Some Studies on leaves Extracts of Olive and Morus alba in experimentally STZ induced diabetes in rats.

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Abstract:

The aim of this study is to investigate the effects of Olive and morus alba leaves extracts(ethanol hydro-alcohol 60%) on anti-oxidant parameters {superoxide dismutase (SOD), glutathione peroxidase (GSH), Catalase (CAT) and Malondialdehyde (MDA)} and on lipid profile {Cholesterol (Cho), Triglyceride (TAG), HDL, LDL and VLDL} in experimentally STZ induced diabetic in rats. Fifty adult male albino rats (weighting 150 -200 g) were used and divided into 5 experimental groups (10 rats in each group). The first group was served as control group. The remaining groups were injected (i.p) by streptozotocin (STZ) at 45 mg/kg b.wt to induce diabetes. The 2nd diabetic group was left 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 leaves and Morus alba leaves extracts (500 mg/kg b.wt, 600 mg/kg b.w respectively orally), respectively. All treatment was given daily for successive 30 days. The levels of some anti-oxidant parameters (SOD, GSH, CAT and MDA) and lipid profile (Cholesterol, TAG, HDL, LDL and VLDL) were measured at 1st day, 1st and 2ndweeks after treatment. The rats were sacrificed at the end of 30 days post treatment. The obtained results demonstrated that, the use of Olive leaves and Morus alba leaves extracts improved the levels of the tested parameters in diabetic rats. Hydro-alcoholic leaves extracts of Olive and Morus alba could improve the anti-oxidant and lipid profile parameters of male rats, and minimized the possible side effects of diabetes mellitus.

Introduction

Phytotherapy is simply defined as the utilization of plants in treatment or prevention of diseases. Any herbal organ used for treatment purposes, such as leave, seed, flower, root or bark is named herbal drug or medicinal herb. Medicinal plants supply a useful source of oral hypoglycemic compounds for the development

of new pharmaceutical leads as well as dietary supplements to existing therapies **(Bailey, and Day. 1989)**. Some of the plants that are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the World Health Organization expert committee on diabetes recommends that this area warrants further attention **(WHO, 1980)**.

Diabetes mellitus (DM) is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that is caused by an absolute or relative lack of insulin and or reduced insulin activity **(Kamtchouing, *et al.*, 2006)**. It is characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, nerves, and blood vessels. Furthermore, with diabetes, several features appear including an increase in lipid peroxidation **(Gumieniczek, 2005)**, alteration of the glutathione reeducates state, a decrease in the content of individual natural antioxidants, and a reduction in the antioxidant enzyme activities.

Nowadays, herbal drugs are gaining popularity in the treatment of diabetes and its complications. As a new strategy for alleviating the oxidative damage in diabetes, a growing interest has been noticed in the usage of natural antioxidants. It has been suggested that many of the negative effects of oxidative stress are diminished upon supplementation with certain dietary antioxidants such as vitamins and other non-nutrient antioxidants such as flavonoids and polyphenols **(Al-Azzawie, and Alhamdani, 2006)**.

Among natural antioxidants, the Olive tree has been usually accepted as one of the species with the highest antioxidant activity via its oil, fruits, and leaves **(Bouaziz, *et al*., 2004)**. It is well known that the activity of the Olive tree byproduct extracts in medicine and food industry is due to the presence of some important antioxidant and phenolic components to prevent oxidative degradations **(Allouche, *et al*.,2004)**. The Olive tree has long been recognized as having antioxidant molecules, such as oleuropein, hydroxytyrosol, oleuropeinaglycone, and tyrosol**(Jemai,*et al.*, 2008)**.

Mulberry (Morus spp. L., Moraceae) has been domesticated over thousands of years and adapted to the wide area of tropical, subtropical, and temperate zones of Asia, Europe, North and South America, and Africa. Mours alba is rich in

polyphenolic compound especially the flavonoids and among the flavonoids quercetin 3-(6-malonylglucoside) is most significant for antioxidant potential of mulberry plant **(Butt, *et al.*, 2008)**. The leaves of mulberry contains higher amount of quercetin which is responsible for decrease of oxidation process in vivo and in vitro **(Iqbal, *et al.*, 2012)**.

Anthocyanin components from Morus alba fruits were isolated and identified by **Chen, *et al.*, (2006)** to check their antioxidant properties and reported that cyanidin 3-glucoside and cyanidin 3-rutinoside are of valuable importance as antioxidants. Mulberroside A is a major stilbene glycoside of Morus alba and It showed inhibitory effects against FeSO4/H2O2-induced lipid per oxidation in microsomes of rat and also found that Mulberroside A have scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical **(Chung, *et al.*, 2003)**. **Rossetto, *et al.*, (2007)** stated that the anthocyanin is present in mulberry extract and it demonstrated antioxidant activity by scavenging the peroxyl radicals in trapping reaction.

This study was aimed to investigate the possible effects of Olive and Morus alba leaves extracts(ethanol hydro-alcohol 60%) on some anti-oxidant parameters {superoxide dismutase (SOD), glutathione peroxidase (GSH), Catalase (CAT) and Malondialdehyde (MDA)} and on lipid profile {Cholesterol (Cho), TAG, HDL, LDL and VLDL} in experimentally STZ induced diabetes in rats.

Material and Methods

1- Materials

Streptozotocin (STZ) was purchased from Sigma Company (USA), Cidophage was obtained from CID Company (Egypt), NaCl 0.9%, sodium citrate, citric acid, ethyl alcohol. 95% were purchased from El- Gomhoria Company. The tests reagent Kits for SOD, GSH, CAT, MDA, purchased from bio diagnostic Company, Germany, while Kits for cholesterol, TAG and HDL were purchased from Linear spain (Barcelona) Company,

Olive leave and Morus alba leave extracts :-

Olive leaves and Morus alba Leaves were collected, cleaned, washed with tap water, dried and stored in dry atmosphere until used. The alcoholic extracts of

Olive and Morus alba Leaves were suspended in distilled water according to the method of **Harborne, (1984**) by the use of Soxhlet apparatus, and were orally administrated to the animals at a dose of 500 mg Olive leave / kg b.wt (**Eidi, *et al*., 2009**), while Morus alba leaves at 600 mg/kg b.wt (**Jamshid, and Prakash, 2012**), by stomach tube daily for30 days.

Cidophage: (Metformin hydrochloride 500 mg) CID Company (CID, Giza, Egypt) and it was administrated orally by stomach tube in a dose 500 mg/kg b.W **(Pagano, *et al*., 1983)**.

Induction of diabetes:

Induction of diabetes was done by using streptozotocin (STZ) at 45mg/kg b.wt in rats according to **El- Seifi, *et. al*., (1993**).

Experimental Animals:

A total of fifty (50) adult healthy males rats(8-10 weeks) with weight ranged between 150-200 grams, were used in this study. Animals purchased from the animal house in Mansoura city, and housed in Department of Pharmacology, Faculty of Veterinary medicine, Mansoura University. Animals were left for one week to acclimatize the place. Animals were kept in cage in a controlled environment, maintained under a 20-25°C and light period of 12 hours daily and 50-70 % humidity. Rats provided with standard diet and water ad-libitum. 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) as follows :

GroupI: (control clinically healthy) treated with 0.2 ml distilled water orally.

GroupII: diabetic non-treated.

GroupIII: diabetic treated with Cidophage (500 mg /kg b.wt orally) orally for 30 successive days **(Pagano, *et al*., 1983)**.

GroupIV: diabetic treated with Olive leaves extract (500 mg /kg b.wt) orally for 30 successive days **(Eidi, *et al*., 2009).**

Group V: diabetic treated with Mours alba leaves extract (600 mg /kg b.wt)orally daily for 30 successive days. **(Jamshid, and Prakash, 2012)**.

Sampling:-

Blood sample:

Blood samples (from 5 rats of each group) were collected after 1 day, 1st week and 2nd week from the last dose into clean centrifuge tubes. The blood samples were centrifuged at 3000 r. p. m for 20 minutes for serum collection. The obtained serum samples were stored at -20^{0}C until assayed.

2- Methods

I- Liver antioxidant Analysis:

a- Determination of superoxide dismutase (SOD):

Determination of SOD by enzymatic colorimetric method by using kits ready made diagnostic provided by Bio-diagnostic Company, Egypt, according to **Nishikimi*et al.*, (1972)**.

b-Determination of glutathione reduced (GSH):

Glutathione reduced was determined by enzymatic colorimetric method by using ready-made diagnostic kits provided by Bio-diagnostic, Egypt according to **Goldberg, and Spooner, (1983)**.

c. Determination of serum catalase activity:

Catalase was determined by enzymatic colorimetric method by using ready-made diagnostic kits provided by Bio-diagnostic, Egypt according to **Aebi, (1984)**.

d. Determination of serum MDA:

The thiobarbituric acid method of **Satoh, (1978**) was used to measure the malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA) to give a pink color that is read at 534 nm.

II- Lipid profile analysis:-

a. Determination of serum total cholesterol:

Total concentration of cholesterol was measured by enzymatic method according to **Allain*et al*., (1974) and Richmond, (1992)**.

b. Determination of serum triglycerides:

Total serum TAG concentration was measured by enzymatic according to method of **Fossati, (1982).**

c. Serum HDL-Cholesterol Concentration:

HDL-cholesterol concentration is measured in serum by enzymatic colorimetric method (**Burstein, *et. al*., 1980).**

D. Determination of serum very low density lipoprotein (VLDL):

Very low density lipoprotein was determined according to the conventional Friedewald equation **(Friedwald, *et al*., 1972**).

E. Serum LDL-Cholesterol Concentration:

LDL cholesterol concentration in serum is calculated by using Friedewald equation **(Friedewald, *et al.*, 1972)**.

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 done. Differences between means of different groups were done using one way ANOVA with Duncan multiple comparison tests. Dissimilar superscript letters in the same column demonstrate a significance (P<0.05) **(Snedecor and Cochran, 1981).**

RESULTS

1. Effect of Olive and Morus alba leaves extracts on serum superoxide dismutase activity:-

It was observed clearly from Table (1) that serum SOD (U/L) activity was significantly decreased (P<0.05) in diabetic group (259.4 ± 0.509) in comparison with the control group (355.4± 0.509) after treatment. Meanwhile serum SOD activity was significantly increased (P<0.05) in all diabetic treated groups and were 312 ± 0.316, 316 ± 0.316, 302.4 ± 0.509 (Cidophage) 308.4 ± 0.748, 311.6 ± 1.288, 312.6 ± 0.244 (Olive leave alcoholic extract) and 312 ± 0.447, 315 ± 0.707, 314.6 ± 0.509 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

2. Effect of Olive and Morus alba leaves extracts on serum glutathione peroxidase GSH activity:-

It was observed clearly from Table (2) that serum GSH (U/L) activity was significantly decreased (P<0.05) in diabetic group (20.36 ± 1.393) in comparison with the control group (28.66 ± 0.833) after treatment. Meanwhile serum GSH activity was significantly increased (P<0.05) in all diabetic treated groups and were 26.9 ± 0.698, 25.80 ± 0.492, 26.42 ± 1.60 (Cidophage), 24.42 ± 0.58, 28.860 ± 0.969, 26.332 ±0.440 (Olive leave alcoholic extract) and 23.564 ± 1.226, 26.9 ± 2.770, 27.5 ± 2.261 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

3. The effect of Olive leave and Morus alba leaves extracts on serum CAT activity:-

It was observed clearly from Table (3) that serum CAT (U/L) activity was significantly decreased (P<0.05) in diabetic group (249 ± 12.81) in comparison with the control group (354.2 ± 10.70) after treatment. Meanwhile serum CAT activity was significantly increased (P<0.05) in all diabetic treated groups and were 322 ± 7.72, 342.2 ± 14.94, 338 ± 8.59 (Cidophage), 328.2 ± 15.56, 335 ± 3.89, 344.6 ± 1.60 (Olive leave alcoholic extract) and 321 ± 1.00, 328 ± 10.68, 388 ± 17.72 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

4. The effect of Olive leave and Morus alba leaves extracts on serum MDA level:-

It was observed clearly from Table (4) that serum MDA ng/ml level was significantly increased (P<0.05) in diabetic group (16.06 ± 1.62) in comparison with the control group (9.66 ± 0.32) after treatment. Meanwhile serum MDA level was significantly decreased (P<0.05) in all diabetic treated groups and were 12.3 ± 0.094, 10.36 ± 0.102, 9.66 ± 0.112 (Cidophage), 15.46 ± 0.89, 13.16 ± 0.32, 10.6 ± 0.37 (Olive leave alcoholic extract) and 14.8 ± 0.301, 12.22 ± 0.320, 11.32 ± 0.152 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

5. The effect of Olive leaves and Morus alba leaves extracts on serum Cholesterol level:-

It was observed clearly from Table (5) that serum Cholesterol mg/dl level was significantly increased (P<0.05) in diabetic group (128.6 ± 2.767) in comparison with the control group (98.4 ± 3.558) after treatment. Meanwhile serum Cholesterol level was significantly decreased (P<0.05) in all diabetic treated groups and were 111.20 ± 2.709, 106.8 ± 3.813, 103 ± 2.024 (Cidophage), 108.2 ± 3.826, 104.6 ± 1.691, 105 ± 2.481 (Olive leave alcoholic extract) and 98.0 ± 1.612, 99.8 ± 0.800, 98.6 ± 2.712 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

6. The effect of Olive leave and Morus alba leaves extracts on serum TAG Level:-

It was observed clearly from Table (6) that serum TAG mg/dl level was significantly increased (P<0.05) in diabetic group (317.2 ± 8.890) in comparison with the control group (229 ± 4.669) after treatment. Meanwhile serum triglyceride level was significantly decreased (P<0.05) in all treated groups and were 285.2 ± 7.137, 248 ± 25.447, 251 ± 25.899 (Cidophage), 263 ± 21.337, 240.4 ± 16.536, 249.2 ± 12.338 (Olive leave alcoholic extract) and 239.4 ± 14.183, 235.2 ± 14.022, 232.6 ± 6.297 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

7. The effect of Olive leave and Morus alba leaves extracts on serum HDL Level:-

It was observed clearly from Table (7) that serum HDL mg/dl level was significantly decreased (P<0.05) in diabetic group (18 ± 1.183) in comparison with the control group (23 ± 1.341) after treatment. Meanwhile serum HDL level was significantly increased (P<0.05) in all diabetic treated groups and were 21 ± 0.707, 24 ± 1.048, 23.4 ± 1.122 (Cidophage), 24.4 ± 0.678, 23.6 ± 1.326, 22.4 ± 0.812 (Olive leave alcoholic extract) and 22 ± 1.140, 23.8 ± 0.800, 22.2 ± 1.157 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

8. The effect of Olive leave and Morus alba leaves extracts on serum LDL Level:-

It was observed clearly from Table (8) that serum LDL mg/dl level was significantly increased (P<0.05) in diabetic group (47.2 ± 2.919) in comparison with the control group (29.6 ± 4.826) after treatment. Meanwhile serum LDL level was significantly decreased (P<0.05) in all diabetic treated groups and were 32.12 ± 3.617, 33.2 ± 2.669, 29.4 ± 5.883 (Cidophage), 31.200 ± 1. 432, 32.92 ± 1.771, 33.16 ± 2.868 (Olive leave alcoholic extract) and 29.92 ± 4.381, 28.96 ± 1.722, 29.88 ± 3.070 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

9. The effect of Olive leave and Morus alba leaves extracts on serum VLDL Level:-

It was observed clearly from Table (9) that serum VLDL mg/dl level was significantly increased (P<0.05) in diabetic group (63.44 ± 1.778) in comparison with the control group (45.8 ± 0.933) after treatment. Meanwhile serum VLDL level was significantly decreased (P<0.05) in all diabetic treated groups and were 57.04 ± 1.427, 49.6 ± 5.089, 50.2 ± 5.179 (Cidophage), 52.6 ± 4.267, 48.08 ± 3.307, 49.84 ± 2.467 (Olive leave alcoholic extract) and 47.88 ± 2.836, 47.04 ± 2.804, 46.52 ± 1.259 (Morus alba leave alcoholic extract) at1day, 1st and 2nd weeks, respectively post dosing, in comparison with control diabetic group.

Table (1): Effect of Olive and Morus alba leaves extracts on serum superoxide dismutase activity of diabetic and non-diabetic rats.(M ± S.E) (n=5) .

No.	parameter / Group	Serum SOD activity U/ml		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	355.4± 0.509 A	355.4± 0.509 A	355.4± 0.509 A
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	259.4 ± 0.509 D	259.4 ± 0.509 D	259.4 ± 0.509 D
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	312 ± 0.316 B	316 ± 0.316 B	302.4 ± 0.509 B
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	308.4 ± 0.748 C	311.6 ± 1.288 C	312.6 ± 0.244 C
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	312 ± 0.447 B	315 ± 0.707 b	314.6 ± 0.509 B

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (2): Effect of Olive and Morus alba leaves extracts on serum glutathione peroxidase activity of diabetic and non-diabetic rats. (Mean ± SE)(n=5)

.No.	Parameter / Group	Serum GSH activity U/ml		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	28.66 ± 0.833 A	28.66 ± 0.833 a	28.66 ± 0.833 A
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	20.36 ± 1.393 C	20.36 ± 1.393 b	20.36 ± 1.393 B
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	26.9 ± 0.698 A	25.80 ± 0.492 a	26.42 ± 1.60 A
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	24.42 ± 0.58 B	28.860 ± 0.969 a	26.332 ±0.440 A
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	23.564 ± 1.226 B	26.9 ± 2.770 a	27.5 ± 2.261 A

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (3): Effect of Olive and Morus alba leaves extracts on serum catalase activity of diabetic and non-diabetic rats. (Mean ± SE) (n=5).

No.	**Parameter / Group**	**Serum CAT activity U/ml**		
		1st day	**1st week**	**2nd week**
1-	G1 (Control given 0.2ml normal saline)	354.2 ± 10.707 A	354.2 ± 10.707 a	354.2 ± 10.707 Ab
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	249 ± 12.814 C	249 ± 12.814 b	249 ± 12.814 C
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	322 ± 7.726 B	342.2 ± 14 944 a	338 ± 8.596 B
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	328.2 ± 15.567 Ab	335 ± 3.898 a	344.6 ± 1.600 B
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	321 ± 1.000 B	328 ± 10.681 a	388 ± 17.722 a

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (4): Effect of Olive and Morus alba leaves extracts on serum MDA level of diabetic and non-diabetic rats (Mean ± SE) (n=5).

No.	parameter / Group	Serum MDA level ng/ml		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	9.66 ± 0.326 C	9.66 ± 0.326 c	9.66 ± 0.326 b
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	16.06 ± 1.626 A	16.06 ± 1.626 a	16.06 ± 1.626 a
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	12.3 ± 0.094 B	10.36 ± 0.102 c	9.66 ± 0.112 b
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	15.46 ± 0.898 Ab	13.16 ± 0.320 b	10.6 ± 0.374 b
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	14.8 ± 0.301 B	12.22 ± 0.320 b	11.32 ± 0.152 b

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (5): Effect of Olive and Morus alba leaves extracts on serum cholesterol level of diabetic and non-diabetic rats(Mean ± SE) (n=5).

No.	parameter / Group	Serum Cholesterol level (mg/dl)		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	98.4 ± 3.558 C	98.4 ± 3.558 b	98.4 ± 3.558 B
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	128.6 ± 2.767 A	128.6 ± 2.767 a	128.6 ± 2.767 A
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	111.20 ± 2.709 B	106.8 ± 3.813 b	103 ± 2.024 B
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	108.2 ± 3.826 B	104.6 ± 1.691 b	105 ± 2.481 B
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	98.0 ± 1.612 C	99.8 ± 0.800 b	98.6 ± 2.712 B

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (6): Effect of Olive and Morus alba leaves extracts on serum TAG level of diabetic and non-diabetic rats. (Mean ± SE) (n=5).

No.	parameters / Group	Serum triglyceride level (mg/dl)		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	229 ± 4.669 C	229 ± 4.669 b	229 ± 4.669 b
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	317.2 ± 8.890 A	317.2 ± 8.890 a	317.2 ± 8.890 a
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	285.2 ± 7.137 Ab	248 ± 25.447 B	251 ± 25.899 b
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	263 ± 21.337 Bc	240.4 ± 16.536 B	249.2 ± 12.338 b
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	239.4 ± 14.183 Bc	235.2 ± 14.022 B	232.6 ± 6.297 b

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (7): Effect of Olive and Morus alba leaves extracts on HDL level of diabetic and non-diabetic rats (Mean ± SE) (n=5).

No.	parameter / Group	Serum HDL level (mg/dl)		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	23 ± 1.341 A	23 ± 1.341 A	23 ± 1.341 a
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	18 ± 1.183 B	18 ± 1.183 B	18 ± 1.183 b
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	21 ± 0.707 B	24 ± 1.048 A	23.4 ± 1.122 a
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	24.4 ± 0.678 A	23.6 ± 1.326 A	22.4 ± 0.812 a
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	22 ± 1.140 a	23.8 ± 0.800 A	22.2 ± 1.157 a

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (8): Effect of Olive and Morus alba leaves extracts on serum LDL level of diabetic and non-diabetic rats. (Mean ± SE) (n=5).

No.	parameters / Group	Serum LDL level (mg/dl)		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	29.6 ± 4.826 B	29.6 ± 4.826 b	29.6 ± 4.826 b
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	47.2 ± 2.919 A	47.2 ± 2.919 a	47.2 ± 2.919 a
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	32.12 ± 3.617 B	33.2 ± 2.669 b	29.4 ± 5.883 b
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	31.200 ± 1. 432 B	32.92 ± 1.771 b	33.16 ± 2.868 b
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	29.92 ± 4.381 B	28.96 ± 1.722 b	29.88 ± 3.070 b

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Table (9): Effect of Olive and Morus alba leaves extracts on serum VLDL level of diabetic and non-diabetic rats. (Mean ± SE) (n=5).

No.	Parameters / Group	Serum VLDL level (mg/dl)		
		1st day	1st week	2nd week
1-	G1 (Control given 0.2ml normal saline)	45.8 ± 0.933 C	45.8 ± 0.933 b	45.8 ± 0.933 b
2-	G2 (Diabetic by 45 mg/kg b. wt STZ)	63.44 ± 1.778 A	63.44 ± 1.778 a	63.44 ± 1.778 a
3-	G3 (Diabetic treated with Cidophage at 500 mg/kg b. wt)	57.04 ± 1.427 A	49.6 ± 5.089 b	50.2 ± 5.179 b
4-	G4 (Diabetic treated with alcoholic extract of Olive leaves at 500 mg/kg b. wt)	52.6 ± 4.267 B	48.08 ± 3.307 b	49.84 ± 2.467 b
5-	G5 (Diabetic treated with alcoholic extract of Morus alba leaves) at 600 mg/kg b. wt)	47.88 ± 2.836 C	47.04 ± 2.804 b	46.52 ± 1.259 b

*Means within the same column bearing different superscript at P<0.05.
*Mean significant change,
*Mean with the same column bearing the same superscript letters
*Means non significant change

Discussion

Recently, much attention has been focused on antioxidants in food that are potential compounds for preventing diseases caused by oxidative including diabetes because of their distinctive biological activity.

The obtained results revealed that, STZ injected rats showed a significant decrease in serum activities of superoxide dismutase (SOD), glutathione peroxidase (GSH), catalase activity and high density lipoprotein(HDL), and a significant increase in total lipids, cholesterols, triglycerides and low density lipoprotein (LDL) levels. These findings are in accordance with those of **Hye, *et al.* (2009)** who mentions that the levels of serum lipids are usually elevated in diabetes mellitus, and this represents the risk of coronary heart disease. The hyperglycemic effect of STZ showed significant increase in oxidative stress markers (lipid peroxidation and protein oxidation) and significant decrease in antioxidants status. Similar changes were already observed in different experimental situations reported by **Rajani, *et al*. (2008)**.

It has been demonstrated that hyperglycemia of diabetes can generate oxidative stress, manifested by the presence of free radicals with the simultaneous decline of antioxidant defense mechanisms observed in diabetic patients, a finding that could promote the development of diabetic complications **(Godin, *et al*., 1988)**. Furthermore, hypercholesterolemia, especially elevated plasma LDL, and hyper glyceridemia are independent risk factors that alone or together can accelerate the progression of atherosclerotic lesions **(McKenney, 2001)**.

Regarding the effects of the orally administered leaves extracts of Olive and Morus alba on serum levels of the tested parameters in diabetic rats. The recorded results showed that, both Olive leaves and Morus alba extracts elicited a significant increase in serum levels of superoxide dismutase (SOD), glutathione peroxidase (GSH), catalase and high density lipoprotein(HDL), and a significant decrease in total lipids, cholesterols, TAG and low density lipoprotein (LDL) levels. These findings are in agreement with that of many authors.

Several reports have indicated that Olive leave has antioxidant properties **(Bianco, *et al*., 2006)**. Recently, high request of whole olive leaves and olive leaves extract has increased for use in food stuff, functional food materials and food additives **(Soni, *et al*., 2006)**. **Khan, *et al*. (2007)**. Explained that Olea europaea leave showed hypolipidimic activity when studied in laboratory animals. This

effect may be due to the presence of the active constituent Oleuropein, with a proposed mechanism of action of potentiation of glucose-induced insulin release, and an increase in peripheral blood glucose uptake. **Manna, *et al*., (2004)** showed that many of these pharmacologic features of oleuropein are due to its potent antioxidant actions. Indeed, both oleuropein and hydroxytyrosol have been shown to be scavengers of superoxide anions and inhibitors of the respiratory burst of neutrophils and hypochlorous acidderived radicals **(Al-Azzawie, and Alhamdani, 2006**).

According to **Andallu, (2009)** mulberry plants contains numerous active compounds which acts as an antioxidant like carotenoids, polyphenols and vitamin A, C, E. They found that these compounds increase the body's antioxidant status and regulate Low-density lipoprotein (LDL) oxidation. The ethanolic extract of Morus alba leaves have oxyresveratrol and 5, 7-dihydroxycoumarin 7-methyl ether which scavenge superoxide and have antioxidant potential **(Oh, *et al*., 2002)**. Similarly aqueous extract of Morus alba leaves showed highest antioxidant activities evaluated through ferric reducing/antioxidant power assay **(Wattanapitayakul, *et al*., 2005)**.

Studies conducted on flavonoid substances, indicated increased activity of glutathione peroxidase, glutathione reductase and total antioxidant levels in diabetic rats that have received these compounds **(Cho, SY.,*et al*., 2002)**. Because Morus alba leaves extract containing abundant isoflavones, it is possible role of extract in increasing glutathione peroxidase activity and plasma antioxidant levels related to neutralize free radicals, especially hydroxyl radicals. **El-Beshbishy *et al*. (2006)**recorded the same effect of Morus alba on cholesterol, T.G, LDL-c and HDL-c. This was due to the presence of flavonoids in Morus alba leaves.

Conclusion: It could be concluded that the use of Olive leaves and Morus alba leaves extracts improved the levels of the tested parameters in diabetic rats. Hydro-alcoholic leaves extracts of Olive and Morus alba could improve the anti-oxidant and lipid profile parameters of male rats, and minimized the possible side effects of diabetes mellitus.

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الملخص العربي

بعض الدراسات علي مستخلصاتأوراق الزيتون و التوت على مضادات الأكسدة والدهون في الجرذان المصابة بالسكري تجريبيا

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الهدف من هذه الدراسة:-

تهدف هذه الدراسة الى معرفة تأثير كل من مستخلصاتأوراق الزيتون والتوت (ethanolic hydro-alcohol 60 %) على بعض مضادات الأكسدة (السوبر أكسيد ديسميوتيزSOD– الجلوتاسيون بيرأوكسيديز GSH– الكاتاليز CAT– المالونداي الدهايد MDA)وعلى مستوى الدهون في الدم (الكولسترول ، الدهون الثلاثية - الليبوبروتين عالي الكثافة HDL– الليبوبروتين منخفض الكثافة LDL - وVLDL) في ذكور الجرذان المصابة بداء السكري تجريبيا باستعمال مادة ستربتوزوتيسين (STZ).

أستخدمت في هذة التجربة عدد خمسين من ذكور الجرذان البيضاء وزنها يتراوح مابين (١٥٠ - ٢٠٠غم) وتم تقسيمها إلى خمس مجاميع (لكل مجموعة ١٠جرذان) : تركت المجموعة الأولى كمجموعة ضابطة. تم حقن المجاميع المتبقية (بالغشاء البريتوني)) بمادة ستربتوزوتيسين (STZ)بجرعة ٤٥ ملغم / كغم من وزن الجسم b.wtلإحداث مرض السكري. المجموعة الثاني اعتبرت مجموعه ضابطة 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) على التوالي. استمرت فترت العلاج يوميا لمدة ٣٠ يوما متتالية.

تم جمع عينات الجرذان بعد انتهاء فترة العلاج ومن ثم تم قياس نشاط مضادات الأكسدة (SOD، GSH، CAT ومستوى MDA) ومستوى الدهون في الدم (الكولسترول، الدهون الثلاثية، HDL، LDL وVLDL) بعد يوم واحد و أسبوع وأسبوعين بعد نهاية فترة ٣٠ يوم من العلاج..

النتائج:-

وأظهرت النتائج أن استخدام مستخلص ورق الزيتون ومستخلص ورق التوت آدا إلى تحسين واضح في مضادات الأكسدة (SOD ، GSH، CAT وMDA) مستوى الدهون في الدم (الكولسترول، الدهون الثلاثية، HDL، LDL وVLDL).

الاستنتاجات:-

المستخلص المائي الكحولية لأوراق الزيتون وأوراق التوت آدا إلى تحسين مضادة للأكسدة ومستوى الدهون من ذكور الفئران، وتخفف من الآثار الجانبية المحتملة لمرض السكري.