The protective effect of hydroalcoholic extracts of olive and *morus alba* leaves on liver enzymes of male rats treated with streptozotosin

O.S.I. Al-Janabi

Pharmacology Department, Faculty of Medicine, Anbar University, Iraq
E-mail:- dr.osin1981@gmail.com – omar_81msc@yahoo.com

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Abstract

The objective of this study is to investigate the effects of *Olive* and *Morus alba* leaves extracts (ethanol hydro-alcohol 60%) on some liver enzyme {Serum aspartate aminotransferase (AST), Serum alanine aminotransferase (ALT)} in experimentally STZ induced diabetes in male rats. Fifty adult male albino rats weighting (150 -200 g) were used and divided equally into 5 experimental groups; the first group was served as a control group. The remaining groups were injected (i.p) by streptozotocin (STZ) at 45 mg/kg B.W to induce diabetes. The 2nd diabetic group was received as control diabetic group. The third diabetic group was treated with Cidophage (500 mg/kg, orally). While, the fourth and fifth diabetic groups were treated with *Olive* and *Morus alba* leaves extracts (500 mg/kg B.W, 600 mg/kg B.W orally) respectively. All treatment groups were given daily for successive 30 days. The levels of (AST and ALT) were measured at 1st day after end treatments. The rats were sacrificed at the end of 30 days of treatment. The obtained results demonstrated the use of *Olive* and *Morus* alba leaves extracts improve of (AST and ALT) levels of diabetic rats. Hydro-alcoholic extract of *Olive* and *Morus* alba leaves could improve the liver enzymes of male rats, an induced diabetes in male rats.

Keywords: Anti Oxidant Medicinal Plants, Liver Enzymes (AST And ALT), *Olive* And *Morus Alba* Leave Extracts.
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التاثير الوقائي لمستخلص المائي الكحولي لاوراق الزيتون والتوت على انزيمات الكبد للجرذان المعاملة ب الستربتوزوتوسين

عمر سالم ابراهيم الجنابي

فرع الادوية، كلية الطب، جامعة الانبار، الانبار، العراق

الخلاصة

تهدف هذه الدراسة الى معرفة تأثير كل من مستخلص أوراق الزيتون والتوت (ethanolic hydro-alcohol 60 %) على بعض انزيمات الكبد (AST and ALT) في ذكور الجرذان المصابة تجريبيا بداء السكري باستعمال مادة الستربتوزوتيسين (STZ). أستخدم في هذة التجربة عدد خمسين من ذكور الجرذان البيضاء وزنها يتراوح مابين (١٥٠ -٢٠٠غم) وتم تقسيمها إلى خمس مجاميع (١٠جرذان لكل مجموعة). تركت المجموعة الأولى كمجموعة سيطرة. تم حقن المجاميع المتبقية (بالغشاء البريتوني) بمادة الستربتوزوتيسين (STZ) بجرعة ٤٥ ملغم / كغم من وزن الجسم لإحداث مرض السكري. المجموعة الثانية اعتبرت مجموعة سيطرة ومصابة بالسكري. عولجت مجموعة السكري الثالثة بدواء السيدوفاج (٥٠٠ ملغم / كغم، فمويا). بينما تم علاج المجموعتين الرابعة (مستخلص أوراق الزيتون بجرعة ٥٠٠ ملغم / كغم من وزن الجسم) والخامسة (مستخلص ورق التوت بجرعة ٦٠٠ ملغم / كغم من وزن الجسم). استمرت فترت العلاج يوميا لمدة ٣٠ يوما. تم جمع عينات الدم بعد انتهاء فترة العلاج ومن ثم تم قياس (AST and ALT) أظهرت النتائج أن استخدام مستخلص ورق الزيتون ومستخلص ورق التوت آدا إلى تحسين واضح في بعض انزيمات الكبد (ALT and AST).

Introduction

Plants are used for prevention or treatment of many diseases satates. Herbal organs used for treatment purposes, like root, seed, flowers and leave or bark is named (herbal drug or herbal medicin). The term of "phytotherapy" is used at the 1st in 1939 by the French Physician, Mr Henri Leclerc (1870-1953) in the title of journal 'La Presse Medical (1).

Diabetes mellitus is defined as metabolic disorder originally characterized by loss of glucose homeostasis by disturbances of carbohydrate, protein and fat metabolism resulting from defects in insulin action, or insulin secretion or both (2). Without enough insulin, the cells of the body cannot absorbtion adequate glucose from the blood, hence the level of blood glucose increase, which is termed hyperglycemia. If the level of glucose in the blood remained high over a long time, this can be resulted in long-term damage for organs, such as the kidneys, eyes, liver, nerves, heart and blood vessels, the complications in some of this organs may be lead to death (3).

Ayurveda and other traditional medicin system for the treatment of diabetes describe a number of plants that used as herbal medications. subsequently, they play an important role in alternative medicine because of less side effects and low cost. The active principles present in the medicinal plants have been reported to possess pancreatic beta cells re-generating, insulin releasing and fighting the problem of insulin resistance. The hyperglycemia is involved in the etiology of development of diabetic complications (4).

Olea europaea L., family Oleaceae, and in particular, its leaves have been used in the treatment of diabetes, wounds, gout, fever, atherosclerosis and hypertension since ancient times (5). It is used to promote immune system, in heart disease and as an antimicrobial agent (6). The folk medicine used in treatment of hypertonia, arteriosclerosis, rheumatism, cardioprotection, gout, diabetes mellitus, and fever (7). hypotensive (8) antiarrhythmic (9), anti-atherosclerotic (10), and vasodilator effects (11). Antimicrobial (12), antiviral (13), anti-tumor effect (14) and anti-inflammatory activity (15).

Morus alba belongs to family sterculiaceae, the plant is usually known as Vagadu in india, and distributed throughout the plains and Africa, Australia and tropical Asia. Ayurveda, all parts of plant are medicinally important (16). It is used as antiphlogistic, diuretic, expectorant and antidiabetic effects (17). It used to treat vitiated conditions rheumatism, asthma, and anti-inflammatory, it contains steroids, tannins, mucilage alkaloid (18) and is rich in polyphenolic compounds especially flavonoids and among the flavonoids quercetin 3-(6-malonylglucoside) is most significant for antioxidant potential of the mulberry plant (19).

Antihyperglycemic activity of *Morus alba* leaves due to the presence the trigonelline and high fibers content in leaves (20). The present study aimed to investigate the effects of both extracts on some liver enzyme in STZ diabetic rats.

Material and methods

Olive and *Morus* alba *leave* extracts

Olive (Olea europaea L.) and *Morus alba* Leaves were collected from Arboretum of Agriculture College, Mansoura University Egypt, cleaned, washed with tap water, dried and stored in dry atmosphere. The alcoholic extract of *Olive* and *Morus alba* Leaves was suspended in distilled water according to the method of (21) by using the soxhlet apparatus, and orally administrated of *Olive* leave to the animals at a dose of 500 mg/ kg B.W (22), while *Morus alba* leave 600 mg/kg B.W (23), by stomach tube daily for 30 days.

Cidophage (Metformin hydrochloride 500 mg) CID Company (CID, Giza, Egypt) was administrated orally by stomach tube in a dose 500 mg/kg B.W (24).

Introduction of diabeties

Streptozotocin (STZ): was purchased from Sigma Company (USA), and used to induce diabetes at dose 45mg/kg B.W STZ was dissolved in 0.9% sodium citrate and injected I/P to rats according to (22). Rats was gave glucose with water for one day after injected with STZ, after three days from injection, diabetes was examind by using Accua chik system to detirmin diabetes.

Experimental Animals

Fifty (50) adult healthy albino male rats was aged range between 8-10 weeks, and their weight range between 150-200 grams, was used in this study. Animals were left for one week to acclimatize the place. Animals were kept in a cage in a controlled environment, maintained under a 20-25°C and light period of 12 hours daily and 50-70 % humidity. Rats were provided with standard diet and water ad-libitum. The animals were housed in plastic cages. Care was taken to avoid any unnecessary stress. The cages were cleaned twice a week. After one week period of acclimatization in cages condition, rats were divided into 5 groups (each of 10 rats).

Experimental design

After one week period of acclimatization in cages condition, rats were divided into 5 groups (each of 10 rats); Group I: (control clinically healthy) treated with 0.2 ml distilled water orally. Group II: diabetic non -treated (45 mg/ kg B.W STZ) intra peritonea (25). Group III: diabetic treated with 500 mg/kg B.W Cidophage orally/day by stomach tube for 30 days (24). Group IV: diabetic treated with 500 mg /kg B.W *Olive* leaves extract orally daily for 30 days (22). Group V: diabetic treated with 600 mg /kg

B.W *Morus alba* leaves extract orally daily for 30 days (23).

Blood sample

Blood samples were collected from (10 rats from each group) after 30 days from teratment the sample is collected than centrifuged at 3000 r. p. m for 20 minutes. The obtained serum samples were stored at -20^{0}C until assayed.

Liver enzyme analysis: determination AST and ALT

Determination of AST and ALT by enzymatic colorimetric method by using linear kits provided by the linear chemicals company, Spain according to (26,27).

Statistical Analysis

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 18, USA). Means and standard error for each variable were estimated. Differences between means of different groups were carried out using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column show a significance (P<0.05) (28).

Results

Effect of *Olive* and *Morus alba* leaves extracts on serum AST levels

It was observed clearly from Table (1) that serum AST level was significantly increased (P<0.05) in the diabetic group (181 ± 5.44) in comparison with the control group (154 ± 7.47) after treatment. Meanwhile serum AST level was significantly decreased (P<0.05) in all diabetic treated groups and were 155.4 ± 1.43 (Cidophage) 156.2 ± 1.52 (*Olive* leave alcoholic extract) and 154.4 ± 1.28 (*Morus alba* leave alcoholic extract) in comparison with control diabetic group.

Effect of *Olive* and *Morus alba* leaves extracts on serum ALT level

It was observed clearly from Table (1) that serum ALT level was significantly increased (P<0.05) in the diabetic group (146.6 ± 4.82) in comparison with the control group (75.4 ± 4.06) after treatment. Meanwhile serum ALT level was significantly decreased (P<0.05) in all diabetic treated groups and were 80.4 ± 0.81 (Cidophage), 82.6 ± 3.55 (*Olive* leave alcoholic extract) and 71.6 ± 1.50 (*Morus alba* leave alcoholic extract) in comparison with control diabetic group.

Discussion

In the recent times, increasing the attention on intake of antioxidants because has potential compounds for preventing diseases caused by oxidative such as diabetes. The results of this study revealed that, STZ injected rats showed a significant increase in levels of serum (AST and ALT) these findings are in agreement with (29) that observed the liver function tests performed for trichloroacetic acid TCA-intoxicated rats showed an increase in the activity of serum ALT and AST compared to their corresponding control values, The rising of both enzymes is presumed to be because of leakage from damaged or necrotic cells. These results were in accordance with (30) that explain the transaminase activities were reduced after 5-fluorouracil (5-FU) treatment. After treatment with Aqueous *Olive* leaves extract (AOLE) significant reduction in both ALT and AST was observed and a highly significant reduction was after treatment with combination of 5-FU and AOLE. The decline in liver enzyme activities showed some proof about liver recovery and hepatoprotective properties of AOLE. The hepatoprotective action of aqueous extract of *Olive* leaves may be due to the antioxidant properties (31). The phenolic structure of *Olive* leave extract helps to decrease the free radicals effect (32).

Table 1: Effect of *Olive* and *Morus alba* leaves extracts on serum AST and ALT level of diabetic and non-diabetic rats

Groups	Parameters	
	AST U/L	ALT U/L
G1 (Control given 0.2ml normal saline)	154 ± 7.47	75.4 ± 4.06
G2 (Diabetic by 45 mg/kg B.W STZ)	181 ± 5.44	146.6 ± 4.82
G3 (Diabetic treated with Cidophage at 500 mg/kg B.W)	155.4 ± 1.43	80.4 ± 0.81
G4 (Diabetic treated with alcoholic extract of *Olive* leaves at 500 mg/kg B.W)	156.2 ± 1.52	82.6 ± 3.55
G5 (Diabetic treated with alcoholic extract of *Morus alba* leaves at 600 mg/kg B.W)	154.4 ± 1.28	71.6 ± 1.50

Means within the same column bearing different superscript at P<0.05, Mean ± SE, N=10.

The free and total polyphenolic compounds extracted from *Olive* leaves are safe on serum (AST and ALT) (33) *Olive* leave is well-know for its antioxidant properties, hypoglycemic, hypotensive, cardiovascular, radio and hepato productive activity (34).

Morus alba is rich in polyphenolic compounds especially flavonoids and among the flavonoids quercetin 3-(6-malonylglucoside) is the most significant for antioxidant potential of the mulberry plant (19).

Morus alba contains coumarine, flavonoids, and stilbene, which possess hepatoprotective effects (35). The aqueous extract of *Morus alba* leaves protects the liver, The hydro alcoholic crude extract of *Morus alba* has hepatoprotective action expermintally (36). For this purpose the hydro alcoholic extract of *Morus alba* was studied against carbon tetrachloride (CCl4) induced the hepatotoxicity in animals, which showed that the extract has the greatest power to capture the free radicals and potent hepatoprotective (37).

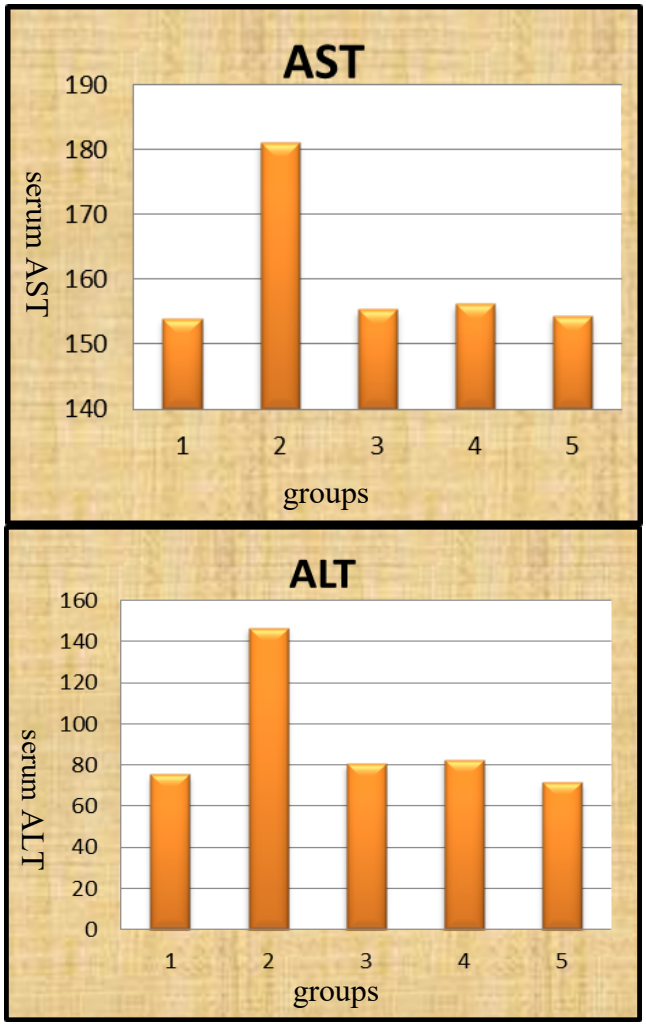

Figure 1: show serum AST and ALT level in diabetes and non diabetic rats. (Mean ± SE) (N=10).

Some studies explain that the liver protective effect of *Morus alba* and *Calendula officinalis* extracts against (CCl4) induced toxicity in isolated hepatocytes from laberatory rats, *Morus alba* and *Calendula. officinalis* extracts prominently reduced levels of the alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and maintained the integrity of isolated hepatocytes. The study confirmed this plants has significant hepatoprotective effects against hepatotoxicity that induced by CCl4 (38).

Conclusion

The study concluded that the hydro-alcoholic extract of *Olive* and *Morus alba* leave could improve the liver enzymes diabetic male rats, and alleviate the possible side effects of diabetes mellitus.

References

1. Capasso F, Gaginella TS, Grandolini G, Izzo AA. "Phytotherapy". Berlin: Springer, 2003;pp:8.
2. Barcelo A, Rajpathak S. Incidence and prevalence of diabetes mellitus in the Americas. Amer J Pub Heal. 2001;10:300-308.
3. Pari L, Saravanan R. Antidiabetic effect of diasulin, a herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. Diabetes. Obes Meta. 2004;6:286–292.
4. Welihinda J, Arvidson G E, Hellman B, Karlsson E. A review on antidiabetic plants. Ada Biol Met L Ger. 1982;41:1229.
5. Janicke C, Grunwald J, Brendler T. Hand buch Phytotherapie. Wissenschaftliche Verlagsgesellschaft Stuttgart 2003.
6. PDR For Herbal Medicine. Physician's Desk References for Herbal Medicines, Fleming T, eds. Medical Economics Company, Montvale, New Jersey, 2000; pp:556-557.
7. Jemai H, Elfeki A, Sayadi S. Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from *Olive* leaves in alloxan-diabetic rats. J Agricul Food Chem. 2009;57:8798-8804.
8. Schefler A, Rauwald HW, Kamp AB, Mann U, Mohr FW, Dhein S. *Oleaeuropaea* leave extract exerts Ltype Ca2+ channel antagonistic effects. J Ethnopharmacol. 2008;120:233-240.
9. Somova LI, Shode FO, Mipando M. Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. Phytomed. 2004;11:121-129.
10. Wang L, Geng C, Jiang L, Gong G, Liu D, Yoshimura H. The anti-atherosclerotic effect of *Olive* leave extract is related to suppressed inflammatory response in rabbits with experimental atherosclerosis. Euro J Nutrit. 2008;47:235-243.
11. Zarzuelo A, Duarte J, Jimenez J, Gonzales M, Utrilla MP. Vasodilator effect of *Olive* leave. Planta Medical. 1991;57:417-419.
12. Pereira AP, Ferreira IC, Marcelino F, Valentao P, Rade PB, Seabra R. Phenolic compounds and antimicrobial activity of *Olive* (Oleaeuropaea L. Cv. Cobrancosa) leaves. Molecules. 2007;12:1153-1162.
13. Micol V, Caturla N, Perezfons L, Esterpa A, Mas V, Perez L. The Olive leaf extract exhibits antiviral activity against viral haemorrhagic septicemia rhabdovirus (VHSV). Antiviral Res. 2005;66:129- 136.
14. Abaza L, Talorete TPN, Yamada P, Kurita Y, Zarrouk M, Isoda H. Induction of growth inhibition and differentiation of human leukemia HL-60 cells by Tunisian gerboui *Olive leave* extract. Bioch Sci Biol Technol Biochem. 2007;71:1306-1312.
15. Pieroni A, Heimler D, Pieters L, Van Poel B, Vlietinck AJ. In vitro anti complementary activity of flavonoids from *Olive* (*Oleaeuropaea* L.) leaves. Pharmazie. 1996;51:765-768.
16. Gogate VM, Ayurvedic pharmacology and theraputic uses of medicinal plants (Dravyaganvigyan)1st Ed. Mubmbi: BhartiyaVidya. Bhavan. 2000.

17. Hikino H, Mizuno T, Oshima Y, Konno C. Isolation and hypoglycemic activity of *Moran alba*, a glycoprotein of *Morus alba* root bark. Planta Medical. 1985;51:159-60.
18. Basu NK, Lamsal PP. Chemical investigation of *Sphaeranthusindicus Linn*. J Amer Pharma Associa.1946;35:274-275.
19. Butt MS, Nazir A, Sultan MT, Schroen K. *Morus alba* L. nature's functional tonic trends. Food Sci Technol. 2008;19:505–512.
20. Watanabe P. Volatile components of mulberry leaves. J Sericul Sci Japan, 1958;20:448–452.
21. Harborne JB, Phytochemical methods. A guide to modern techniques of plat analysis. Chaman and Hall, 2nd ed., New York, 1984;PP: 288.
22. Eidi A, Eidi M, Darzi R. Antidiabetic effects of oleaeuropaea L. in normal and diabetic rats. Phytoth Res. 2009;23:347-350.
23. Jamshid M, Prakash R. The histopathologic effects of *Morus alba* leave extract on the pancreas of diabetic rats. Turk J Biolo. 2012;36:211-216.
24. Pagano G, Tagliaferro V, Carta Q, Caselle MT, Bozzo C, Vitelli F. Metformin reduces insulinrequirement in Type 1 (insulin-dependent) diabetes Diabetolo. 1983;24:(5)351–354.
25. El-Seifi S, Abdel- Moneim A, Badir N. The effect of Ambrosia maritima and Cleome droserfolia on serum insulin and glucose concentrations in diabetic rats. J Egyp German Soci Zool. 1993;12(A):305-328.
26. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 1957; 28: 56–63.
27. Young DS. Effects of Drugs on clinical laboratory tests. 4th edition. AAcc press. 1995.
28. Snedecor GW, Cochran WG. Statistical Methods. 7th Ed. The Iowa State University, Ames, Iowa, USA. 1981.
29. Kew MC. Serum aminotransferase concentration as evidence of hepatocellular damage. Lancet. 2000;35:(5)591-592.
30. Leung, TW, Tang AM, Zee B, Yu SC, Lai PB, Lau WY. Factors predicting response and survival in 149 patients with unresectable hepatocellular carcinoma treated by combination cisplatin, interferon-alpha, doxorubicin and 5-fluorouracil chemotherapy. Cancer. 2002;94:421-427.
31. Peirce A. Practical guide to natural medicines. New York. William Morrow and Co, 1999;pp:469.
32. Briante R, Patumi L, Tereniziani S, Bismuto E, Febbraio F. *Olea europaea* L. leave extract and derivatives: antioxidant properties. Jo Agrical Food Chem. 2002;50(17):4934.
33. Farag RS, El-Baroty GS, Abasuny AM. Safty evaluation of *Olive* phenolic compounds as natural antioxidants. Interna J Food Sci Nutrt. 2003;64(3):159.
34. Poudyal H. Campbell F. Brown *Olive* leave extract attenuates cardiac metabolic changes in high carbohydrate, high fat-fed rats. J Nutri. 2010;140:946-953.
35. Oh H Ko EK, Jun JY, Oh MH, Park SU, Kang KH. Hepatoprotective and free radical scavenging activities of prenylflavonoids coumarin and stilbene from *Morus alba*. Planta Med. 2002;68:932–934.
36. Zeni ALB, Molin MD. Hypotriglyceridemic effect of *Morus alba* L., Moraceae, leaves in hyperlipidemic rats. *Braz* J Pharmaco. 2010;20:130–133.
37. Kalantari H, Aghel N. Bayati M. Hepatoprotective effect of *Morus alba* L. in carbon tetrachloride- induced hepatotoxicity in mice. J Saudi Pharmaceut. 2009;17:90–94.
38. Hussein MS, El-Tawil OS, Yassin NEH, Abdou KA. The protective effect of *Morus alba* and *Calendula officinalis* plant extracts on carbon tetrachloride- induced hepatotoxicity in isolated rat hepatocytes. J Amer Sciocial. 2010;6:762–773.