac{dy}{dt}\frac{dt}{dx}$ \Rightarrow $x\frac{dy}{dx} = \frac{dy}{dt}$, $x^2\frac{d^2y}{dx^2} = \frac{d^2y}{dt^2} - \frac{dy}{dt}$

Thus, $x^2y'' + 4xy' + 2y = x^2$ will become

$$\frac{d^2y}{dt^2} + 3\frac{dy}{dt} + 2y = e^{2t}, \quad 0 \le t \le 2, \quad y(0) = 0, \ y'(0) = 1$$
 (30)

Therefore, the exact solution for the differential eq.(30) will be given

$$y(t) = \frac{2}{3}e^{-t} - \frac{3}{4}e^{-2t} + \frac{1}{12}e^{2t}$$

The reducing of the IVP into system of first order takes the form

$$y_1' = y_2$$
 $y_1(0) = 0$ (31)
 $y_2' = e^{2t} - 3y_2 - 2y_1$ $y_2(0) = 1$ $\sim 157 \sim$

Therefor, the classical Picard iteration method takes the form

$$y_{1,n} = \int_0^t y_{2,n-1} d\eta, \qquad n = 1, 2, ...$$

$$y_{2,n} = 1 + \int_0^t (e^{-2\eta} - 3y_{2,n-1} - 2y_{1,n-1}) d\eta$$
(32)

Accordingly, the corresponding modified Picard iteration with Green's function integral of (31) takes the form

$$y_{1,n} = \int_0^t y_{2,n-1} d\eta, \qquad n = 1, 2, ...$$

$$y_{2,n} = e^{-3t} + \int_0^t e^{3(\eta - t)} (e^{-2\eta} - 2y_{1,n-1}) d\eta$$
(33)

and the corresponding Picard iteration modified by Gauss Seidel with Green's function takes the form

$$y_{1,n} = \int_0^t y_{2,n-1} d\eta, \qquad n = 1, 2, \dots$$

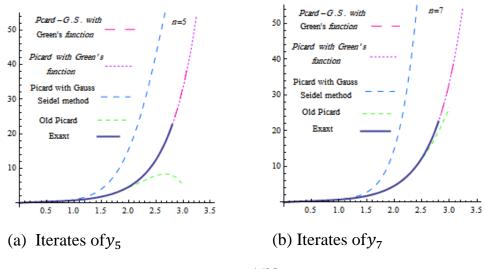
$$y_{2,n} = e^{-3t} + \int_0^t e^{3(\eta - t)} (e^{-2\eta} - 2y_{1,n}) d\eta$$
(34)

We introduce the comparison between the Picard iteration as given by the procedure (32), the Picard method modified with Green's function as given by the procedure (33), and the Picard iteration modified by Gauss-Seidel method with Green's function as given by the procedure (34), which are illustrated in the following tables (7 - 8) on the interval [0,2], where the procedure (34) for the proposed method gives us good consequences. Also, the comparisons for all procedures with exact solution are shown in Figure 3.

t_i	Exact	Picard	Picard with	Picard G-S.	Absolute Error
ι_i	y	<i>y</i> _{1,7}	<i>Gr. F.</i> y _{1,7}	with $Gr. F. y_{1,7}$	Hosoinie Error
0.0	0	0	0	0	0
0.2	0.167399	0.167399	0.167399	0.167399	0.000000
0.4	0.295345	0.295347	0.295345	0.295345	0.000000
0.6	0.416655	0.416695	0.416652	0.416655	0.000000
0.8	0.560883	0.561238	0.560859	0.560883	0.000000
1.0	0.759506	0.761369	0.759396	0.759506	0.000000
1.2	1.05136	1.05828	1.05099	1.05136	0.000000
1.4	1.48918	1.50916	1.48819	1.48918	0.000000
1.6	2.1484	2.19541	2.14619	2.1484	0.000000
1.8	3.13956	3.23075	3.13519	3.13956	0.000000
2.0	4.62633	4.76737	4.61866	4.62633	0.000000

Table 7, the comparison between exact solution and the numerical approximations

From table (7), we estimate that the procedure (34) give results analogous to the exact solution after only seven steps, and very accurate convergence to the exact solution.



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Figure 3 Comparison between exact solution and the numerical approximations

<i>t</i> .	Exact	Picard	Picard with	Picard G-S.
t_i	у	$y_{1,16}$	<i>Gr. F.</i> $y_{1,12}$	with $Gr. F. y_{1,7}$
0.0	0	0	0.	0
0.2	0.167399	0.167399	0.167399	0.167399
0.4	0.295345	0.295345	0.295345	0.295345
0.6	0.416655	0.416655	0.416655	0.416655
0.8	0.560883	0.560883	0.560883	0.560883
1.0	0.759506	0.759506	0.759506	0.759506
1.2	1.05136	1.05136	1.05136	1.05136
1.4	1.48918	1.48918	1.48918	1.48918
1.6	2.1484	2.1484	2.1484	2.1484
1.8	3.13956	3.13956	3.13956	3.13956
2.0	4.62633	4.62632	4.62633	4.62633

Table 8, the comparison between exact solution and the numerical approximations

Table (8) is summarize the comparison between all procedures, where the Picard iteration is not get the fixed point, while the Picard iteration modified with Green's function is arrive to fixed point after 6 steps from seventh step, whilst the Picard iteration modified by Gauss Seidel method with Green's function is still save the fixed point, also will do on decreasing of the steps of the iterations, and is give the accurate results, moreover, gets rapid convergency to the exact solutions.

5. Discussion

The fundamental objective of this work is to find some multipliers that can be used to accelerate the convergence of the Picard iteration method. We find that the ideas of Green's function integration can be used to collect some terms in a single perfect differential term. We used the ideas introduced by Yildiz [7] to decompose the equation, and define Green's

function integration to the linear part of the decomposed equation. Also, we considered the Gauss Seidel treatment introduced in Youssef [8]. The new modified Picard iteration method is relatively straightforward to apply at least with the assistance of powerful computer algebra packages and in simple cases it gives exact solutions and in most cases it gives a series that converges rapidly to the unique solution.

The accuracy of the new modified Picard iteration method has been confirmed by comparison with the exact solution as shown in the tables. Therefore, the method of successive approximations is generally speaking, more widely used: it also used when the expansion of the solution of a differential equation in a power series is impossible, [8]. But this method, unfortunately, has its own shortcoming, which consists in that it calls for the necessity to compute more and more cumbersome integrals.

In a next subsequent work we will try to use this approach to comparison between different numerical methods for solution of differential equations.

REFERENCES

- [1] EL-Arabawy H. A., Youssef I. K., A symbolic Algorithm for Solving Linear Two Point Boundary Value Problems by Modified Picard Technique, 49 (2009). Mathematical and Computer Modally.344-351. DOI:10.1016/j.mcm.2008.07.030
- [2] Gockenbach M. S., Partial Differential Equations: Analytical and numerical methods. pp: 123 125.
- [3] Ha S. N., Lee C.R., Numerical Study for Two-Point Boundary Value Problems Using Green Function, (2002). Com. Math. with appl. 1599-1608. DOI:10.1016/S0898 1221(02) 00282-1
- [4] Peterson B.E., The Picard Iteration, (2007). Mth 480 1-14.
- [5] Ravi P. A., O'Regan D., Ordinary and Partial Differential Equations, With Special Functions, Fourier Series, and Boundary Value Problems. Springer Sci.+Business Media, LLC (2009).
- [6] Tyn M., Lokenath D., Linear Partial Differential Equations for Scientists and Engineers, Fourth Edition, Birkhäuser Boston (2007).

- [7] Yildiz B., H. Simsek, On the Contraction Mapping, (2000). Appl. Math. Com. 91-101. DOI:10.1016/S0096-3003(98)10144-3
- [8] Youssef I. K., EL-Arabawy H. A., Picard Iteration Algorithm Combined with Gauss-Seidel Technique for Initial Value Problems, (2007). Appl. Math. Comp. 345-355, DOI:10.1016/j.amc.2007.01.058.
- [9] Green Functions for First Order Equations, Report, http://www.nada.kth.se/~annak/greens1d_odes.pdf.