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Highlights

Sildenafil Citrate Solid

Pistacia Lentiscus Shoot

Profile of Dengue Fever

Skin Penetration Enhancer

Discovering Thoughts, Inventing Future

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Preparation and Evaluation of Inhalable Sustained Release Sildenafil Citrate Solid Lipid Microparticles Dispersions

By AA Yas
Tikrit University

Abstract- This is a preliminary study utilizing drug targeting approach for developing sildenafil citrate (SFC) pulmonary delivery system, a first – line for pulmonary arterial hypertension (PAH) treatment and hence reducing the dose and the side effects. The closed melt method was employed for preparing SFC – solid lipid microparticles dispersions (SFC – SLMDs), a non – solvent technique aid in the production of drug – matrix dispersions with sustained release properties. Glyceryl behenate (GB) (Compritol® 888 ATO) was used as the retarding matrix and the results shown that as its ratio increase there was a decrease in the fine particle fraction, an increase in the drug content and a prolong drug release pattern. The best model fit the release data was Higuchi – Matrix model which indicates drug diffusion – controlled releasing mechanism. Thus, inhaled SFC – SLMDs dry powder will improve PAH treatment via drug localization at low doses and reducing the administration frequency.

Keywords: sildenafil citrate, glyceryl behenate, closed melt method, solid lipid microparticles dispersions, dry powder inhaler.

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Abstract- This is a preliminary study utilizing drug targeting approach for developing sildenafil citrate (SFC) pulmonary delivery system, a first – line for pulmonary arterial hypertension (PAH) treatment and hence reducing the dose and the side effects. The closed melt method was employed for preparing SFC – solid lipid microparticles dispersions (SFC – SLMDs), a non – solvent technique aid in the production of drug – matrix dispersions with sustained release properties. Glyceryl behenate (GB) (Compritol® 888 ATO) was used as the retarding matrix and the results shown that as its ratio increase there was a decrease in the fine particle fraction, an increase in the drug content and a prolong drug release pattern. The best model fit the release data was Higuchi – Matrix model which indicates drug diffusion – controlled releasing mechanism. Thus, inhaled SFC – SLMDs dry powder will improve PAH treatment via drug localization at low doses and reducing the administration frequency.

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I. Introduction

Pulmonary arterial hypertension (PAH) is a chronic disease characterized by increased pulmonary vascular resistance and pulmonary arterial pressure resulting from blood flow restriction in the pulmonary arterial circulation, and hence shortens lifespan by leading to right – sided heart failure. The most common form is idiopathic with unknown risk factor. Although, PAH pathobiology is not well understood, the pathologic abnormalities of vascular endothelial and smooth muscle cells result from excess cellular proliferation and apoptosis resistance together with inflammation, vasoconstriction and in situ thrombosis contribute to the distal pulmonary arterioles narrowing [1]. Sildenafil citrate (SFC) has been approved by the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) as first – line for PAH treatment. It acts on the NO pathway by inhibition of phosphodiesterase type 5 (PDE – 5) responsible for cyclic guanosine monophosphate (cGMP) degradation which play a role in vasodilatation. NO acts intracellularly within the smooth muscle cells by allosteric binding to the soluble guanylate cyclase (sGC) prosthetic heme group. The subsequent sGC activation catalyzes the conversion of guanosine triphosphate (GTP) to cGMP leading to vasodilatation. SFC is administered for PAH treatment as 20 mg oral tablet, 10 mg / ml oral suspension and 0.8 mg / ml intravenous (IV) formulation. They will produce the same SFC plasma concentration at their usual doses [2].

Pulmonary targeting of SFC will be promising for local treatment of PAH due to skipping liver first – pass effect, reduction in the dose and side effects [3], and improving pediatric patients' compliance [4]. Pulmonary route offers many advantages over other routes, such as high surface area and vascularization. Solid lipid nanoparticles (SLNs) consist of phospholipid: triglyceride 30: 70 ratio aqueous nanoscale suspensions is one of the colloidal drug delivery systems that is ideal platform for hydrophobic drugs, physiologically compatible and with typical pulmonary applications [5]. In vitro and ex vivo toxicological testing of SF – loaded SLNs support system suitability for the PAH treatment via pulmonary delivery [6]. Nanosuspensions formulation prepared by SFC monohydrate being complexed with cyclodextrins (α – CD, HP – β – CD and γ – CD respectively), where SF piperazine moiety formed an inclusion in the cavity of the CDs, enhancing its water solubility by a bottom – up process using dried ethanol as a solvent and HFA – 134a as an antisolvent and propellant in order to form pressurized metered – dose inhaler (pMDI) [7].

Pulmonary localizing drug release by preparing dry powder inhaler (DPI) formulation containing particles that microscaled enough to be inhalable, in the same time a release – modifying matrix should exist in order to control drug release after delivery. The difficulty inherited in the micro – sized particles production, where a size reduction always accompanied by an increment in surface areato – mass ratio; subsequently the difficulty will be escalated in the production of a controlled release profile and efficient release agent to be incorporated [8]. The present study aim is to improve SFC delivery characteristics using solid lipid microparticles dispersions (SLMDs) that utilize glyceryl behenate (Compritol ® 888 ATO) as the lipid matrix and closed melt method as a technique for dispersing / incorporating SFC, i.e.; SFC – loaded SLMDs

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formulation in vitro aerodynamic and release profile evaluation.

II. Materials and Methods

a) Materials

Sildenafil citrate (SFC) was obtained from the State Company for Drug Industries and Medical Appliances (SDI) (Samarra / Iraq). Gattefosse´ (Lyon / France) kindly donated glyceryl behenate (GB) (Compritol® 888 ATO). All other chemicals / solvents used were of analytical grade.

b) High – Performance Liquid Chromatography (HPLC)

The HPLC instrument (Shimadzu LC 20A / Japan) was equipped with a reversed – phase C_{18} column (25 cm X 4.6 mm; particle size = 5 μm). The isocratic mobile phase, acetonitrile: 0.2 M phosphate buffer (70: 30, v / v, pH 7.4) was run at a flow rate of 1 ml / min at 25 ˚C and the column effluent was monitored by UV detector at 293 nm. A 20 μl of each sample was injected manually into the analytical column. The calibration curve of peak area versus SFC concentration was (Y = 1351313.56 X – 31213.43) under SFC concentration of 2 – 10 mg %. The retention time was 4.077 ± 0.32 min (R^2 = 0.999; limit of quantification = 2 – 10 μg / ml; accuracy = 99.85 %) [9].

c) Preparation of Physical Mixtures

Physical mixtures (PMs) of SFC and GB in powder form were mixed in mortar and passed through 60 – mesh screen (Retsch / Germany). The PMs were prepared in the following ratios; SFC: GB of 0.1: 1, 0.1: 2, 0.1: 3, 0.1: 4, and 0.1: 5.

d) Sildenafil Citrate Solid Lipid Microparticles Dispersions (SFC – SLMDs) Prepared by Closed Melt Method

The closed melting technique was employed in the preparation of solid dispersions (SDs). Weight of 2 gm from each PM was placed into an ampoule, sealed, heated at 80 °C for 10 minutes and then opened and dried for another 10 minutes at the heating temperature to remove the moisture. The collected sample from each ampoule kept overnight, triturated and passed through 625 – mesh screen (Retsch / Germany). The SDs were then stored in well closed containers until further use [10].

e) In – Vitro Microparticles Aerodynamic

Andersen cascade impactor (ACI) (Graseby – Andersen / USA) is employed in the fine particle fraction (FPF) determination in order to evaluate the in vitro deposition profiles of SFC. Samples of 30 mg were manually loaded into Rotahaler® and the ACI was operated at flow of 28.3 l / min for 10 seconds. The ACI stages effective cutoff aerodynamic diameter are as follows; stage 0, 9 μm; stage 1, 5.8 μm; stage 2, 4.7 μm; stage 3, 3.3 μm; stage 4, 2.1 μm; stage 5, 1.1 μm; stage 6, 0.65 μm; and stage 7, 0.43 μm. The definition of FPF is the amount of powder with an aerodynamic size ≤ 5 μm divided by the nominal dose [11].

f) Drug Content and Percent Yield

Accurately weighed SLMDs equivalent to 10 mg of SFC were added to 1000 ml of distilled water, heated up to 10 ˚C above excipients melting point on hotplate magnetic stirrer and then stir at 1500 rpm for 5 min to extract SFC. After being cooled to room temperature, the extract is filtered through 0.2 μm millipore filter, the drug content was determined using the previously detailed HPLC method and the percentage yield of SDs was also determined [12].

g) In Vitro Release Study

The conventional dissolution procedures utilizing large dissolution medium volumes will results in uncorrelated data in case of inhaled drugs, because the volume of surface liquid in the respiratory tract is relatively low. Therefore; in order to study SFC release from the SLMDs a dispersion method is being employed. Test tubes each contain 10 mg of each formulation suspended in 10 ml phosphate buffer pH 7.4 and incubated in a shaker at 37 ˚C on 50 rpm. Samples were withdrawn at time intervals of 0.25, 0.5, 1, 2, 4, 8 and 12 hours and SFC concentration was determined according to the HPLC method above [13].

III. Results and Discussions

a) In – Vitro Microparticles Aerodynamic

There is a decline in the fine particle fraction as the GB ratio increases, as shown in table 1. The reason for the initial increment is due to the SFC amount add to the zeta potential, but as the GB amount further increased, the zeta potential is reduced [14]. In addition, because GB microparticles undergo phase transformations at low temperatures and their irregular morphologies, results in an instability state and higher interparticulate adhesion [15].

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Table 1 : Effect of SFC: GB – PMs Ratios on the SFC – SLMDs Fine Particle Fraction

SFC: GB – PMs	SFC – SLMDs	% FPF
PM 1 = 0.1: 1	SLMD 1	23.32 ± 5.46
PM 2 = 0.1: 2	SLMD 2	19.33 ± 3.58
PM 3 = 0.1: 3	SLMD 3	14.60 ± 1.15
PM 4 = 0.1: 4	SLMD 4	11.20 ± 1.19
PM 5 = 0.1: 5	SLMD 5	8..27 ± 0.34

b) Drug Content and Percent Yield

Although SFC is an amphoteric drug and has pH – dependent characteristics, i.e. different level of ionization will affect its partition coefficient in both aqueous and oil phases, the entrapped amount of SFC was high and increased as the GB ratio increased, as shown in table 2. This is due to the method of preparation employed, where SFC solubility further increased in the melted GB resembling its solubility in oils which is higher than in solid lipids [9]. Also, the complexity feature of the GB which consists of varying 12 – 18 % mono -, 52 – 54 % di – and 28 – 32 % tri – esters of glycerol and behenic acid provides less ordered lipid crystals and hence high SFC quantity loaded [16].

Table 2 : Effect of SFC: GB – PMs Ratios on the SFC – SLMDs Drug Content and Percent Yield

SFC: GB – PMs	SFC – SLMDs	% Drug Content	% Yield
PM 1 = 0.1: 1	SLMD 1	95.24 ± 2.45	96.67 ± 1.37
PM 2 = 0.1: 2	SLMD 2	96.45 ± 1.46	97.30 ± 2.65
PM 3 = 0.1: 3	SLMD 3	97.51 ± 1.57	98.34 ± 1.89
PM 4 = 0.1: 4	SLMD 4	98.24 ± 0.53	99.32 ± 1.87
PM 5 = 0.1: 5	SLMD 5	99.41 ± 0.74	99.24 ± 1.08

c) In Vitro Release Study

The release profiles of SFC from SLMDs are sustained as shown in figure 1. They all have an initial slight burst followed by a sustained release over the 12 hours period. The reason for the former burst release is due to the free non – incorporated SFC amount accumulates on the surface of the SLMDs particles, whereas the reason for the latter sustained release is due to the closed melt technique employed in the SLMDs preparation which results in a drug solid solution incorporation model in a low crystallization degree GB matrix [17]. The cumulative percentage SFC released decrease as the GB ratio increased and hence more prolonged sustained release due to the steps govern the drug release from the SLMDs; entrance of the dissolution medium into the SLMDs matrices, dissolution of the dispersed SFC and diffusion of the dissolved SFC through the inert SLMDs matrices [18]. The release data were fitted to the zero – order, first – order, Higuchi – Matrix, Hixson – Crowell and Korsmeyer – Peppas release kinetic models to find the best fitting equation using DDSolver program [19]. The best fit was Higuchi – Matrix model with the highest correlation coefficient which predicts the drug diffusion – controlled releasing mechanism [20].

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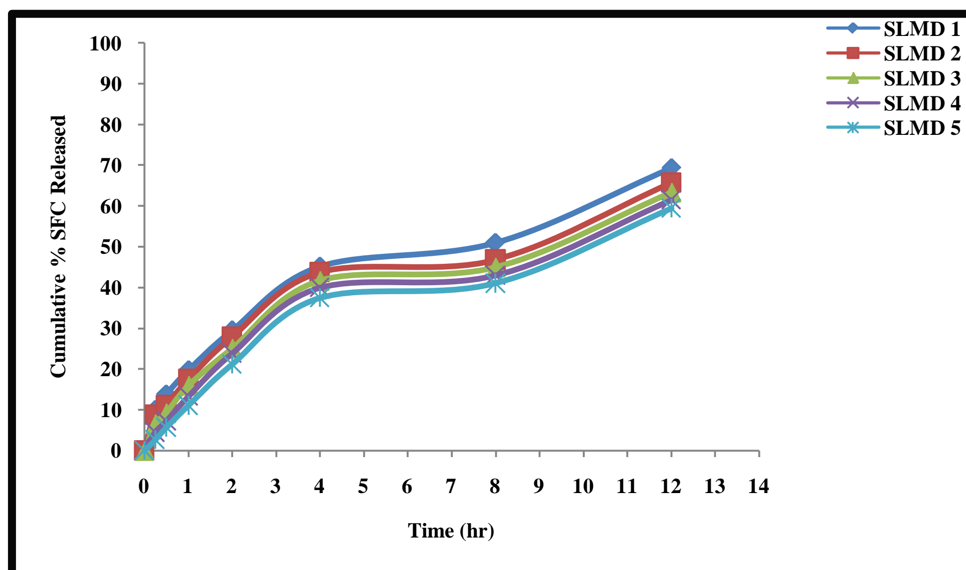

Figure 1 : In Vitro Cumulative Release Profiles of SFC from SFC – SLMDs in Phosphate Buffer pH 7.4/37 °C

IV. Conclusions

The solid solution incorporation model of SFC within the GB matrix improves the prolonged release phenomenon which aid in the reduction of the dose used and the side effects. The varying percentages of mono -, di – and – tri – glycerides in GB produce less ordered lipid crystals which aid in SFC loading capacity and release retardation. Also, this GB complexity affects fine particle fraction and drug content and release. The sustained release of SFC was further improved by the closed melt technique that employed in the preparation of SLMDs which create a dry powder inhaler that best suited for PAH treatment.

References Références Referencias

1. Frumkin LR. The Pharmacological Treatment of Pulmonary Arterial Hypertension. Pharmacological Reviews 2012; 64(3): 583 – 620.
2. Chaumais M – C, Perrin S, Sitbon O, Simonneau G, Humbert M, Montani D. Pharmacokinetic Evaluation of Sildenafil as a Pulmonary Hypertension Treatment. Expert Opinion in Drug Metabolism and Toxicology 2013; 9(9): 1193 – 1205.
3. Labiris NR, Dolovich MB. Pulmonary Drug Delivery. Part I: Physiological Factors Affecting Therapeutic Effectiveness of Aerosolized Medications. British Journal of Clinical Pharmacology 2003; 56(6): 588 – 599.
4. Beck – Broichsitter M, Kleimann P, Gessler T, Seeger W, Kissel T, Schmehl T. Nebulization Performance of Biodegradable Sildenafil – Loaded Nanoparticles using the Aeroneb® Pro: Formulation Aspects and Nanoparticle Stability to Nebulization. International Journal of Pharmaceutics 2012; 422(1 – 2): 398 – 408.
5. Paranjpe M, Müller – Goymann CC. Nanoparticle – Mediated Pulmonary Drug Delivery: A Review. International Journal of Molecular Sciences 2014; 15(4): 5852 – 5873.
6. Paranjpe M, Neuhaus V, Finke JH, Richter C, Gothsch T, Kwade A, Buttgenbach S, Braun A, Muller – Goymann CC. In Vitro and Ex Vivo Toxicological Testing of Sildenafil – Loaded Solid Lipid Nanoparticles. Inhalation Toxicology 2013; 25(9): 536 – 543.
7. Sawatdee S, Phetmung H, Srichana T. Sildenafil Citrate Monohydrate – Cyclodextrin Nanosus pension Complexes for use in Metered – Dose Inhalers. International Journal of Pharmaceutics 2013; 455(1 – 2): 248 – 258.
8. Cipolla D, Shekunov B, Blanchard J, Hickey A. Lipid – Based Carriers for Pulmonary Products: Preclinical Development and Case Studies in Humans. Advanced Drug Delivery Reviews 2014.
9. Elnaggar YSR, El-Massik MA, Abdallah OY. Fabrication, Appraisal, and Transdermal Permeation of Sildenafil Citrate-Loaded Nanostructured Lipid Carriers versus Solid Lipid Nanoparticles. International Journal Nanomedicine 2011; 6: 3195 – 3205.
10. Jagdale SC, Patil SA, Kuchekar BS, Chabukswar AR. Preparation and Characterization of Metformin Hydrochloride – Compritol 888 ATO Solid Dispersion. Journal of Young Pharmacists 2011; 3(3): 197 – 204.
11. Tsifansky MD, Yeo Y, Evgenov OV, Bellas E, Benjamin J, Kohane DS. Microparticles for Inhalational Delivery of Antipseudomonal Antibiotics. The AAPS Journal 2008; 10(2): 254 – 260.
12. Fini A, Cavallari C, Ospitali F, Gonzalez-Rodriguez ML. Theophylline – Loaded Compritol Microspheres

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13. Daman Z, Gilani K, Najafabadi AR, Eftekhari HR, Barghi MA. Formulation of Inhalable Lipid – Based Salbutamol Sulfate Microparticles by Spray Drying Technique. DARU Journal of Pharmaceutical Sciences 2014; 22(50).
14. Ahmed TA. Preparation of Transfersomes Encapsulating Sildenafil Aimed for Transdermal Drug Delivery: Plackett – Burman Design and Characterization. Journal of Liposome Research 2014.
15. Mezzena M, Scalia S, Young PM, Traini D. Solid Lipid Budesonide Microparticles for Controlled Release Inhalation Therapy. The AAPS Journal 2009; 11(4): 771 – 778.
16. Seetapan N, Bejrapha P, Srinuanchai W, Puttipipatkhachorn S, Ruktanonchai U. Nondestruct tive Rheological Measurement of Aqueous Dispersions of Solid Lipid Nanoparticles: Effects of Lipid Types and Concentrations on Dispersion Consistency. Drug Development and Industrial Pharmacy 2010; 36(9): 1005 – 1015.
17. Üner M, Yener G. Importance of Solid Lipid Nanoparticles (SLN) in Various Administration Routes and Future Perspectives. International Journal of Nanomedicine 2007; 2(3): 289 – 300.
18. Abdul Qadir M, Rahman MS, Karim MZ, Akter S, Bin Awkat T, Reza MS. Evaluation of Hydrophobic Materials as Matrices for Controlled – Release Drug Delivery. Pakistan Journal of Pharmaceutical Sciences 2003; 16(2): 17 – 28.
19. Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S. DDSolver: An Add-In Program for Modeling and Comparison of Drug Dissolution Profiles. The AAPS Journal 2010; 12(3): 263–271.
20. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic Modeling on Drug Release from Controlled Drug Delivery Systems. Acta Polonniae Pharmaceutica – Drug Research 2010; 67(3): 217 – 223.

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A Study of Clinical and Laboratory Profile of Dengue Fever in Tertiary Care Hospital in Central Karnataka, India

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Abstract- Objective: To evaluate the clinical and laboratory profile of dengue in the central Karnataka region of South India.

Materials and Methods: It is a prospective study was carried out between July-October, 2013 in BMCH & RC in central Karnataka. The study included seropositive dengue fever in-patients admitted in the medical wards in the age group of 18- 75 yrs. The test kit used for the sero diagnosis of dengue was “Dengue day 1 test kit (J. Mitra & co. Pvt. Ltd.)” which shows NS1, IgM and IgG reactivity towards dengue fever.

Result: Out of 146 seropositive cases, 92 were males and 54 were females. Most of the cases reported in young age groups (i.e. 20-30 years) compared to other age groups. NS1 antigen, IgM and IgG antibody was found reactive in 112 (76.71%), 2 (1.36%) and 6 (4.10%) patients respectively.

Keywords: *dengue, dengue virus, seropositive, bleeding manifestations.*

GJMR-B Classification : *NLMC Code: WC 528, QV 190*

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A Study of Clinical and Laboratory Profile of Dengue Fever in Tertiary Care Hospital in Central Karnataka, India

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Result: Out of 146 seropositive cases, 92 were males and 54 were females. Most of the cases reported in young age groups (i.e. 20-30 years) compared to other age groups. NS1 antigen, IgM and IgG antibody was found reactive in 112 (76.71%), 2 (1.36%) and 6 (4.10%) patients respectively. The commonest presenting clinical symptoms in patients are fever (in all patients, 100%), severe headache (n=110, 75.34%), Nausea/Vomiting (n=84, 57.53%) and Fatigue (n=68, 46.57%). The bleeding manifestations were found in 14 patients (9.58%) which includes Gum bleeding, Hematuria, Hematemesis and Malena. Bleeding manifestations was associated with severe thrombocytopenia, were in 33.33% of patients.

Discussion: Early recognition and prompt management is essential to reduce the morbidity and mortality associated with disease.

Keywords: dengue, dengue virus, seropositive, bleeding manifestations.

I. Introduction

The word "dengue" is derived from Swahili phrase ka-dinga pepo means "cramp like seizure". First clinical case report was by Benjamin Rush in Philadelphia, who describes dengue as "Back born fever" because of symptoms of myalgia and arthralgia.[1] Dengue fever is currently the second most prevalent vector born disease in the world,[2]posing threat to nearly half of world population. Each year has been as many as 100 million cases of dengue fever with 500000 cases of DHF and an estimated 22000 dengue related deaths. Annually in more than 100 countries including South America, Central America, Caribbean, India, South east Asia and Africa.[3] Increased urbanization and population growth facilities have contributed to the increased occurrence of Dengue fever.[4] The seasonally of transmission of dengue are more in monsoon and post monsoon.[5] In India dengue is prevalent since last two centuries and first evidence of occurrence is from Vellore district in Tamil Nadu during 1956. Every year there has been upsurge in occurance.[6] In last decade, major outbreaks and death have occurred in Northern India (Haryana, Punjab, Utter Pradesh), Southern India (Andhra Pradesh, Tamil Nadu and Karnataka), Western India (Gujarat, Rajasthan) and Eastern India (West Bengal). The case fatality has increased to above 1% are last 10 years.[7]

Dengue fever is an acute viral illness, prevailing in tropical and subtropical countries caused by fare distinct serotypes- Dengue virus 1, dengue virus 2, dengue virus 3 and dengue virus 4.[8] Serious manifestations occur more frequently in reinfections with a co-circulation of second serotype also reported.[9]Dengue fever is transmitted by Aedes Egypti mosquitoes and also by Aedesalbopictus and Aedespolynesiensis. Clinical manifestations range from self-limiting flu like illness called Dengue fever to severe often with unpredictable symptoms in DHF/DSS.[10] DHF is characterized by onset of dramatic haemorrhagic manifestations. DSS is most severe form of DHF that is due significant intravascular volume depletion, haemodynamic compromise poor organ and tissue perfusion.[11]Hence clinicians must be able to identify the warning signs of dengue fever like severe abdominal pain, tenderness, persistent vomiting, mucosal bleeding, liver enlargement > 2 cm, clinical fluid accumulation, lethargy, restlessness, increase hemocrit with rapid decrease in platelet counts for the better management of dengue cases.[12]

The present study was conducted to evaluate the clinical and laboratory profile of dengue in the central Karnataka region of South India.

II. Materials and Methods

The present prospective study was carried out between July-October, 2013 in BMCH & RC in central Karnataka. The study included seropositive dengue fever in-patients admitted in the medical wards in the

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age group of 18-75 yrs. The test kit used for the sero diagnosis of dengue was “Dengue day 1 test kit (J. Mitra & co. Pvt. Ltd.)” which shows NS1, IgM and IgG reactivity towards dengue fever. Patients were assessed for clinical manifestations such as fever, along with other cardinal symptoms like headache, anorexia, nausea/vomiting, myalgia, joint pain and retro-orbital pain. Complications at any stage of dengue fever were recorded. The patient were subjected to routine laboratory tests such as complete hemogram, liver function test, renal function test, serum electrolytes and urine microscopy test. Serial platelet count and hematocrit levels were monitored during the hospital stay. The patients were also subjected to radiological and other investigations when clinically warranted. The patients were also investigated for other common causes of fever endemic in our region such as Malaria, Typhoid and Leptospirosis. The collected data was analyzed by using Microsoft excel and Microsoft access.

III. Results

Out of 146 seropositive cases, 92 were males and 54 were females. Most of the cases reported in young age groups (i.e. 20-30 years) compared to other age groups. Majority of patients were from Chitradurga city area followed by Hiriyur and other different area of central Karnataka. People who are working outdoor, schooling and spending more time outside than home were more affected. (Table 1)

Table 1 : Socio-demographic characteristic of Patient (n=146)

Characteristic	No. of patients	Percentage (%)
Age group (Years)		
18-30	100	68.49
31-40	24	16.43
41-50	14	9.58
51-60	8	5.47
61-75	0	0.00
Sex		
Male	92	63.01
Female	54	36.98
Place of Residence		
Chitradurga	82	56.16
Hiriyur	42	28.76
Others	22	15.06
Occupation		
Farmer	50	34.24
Labour	20	13.69
Student	38	26.02
Housewives	14	9.58
Business	24	16.43

The figure 1 shows that pattern of Seropositivity of dengue in central Karnataka region. We notified that NS1 antigen positive patients are more during our study.

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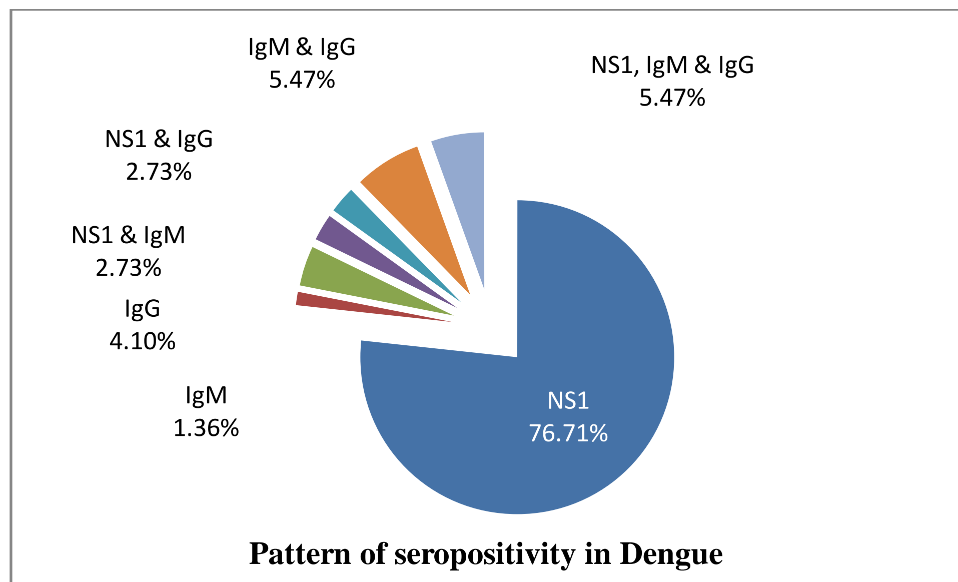

Figure 1 : Pattern of Seropositivity in dengue fever

The commonest presenting clinical symptoms in patients are fever (in all patients, 100%), followed by severe headache, Nausea/Vomiting and Fatigue. The other common symptoms include Backache, Myalgia, Anorexia and pruritus. The bleeding manifestations were found in 14 patients (9.58%) which includes Gum bleeding, Hematuria, Hematemesis and Malena. (Table 2)The complications have been found in 45 patients (30.82%) which include Pleural effusion, Hypotension, Pneumonia, Cholecystitis, ARDS, Renal failure, Encephalopathy, and Multi-organ failure. (Table 2)

Table 2 : Distribution of clinical manifestations and complications of Dengue fever (n=146)

Symptoms	No. of patients	Percentage (%)
Fever	146	100
Headache	110	75.34
Backache	50	34.24
Myalgia	48	32.87
Anorexia	20	13.69
Nausea/Vomiting	84	57.53
Abdominal pain	38	26.02
Fatigue	68	46.57
Pruritus	4	2.73
Retro-orbital pain	18	12.32
Joint pain	20	13.69
Epistaxis	4	2.73
Gum bleeding	2	1.36
Hematuria	3	2.05
Hematemesis	3	2.05
Malena	2	1.36
Complications		
▪ Dengue with Pleural effusion	20	13.69
▪ Dengue with Hypotension	8	5.47
▪ Dengue with Pneumonia	6	4.10
▪ Dengue with Cholecystitis	3	2.05
▪ Dengue with Renal failure	2	1.36
▪ Dengue with ARDS	2	1.36
▪ Dengue with Encephalopathy	2	1.36
▪ Dengue with Multi-organ failure	2	1.36

Out of 146 cases reviewed, patients with anemia were observed very less. Leukopenia was found more than leukocytosis. Other laboratory findings are illustrated in Table 3

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Table 3 : Distribution of laboratory investigations in Dengue fever

Lab test	No. of Patients	Percentage (%)
Hemoglobin (<10)	6	4.10
Hematocrit (>40)	84	57.53
Leukocytosis	16	10.95
Leukopenia	64	43.83
Platelet <100000	126	86.29
SGOT (>40 u/l)	40	27.39
SGPT (>40 u/l)	36	24.65
Deranged RFT	2	1.36

Table no 4 illustrates that 33.33% of bleeding manifestations were seen in patients with platelet count <20,000 cells/cumm and 10.34% with platelet count between 20000 to 50000 cells/cumm. So there was significance difference found in between bleeding manifestations and thrombocytopenia.

Table 4 : Correlation of bleeding manifestations with Thrombocytopenia

Platelet count	<20,000 (n=12) (Severe)	20000-50000(n=58) (Moderate)	50000-100000 (n=56) (mild)
Bleeding manifestations	4 (33.33%)	6 (10.34%)	4 (7.14%)
Without Bleeding manifestations	8 (66.66%)	52 (89.65%)	52 (92.85%)

IV. Discussion

Dengue is an important emerging disease of the tropical and sub-tropical regions today. Since the first confirmed case of dengue in India, during the late 1940s.[13]In the present study maximum number of patients were admitted in the rainy season (August to October) that is related to favourable conditions for growth of vector Aedesaegypti.[14]Transmission of dengue increases during monsoon.[4]the correlation between occurrence of dengue and monsoon is clearly evident in this study and previous studies conducted.[13] In the present study maximum number of patients who suffered were in the age group between 20-30 years, Our findings were related with Doke et al, as maximum number of patients occurred in age group 15-44 years.[15] The male to female ratio is found to 1.7:1, the study conducted by ashwini kumar et al reveals similar ratio 1.8:1.[13]where as another study showed slight difference in ratio was 1.3:1 by anagha G kinikar et al.[16] Almost all the studies had male preponderance among affected individuals.

In our present study, NS1 antigen reactive patients found more in number when compared to seropositive IgM and IgG antibody patients. A similar study was conducted by Anugha G. Kinikar et.al shows alike results.[16]

The clinical profile of dengue shows that fever was the most common presenting symptom in 146 (100%) patients. Abdominal symptoms/signs such as abdominal pain, nausea/vomiting, anorexia, abdominal tenderness, hepatomegaly and splenomegaly were found to be present 83.55% of study population which shows identical result statistically[13] where as another study was conducted by Satya sudhish Nimmagadda which shows less number of patients are affected with abdominal symptoms.[17] In the present study, the other symptoms which were found frequently such as headache followed by fatigue, myalgia and backache whereas Mavilla anuradha et al, shows frequently affected symptoms in their study population are myalgia followed by headache, vomiting etc.[14]which shows vise- verse result but M. Neeraja et al, reported similar frequency of all symptoms related to our study.[18] Retro-orbital pain was observed in 12.32% of patient whereas Denys Eiti Fugimoto was reported 16.1% of patients.[19]

Bleeding manifestations were revealed in 9.58% of patients while Ashwini Kumar et al, reported in 26.6%[13] and Tejashree .A et al, were reported in 3.84% of patients.[20]

Our study shows pleural effusion was found in 13.69% patients where other study displayed ARDS (33.33%) as a significant complication [13] but our study revealed that ARDS was found to be least. Other complications such as renal failure and encephalopathy, each was observed in 1.36% patients in our study whereas other study shows renal failure and encephalopathy was found in 40.6% and 0.66% patients respectively. So both study shows that encephalopathy was associated very rare compared to renal failure. In our study, Hypotension was observed in 8 (5.47%) of patients but no death was found whereas other study was reported 3 deaths due to hypotension in seropositive patients.[17] A similar study was conducted by Ashwini kumar et al, shows statistically significant result as our study in complications of pneumonia, renal

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failure and multi-organ failure.[13] The laboratory investigations are evaluated in our study, the finding shows that anemia was associated in least patients compare to other study was conducted by Tejushree .A et al. which shows significant difference in both study.[20] Increased hematocrit was observed in 57.53% of patients whereas Mavilla Anuradha et al, were reported in 30.00% of patients. 126 (80.29%) patients had platelet count < 100000 cells/cumm but Rashmi K.S et al reported 72.77% of patients had platelet count <100000 cells/cumm. So our study reflected that more patients are encountered with thrombocytopenia.[21] Leukopenia was observed in 43.83% of patients whereas Prafulla Dutta et al, were reported 30.00% of patients presented with leukopenia.[22] Leukopenia was mainly found in NS1 seropositive patients.Liver enzymes like AST was found in 1/4th of study population whereas Prafulla Dutta et al, were reported in 1/3rd of study population[22] and ALT were in 1/4th of study population whereas other study shows half of the patients.[14] So AST and ALT was less affected in the region of central Karnataka. Table 4 illustrates the correlation between bleeding manifestation and thrombocytopenia in our study whereas Satya Sudish Nimmagada et al, were reported correlations between bleeding manifestations and thrombocytopenia but in both study shows no significant difference in bleeding manifestations and platelet count <20000 cells/cumm but there was significant difference was found in bleeding manifestations and platelet count 20000 to 50000 cells/cumm in two studies.[17] The various factors were responsible for thrombocytopenia such as platelet dysfunction, consumption coagulopathy and endothelial dysfunction which are not related to severity of bleeding. The patients were also investigated for other causes of fever endemic in our region such as malaria, typhoid and leptospirosis which causes the thrombocytopenia and often lead to delay in diagnosis of dengue. No deaths were found in our prospective study.

After comparing different studies, it can be deduced that clinical presentation of dengue varies from region to region.

V. Conclusion

Dengue fever is an important public health problem in tropical countries like India. It can present with varied clinical manifestations. Early recognition and prompt management is essential to reduce the morbidity and mortality associated with dengue.

References Références Referencias

1. Nivedita G, Sakshi S, Amita J, Umesh CC. Dengue in India. Indian J Med Res 2012; 136:373-390.
2. Thomas J. Dengue fever in international travelors. CID 2000; 31: 144-147.
3. Kim K, Gina S, Miriam RE. Fever pitch: Mosquito-Borne dengue feverthreat spreading in the Americas. NRDC 2009.
4. Gunusekaran P, Kaveri K, Mohana S, Kavita A, Sureshbabu BV, Padmapriya P, Kiruba R, et al. Dengue disease status in Chennai (2006-2008): A Retrospective analysis. Indian J Med Res 2011; 133: 322-325.
5. Zainab G, Anuradha HV, Shivamurthy MC. Pattern of management and outcome of dengue fever in pediatric in-patients in a tertiarycare hospital: A prospective observation study. Int J Basic Clin Pharmacol 2014; 3(3): 534-538.
6. Sharma SK, Gautham A. Dengue fever in India: An overview. Medicine update 2010; 20: 657-659.
7. Dengue: Guidelines for diagnosis, treatment, prevention and control- New edition. A joint publication of the WHO and TDR 2009.
8. World Health Organization. Dengue Hemorrhagic Fever; Diagnosis, Treatment, Prevention and Control. Geneva: World Health Organization; 1997.
9. Ahluwalia G, Sharma SK. Dengue: current trends and challenges – An Indian perspective. JAPI 2004; 52: 561- 563.
10. Siddharth NS, Yash PM, Surendra KS, et al. API Textbook of Medicine. 7th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2003.
11. Changa K, Fredrico D, Diana LTT, David CL. When less is more: can we abandon prophylactic platelet transfusion in Dengue fever? Ann Acad Med Singapore 2011; 40: 539-545.
12. http://www.cdc.gov/dengue/redources/dengueclinicianguide508pdf
13. Ashwini K, Chythra RR, Vinay P, Seema S, Channuveerappa B, Charmaine MS. Clinical manifestations and trend of dengue cases admitted in a tertiary care hospital, udupi district, Karnataka. Indian Journal of Community Medicine 2010; 35(3): 386-390.
14. Mavilla A, Rahul HD. Screening and manifestations of seropositive dengue fever patients in perambalur: A hospital based study. International journal of Medical Science and Public Health 2014; 3(6): 745-748.
15. Doke P, Pawar S. Profile of Dengue fever outbreaks in Maharashtra. Indian J Community Med 2000; 25:170-6.
16. Saini S, Anagha GK, Sachin D, Deepika B, Roushni SB. Epidemiology and Seropositivity of dengue cases in a rural tertiary care hospital of western Maharashtra, India. IJBAR 2013; 4(7): 473-77.
17. Satya SN, Chakrapani M, Archit B, Pavan MR, Akshatha NU. Atypical manifestations of Dengue Fever (DF) – Where do we stand today?. J Clin Diag Res 2014; 8(1): 71-73.
18. Neeraja M, Lakshmi V, Teja VD, Umabala P, Subbalakshmi MV. Serodiagnosis of dengue virus

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infection in patients presenting to a tertiary care hospital. Indian J Med Microbiol 2006; 24(4): 280-282.

19. Denys EF, Sergio K. Clinical and laboratory characteristic of patients with dengue hemorrhagic fever manifestations and their transfusion profile. Rev Bras Hematol Hemoter 2014; 36(2): 115-120.
20. Tejushree A, Thejaswini HS, Madhuri K. A serological study of Dengue and Hanta virus in acute febrile patients in a tertiary care hospital. International Journal of Pharmaceutical Science Invention 2014; 3(7): 22-25.
21. Rashmi KS, Jagdeesh, Ravikumar KL, Pratibha MJ, Giridhar UP, Arun KB. Serological markers prevalence and trend of probable dengue infection at a tertiary care hospital in Banglore. Journal of Evolution of Medical and Dental Sciences 2013; 12(36): 6968-6976.
22. Prafulla D, Siraj AK, Jani B, Jagadish M. Demographic and clinical features of patients withdengue in Northeastern region of India: A Retrospective cross-sectional study during 2009-2011. Journal of virology and Microbiology 2012; 2012: 1-11.

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Reducing Topical Mometasone Furoate Doses by Applying Hyaluronic Acid as a Skin Penetration Enhancer

By Khaled Aly Khaled, Usama Farghaly Aly & Doaa Amal Tawfik

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Abstract- The objective of the present study was to investigate the possibility to add hyaluronic acid (HA) as skin penetration enhancer to mometasone furoate (MF) to enhance its skin absorption, and so decrease the dose and side effects in different types of topical formulations including absorption ointment base, oil in water emulsion base and water in oil emulsion base in addition to alcoholic gel base. MF was introduced into the bases with and without the addition of 0.1% HA. The prepared formulations were evaluated for physical appearance, rheological behavior, drug release through a standard cellophane membrane and antiinflammatory effects in carrageenan-induced oedema in male albino rats. Results showed that all formulations showed good and acceptable physical properties. The in-vitro release rate of each base and its corresponding one, with 0.1% HA, showed no statistical differences.

Keywords: hyaluronic acid, mometasone furoate, topical, rheology, release, anti-inflammatory, dose.

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Reducing Topical Mometasone Furoate Doses by Applying Hyaluronic Acid as a Skin Penetration Enhancer

Khaled Aly Khaled [α], Usama Farghaly Aly [σ] & Doaa Amal Tawfik [ρ]

Abstract- The objective of the present study was to investigate the possibility to add hyaluronic acid (HA) as skin penetration enhancer to mometasone furoate (MF) to enhance its skin absorption, and so decrease the dose and side effects in different types of topical formulations including absorption ointment base, oil in water emulsion base and water in oil emulsion base in addition to alcoholic gel base. MF was introduced into the bases with and without the addition of 0.1% HA. The prepared formulations were evaluated for physical appearance, rheological behavior, drug release through a standard cellophane membrane and anti-inflammatory effects in carrageenan-induced oedema in male albino rats. Results showed that all formulations showed good and acceptable physical properties. The in-vitro release rate of each base and its corresponding one, with 0.1% HA, showed no statistical differences. The data obtained revealed that the total amount of drug released was affected by the nature and the composition of bases. Animal studies showed that the differences in decrease in oedema diameter between the full dose of MF 0.1% and the half and the quarter dose of MF with hyaluronic acid sodium salt 0.1% added were unnoticed and the statistical analysis showed that the difference was insignificant ($p>0.05$).

Keywords: hyaluronic acid, mometasone furoate, topical, rheology, release, anti-inflammatory, dose.

I. Introduction

Corticosteroids are derivatives of the natural corticosteroid hormones that are produced by the adrenal glands. These have many important functions in the body, including control of inflammatory responses. Corticosteroid medicines are mainly used for their effect in controlling inflammation, and topical corticosteroids are applied to the skin for the localized treatment of various inflammatory skin disorders (***warner et al, 2001***). While topical steroids have tremendous benefit in reducing inflammation, they also have significant side effects. Most of these side effects are seen with long-term use, but some may be noticed within days of starting therapy (***Wolverton 2001a, Wolverton 2001b, Maibach et al, 1962***). Local steroid use may induce a typical or extensive crusted scabies. Hypertrichosis, hypopigmentation from high- and super-potency steroids is a possible consequence when used on a dark skinned person. Repeated use of topical steroids in the same area can cause thinning of the epidermis and changes in the connective tissue of the dermis, and topical steroid allergy (***Wester et al, 1991***).

Mometasone furoate (9α, 21-dichloro-11β, 17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate)) is a synthetic corticosteroid which is non-fluorinated and containing a furoate moiety. Mometasone furoate is used topically to reduce inflammation of the skin or in the airways. It is a prodrug of the free mometasone. It is used in the treatment of inflammatory skin disorders such as eczema and psoriasis. It is also used in the treatment of allergic rhinitis and asthma (***Bousquet, 2009***). It reduces inflammation by causing several effects such as reversing the activation of inflammatory proteins, activating the secretion of anti-inflammatory proteins, stabilizing cell membranes and decreasing the influx of inflammatory cells.

Of the various skin layers, it is the stratum corneum that is the rate-limiting barrier to percutaneous drug transport. In fact, the stratum corneum is a remarkably more formidable barrier to drug transport than the epithelial barriers of gastrointestinal, nasal, buccal, vaginal, or rectal delivery routes. Ideally, penetration enhancers reversibly reduce the barrier resistance of the stratum corneum without damaging viable cells (***Hoogstrate et al, 1991***). Some of the more desirable properties for penetration enhancers have been given such as, being non-toxic, non-irritating and non-allergenic. They would ideally work rapidly; the activity and duration of effect should be both predictable and reproducible. They should have no pharmacological activity within the body.

Hyaluronic acid (HA) has been introduced as a vehicle for topical application of drugs to the skin (***Tracey et al, 1999***). It is a naturally occurring polyanionic, polysaccharide that consist of N-acetyl glucosamine and glucoronic acid. It is present in the intercellular matrix of most vertebrate connective tissues especially skin.It is most frequently referred to as hyaluronic acid due to the fact that exists in vivo as a polyanion and not in protonated acid form. Commercially produced hyaluronic acid is isolated either from animal sources, within the synovial fluid, umbilical cord, skin, and rooster comb or from bacteria

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through a process of fermentation or direct isolation. (*Brown et al, 2005*).

The objective of the present study was to investigate the possibility to add hyaluronic acid to mometasone furoate to enhance its skin absorption, and so decrease the dose and its side effects in different types of pharmaceutical topical formulations including ointment bases such as absorption ointment base, oil in water emulsion base and water in oil emulsion base in addition to alcoholic gel base It was also introduced into the same bases with addition of 0.1% HA. The prepared formulations were evaluated for physical appearance, rheological behavior, drug release through a standard cellophane membrane and anti-inflammatory effects in carrageenan induced oedema in male albino rats.

II. Material and Methods

a) Materials

Mometasone furoate was kindly supplied by Sigma Pharmaceutical Industries, Egypt. Hyaluronic acid sodium salt from streptococcus, Sigma Aldrich, USA. Dialysis sacks, Sigma Aldrich, USA. Hydroxy propylmethylcellulose, winlab, UK. Carbopol 941, Sigma-Aldrich, USA. Stearyl alcohol, Fisher scientific, UK. Anhydrous lanolin, Elnasr pharmaceutical chemicals, Egypt, Tween 40, Columbus chemicals industries, USA. Span 60, Oxford laboratory reagents, Mumbai, India. All other ingredients were of analytical grade.

b) Preparation of Topical Formulations

Mometasone furoate (0.1%w/w) was introduced into various topical formulations including ointment bases such as absorption base, water in oil emulsion base and oil in water emulsion base in addition to alcoholic gel base. It was also introduced into the same bases with addition of 0.1% HA.

c) Absorption Base

Hard paraffine was added to anhydrous wool fat and the white soft paraffine, the all were heated up to 70±2°c in a water bath then added to liquid paraffin in which 0.1% MF was levigated at the same temperature then water was added with stirring and cooled down at room temperature (F1). The same base was prepared by the same manner with the addition of 0.1% HA that was previously dissolved in the water portion of the base (F2).

d) Oil in Water Emulsion Base

Stearyl alcohol and white soft paraffine were heated up to 70±2°c in a water bath then tween 40, propylene glycol and 0.1% MF previously dissolved in ethyl alcohol were added. Water was added with stirring and left to cool down at room temperature (F3). The same base was prepared by the same manner with the addition of 0.1% HA that was previously dissolved in the water portion of the base (F4).

e) Water in Oil Emulsion Base

Cetostearyl alcohol and white soft paraffine heated up to 70 ±2°c in a water bath, span 60 and 0.1% MF previously dissolved in ethyl alcohol were added. Water was added with stirring and left to cool down at room temperature (F5). The same base was prepared by the same manner with the addition of 0.1% HA that was previously dissolved in the water portion of the base (F6).

f) Alcoholic Gel Base

Hydroxypropylmethyl cellulose (HPMC) was soaked in distilled water till the polymer was fully hydrated. Then ethyl alcohol with 0.1% MF was added. Carbomer 941 and glycerin was added to the mixture and kept under magnetic stirrer for 5 hours (F7). The same base was prepared by the same manner with the addition of 0.1% HA that was previously dissolved in the water portion of the base (F8). The compositions of the prepared formulations were illustrated in table (1).

g) Physical Examination

The prepared formulations were inspected visually for their color and homogeneity. The spreadability of the formulations was determined by measuring the spreading diameter of 1 g of each formula between two horizontal plates (20 cm × 20 cm) after one min. The standardized weight tied on the upper plate was 125 g. The results obtained were average of three determinations. The pH of all formulations was checked by using a digital pH meter at constant temperature. The electrode was directly dipped into 1 gram of each formulation previously dissolved in appropriate volume of distilled water to produce concentration 10% w/v and readings were taken.

h) Rheological Studies

For the rheological measurements, the samples of all the 8 formulations , in addition to the commercial product, were examined using cole-parmer 98936 series viscosity centipoise (Vernon Hillss, IL 60061, USA), at 0.5, 1, 2.5, 5, 10, 20, 50 and100 rpm. Each reading was taken after equilibration of the sample, for 1 minute and temperature 25°C using 20 gram sample. The flow curves of all formulations were obtained by directly reading the viscosity (cps) and shear stress (rpm) from the viscometer.

i) In Vitro Drug Release

The release studies were carried out in a modified franz-diffusion cell. A sample of 2 grams of each formula was accurately weighed and placed on a semipermeable standard cellophane membrane previously immersed in distilled water for 24 hours. The loaded membrane was stretched over the lower open end of a glass tube of 3 cm diameter and sealed with a rubber band. The glass cylinder was then immersed in 250 ml beaker containing 150 ml of phosphate buffer (pH 7.4) in such a manner that the membrane was

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located just below the surface of the sink solution. The whole dialysis unit was placed in a thermostatically controlled shaker water bath adjusted at 37±0.1°c with a constant stirring at 30 rpm to avoid development of concentration gradient. Each 15 minutes an aliquot, 2 ml, was collected and replaced by equal volume of the buffer at the same temperature to make the volume of the sink solution constant during the 2 hours of the experiment. Samples were then assayed spectrophotometrically. Concentration of MF in each sample was determined from the standard curve previously constructed. Blank samples were carried out to check any interference simultaneously.

j) Kinetic Studies

To analyze the mechanism of MF release from the prepared formulations, the following plots were made: cumulative % drug release vs. time (zero order kinetic model: $C = k_0t$, where k_0 is the zero-order rate constant expressed in units of concentration/time and t is the time); log of cumulative % drug remaining vs. time (first order kinetic model, as log cumulative percent drug remaining versus time $\text{Log } C = \text{Log } C_0 - kt/2.303$, where C_0 is the initial concentration of drug and k is the first-order constant; and cumulative % drug release per surface area of membrane vs. square root of time (Higuchi model Q = kt , where k is the constant reflecting the design variables of the system).

k) Animal study

The in-vivo experimental protocol was approved by the ethical committee of faculty of pharmacy, El-Minia university. Male albino rats (120-170 g) were purchased from the animal house of faculty of medicine (Assuit University, Egypt). The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay.

The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw edema assay.

Rats were randomly classified into 14 groups. Each group contains 5 rats.

- Group 1: the rats were served as untreated group.
- Group 2: the rats were treated topically with absorption ointment base of 0.1% mometasone furoate (**F1**).
- Group 3: the rats were treated topically with absorption ointment base of 0.05% mometasone furoate (the half dose) combined with 0.1% hyaluronic acid sodium salt (**F2b**[1]).
- Group 4: the rats were treated topically with absorption ointment base of 0.025% mometasone furoate (the quarter dose) combined with 0.1% hyaluronic acid sodium salt (**F2c**[2]).
- Group 5: the rats were treated topically with oil in water emulsion base of 0.1% mometasone furoate (**F3**).
- Group 6: the rats were treated topically with oil in water emulsion base of 0.05% mometasone furoate (the half dose) combined with 0.1% hyaluronic acid sodium salt (**F4b**).
- Group 7: the rats were treated topically with oil in water emulsion base of 0.025% mometasone furoate (the quarter dose) combined with 0.1% hyaluronic acid sodium salt (**F4c**).
- Group 8: the rats were treated topically with water in oil emulsion base of 0.1% mometasone furoate (**F5**).
- Group 9: the rats were treated topically with water in oil emulsion base of 0.05% mometasone furoate (the half dose) combined with 0.1% hyaluronic acid sodium salt (**F6b**).
- Group 10: the rats were treated topically with water in oil emulsion base of 0.025% mometasone furoate (the quarter dose) combined with 0.1% hyaluronic acid sodium salt (**F6c**).
- Group 11: the rats were treated topically with alcoholic gel base of 0.1% mometasone furoate (**F7**).
- Group 12: the rats were treated topically with alcoholic gel base of 0.05% mometasone furoate (the half dose) combined with 0.1% hyaluronic acid sodium salt (**F8b**).
- Group 13: the rats were treated topically with alcoholic gel base of 0.025% mometasone furoate (the quarter dose) combined with 0.1% hyaluronic acid sodium salt (**F8c**).
- Group 14: the rats were treated topically with commercial product of mometasone furoate (Elcon, Schering-plough) of 0.1% mometasone furoate.

[1] Fb: the half dose of MF (0.05%) combined with HA (0.1%)

[2] Fc: the quarter dose of MF (0.025%) combined with HA (0.1%)

After 1 hour, 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured at hourly interval for 5 hours using paw edema meter (vernier caliper). Anti-inflammatory activity was measured as the reduction in edema diameter when drug was present in full dose or fraction dose combined with hyaluronic acid sodium salt relative to the control group.

l) Statistical analysis

All values were expressed as Mean ± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA). The value of p less than 5% ($p < 0.05$) was considered statistically significant.

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III. Results and Discussion

a) *Physical Examination*

The physical properties of all formulations are shown in Table 2. All formulations showed good homogeneity and spreadability. The physical appearance of most formulations was white to off white except the alcoholic gel base was transparent. The viscosities of all formulations have shown shear thinning/pseudoplastic behavior at ambient temperature where there is decrease in viscosity by increasing shear rate this shear thinning behavior is a desirable property for topical preparations as they should be thin during application and thick otherwise. The viscosity data obtained has been shown graphically in figures 1-4. The rheological properties of topical pharmaceutical formulations, and hence the patient's compliance, would be accepted. Being a shear-thinning polymer, (HA) can be easily spread on the surface of the skin. It could be also observed that the presence of HA did not affect the rheological behaviors of the prepared bases. The pH of all formulations was in range (5.9±0.159 to 7.8±0.057) with lowest pH value with oil in water emulsion base and the highest value was observed with alcoholic gel base that contains 0.1% HA. This pH range was expected not to produce any skin irritation.

b) *Release of mometasone furoate from the prepared topical formulations*

The release data of MF from the all formulations were obtained and displayed in table 3. The release of MF from the different formulations could be ranked in a descending order as: F1>commercial>F7>F3>F5. It could be noticed that the absorption ointment base showed the highest release pattern as compared to the other selected formulations. This could be due to the hydrophilic or water absorbing property of the absorption base and, this base is known to take up several times their own weight of water due to the effect of anhydrous lanolin (*sandhu, 2012*). The statistical analysis showed that the absorption ointment base has a significant higher release of MF than both oil in water and water in oil emulsion base ($p<0.001$), but also showed a statistically insignificant higher release rate than both the alcoholic gel base and the commercial mometasone furoate ($p>0.05$).

The table also demonstrated that the release of MF oil in water emulsion base was higher than its release from water in oil emulsion base but the statistical studies showed that the difference was insignificant ($p>0.05$). The stearyl alcohol present in oil in water emulsion base caused greater potentiating effect on water number of petrolatum over cetostearyl alcohol. Accordingly, the presence of stearyl alcohol increased the hydrophilic properties of this formulation over the one that contain cetostearyl alcohol. This increased the affinity of the base to absorb water from the release medium and subsequently increased the drug diffusion and release, this explanation was previously discussed by (*Aml et al, 2013*).

It could be observed also that the release of MF from alcoholic gel base which exhibited a higher release rate than the oil in water and water in oil emulsion base. Statistical studies showed that the difference was insignificant ($p>0.05$), this higher release rate could be attributed to the effect of excessive amount of alcohol that may facilitate the partitioning of drug into the receptor solution and decreasing the viscosity of the gel. These effects were previously suggested by (*Chi et al, 1991*). The commercial product containing 0.1% MF was in the second after the absorption ointment base in the order of the amount released but also the statistical studies showed that the difference was insignificant ($p>0.05$). Statistical analysis showed also a significant higher release of commercial product than both the oil in water emulsion base ($p<0.05$) and the water in oil emulsion base ($p<0.01$), while the release rate was insignificant as compared to the alcoholic gel base ($p>0.05$).

Table 3 demonstrated that the release of MF from all formulations that contains HA as skin penetration enhancer (F2, F4, F6, F8) was slightly higher than its release from the same bases but without HA (F1, F3, F5, F7). The statistical analysis showed that the difference was insignificant ($P>0.05$). This means, the drug release through synthetic membrane was mainly influenced by the rheological properties of the vehicles and diffusion ability through cellulose acetate membrane and HA had no penetration enhancing effect through the membrane.

c) *Kinetic analysis of the release data*

The kinetic analysis of the in vitro release data of MF from all the prepared formulations is presented in table 4 which listed the correlation coefficients (r^2) of the release profiles when different mathematical models for the analysis of the release kinetics were applied. The preference between the release mechanisms was dependent on the correlation coefficients. As shown in the table, r^2 indicated that the release of MF from w/o emulsion bases (F5 and F6) and the alcoholic gel base (F8) followed zero order kinetics. While the drug release from the other bases followed the Higuchi model.

d) *Anti-inflammatory effect of 0.1% MF and (0.05% and 0.025% MF) combined with 0.1% HA formulated in all selected formulations on carrageenan induced paw oedema in rats*

Figures 1-8 showed that after 3 and 5 hours, the reduction in oedema thickness produced by the formulations contain 0.1% of MF (the full dose), was

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nearly the same as the formulations that contain 0.05% of MF (the half dose) combined with 0.1% HA and those contain 0.025% MF (the quarter dose) combined with 0.1% HA. The statistical analysis showed that no significant difference was produced ($P>0.05$), between the formulations with full dose of MF and the others with half and the quarter dose of MF combined with HA. While the reduction in oedema diameter produced with all formulations was statistically significant when compared to the control group ($p<0.05$). Results also showed that no significant difference was observed between those formulations and the commercial one.

IV. Conclusion

In conclusion, the diffusion of mometasone furoate from different topical bases through a synthetic cellophane membrane depends on the nature and the composition of the bases. So, the release rate can be altered by changing the nature and the composition in addition to the viscosity of the bases and also by adding the HA. The rheology of all bases were affected by the addition of HA due to the viscoelastic nature of hyaluronic acid that when binds to water gives it a stiff viscous quality similar to "Jello and being a shear-thining polymer the hyaluronic acid also improves the spreadability of the different topical bases. From the in-vivo anti-inflammatory studies, it could be included that the difference in decrease in the oedema diameter in case of using formulation with(full dose) of MF and the same formulation of (half dose) and (quarter dose) MF combined with the skin penetration enhancer 0.1% HA was statistically insignificant (P>0.05). These results explain the effect of HA when absorbed from the surface of the skin and passes rapidly through epidermis, which may allow associated drugs to be carried in relatively high concentration at least as far as the deeper layers of the dermis. This effect was previously suggested by (*Tracey et al, 1999*).

References Références Referencias

1. Aml I, Fathy M, Sohair el-shanawany (2013) Study of fluconazole release from o/w cream and water soluble ointment bases. Brit J of Pharm Res 3(4):686-696.
2. Barbucci R, Lampeni S, Barzacchiello A, Ambrosio L, Fini M, Torricelli P, Giardino R.(2002) Hyaluronic acid hydrogel in the treatment of osteoarthritis. Biomaterials 23: 4503-4513.
3. Bousquet J. (2009) Mometasone furoate: an effective anti-inflammatory with a well-defined safety and tolerability profile in the treatment of asthma. Intl J Clin Practice 63 (5): 806–19.
4. Brown MB, Jones SA (2005) Hyaluronic acid: a unique topical vehicle for the localized delivery of drugs to the skin. J Eur Aca Derm and Ven, 19: 308-318.
5. Chi S, Jun H (1991) Release rate of ketoprofen from poloxamer gel in a membranous diffusion cell. J Pharm Sci 80 (3): 280-283.
6. Hoogstrate AJ, Verohoef J (1991) Kinetic intrastructural aspects and molecular modeling of transdermal peptide flux enhancement by N-alkylazocylo heptan. Int J Pharm 76: 37-47.
7. Maibach HI, Kligman AM (1962) The biology of experimental human cutaneous moniliasis (candida albicans). Arch of Dermato 85: 113-134.
8. Sandhu P, Bilandi A, Kumar S, Kapoor B, Kataria S, Rathore D, Bhardwaj S (2012) Additives in topical dosage forms. Int J Pharml, Chem and Bio Sci 2(1): 78-96.
9. Tracey JB, Dine A, Robert TE (1999) AnAbsorption of hyaluronan applied to the surface of intact skin. J Inves dermato 113: 740-746.
10. Warner M, Camisa C (2001) Topical corticosteroids. Comprehensive Dermatologic Drug Therapy 27: 548-577.
11. Wester RC, Maibach HI (1991) In vivo percutaneous penetration. Dermatotoxicology 4: 75-96.
12. Wolverton, Stephen E. (2001). Comprehensive Dermatologic Drug Therapy. Philadelphia, PA: W.B. Saunders Company. pp. 562–3.
13. Wolverton, Stephen E. (2001). *Comprehensive Dermatologic Drug Therapy*. Philadelphia, PA: W.B. Saunders Company. p. 563.

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Table 1 : Composition of the prepared topical bases

Component	F1	F2	F3	F4	F5	F6	F7	F8
MF (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HA (%)	-	0.1	-	0.1	-	0.1	-	0.1
Hard paraffin(g)	22	22	-	-	-	-	-	-
Anhydrous wool fat(g)	10	10	-	-	-	-	-	-
White soft paraffin(g)	8	8	25	25	18.5	18.5	-	-
Liquid paraffin(ml)	50	50	-	-	-	-	-	-
Stearyl alcohol(g)	-	-	25	25	-	-	-	-
Tween 40(ml)	-	-	2	2	-	-	-	-
Propylene glycol (ml)	-	-	12	12	-	-	-	-
Cetostearyl alcohol(g)	-	-	-	-	25	25	-	-
Span 60(g)	-	-	-	-	2	2	-	-
HPMC(g)	-	-	-	-	-	-	0.75	0.75
Carbomer 941(g)	-	-	-	-	-	-	0.1	0.1
Glycerin(ml)	-	-	-	-	-	-	2	2
Ethyl alcohol(ml)	-	-	10	10	10	10	70	70
Distilled water to(g)	100	100	100	100	100	100	100	100

Table 2 : Physical properties of the prepared formulations

Formulation	pH	Spreading diameter after 1 min (cm)	Color	Transparency	Grittiness
F1	7.7±0.1	3.2±0.2	Yellowish white	Opaque	Smooth
F2	7.2±0.2	2.2±0.057	Yellowish white	Opaque	Smooth
F3	5.9±0.15	3.4±0.1	White	Opaque	Smooth
F4	6.4±0.1	2.7±0.1	White	Opaque	Smooth
F5	6.7±0.12	3±0.1	White	Opaque	Smooth
F6	6.1±0.15	2.5±0.15	White	Opaque	Smooth
F7	7.5±0.15	6.7±0.15	Colorless	Transparent	Smooth
F8	7.8±0.06	5.8±0.15	Colorless	Transparent	Smooth
Commercial	7.4±0.00	4.6±0.15	White	Opaque	Smooth

Table 3 : Mean cumulative amount released

Time (minute)	Mean cumulative amount released (µg) ± standard deviation								
	F1	F2	F3	F4	F5	F6	F7	F8	Com-mercial
15	485.26 ±0.012	485.25 ±0.006	73.54 ±0.001	196.96 ±0.006	38.24 ±0.001	44.12 ±0.001	205.81 ±0.02	205.81 ±0.02	323.54 ±0.011
30	497.59 ±0.02	502.42 ±0.002	103.93 ±0.001	205.59 ±0.006	44.63 ±0.002	56.47 ±0.001	237.94 ±0.001	223.24 ±0.001	379.31 ±0.001
45	507.12 ±0.001	524.97 ±0.002	128.84 ±0.001	225.95 ±0.001	77.58 ±0.001	60.16 ±0.001	246.94 ±0.005	235.03 ±0.001	397.54 ±0.001
60	519.56 ±0.001	531.82 ±0.015	159.94 ±0.002	240.65 ±0.002	110.95 ±0.001	105.07 ±0.001	273.69 ±0.001	258.64 ±0.001	407.13 ±0.001
75	532.07 ±0.001	537.60 ±0.015	191.43 ±0.001	249.57 ±0.001	135.93 ±0.001	141.74 ±0.001	291.97 ±0.04	288.52 ±0.001	419.71 ±0.001
90	541.82 ±0.002	560.23 ±0.002	211.55 ±0.001	263.11 ±0.001	149.47 ±0.001	149.47 ±0.001	351.59 ±0.001	433.37 ±0.006	438.28 ±0.001
105	563.28 ±0.015	581.95 ±0.001	223.08 ±0.02	284.07 ±0.001	166.09 ±0.001	163.13 ±0.05	361.92 ±0.001	565.38 ±0.001	448.20 ±0.001
120	584.97 ±0.005	598.01 ±0.001	252.32 ±0.001	290.56 ±0.001	177.03 ±0.001	176.99 ±0.001	537.01 ±0.005	579.68 ±0.001	461.12 ±0.001

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Table 4 : Kinetic data of the release studies

Formula	Zero Order		First Order		Diffusion Model (Higuchi)	
	r^2	K *µg/min*	r^2	K *min⁻¹*	r^2	K *µg. t^0.5*
F1	-0.41	16.01	-0.85	-0.02	**0.57**	22.2
F2	-0.35	16.41	-0.85	-0.02	**0.6**	22.7
F3	0.91	5.8	-0.85	-0.02	**0.98**	7.6
F4	0.12	7.66	-0.85	-0.02	**0.82**	10.5
F5	**0.97**	4.04	-0.3	-0.02	0.91	5.23
F6	**0.95**	4.01	-0.85	-0.02	0.92	5.2
F7	0.73	10.53	-0.85	-0.02	**0.88**	13.9
F8	**0.88**	12.3	-0.85	-0.02	0.85	15.9
Commercial	-0.12	12.6	-0.85	-0.02	**0.72**	17.4

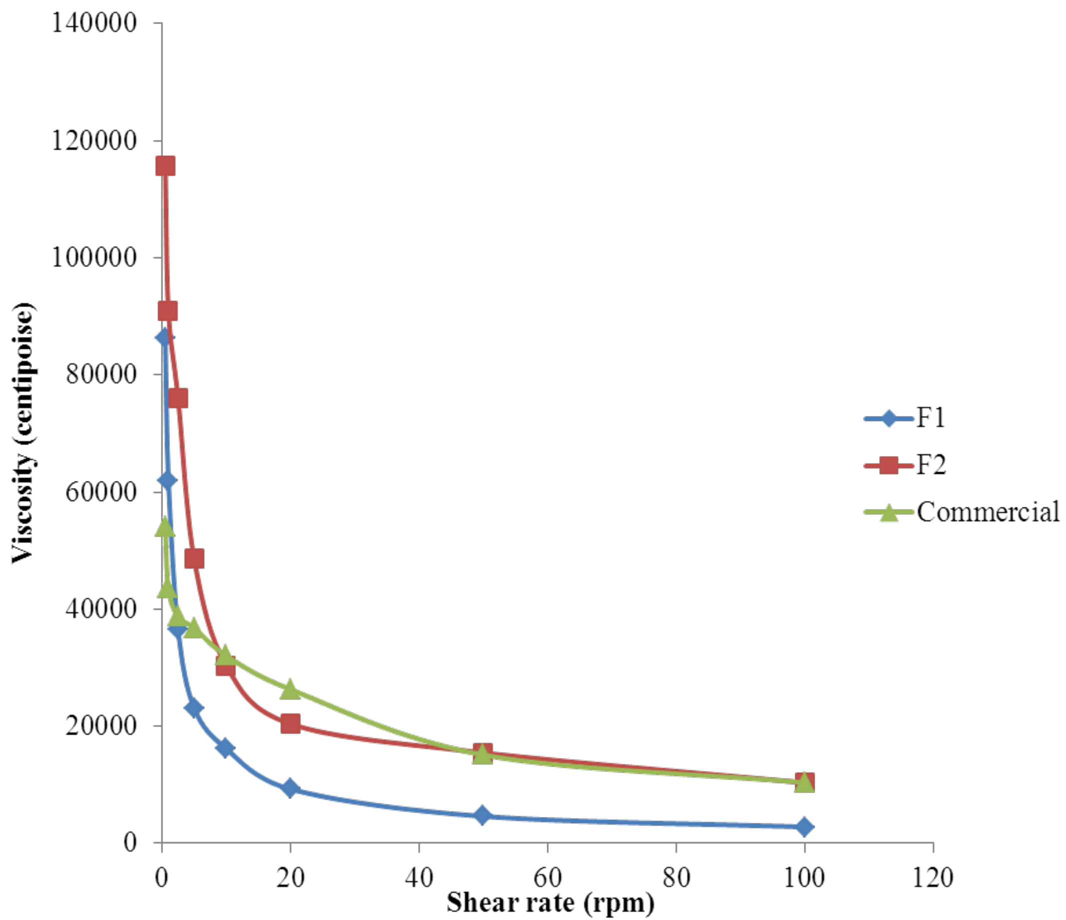

Figure 1 : Rheogram of absorption ointment base

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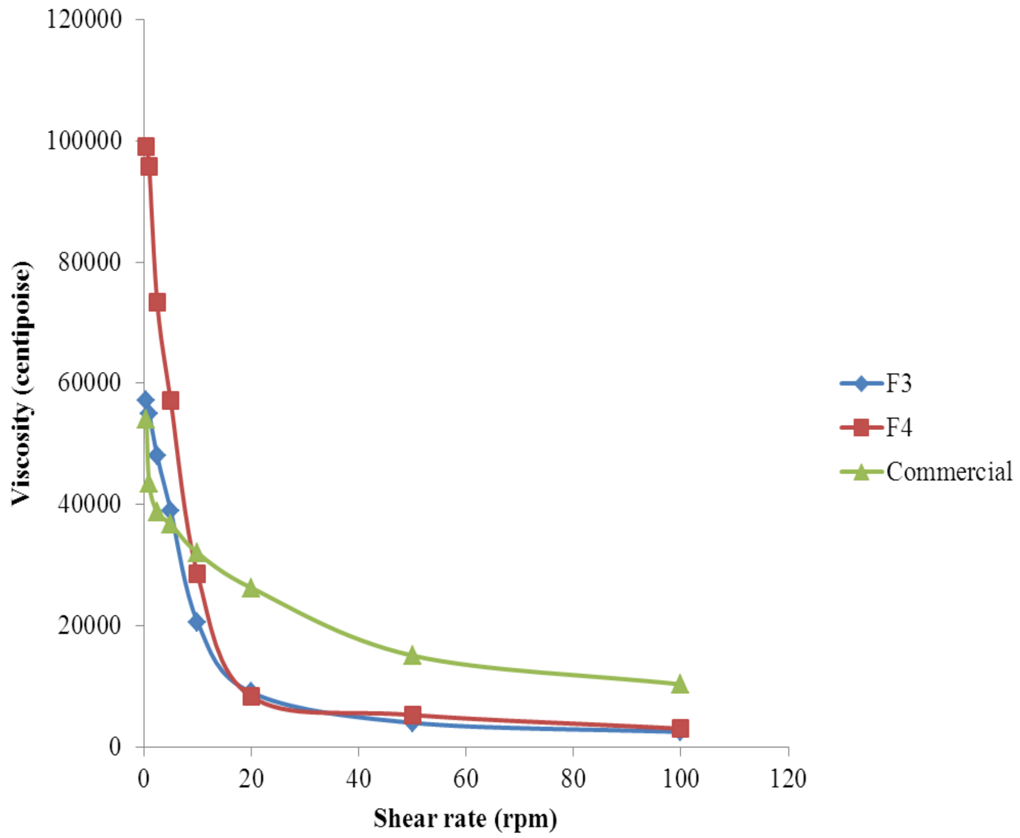

Figure 2 : Rheogram of oil in water emulsion base

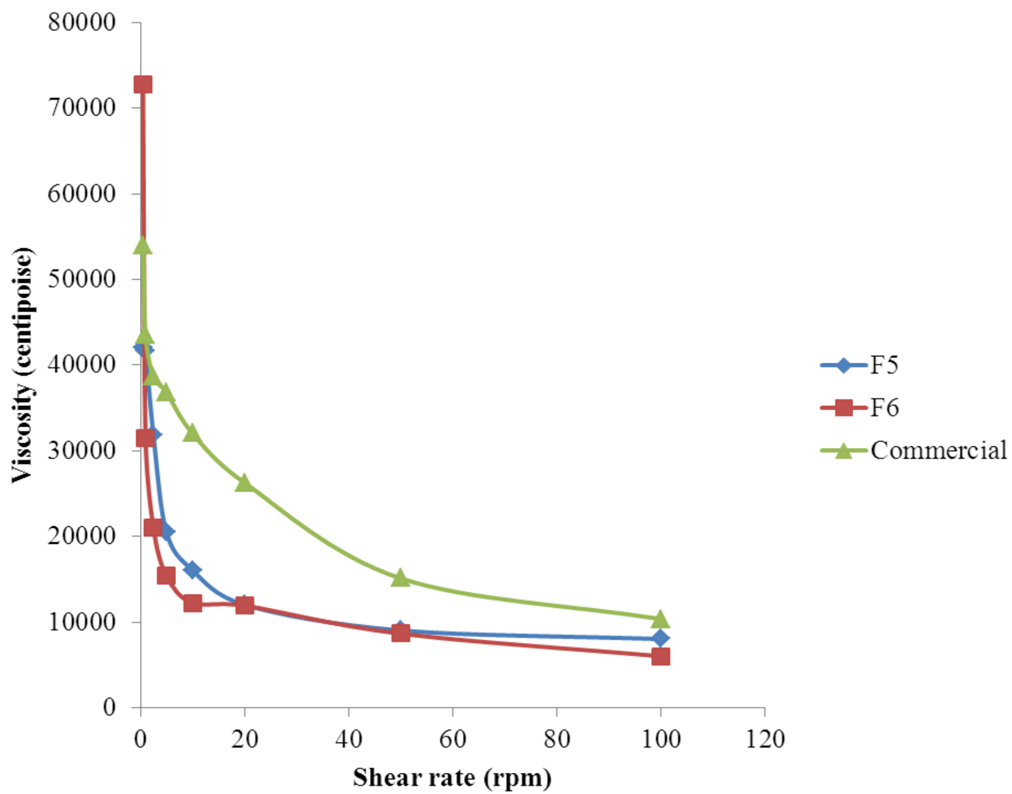

Figure 3 : Rheogram of water in oil emulsion base

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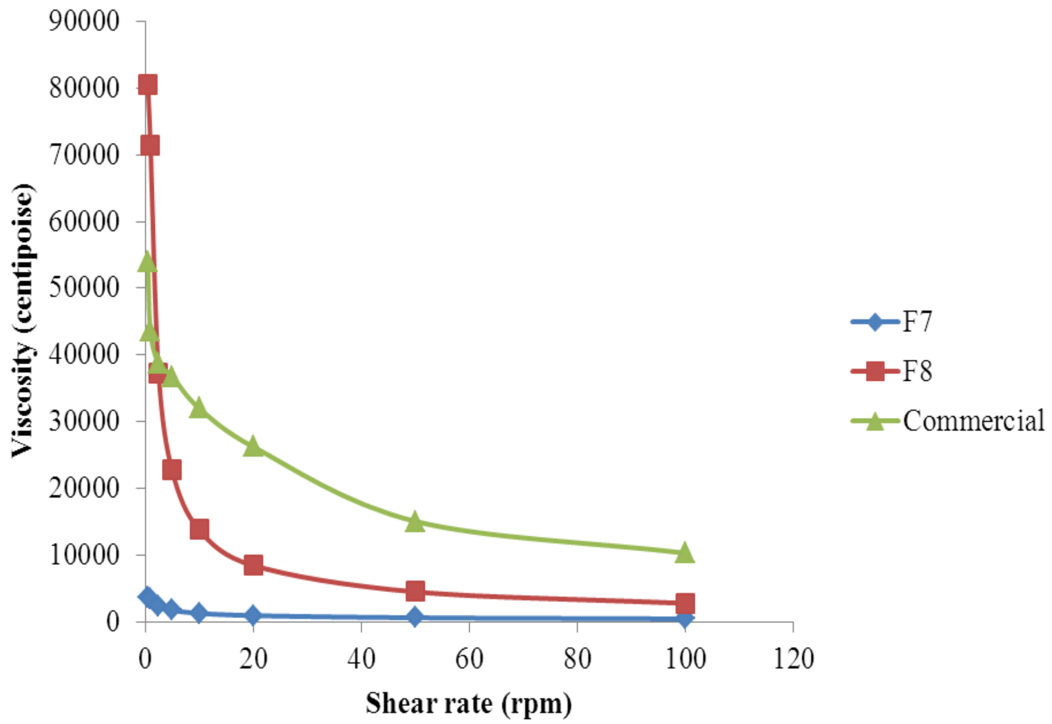

Figure 4 : Rheogram of alcoholic gel base

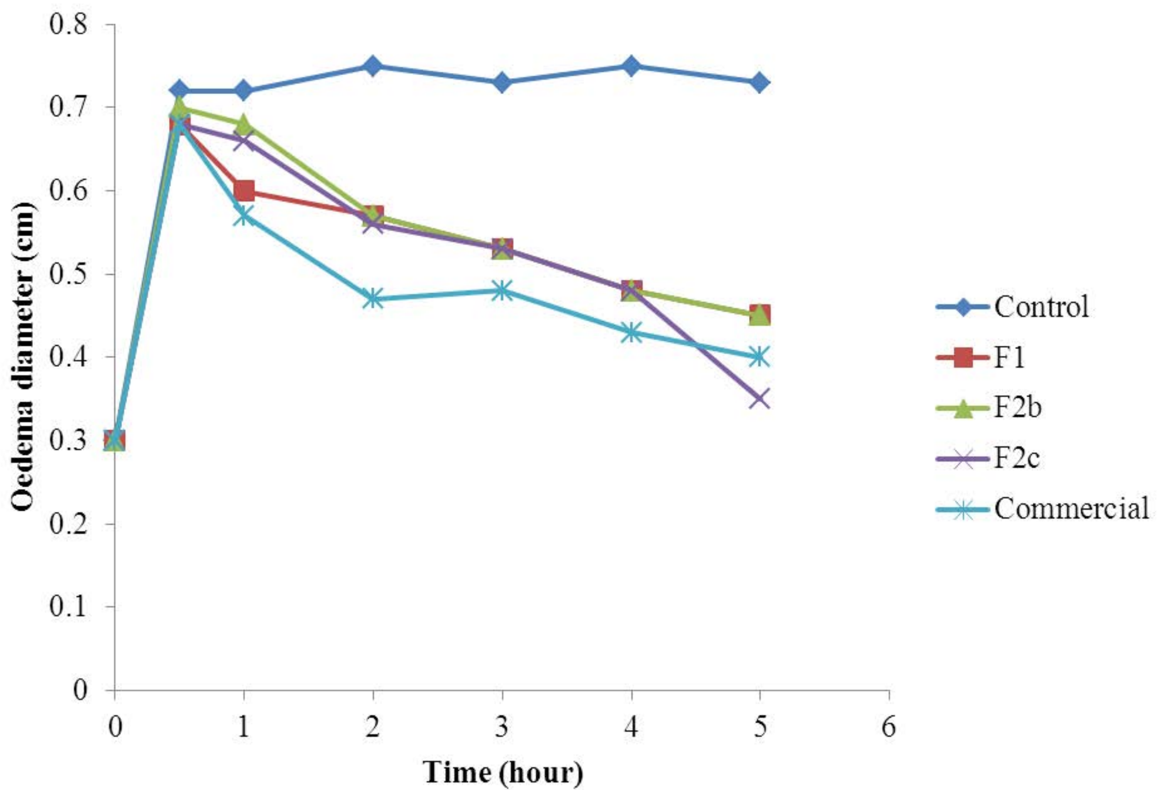

Figure 5 : Anti-inflammatory effect MF using absorption ointment base (F1, F2b and F2c)

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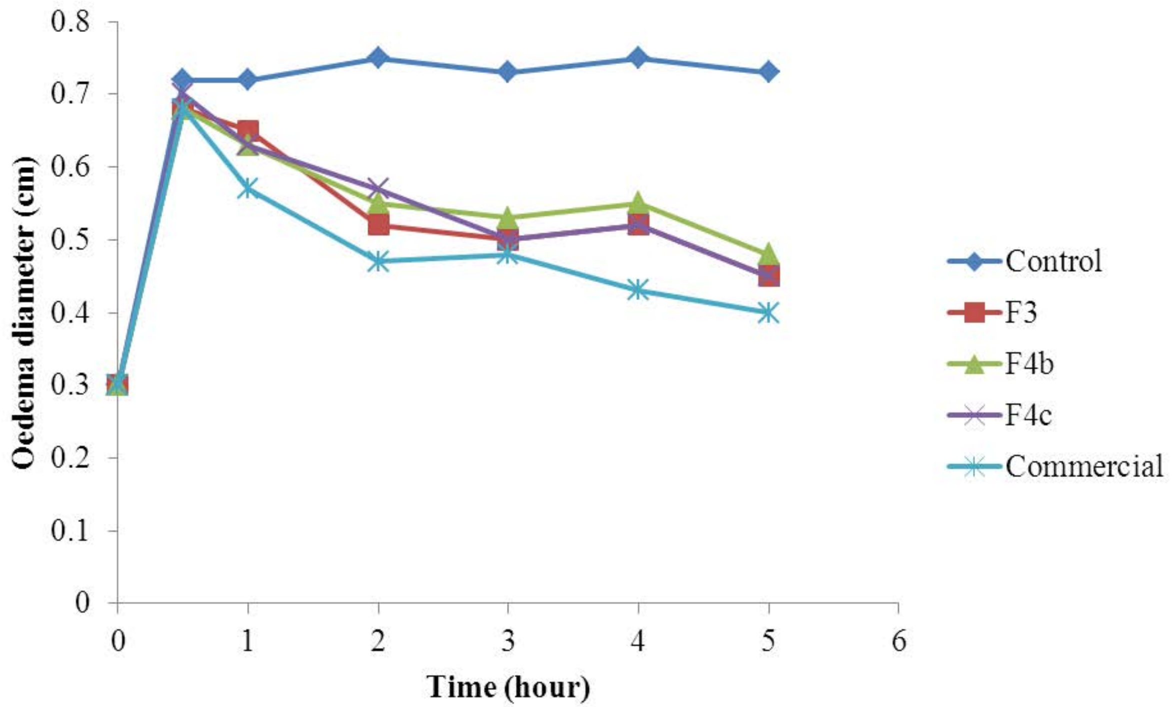

Figure 6 : Anti-inflammatory effect of MF oil in water emulsion base (F3, F4b and F4c)

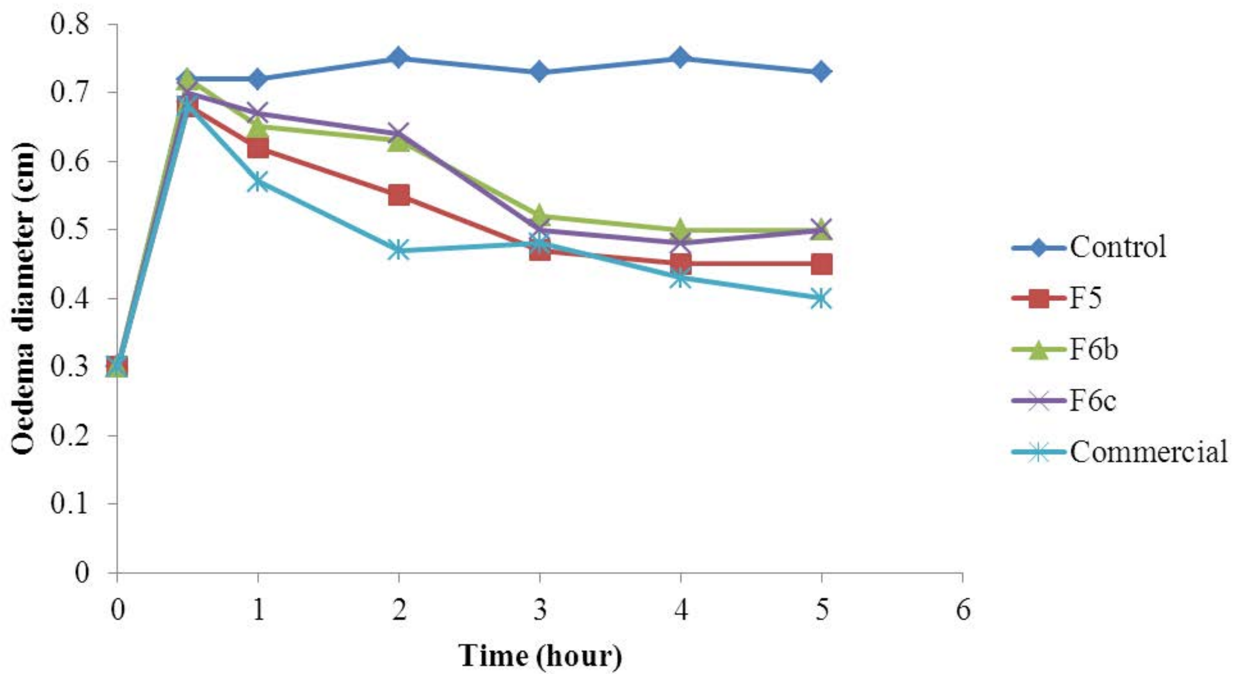

Figure 7 : Anti-inflammatory effect of MF using water in oil emulsion (F5, F6b and F6c)

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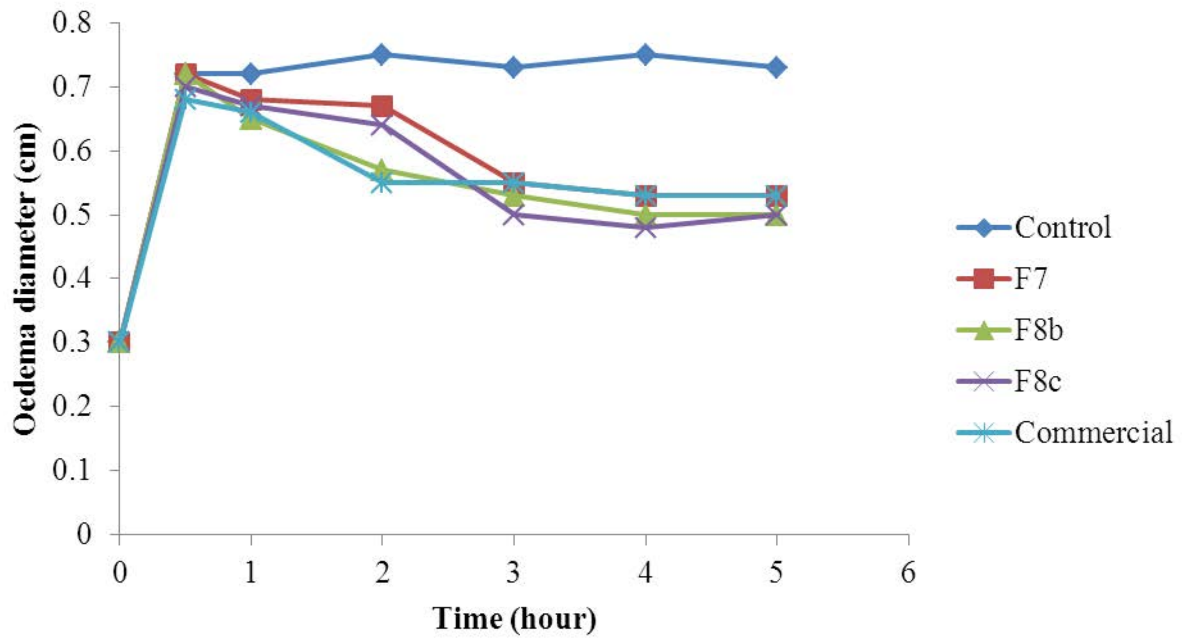

Figure 8 : Anti-inflammatory effect of MF using alcoholic gel base (F7, F8b and F8c)

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Effect of Cabergoline added to Metformin on Glycemic Control, Insulin Resistance and Beta Cell Function in Obese type 2 Diabetic Patients

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Abstract- The aim of this study is to examine the effect of cabergoline added to metformin on glycemic control, insulin resistance and B-cell function in obese type 2 diabetic patients. Forty obese patients with newly diagnosed type2 diabetes were enrolled in this study and randomized by 1:1 ratio into group (I) receives metformin and group (II) receives metformin plus cabergoline for 12 week. We evaluated fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) every 4 week while body weight, glycosylated hemoglobin, fasting plasma insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and B-cell function (HOMA-B) at the baseline and after 12 week.

Keywords: cabergoline, glycemic control, insulin resistance, beta-cell function, obesity, type 2 diabetes

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Effect of Cabergoline added to Metformin on Glycemic Control, Insulin Resistance and Beta Cell Function in Obese type 2 Diabetic Patients

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Abstract- The aim of this study is to examine the effect of cabergoline added to metformin on glycemic control, insulin resistance and B-cell function in obese type 2 diabetic patients. Forty obese patients with newly diagnosed type2 diabetes were enrolled in this study and randomized by 1:1 ratio into group (I) receives metformin and group (II) receives metformin plus cabergoline for 12 week. We evaluated fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) every 4 week while body weight, glycosylated hemoglobin, fasting plasma insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and B-cell function (HOMA-B) at the baseline and after 12 week. At the end of the study, Cabergoline plus metformin significantly improved FPG, PPG and HOMA-IR more than metformin alone. Beta-cell functions significantly improved by cabergoline plus metformin but not by metformin alone after 12 week compared with baseline. We can conclude that cabergoline added to metformin improved glycemic control and insulin resistance better than metformin alone.

Keywords: cabergoline, glycemic control, insulin resistance, beta-cell function, obesity, type 2 diabetes.

I. Introduction

Type2 diabetes is a complex heterogeneous metabolic disorder of glucose homoeostasis characterized by insulin resistance and impaired B-cell function, as well as dysfunction in multiple other organs or tissues[1]. There is strong association between obesity and T2D development[2]. The incidences of T2D have tripled over the past 30 years mainly because of the global prevalence of obesity[3]. Al though insulin resistance and B-cell dysfunction represent the core defect in pathophysiology of T2D, the Ominous Octet theory of de Fronzo implicates multiple abnormalities in T2D[4]. The brain, as seat of cerebral insulin resistance and neurotransmitter dysfunction, is described as eighth pathophysiologic factor this theory[4]. Plethora of evidence indicated that reduced dopaminergic neurotransmission in the hypothalamus and subsequently enhanced noradrenergic activities in the ventromedial hypothalamic nuclei are directly and casually involved obesity and Insulin resistance[5]. It is fact that obesity and T2D appear to be important side effects dopamine D2 receptorsblocker[6]. Additionally obese individuals have significantly lower D2/D3 receptor levels, which make them less sensitive to reward stimuli and put them at risk for overeating[7]. Chronic over nutrition can trigger Hypothalamic neuroinflammation and stressors like ER stress which impaired insulin signaling in the CNS, central insulin resistance,leads to hyperphagia, weight gain and consequently to hyperinsulinemia as well as hyperglycemia[8,9]. Because of the complex and multifactorial pathogenesis, it is difficult to restore normoglycemia and unlikely to achieve glycemic target by single antidiabetic agent. Therefore there is continuous need to develop new antidiabetic agents that have different mechanism of action targeting known pathogenic abnormalities and can be use in combination to produce an additive effect[4]. Timed-release bromocriptine is the first centrally acting dopamine agonist used for the treatment of T2D as monotherapy and combination with metformin[10]. Cabergoline is a centrally acting dopamine agonist with high specificity for dopamine D2 receptors and binding affinity lasting up to 72 hours. It is more effective, better tolerated and four times more potent than bromocriptine[11]. Some clinical studies reported direct beneficial metabolic effects of cabergoline on glucose level, insulin resistance and inflammation[12, 13]. Therefore this study performed to examine the effect of cabergoline added to metformin on glycemic control, insulin resistance and B-cell function in patients with obesity and T2D.

II. Patient and Methods

a) Study design

The present study is prospective randomized control clinical trial. The study is conducted from March to December /2013 in Obesity Research and Therapy Centre /Al Kindi College of medicine and in Al kindi Specialized Center for Endocrinology and Diabetes in Baghdad. This study is approved by Institutional Ethics Committee. Fasting plasma glucose (FPG) and post prandial plasma glucose (PPG) level were measured every four week during the treatment period while

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HbA1c, fasting insulin and HOMA-IR and HOMA-B, were measured at baseline and after 12 weeks.

b) Patients and study group

Forty patients were recruited and enrolled in this study. The included patients were men and women with BMI (BMI ≥ 30 kg/m^2)and with newly diagnosed ofT2D according to ADA guidelines criteria[2]. Patients excluded from the study were: (1) Patients on oral hypoglycemic agent or insulin; (2) patient with impaired renal or hepatic function; (3) Pregnancy or breastfeeding; (4) Patients with chronic cardiovascular or inflammatory diseases (5) hypersensitivity to ergot derivatives. The patient randomized by 1:1 ratio into two group: Group (I) treated with Metformin 500-850mg three time daily(N=20) and Group (II) treated with metformin 500-850mg three time daily and cabergoline 0.5mg twice weekly (N=20).The treatment and follow up period was 12 week. All patientswere advised for standard dietary therapy and life style modifications.

c) Measurements

Height and weight were obtained using a standard stadiometer and electronic scale, respectively. Body mass index was calculated using the standard formula, weight (kg)/height (m)^2. Plasma glucose was assayed by glucose-oxidase method (Cromatest Linear Chemicals.S.L Spain). Glycosylated hemoglobin level was measured by a high performance liquid chromatography (Bio-Rad VARIANT™, USA). Insulin was measured ELISA (Demeditec Diagnostics Gmbh, Germany). Insulin resistance and β-cell function were evaluated by the homeostasis model assessment (HOMA) method which has been suggested as a method to assess insulin resistance (HOMA-IR) and β-cell function (HOMA-β) from the fasting glucose and insulin concentration according to the following formula[14]:HOMA-IR = (glucose × insulin)/405 and HOMA β-Cell=360 ×Fasting insulin (mU/ml)/ (Fasting glucose (mg/dl) — 63)

d) Statistical analysis

Paired Student's t test was used to compare values in same group at different time with baseline. Independent sample t-test was used to compare changes in variables between the two groups. Data are presented as mean ± Standard error mean (SEM). Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 16.0 (SPSS, Chicago, IL)

III. Result

a) Patient's characteristics

Out of the total enrolled patients, 8 patients did not complete the study due to many reasons noncompliance (1), lost to follow up (4), start oral hypoglycemic agent (2) and develop adverse event (1). The remaining 32 patient (17 patients in metformin treated group and 15 patients metformin plus cabergoline treated group). The demographic and baseline clinical characters were not different between the two. Table (1)

Table 1 : Baseline characteristics

Parameters	Metformin (n=17)	Metformin+ Cabergoline (n=15)
Gender(M/F)	(6/11)	(5/10)
Age(years)	44.35±2.5	47.6±2.8
WT(Kg)	99.6±2.4	101±3.0
Height (m)	1.66±0.3	1.65±0.2
BMI(kg/m^2)	36.5±0.89	36.8±0.61
FPG(mg/dl)	161.5±4.7	165.5±5.4
PPG(mg/dl)	205.3±6.9	211.1±6.4
HbA1c	7.95±0.29	8.39±0.34
Fasting Insulin(mU/ml)	16.76±1.3	17.8±1.6
HOMA-IR	6.8±0.67	7.4±0.9
HOMA-B%	62.±4.3	63.3±5.2

b) Effect body weight and BMI

Both group demonstrated a significant decrease in the body WT and BMI at the end of 12 weeks compared with the baseline. But the change was not significant between the two groups. Table (2)

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Table 2 : Body wiegth (WT), body mass index (BMI) before and after 12 week treatments and the change from baseline

Parameters	Time	Metformin	Metformin+ cabergoline
WT	Baseline	99.6±2.4	101±3.0
	12week	95.5±2.3*	95.4±3.1*
	Change	4.1±1.1	5.6±1.5
BMI	Baseline	36.5±0.89	36.8±0.61
	12week	35.0±0.72*	34.9±0.78*
	Change	-1.5±0.47	-1.9±0.54

**=p< (0.05) comparing with baseline*

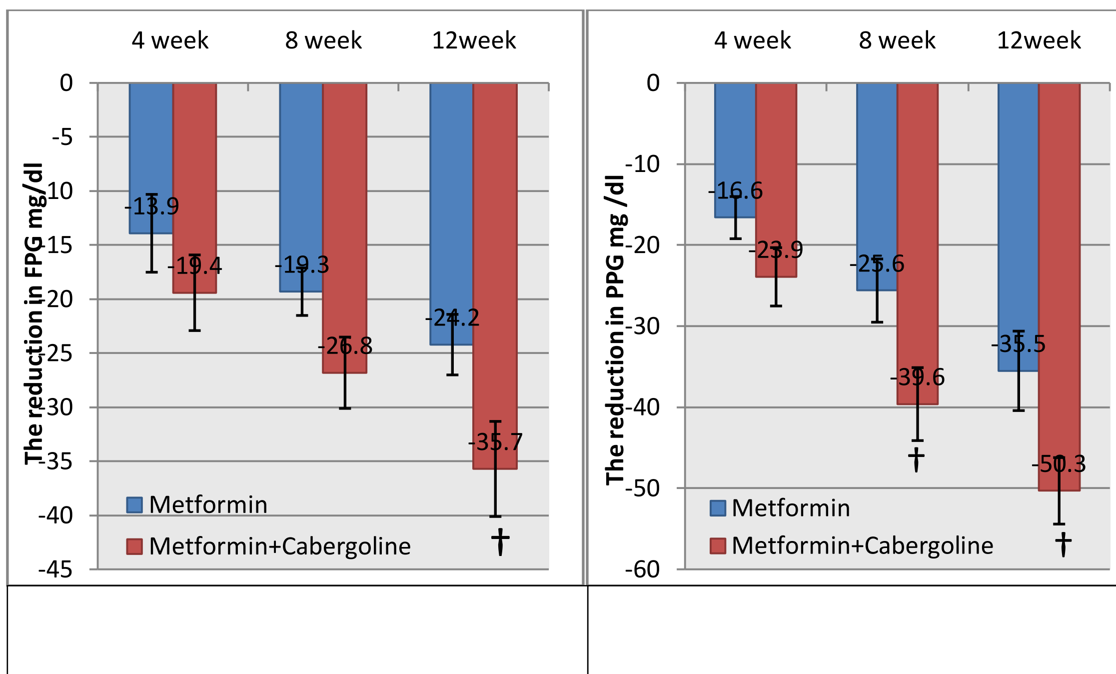
c) *Effect of study treatment on glycemic parameters (FPG &PPG)*

Both group significantly improved FPG and PPG over time compared with baseline Table(3). The reduction in FPG was significntly greater in metformin plus cabergoline than metformin alone at 12 weeks. Also Metformin plus cabergoline reduced PPG significantly greater than metformin alone at week 8 and 12.

Table 3 : Treatment effect on FPG (mg/dl) and PPG (mg/dl) at the different duration of the study

Parameters	Time	Metformin	Metformin+ cabergoline
FPG (mg/dl)	0week	161.5±4.7	165.5±5.4
	4week	147.7±5.4*	145.7±6.9**
	8week	142.1±3.7**	138.5±5.0**
	12week	137.4±5.9**	129.7±4.5**
PPG (mg/dl)	0week	205.3±6.9	211.1±6.4
	4week	183.4±7.0*	187.6±6.5**
	8week	174.4±6.2*	171.5±5.4**
	12week	169.8±4.8**	160.8±4.6**

**=p< (0.05) and **=P (<0.001) comparing with baseline*



†=(p<0.05) in comparing of metformin plus cabergoline group with metformin group

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d) Effect on HbA1c

Highly significant (P<0.001) decrease was observed in the two groups after 12 weeks compared to baseline. However, the reduction in HbA1c was not statistically significant between them. Interestingly, the percentage of patients achieving HbA1c <7.0% was 60% by adding cabergoline to metformin vs 41 % by metformin alone.

Table 4 : HbA1c before and after 12 week and the change from baseline

Parameters	Time	Metformin	Metformin+ cabergoline
HbA1c	Baseline	7.95±0.29	8.39±0.34
	12week	7.05±0.23**	7.17±0.29**
	Change	-0.9±0.16	-1.22±0.14

***=P (<0.001) comparing with baseline*

e) Effect of the study treatment on fating insulin level, HOMA-IR and HOMA-B

The decrease in fasting insulin level was significant in metformin group (P<0.05) and highly significant in metformin plus cabergoline after 12 week (P<0.001) compared to the baseline however there was no significant differences between the two group. HOMA-IR decreased significantly in both group after 12 week compared with the baseline (P<0.001). The change in HOMA-IR was significantly greater in metformin plus cabergoline compared with metformin group (P<0.05).HOMA-B% significantly increased by adding cabergoline with metformin (P<0.05) but not by metformin alone (P>0.05) after 12 weeks compared with the baseline However the change in HOMA-B% between the two group was not significant.

Table 5 : Fasting insulin level, HOMA-IR and HOMA-B before and after 12 week and the change from baseline

Parameters	Time	Metformin	Metformin+ cabergoline
Insulin mU/ml	Baseline	16.76±1.3	17.8±1.6
	12week	14.47±1.8*	13.9±1.3**
	Change	2.3±0.95	3.9±0.52
HOMA-IR	Baseline	6.8±0.67	7.4±0.9
	12week	5.2±0.79**	4.5±0.48**
	Change	-1.6 ±0.35	-2.88±0.45†
HOMA-B	Baseline	62.±4.3	63.3±5.2
	12week	69.1±5.7	78.5±7.8*
	Change	7.1±4.5	15.2±5.4

**=p< (0.05) and **=P (<0.001) comparing with baseline, †= (p<0.05) in comparing of metformin plus cabergoline group with metformin group.*

IV. Discussion

a) Effect of cabergoline on glycemic control

This is the first study that examined the effect of cabergoline on glycemic control in treatment naïve T2D with obesity. This study demonstrated a beneficial effect of cabergoline in reducing the hyperglycemia in patient with obesity and newly onset diabetes because add on therapy of cabergoline with metformin improved FPG after 12 week and PPG after 8 and 12 week to significantly greater degree than metformin alone. Although the decrease in HbA1c was higher by adding cabergoline to metformin than metformin (1.22±0.14 and -0.9±0.16 respectively), the difference between them was not significant which might be attributed to the slow effect of cabergoline in achieving glycemic control and the short period of the study. However the percentage of patient reaching to target HbA1c <7.0% was 60% by taking cabergoline along with metformin vs 41 % by metformin monotherapy. At the present time, there is only one published clinical study demonstrated the effect of cabergoline on glycemic control in T2D in which 3 month cabergoline treatment reduced both FPG and PPG as well as caused 0.45–1.11 reduction in HbA1c in patient with failure to oral antidiabetic agent[15]. Also 16 weekcabergoline treatment decreased PPG overtime in healthy obese[12]. Similarly, short term bromocriptine treatment 2.5mg BID significantly reduce FPG and diurnal glucose concentration in obese women[16]. Interestingly, The HbA1c level of a ten patient with acromegaly decreased significantly in the six diabetic patients (from 8.4 % to 6.7 %) compared to no significant reduction of the four non diabetics after 16 week of cabergoline therapy[17]. Furthermore, cabergoline treatment improved glycemic tolerance and decreased

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HbA1c in patients with prolactinoma regardless of the degree of reduction in prolactin levels[18]. Most recently, cabergoline was superior tobromocriptine in reducing 2-hr post-challenge plasma glucosedespite a similar reduction in plasma prolactin levels[19]. More over the findings of the present study are in fundamental agreement with responses of centrally acting dopamine agonist, bromocriptine, obtained in T2D[10, 20]. More recently, the combination of bromocriptine with metformin significantly decreased FPG, PPG, and HbA1c compared with metformin alone in T2D[21]. The mechanism by which dopamine agonist therapy improve glycemic control can be explained by

1. Activation of dopamine receptor D1& D2 in the hypothalamus normalizes multiple hypothalamic neurophysiological derangements through enhancing hypothalamic dopaminergic tone and consequently preventing ventromedial hypothalamic noradrenergic and serotonergic over activity, as well as reverting elevated paraventricular hypothalamic neuropeptide Y and corticotrophin-releasing in obese T2D, thus improving peripheral glucose disposal and insulin resistance as well as suppressing of hepatic glucose production[22,23].
2. Regulation food intake by modulating food reward and motivation via the meso-limbic circuitry of the brain, thus suppressing hunger and improving satiation and satiety[7].
3. Activation D2 receptors present on pancreatic beta cells lead to increase the islet insulin content and restores the link between glucose sensing and insulin secretion, thus improving beta cell response to hyperglycemia[24].

b) Effect of cabergoline on insulin resistance and beta-cell function

The relationship between insulin resistance and beta cell dysfunction is dynamic and largely dependent on the metabolic state that is primarily determined by glycemic status and consequently insulinemic status[25].The Homeostasis Model Assessment (HOMA)has considered as a robust clinical tool for the assessment of insulin resistance and has been reported in > 500 publications[26]. Therefore the present study used this model to assess insulin resistance and B-cell function.

Cabergoline therapy profoundly improved the metabolic abnormalities; such as Obesity, hyperinsulinemia, insulin resistance and glucose intolerance associated with hyperprolecteno main dependent from the changes in BMI and normalization of prolactin level. Several Recent studies demonstrated a significant reduction in fasting insulin and HOMA-IR[13,18]as well as a significant improvement in insulin sensitivity indexassessed by both ISI Matsuda and clamp[27,28]. Furthermore, Gibson et al demonstrated tendency towards stabilization or improvement in HOMA-IR and insulin AUC after 16 week of cabergoline treatment in health obese person[12]. Moreover, two week of Bromocriptine treatment reduced fasting plasma insulin level by 35.0% and insulin resistance (HOMA-IR) by 38% and also considered as unique postprandial insulin Sensitizer[29]. All these findings are suggesting a direct beneficial effect of dopamine agonist on insulin resistance.The results of present study further supported this effect of cabergoline because the reductions in fasting insulin and insulin resistance (HOMA-IR) were higher by taking cabergoline with metformin than metformin alone.

Basal hyperinsulinemia associated with obesity and T2D, generates and sustains insulin resistance in all tissue having insulin receptor including pancreatic B-cell and the brain by several mechanisms, reduction in number of insulin receptor, serine phosphorylation of IRS-1 and elevated level of inflammatory markers, including cytokines and C-reactive protein[30,31]. Endogenous dopamine regulates insulin release by acting D2 receptors expressed on pancreatic B-cell[32]. It was found that the administration of neuroleptic drugs, D2R-blocker, causes hyperinsulinemia in normal subjects[33].Thus activation of D2R on islet Beta-cells by dopamine agonist result in inhibition of insulin secretion[34].Counterintuitively, the ability of dopamine agonist to suppress insulin secretion might be at the basis of its beneficial effect on glucose homeostasis by preventing long-lasting hyperinsulinemia and therefore prevent subsequent development of insulin resistance and beta cell failure[35]

Pancreatic B-cell dysfunction associated with the obesity and insulin- resistant state is characterized by an increased basal insulin secretory rate and a blunted GSIS. Preclinical studies have suggested that treatment with dopamine agonist normalizes basal insulin secretory rate and GSIS and increases the islet insulin content thus improving pancreatic beta cell function[24,34]. The mechanism by which dopaminergic therapy improves islet function in the obese diabetic condition may involve improving B-cell glucokinase (GK), an integral modulator of GSIS, and/or GLUT2 as well as enhancing insulin storage and/or retention, and stabilizing B-cell hyperplasia, thus reducing basal insulin levels[36]. In the present study, interestingly, the combination of cabergoline with metformin significantly improved HOMA-B after 12 week compared with baseline but not by metformin alone. Currently, only two clinical studies in which cabergoline effect on HOMA-B was evaluated. Cabergoline did not show significant effect on HOMA-B in patient with Cushing syndrome[37]while HOMA-B was significantly improved after 24 month of cabergoline treatment compared to baseline in patient with hyperprolactenoma[37]. In contrast to HOMA-IR, it is controversial whether HOMA-B is an accurately reflected pancreatic β-cell function[39]. In

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general HOMA model is used 20 times more frequently for the estimation of IR than β-cell function because B-cell dysfunction is longer model and hence the use of HOMA-B associated with some limitations[25]. Therefore the period of the present study is a major limitation to accurately assess the effect on beta cell function.

V. Conclusion

The combination of cabergoline with metformin significantly improved glycemic control and insulin resistance better than metformin alone in patient with obesity and diabetes. Also the combination might have beneficial protective effect on B-cell of pancreas.

VI. Acknowledgement

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References Références Referencias

1. Lin Y, Sun Z (2010): Current views on type 2 diabetes.J of Endocrinol; 204, 1–11.
2. Astrup A, Finer N (2000): Redefining type 2 diabetes: 'diabesity' or 'obesity dependent diabetes mellitus?Obes Rev;1(2):57-9.
3. Khaodhiar L, Cummings S, Apovian CM (2009). Treating Diabetes and Prediabetes by Focusing on Obesity Management. CurrDiab Rep. 9(5): 348–354.
4. DeFronzo R. (2009): From the Triumvirate to the Ominous Octet: A New Paradigm for the Treatment of Type 2 Diabetes Mellitus. Diabetes; 4 :(58).
5. Pijl H(2003): Reduced dopaminergic tone in hypothalamic neural circuits: expression of a ‘‘thrifty’’genotype underlying the metabolic syndrome. European Journal of Pharmacology; 480 :125– 131.
6. Tschoner A, Engl J, Laimer M et al(2007): Metabolic side effects of antipsychotic medication. Int J ClinPract; 61: 1356-70.
7. Wang GJ, Volkow ND, Thanos PK et al(2009): Brain Dopamine Pathways and Obesity .J Addict Med;3(1):8-18.
8. Pagotto U, (2009): Where Does Insulin Resistance Start? The brain. Diabetes care;32(Suppl 2): S174-S177.
9. Purkayastha S, Cai D (2013). Neuroinflammatory basis of metabolic syndrome. Molecular Metabolism 2:356–363.
10. Scranton RE, Ezrokhi M, Gaziano JM et al (2008): Quick release bromocriptine (Cycloset TM) improves glycaemic control in patients with diabetes failing metformin/sulfonylurea combination therapy. Diabetologia; 51:S372–3.
11. Brunton LL, Parker LL and Blumenthal DK, Goodman & Gilman’s. Manual of pharmacology and therapeutics, McGraw-Hill, 2008; 1st ed: 973-974.
12. Gibson CD, Karmally W, McMahon DJ et al (2012). Randomized Pilot Study of Cabergoline, a Dopamine Receptor Agonist: Effects on Body Weight and Glucose Tolerance in Obese Adults Diabetes Obes Metab.2012; 14 (4): 335–340.
13. Inancli SS, Usluogullari A, Ustu et al (2013). Effect of cabergoline on insulin sensitivity, inflammation, and carotid intima media thickness in patients with prolactinoma.Endocrine; 44:193–199.
14. Muniyappa R, Lee S, Chen H (2008): Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J PhysiolEndocrinolMetab; 294: E15–E26.
15. Taghavi SM, Fatemi SS, Rokni H (2012). Cabergoline Effect on Blood Sugar in Type 2 Diabetic Patients with Oral Agent Failure. Med J Malaysia; 67 (4):390-2.
16. Kok P, Roelfsema F, Frolich M et al (2006):Activation of dopamine D2 receptors simultaneously ameliorates various metabolic features of obese women. AJP-Endocrinol Metab;291.
17. Merican N, Sukor N, Razali A et al (2013): The Effects of Short Term Cabergoline Therapy on Disease Activity and Metabolic Parameters in Acromegaly .Journal of Endocrinology and Metabolism.;3(1): 48-56.
18. Ciresi A, Amato M, Guarnotta V etal (2013): Higher doses of cabergoline further improve metabolic parameters in patients with prolactinoma regardless of the degree of reduction in prolactin levels. Clinical Endocrinology. 79: 845–852.
19. Krysiak Rand Okopien B (2014): Different Effects of Cabergoline and Bromocriptine on Metabolic and Cardiovascular Risk Factors in Patients with Elevated Prolactin Levels. Basic ClinPharmacol Toxicol; 13(8).
20. Cincotta AH, Meier AH, Cincotta MJ (1999): Bromocriptine improves glycaemic control and serum lipid profile in obese Type 2 diabetic subjects: A new approach in the treatment of diabetes. Expert OpinInvestig Drugs. 8:1683–707.
21. Ghosh A, Sengupta N, Sahana P et al. (2014): Efficacy and safety of add on therapy of bromocriptine with metformin in Indian patients with type 2 diabetes mellitus: A randomized open labeled phase IV clinical trial. Indian J Pharmacol. 46(1): 24–28.
22. Bina KG, Cincotta AH (2000): Dopaminergic agonists normalize elevated hypothalamic neuropeptide Y and corticotropin-releasing

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hormone, body weight gain, and hyperglycemia in ob/ob mice. Neuroendocrinology 71:68-78.
23. DeFronzo RA (2011): Bromocriptine: A Sympatholytic, D2-Dopamine Agonist for the Treatment of Type 2 Diabetes. Diabetes Care, 4:(34).
24. Liang Y, Lubkin M, Sheng H et al(1998): Dopamine agonist treatment ameliorates hyperglycemia, hyperlipidemia, and the elevated basal insulin release from islets of ob/ob mice. BiochimBiophysActa 1405:1-13.
25. Turner RC, Rudenski AS, Matthews DR, et al. (1990): Application of structural model of glucose-insulin relations to assess beta-cell function and insulin sensitivity. HormMetab Res. 24(Suppl):66–71.
26. Wallace TM, Levy JC, Matthews DR (2004): Use and abuse of HOMA modeling. Diabetes Care; 27: 1487–1495.
27. Berinder K, Nystrom T, Hoybye C et al (2011): Insulin sensitivity and lipid profile in prolactinoma patients before and after normalization of prolactin by dopamine agonist therapy. Pituitary; 14 (3):199-207.
28. Dos Santos Silva CM, Barbosa FR, Lima GA, et al. (2011): BMI and Metabolic Profile in Patients with Prolactinoma before and After Treatment with Dopamine Agonists. Obesity (Silver Spring).19:800–805.
29. Ezrokhi M, Luo L and Cincotta A et al. (2012): Weighted effects of bromocriptine treatment on glucose homeostasis during hyperglycemic versus euglycemic clamp conditions in insulin resistant hamsters: bromocriptine as a unique postprandial insulin Sensitizer. J Diabetes Metab. S:2.
30. Anan F, Takahashi N, Nakagawa M et al (2005).Metabolism; 54(4):552-8.
31. Corkey BE, (2012):Hyperinsulinemia: Cause or Consequence? .Diabetes; 61(1):4-13.
32. Ustione A, Piston D and Harris P et al (2013). A Mini review: Dopaminergic regulation of Insulin Secretion from the Pancreatic Islet. MolEndocrinol; 27(8): 1198–1207.
33. Sowell MO, Mukhopadhyay N, and Cavazzoni P et al. (2002): Hyperglycemic clamp assessment of insulin secretory responses in normal subjects treated with olanzapine, risperidone, or placebo. J ClinEndocrinolMetab 87:2918–2923.
34. Garcia-Tornado I, Ornstein AM, Chamson-Reig A, et al (2010): Disruption of TEJ dopamine D2 receptor impairs insulin secretion and glucose intolerance. Endocrinology 151 (4): 1441-50.
35. Brown RJ, Rother KI (2008): Effects of beta-cell rest on beta-cell function: a review of clinical and preclinical data. Pediatr Diabetes; 9(3 Pt 2):14–22.
36. Jetton LT , Liang Y, and Cincotta AH et al (2001).Systemic Treatment With Sympatholytic Dopamine Agonists Improves Aberrant B-Cell Hyperplasia and GLUT2, Glucokinase, and Insulin Immunoreactive Levels in ob/ob Mice. Metabolism, 50(11) :1377-1384.
37. Pivonello R, De Martino C, Cappabianca P et al (2009): The Medical Treatment of Cushing's disease: Effectiveness of Chronic Treatment with the Dopamine Agonist Cabergoline in Patients Unsuccessfully Treated by Surgery. J ClinEndocrinol Metab;94(1):223–230.
38. Galdiero M, Auriemma R, Vitale P et al. (2014): Effect of chronic treatment with cabergoline and testosterone replacement on metabolic parameters in male patients with prolactinomas and hypogonadism. Endocrine Abstracts 35: P865.
39. Sung KC, Reaven GM, and Kim SH et al (2010): Utility of Homeostasis Model Assessment of B-Cell Function in Predicting Diabetes in 12,924 Healthy Koreans. Diabetes Care 33:200–202.

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Phytochemical, Mineral Compounds and Anti- Oxidation Studies on Pistacia Lentiscus Shoot Extract

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Abstract- Shoot extracts of Pistacia lentiscus (P. lentiscus) were investigated for its medicinal importance, by valorizing of some chemical characterization, mineral composition, and study of the antioxidant activity. The photochemical screening of the plants constituents were assessed by using qualitative tests were conducted for the presence of the following active components: alkaloid, Hydrolysable tannins, tannins, phlobatannins, phenol, flavonoids, glycoside, saponins, volatile oil, hydrolysable tannin, protein, cortisone and Anthracanens (anti-oxidation compound) . All were present. Mineral contents of shoot extract were determined by Atomic Absorption Spectrophotometer. The highest levels of potassium (K) and Iron (Fe) were found in Shoot of P. lentiscus from Libya. These findings suggest that shoots of P. lentiscus are potential sources of Iron, potassium and antimicrobial compounds. Also it has a great medicinal value due to the presence of anti-oxidation compound and cortisone.

Keywords: pistacia lentiscus L., phytochemical screen-ing; cortisone, mineral composition, antioxidant activity. tannins, phlobatannins, volatile oil, active components, shoot extract, alkaloid.

GJMR-B Classification : NLMC Code: QV 37.5, WA 730

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Phytochemical, Mineral Compounds and Anti-Oxidation Studies on *Pistacia Lentiscus* Shoot Extract

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Abstract- Shoot extracts of *Pistacia lentiscus (P. lentiscus*) were investigated for its medicinal importance, by valorizing of some chemical characterization, mineral composition, and study of the antioxidant activity. The photochemical screening of the plants constituents were assessed by using qualitative tests were conducted for the presence of the following active components: alkaloid, Hydrolysable tannins, tannins, phlobatannins, phenol, flavonoids, glycoside, saponins, volatile oil, hydrolysable tannin, protein, cortisone and Anthracanens (anti-oxidation compound) . All were present. Mineral contents of shoot extract were determined by Atomic Absorption Spectrophotometer. The highest levels of potassium (K) and Iron (Fe) were found in Shoot of *P. lentiscus* from Libya. These findings suggest that shoots of *P. lentiscus* are potential sources of Iron, potassium and antimicrobial compounds. Also it has a great medicinal value due to the presence of anti-oxidation compound and cortisone.

Keywords: pistacia lentiscus L., phytochemical screening; cortisone, mineral composition, antioxidant activity. tannins, phlobatannins, volatile oil, active components, shoot extract, alkaloid.

I. Introduction

Pistacia lentiscus (mastic tree), Family Anacardiaceae, an important medicinal plant is widely distributed in Mediterranean Europe, Morocco and Iberian peninsula and in the west through southern France, Turkey, Iraq and Iran. It has a great medicinal value and already has been used in traditional system of medicines. The pharmacology and medicinal use of mastic is diverse. It has been used in cancer, infection, surgical wound adhesion, and ulcers. Studies also document its use as an antioxidant, antiatherogenic, an insecticide, and anti-inflammatory, anti-microbial and for treatment of hypertension and relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Ahida, et al, 2012, Barra, et. al., 2007, Dedoussis et al., 2004; Lamiri et al., 2001, Bonsignore, et al. 1998, Al-Habbal et al., 1984 and Bentley et al., 1980). In addition to its medicinal usage, it has been re- evaluated as a flavoring in chewing gum (Fernandez et al., 2000).

Phytochemicals are chemical compounds that occur naturally in plants. study done by Kumar and Singh (1976) refereed to that phytochemicals are secondary metabolites and are often found in stems, roots, barks, leaves, flowers, fruits and seeds. Common phytochemicals found in plants include tannins, phlobatannins, quinines, alkaloids, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, sugar. They are having potential to affect diseases such as cancer, gout, rheumatism, arthritis (Berboucha et al., 2009; Dimaset al., 2009; Peksel, 2008, Balan, etal, 2007 and Baytop, 1999). Cortisone is a steroid hormone and is used for a variety of ailments. These include endocrine disorders, rheumatic disorders, collagen diseases, dermatologic diseases, allergic states, ophthalmic diseases, respiratory diseases, hematologic disorders, neoplastic diseases, edematous diseases.

Antioxidant - Most phytochemicals have antioxidant activity and protect the cells against oxidative damage and reduce the risk of developing certain types of cancer activity includes: flavonoids, polyphenols.

Anthracene is glycosidic compounds of formula $C_{14}H_{10}$. Anthracene is converted mainly to anthroquinone, a precursor to dyes (Gerd, et al., 2006). It uses in pesticides (Agency for Toxic Substances, 1999). Study by Dembitsky VM (2005) reported that Anthracene and its derivatives, isolated from and identified in plants and microorganisms that demonstrate different biological activities. They are of great interest, especially for the medicinal and/or pharmaceutical industries. These biologically active natural surfactants are good prospects for the future chemical preparation of compounds useful as antioxidant, anticancer, antimicrobial, and anti-bacterial agents (Dembitsky, 2005). This study was conducted to determine some photochemical present in *Pistacia lentiscus* shoot extract. We also evaluated its mineral composition.

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II. Material and Methods

a) Plant material

Shoot of P. lentiscus was collected around from Msallata (northwestern part of Libya). Plants were identified according to (Ali, et al., 1977).

b) Preparation of plant extracts

Leaves and parts of stem (10gm) were subjected to sohxlet extraction using 200ml of water and ethyl-acetate (individually) as solvents. Samples kept for 6 hours at 90◦C for water and 70◦C for Ethyl- acetate.

c) Phytochemical tests

Plant extracts, of both solvent water and ethylactate, were screened for the presence of biologically active compounds like alkaloids, flavanoids, Protenis, Phenol, Phlobatannin, Volte oil, Hydrolysable Tannins, Tannins, Saponins, Glycosides, Anthracanens and Cortisone.

d) Procedure for Phytochemical Analysis

i. *Alkaloid Test*

Equal volumes of the solvent extract (5ml) and the Wagner's reagent, described by Imohiosen et al.(2014), were placed into a clean test tube and observed for some minutes. The presence of alkaloid was indicated by a brown precipitate.

ii. *Saponins Test:* (Froth test)

1g of the sample was weighed into a conical flask in 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

iii. *FlavonoidsTest*

Flavonoids was determined by placing 5ml of the solvent extracts into a test tube and few pieces of magnesium chips were added, followed by concentrated hydrochloric was added drop wise. Appearance of Reddish colouration demonstrated the presence of Flavonoids.

iv. *Proteins Test*

1ml of plant extracts was placed into a test tube then 4ml of foline reagent was add. Appearance of blue colouration demonstrated the presence of proteins.

v. *Phenol Test*

2ml of extract was added to 2ml of ferric chloride solution ($FeCl_3$); a deep bluish green solution is formed with presence of phenols.

vi. *Phlobatannin Test*

3ml of hydrochloride acid and 2ml of solvent extract were placed into a clean test tube and the tube was heated for about 10 minutes. Reddish green coloration indicates the presence of phlobatannins.

vii. *Hydrolysable tannins*

4 ml of the extract was placed and shaken in a test tube. 4ml of 10% ammonia solution was added. Formation of an emulsion on shaking indicated the presence of hydrolysable tannins.

viii. *Tannins Test*

3ml of sample extract was boiled in 50ml distilled water, warmed and filtered. A portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. Green colour indicates the presence of tannins.

ix. *Volite Oil Test*

2.0ml of extract solution was mixed with 0.1 ml dilute sodium hydroxide and a small quantity of dilute HCl. A white precipitate was formed with volatile oils.

x. *Anthracanens test*

3g of sample powder was mixed with 4ml of ammonia solution, heated for 15 min. Red color indicated the presence of Anthracanens.

xi. *Glycosides Test*

25 ml of dilute sulphuric acid was added to 5 ml of the extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5 ml of Fehling solution A and B was added. A brick red precipitate of reducing sugar indicates the presence of glycosides.

xii. *Detection of Cortisone*

a. *Preparation of plant extraction*

10g of leaves and stem were extracted with 250ml of water, petroleum ether, hexan and finally ethyl acetate (respectively) using a soxhlet extractor. Each extraction was carried out for 6 h continuously at 90˚C, 70˚C, 50˚C and 30˚C with water, ethyl acetate, and hexan and petroleum ether (respectively).

b. *IR Spectra*

A small amount of each extraction was placed on the diamond attenuated total reflectance (ATR) crystal of the Agilent Cary 630 ATR-FTIR analyzer. The samples were pressed against the diamond crystal using the attached pressure clamp. FTIR spectra were acquired in less than 30 seconds. The spectra of reference storied (cortisone) samples were automatically stored in a dedicated spectral database. The spectra of extractions samples were then analyzed using an automated output pass/fail or percentage (%) similarity. 10 ug of pure cortisone was dissolved in different solvent (water, petroleum ether, hexan and ethyl acetate, respectively) and were used for comparison

c. *UV-Visible spectrophotometer*

5g of leaves and stem was extracted with 125ml of ethyl acetate and ethanol (individually). Cortisone was detected in sample by UV-Visible spectrophotometer (Agilent Cary 60 UV-Vis). Cortisone standard was dissolved in ethyl acetate or ethanol then loaded for comparison.

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e) Thin Layer Chromatography (TLC)

i. *TLC analysis*

Chromatography plates were prepared using silica Gel, 60 F254 TLC Aluminum Sheet 20x20cm Merck- Germany. The samples were spotted on the plates with graduated capillary tubes (5 μL). The standard of cortisone was dissolved in different solvents (water, petroleum ether, hexan and finally ethyl acetate) then spotted for comparison. Toluene- ethyl acetate-diethyl-amine (14:2:2) were used as mobile phase.

ii. *Detection*

Cortisone band was observed as follows: * Without treatment using UV (254-356nm). *Universal detection reagents: using both concentrated sulphoric acid and iodine vapor from crystal then comparing its rate of flow (R_f) value with standard.

f) Mineral Compounds Analysis

The dried plant was wet oxidized and the elements were determined by Atomic Absorption Spectrophotometer (Perkin-Elmer model 403, Norwalk Ct, USA). The minerals were reported in ppm. The minerals include sodium, potassium, calcium, magnesium, iron, copper and Zn, Pb, Cd and Cr. Values for, Fe, Cu and Mg were read on Atomic Absorption Spectrophotometer (180-30 Hitachi) after standardizing with respective elements (American Association of Cereal Chemists, 1984).

III. Results and Discussion

a) Phytochemicals analysis

The results illustrated in Table I; the phytochemical analysis conducted on water and ethylacetate shoot extracts of *P. lentiscus* revealed the presence of some bioactive components such as alkaloids, tannins, hydrolysable tannins, phlobatannins, phenol, volatile oil, saponins, glycosides, flavonoids, protein and Anthracanens.

Table 1 : Photochemical compound of water and ethyl acetate shoot extracts of *P. lentiscus*

Phytochemical Compounds	Test	Water and ethyl-acetate shoot extract	color develop
1-Alkaloids	Wagner's	A++ B+	Brown precipita te
2-Portein	Folin test	A+ B++	deep blue
3-Phenol	1% aqueous ferric chloride	A+ B+	deep bluish green
4-Phlobatannin	HCl Heating in 10 minutes	A++ B+	Reddish green
5-Flavonoids	pieces of magnesium chips and HCl	A+ B+	red
6- Volite Oil	0.1 % NaOH + HCl	A+ B+	layer oil
7- Hydrolysable tannins	Amonia solution	A+ B++	Formati on of an emulsion
8- Tannin	10% ferric chloride, 50ml distilled water heating 30 min.	A+ B++	Green precipita te
9- Saponins	Froth test	A+ B+	Honeyc omb froth
10-Glycosides	Fehlange	A+ B+	red
11- Anthra - canens	Amonia solution	A+ B+	red

A=water extract, B=Athylacetate extract, + Positive, ++ = Abundant, - = Negative.

The presence of some of these bioactive components like Alkaloids and Flavonoids confirms similar research conducted by Rhouma, et al..(2009), while the result obtained showed the presence of: glycosides, phenols, saponins tannins, volatile oils, hydrolysable tannins, protein, Hydrolysable tannins and Anthra-canens.

b) Detection of Cortisone

i. *IR Spectra*

The compatibility studies of cortisone standard alone and along with *P. lentiscus* shoot extractions water; ethyl-acetate; hexan and petroleum-ether) are carried out by using FT-IR. The data of study were shown in Fig. (1). The peaks were recorded in the range of 1000 to 3500 cm^{-1}.

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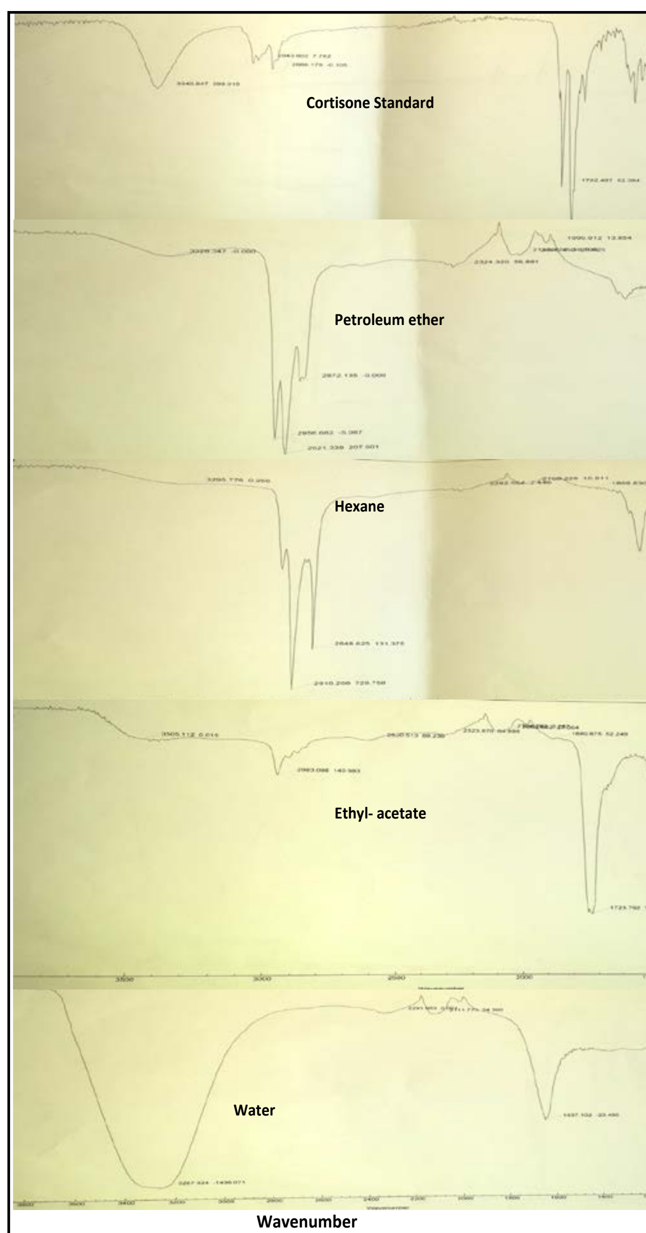

Figure 1 : FT-IR spectra of pure cortisone, Petroleum ether, Haxen, ethyl-acetate and water shoot extracts of P*. lentiscus shoot*

Cortisone isolated from the shoot of *P. lentiscus* corresponds to the infrared spectrum to him by petroleum ether, hexane and ethyl acetate as shown in Figure (1) closely with spectrum of cortisone standard (pouchert, 1981). It pointed out (Nimit et al., 2011 and Harborne, 1973) to the importance of the comparison between the compounds prepared chemically (cortisone and hydrocortisone standard) and those isolated from natural sources as the diagnosis full for several types of plant components, especially when the area fingerprint (Fingerprint region) which is located on the frequencies less than 3000 cm^{-1} and the advantage of this topic area are complex because it produces movement vibratory compound as a whole, well as match frequencies packages described in Fig. (1) with those mentioned by (Nimit et al., 2011 and Tripathi, et.al., 1981) as stated that vibration frequencies of infrared compound effectively isolated from the shoots of *P. lentiscus* intentions and dissolved by petroleum ether and hexane are: (2956.682, 2921.339 , 2872.135 cm^{-1} and2916.208, 2848.625 respectively) either dissolved by and ethyl acetate was so close (2983.098cm^{-1}) and usually show Aliphatic C-H stretch of hydroxy -cortisone and hydrocortisone - at frequency 2943.802cm^{-1} 2916.554 , as shown in Table 2.

Table 2 : Peak of cortisone in FT-IR Spectra

Frequency (cm^{-1}) Cortisone standard	Frequency (cm^{-1}) Shoot extract by petroleum ether	Frequency (cm^{-1}) Shoot extract by haxen	Frequency (cm^{-1}) Shoot extract by ethyl acetate	Description
3340.8847-2900	3328.347	3295.776	3505.112	O-H stretch
2943.802	2921.339	2916.554	2983.098	Aliphatic C-H stretch
1664-1702	1707.808	1701.653	1723.792	C=O stretch
1047.001	1088.545	1048	1043.495	C-O-C bending

The result also illustrated that water extract of *P. lentiscus* did not reveal the presence of cortisone, where infrared absorption peak at 3267.924 and unlike pure cortisone (standard).

ii. *UV-Visible Spectrophotometer*

Ethanol and ethyl acetate extract of *P. lentiscus* shoot were analyzed for cortisone detection using UV-Visible spectrophotometer. The result suggested that cortisone was detected at 284 nm, in ethanol extract and ethyl acetate extract. This result in agreement with study done by Christian, M. which referred that absorption spectra of total 7 steroids at 200 nm, 7 at 254 nm, 6 at 366 nm were obtained from P. fraternus.

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iii. *Thin Layer Chromatography (TLC)*

Standard cortisone and *P. lentiscus* shoot extracts (with petroleum ether, haxen and ethyl acetate, individually) were spotted on TLC plates then developed using 18Toluene: 2 ethyl acetate: 2 diethyl amine as a solvent. Spots were detected using iodine vapor, UV and concentrated sulphoric acid (Heftmann, et al., 1966). The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of RF value (Mendham, et. al., 2002). Result of this study, as shown in Table 3 and Fig. 2, indicated that there was match between standard cortisone and plant extracts in terms of spots number and RF values.

Table 3 : TLC results of *P. lentiscus* shoot extracts and cortisone standard

Spots number	Cortisone Standard R_F values	Shoot extract by petroleum Ether RF values	Shoot extract by hexan R_F values
Spot 1	0.93	0.93	0.93
Spot 2	0.84	0.84	0.84
Spot 3	0.72	0.72	0.72
Spot 4	0.62	0.23	0.62
Spot 5	0.51	0.12	0.23
Spot 6	0.45	0.06	0.12

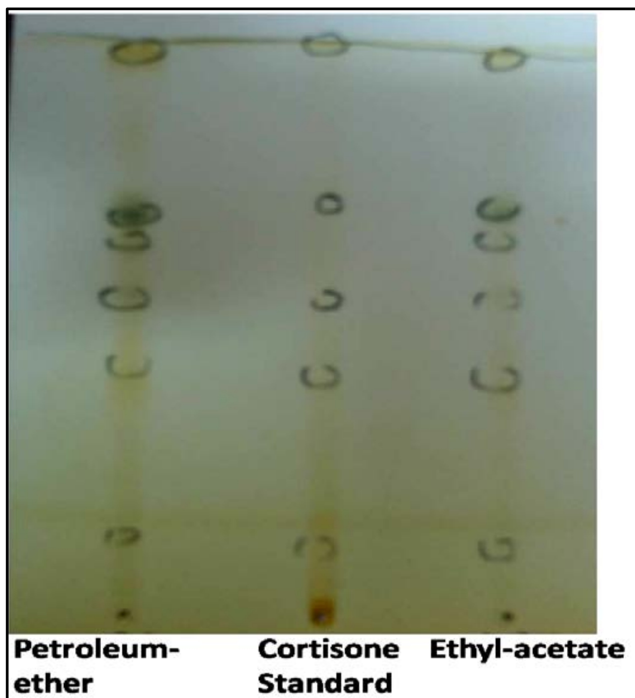

Figure 2 : TLC results of *P. lentiscus* shoot extracts and cortisone standard detected by iodine vapor

TLC profiling of *P. lentiscus* shoot extracts in different solvent system confirms the presence of diverse group of phytochemicals (steroids). Different R_F values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from *P. lentiscus* shoot extract (Das Talukdar et al., 2010).

c) *Mineral components*

Investigated elements were chosen (Na, Zn, Fe, K, Cu, Ca, Mg, Cd, Cr, and Pb) according to their role and importance in many biological mechanisms. Quantitative determinations were made of the mentioned elements in the shoots of *P. Lentiscus.* The composition in major and minor minerals of the shoots of *P. lentiscus* is detailed in Fig. 3 and 4.

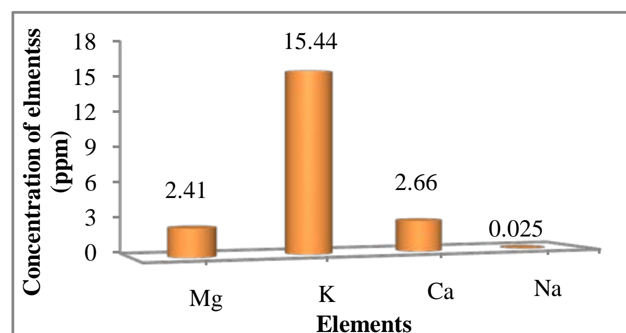

Fig. (3) *P. lentiscus* shoot majer mineral composition.

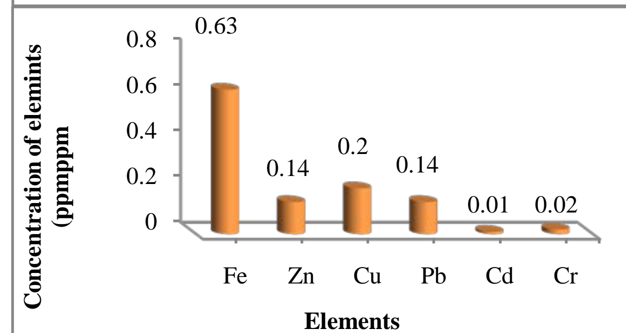

Fig. (4) *P. lentiscus* shoot minor mineral composition

The mineral composition of *P. Lentiscus* shoot revealed its nutritional value for human and/or animal (Omri et. al., 2013).

The importance of mineral composition is due to their nutritional properties and beneficial health effects, as well as their meeting of dietary guidelines required for a healthy diet (Welna et al., 2008). Results of this study revealed to that Potassium had the highest content of (15.44ppm) dry weights this result in agreement with study done by (Aouinti et al., 2014). The level of Ca in the shoots of *P.lentiscus* in this work was found to be higher (2.66 ppm) dry weight compared to Na (0.25 ppm). Calcium is the major component of bone and assists in teeth development (Brody, 1994).

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Magnesium is not only essential, but it is also a constitutive element of chlorophyll, so that its highest concentration was found in leaves (Aouinti et al., 2014). In this study, the average concentration of Magnesium in the *P.lentiscus* shoot was 2.41 ppm. **Iron** functions as hemoglobin in the transport of oxygen. Iron functions as essential component of enzymes involved in biological oxidation such as cytochromes (Malhotra, 1989). It is an important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb) and the cytochromes (Chandra, 1990). Though it is an essential element, excess intake can lead to iron toxicity and can damage lipids and proteins (Bothwell, et al., 1979 and Fraga, et al., 2002). Result of this study revealed to that shoots of *P. lentiscus* are reach in iron with a concentration of 0.63 ppm. This result is consistent with the (Aouinti et al. 2014).

Cupper and Lead has been detected in the shoot of *P. lentiscus* with concentration of 0.14ppm. Cupper is component of many redox and lignin-biosynthetic enzymes. In plant, its deficiency symptoms include Chlorosis, dead spots in leaves, stunted growth, terminal buds die, necrosis in young leaves (Soetan, et al., 2010). There are also inter-relationships of iron, copper and cobalt (in vitamin B12) in hemoglobin synthesis and red blood cell formation (Hays, et. al., 1985). Lead is best known for its toxicological properties (Macrae, et al, 1993) there are increased in depressives and schizophrenics but reduced in manic patients (Stanley, et al., 2002). Lead is an ubiquitous environmental and industrial pollutant that has been detected in every facet of environmental and biological systems.

Zinc is distributed widely in plant and animal tissues and occurs in all living cells. Zn dependent enzymes are involved in macronutrient metabolism and cell replication (Hays, et al., 1985). In humans, deficiency disease or symptoms include hypogonadism, growth failure, impaired wound healing, and parenteral nutrition (Murray *et al.,* 2000).

Cadmium and chromium have detected in the shoots of *P. Lentiscus* with low concentration (0.01 and 0.02 respectively. The possible effects of the general population of long term, low level exposure to cadmium have been of concern recently (Asagba, 2009), because cadmium readily distributed in tissues after exposure and it inhibits antioxidant enzymes (Chater et al.,2009; Asagba and Eriyamremu, 2007; Bagchi et al.,1996; Gupta et al., 1991) and this inhibition can lead to increased oxidative stress which may result in membrane damage and loss of membrane-bound enzymes like ATPases (Galazyn-Sidorczuk et al., 2009; Asagba and Obi, 2005; Asagba et al., 2004 and Figueiredo- Pereira et al., 1998). Chromium is an essential element for animals and humans (Frieden, 1984). It has been found in nucleoproteins isolated from beef liver and also in RNA preparations (Uppala et al., 2005). It could play a role in maintaining the configuration of the RNA molecule, because Cr has been shown to be particularly effective as a cross-linking agent for collagen (Eastmond et al., 2008).

IV. Conclusion

The phytochemical tests performed on the shoot extracts of *P. lentiscus* shows the presence of alkaloids, tannins, hydrolysable tannins, phlobatannins, phenol, volatile oil, saponins, glycosides, flavonoids, , protein and Anthracanens. The present study revealed the presence of cortisone in *P.lentiscus* shoot extracts which were confirmed by various techniques studies. Since cortisone contains a wide range of medicine and pharmacological properties, they can be exploited more storied in future for further studies.

V. Acknowledgements

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References Références Referencias

1. Agency for Toxic Substances and Disease Registry (ATSDR). Public Health Statement, Polycyclic Aromatic Hydrocarbons. 1990. Atlanta, GA: U.S. Department of Health and Human Services.
2. Al-Habbal, M.J., Al-Habbal, Z., and Huwez, F.U. (1984). A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer, J. Clin. Exp. Pharmacol. Physiol. 11, 541-544.
3. Ahida, S.H., Ansari, A.N. and Siddiqui (2012) Pistacia Lentiscus: a Review on Phytochemistry and Pharma cological Properties. International Journal of Phar-macy and Pharmaceutical Sciences. Vol (4):1-20.
4. American Association of Cereal Chemists (AACC). Method 08-01. The Association St.Paul, M.N. 1984.
5. Aouinti, F., Zidane1, H. Tahri1, M., Wathelet, P.j.and El Bachiri1, A. (2014).*Pistacia lentiscus* L. from Eastern Morocco. J. Mater. Environ. Sci. 5 (1) 199-206.
6. Asagba SO, Eriyamremu GE, Adaikpoh MA, Ezeoma A (2004). Levelsof lipid peroxidation, superoxide dismutase and Na+/K+- ATPase insome tissues of rats exposed to a Nigerian diet and cadmium. Biol.Trace Elem. Res. 100(1): 075-086.
7. Asagba, S.O. and Obi, F.O. (2005). A comparative evaluation of the biological effects of environmental cadmium contaminated control diet and laboratory cadmium supplemented test diet. Biometals 18: 155-161.
8. Asagba, S.O. and Eriyamremu, G.E. (2007). Oral cadmium exposure alters-haematological and liver function parameters of rats fed a Nigerian like diet.

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J. Nutr. Envt. Med. 16(1-3): 267-274.
9. Asagba, S.O. (2009). Role of diet in absorption and toxicity of oralcadmium. Afr. J. Biotechnol. 8(25): 7428-7436.
10. Balan K.V, Prince J, Han Z, Dimas K, Cladaras M, Wyche J. H. (2007). Antiproliferative activity and induction of apoptosis in human colon cancer cell treated in vitro with constituents of a product derived from Pistacia lentiscus var chia. Phytomedicine. 14(4); 263-272.
11. Bagchi, D., Bagchi, M., Hassoun, E.A. and Stohs, S.J. (1996). Cadmium-induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague-Dawley rats. Biol. Trace Elements Res. 52(2): 143-154.
12. Barra, A., Coroneo, V., Dessi, S., Cabras, P., Angioni, A. (2007). Characterization of the Volatile Constituents in the Essential Oil of Pistacia lentiscus L. From Different Origins and its Antifungal And Antioxidant Activity. J. Agric. Food Chem., 55, 7093-7098.
13. Baytop, T. (1999). Therapy with Medicinal Plants in Turkey (past and present), Nobel Tip Kitabevleri, Istanbul, edn. 2.
14. Bentley, R. Y. and Trimen, H. (1980). Medicinal plants. J. and A Churchill, London.
15. Berboucha M., Ayouni K., Atmani D., Atmani D., Benboubetra, M. (2009). Kinetic study on the inhibition of xanthine oxidase by extracts from two selected Algerian plants traditionally used for the treatment of inflammatory diseases. J Med Food. 13(4); 896-904.
16. Bonsignore, L., Cottiglia, F., Loy, G. (1998). Antimicrobial activity of Pistacia lentiscus aerial part. Fitoterapia, 69, 537–538.
17. Bothwell, T. H., Charlton, R. W., Cook, J. D., Finch, C. A., Oxford: Blackwell Scientific Publications, (1979).
18. Brody, T. (1994). Nutritional Biochemistry. San Diego, CA: Academic Press.
19. Chater S, Douki T, Favier A, Sakly M, Abdelmelek H (2009). Changes in antioxidant status and biochemical parameters after oral cadmium dministration in female rats. Acta. Biol. Hung. 60(1): 79-88.
20. Chandra, R. K. (1990). Micro-nutrients and immune functions: An overview. Annal New York Acad. Sci. 587:9-16.
21. Christian, M. Steroids – Chemical Constituents of Phyllanthus Fraternus Webster Through TLC And HPTLC. Nternational Research Journal Of Chemistry, ISSN 2321 – 2845(Online).
22. Das Talukdar, A., Dutta Choudhury, M. Chakraborty, M., Dutta, B.K. (2010). Photochemical screening and TLC profiling of plant extracts of Cyathea gigantea (Wall. Ex. Hook.) Haltt. and Cyathea brunoniana. Wall. ex. Hook. (Cl. & Bak.). Assam University Journal of Science & Technology Biological and Environmental Sciences.Vol. 5, No (I) -70-74.
23. Dedoussis GV.Z., Kaliora A.C., Psarras S., Chiou A., MylonaA., Papadapoulos N.G., Andrikopoulos N.K.(2004). An-tiatherogenic effect of Pistacia lentiscus via GSH restora-tion and down regulation of CD36 mRNA expression. Atherosclerosis 174: 293-303.
24. Dembitsky, V.M.(2005). Astonishing diversity of natural surfactants: 5. Biologically active glycosides of aromatic metabolites. Lipids. 40 (9):869-900.
25. Dhifi1, W., Jelali, N Chaabani1, E., Beji1, M., Fatnassi, S. Omri, S. and Mnif1, W. (2013). Chemical composition of Lentisk (Pistacia lentiscus L.) seed oil. African Journal of Agricultural Research. Vol. 8(16), pp. 1395-1400.
26. Dimas, K., Hatziantoniou, S., Wyche, J.H. and Pantazis, P. (2009). A mastic gum extract induces suppression of growth of human colorectal tumour xenografts in immunodeficient mice. In vivo; 23; 63-68.
27. Eastmond, D.A., MacGregor, J.T.and Slesinki, R.S. (2008). Trivalent Chromium:Assessing the genotoxic risk of the essential trace element and widely used human and animal nutritional supplement. Crit. Rev. Toxicol. 38: 173-190.
28. Fernandez A., Camacho, A., Fernandez, C. Perez P. and Altarejos, J. (2000). Composition of the essential oils from galls and aerial parts of Pistacia lentiscus L. J. Essent. oil Res., 12, 19-23.
29. Figueiredo-Pereira, M.E., Yakushin, S.and Cohen, G. (1998). Disruption of the intracellular sulhydryl homeostasis by cadmium-induced oxidative stress leads to protein thiolation and ubiquitination in neuronal cells. J. Biol. Chem. 273: 12703-12709.
30. Fraga, C.G., Ote iza, P.I., Toxicology, 180(2002)23.
31. Frieden, E. (1984). Biochemistry of the essential ultratrace elements.Plenum press, New York.
32. Galazyn-Sidorczuk, M., Brzoska, M.M., Jurczuk, M. and Moniuszk-Jaconiuk, J. (2009). Oxidative damage to proteins and DNA in rats exposed to cadmium and or ethanol. Chem. Biol. Interac. 180(1): 31-38.
33. Gerd, C., Hartmut, H. and Jörg, T. (2006). Anthracene" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim.
34. Gupta, S., Althar, M., Behari, J.R. and Srivastava, R.C.(1991). Cadmium-mediated induction of cellular defence mechanism: a novel example for the development of adaptive response against a toxicant. Health 29: 1-9.
35. Heftmann, E., Ko, S.T., Bennett, R.D. J. (1966). Chromatogr. (21)- 490.

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36. Jafri, and Ali, S. I. (1977). Flora of Libya. al Faateh University, Faculty of Science, Department of Botany, Vol.145.
37. Jafri, M. H. S. and El-Gadi, A. (1978). Flora of Libya.Vol.52. 1-12, Department of Botany, Al-Faateh Univ.,Tripoli, Libya.
38. Harborne, J.B. (1973). Phytochemical methods, Science paper black. Chapman and Hall, London.
39. Hays, V.W. and Swenson, M.J.(1985). Minerals and Bones. In: Dukes' Physiology of Domestic Animals, Tenth Edition pp. 449-466.
40. Lamiri, A. Lahloui, S. Benjilali, B. and Berrada M. (2001). Insecticidal effects of essential oils against Hessian fly, Mayetiola estructor (Say). Field Crops Res. 71, 9-15.
41. Malhotra, V. K. (1998). Biochemistry for Students. Tenth Edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.
42. Macrae, R., Robinson, R.K. and Sadler, M.J. (1993). Ncyclopaedia of Food Science, Food Technology and Nutrition, San Diego, CA: Academic Press INC vol. 7.
43. Mendham, J., Denney, R.C, Barnes, J.D. and Thomas M.J.K. (2002). Vogel's Textbook of Quantitative Chemical Analysis, Pearson Education Pvt. Ltd., Six Edition. 256-57.
44. Murray, R. K., Granner, D. K., Mayes, P. A., Rodwell, V. W. 2000. Harper's Biochemistry, 25th Edition,McGraw-Hill, Health Profession Division, USA.
45. Peksel, A. (2008). Antioxidative Properties of Decoction of Pistacia atlantica Desf. Leaves. Asian Journal of Chemistry. Vol. 20, No. 1 681-693.
46. Pouchert, c.j. (1981).the aldrich library of infrared spectra .3rdedition.aldich chemical company, inc.
47. Rhouma1, A., Ben Daoud, H., Ghanmi, S., ben Salah, H., Romdhane, M. and Demak, M. (2009). Ntimicrobial Activities of Leaf Extracts of Pistacia and Schinus Species Against some Plant Pathogenic Fungi and Bacteria. Journal of Plant Pathology. 91 (2), 339-345.
48. Soetan, O.K., Olaiya, O. C. and. Oyewole, O.E. (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science Vol. 4(5) pp. 200-222.
49. Stanley, P.C., Wakwe, V.C. (2002). Niger Postgrad Med J; 9 199.
50. Tripathi, R.D. Dixit, S.N.(1981). 3rd International sympo., Plant Pathology., IARI.cited from Ali, M. 1996. Chemical and medicinal evaluation of Lawsonia inermis (henna). Hamdadrd Medicus., 39: 43-48.
51. Uppala, R.T., Roy, S.K., Tousson, A., Barnes, S., Uppala, G.R. and Eastwood, Da .(2005). Induction of cell proliferation micronuclei and hyper-diploidy. /diploidy in the mammary cells of DDT and DMBA-treated pubertal rats. Environmental and Molecular Mutagenesis 46: 43-52.
52. Welna, M., Klimpel, M. and Zyrnicki, W. (2008). Investigation of major and trace elements and their distributions between lipid and non-lipid fractions in Brazil nuts by inductively coupled plasma atomic optical spectrometry. Food Chem. 111:1012-1015.

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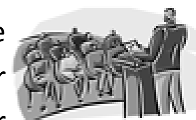

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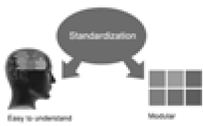

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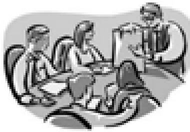

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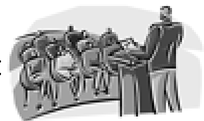

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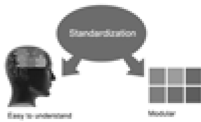

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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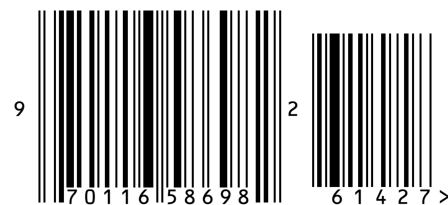

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