Influence of Olive Leaves and its Extracts by Two Methods on Diabetic Rats.

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ABSTRACT

The present review was directed to research the impact of olive leaves and its concentrates by two strategies on diabetic rats, moreover, to decide the fundamental phenolic mixes display in dried olive takes off. The results showed that, the highest concentrations of phenolic compounds in dried olive leaves were Rutin, Quercetin and Oleuropein. Liver and kidney (weights), serum glucose, lipid profile except HDL-c, kidney functions and liver enzymes increased in diabetic group, than these of the control negative group., On the other hand food intake, body weight gain (BWG%), HDL-c, superoxide dismutase (SOD) and glutathione peroxidase (GPx) decreased in diabetic group, than these of the control negative group. All treatments, with the two dosages of decoction extract, aqueous extract and olive leaves powder showed improve in all parameters, especially the groups which were treated with 4ml aqueous extract of olive leaves and 10% of olive leaves powder. In conclusion, olive leaves powder and olive leaves extracts improved the nutritional and biological status of diabetic rats.
Keywords: olive leaves – phenolic compounds – extraction - rats – diabetes.

INTRODUCTION

Diabetes is the most well-known endocrine issue influencing a huge number of individuals around the world. More than 346 million of individuals overall experience the ill effects of this ailment (Danaei , 2011). Articulated changes in the way of life, use of imperativeness rich eating routine and weight are critical reasons for the ascent of diabetes at a disturbing rate (Sikarwar and Patil , 2010).In 2010, diabetes mellitus was assessed to influence around 6.4% of the world's grown-up populace, or 285 million individuals. It is assessed that by 2030, the populace influenced by diabetes will increment to 7.7% of all grown-ups, or 439 million individuals (Al-Azzawie and Alhamdani , 2006). Then again, hindered glucose-instigated insulin emission with a lessening in pancreatic β cell mass will in the end prompt to perpetual hyperglycemi (Sebbagh *et al.*, 2009). Regardless of the hypoglycemic specialists, diabetes and the related entanglements keep on being a noteworthy medicinal issue (Srinivas *et al.*, 2003).

Diabetes is brought about by a flat out or relative absence of insulin as well as breakdown of insulin activity (Balkau *et al.,* 2000 and Rasineni *et al.,* 2010). It is portrayed by hyperglycemia, aggravations in starch, protein, and fat digestion systems, notwithstanding long haul intricacies influencing the eyes, kidneys, nerves, heart and veins (Hung *et al.,* 2005 , Thripathi and Sivastava 2006 and Gupta, 2008).

Olive leaves from Oleaeuropaea, is local to the Mediterranean and has been guaranteed to have restorative qualities including hostile to diabetic and cancer prevention agent exercises (Gonzalez *et al.,* 1992 , Wojcikowski *et al.,* 2007 and Eidi *et al.,* 2009).

The substantial number of phenolic mixes exhibit in olive leaves stirred the enthusiasm of analysts around the globe and the reviews with creatures and people have announced helpful wellbeing impacts, for example, the limit of cell reinforcement (Benavente-Garcia *et al.*, 2000), anti-hipertensive (Susalit *et al.,* 2011), hipoglicemiant (Kontogianni *et al.,* 2013), hypocholesterolemic (Jemai *et al.*, 2009), cardioprotective (Nekooeian *et al.,* 2014) and as co-adjuvant in the treatment of obesity (Santiago-Mora *et al.,* 2011).

Olive leaf removes seem, by all accounts, to be very sheltered. In creature tests, specialists watched no lethality in rats, even at high dosages (1g/kg body weight) for seven days (Petkov and Manolov, 1972). In vitro considers on human cell lines found no poisonous quality at 1 mg/ml of concentrate (Lee-Huang *et al.*, 2003). Therefore, the present study was carried out to assess the effects of olive leaves and its extractions by two methods on diabetic rats.

MATERIALS AND METHODS

Materials:
- Olive leaves *(Olea europaea L.)* were gotten from Agricultural Research Center, Giza Egypt.
- Casein, vitamins, minerals, cellulose, alloxan and choline chloride were purchased from El-Gomhoria Company, Cairo Egypt.
- Corn starch, soybean oil, sucrose were purchased from local market, Cairo, Egypt.
- Forty two male Albino rats (Sprague Dawley Strain) (150 ± 10g) were obtained from National Research Center, Dokki, Egypt.

Methods:
Dried olive leaves

Olive leaves were washed thoroughly with tap water dehydrated into air circulated oven at 40-50˚C for 24 hrs. The dried samples were finely powdered by using a coffee grinder and stored in polyethylene bags at - 20˚C until used.

Preparation of decoction

First, cleaning 150 g of olive leaves with water to clear the tidy and sand, heating up the leaves in one liter and a half of water. The water bubbles for three minutes and takes the blend off and let it deplete. At that point, gather the liquid and spare the blend in a glass holder for the use (Abu-zaiton and Abu-Albasal, 2012).

Preparation of olive leaves watery extract

Watery extract was prepared by boiling olive leaves (300g) in water (3000 ml) for 60 minutes, then filtered through Whatman filter paper No.2 and the filtrate was dried, the yield of dried extract was about

30gm when dissolved in 300ml water (Hung *et al.,* 2006).

Determination of phenolic compounds

Phenolic compounds of olive leaves was resolved by the strategy portrayed by (Crozier *et al*., 1997) by using High performance liquid chromatography analysis HPLC.

Growth parameters

Liver and kidney were separated from each rat and weighted to calculate the liver and kidney weights to body weights %.

Biochemical Analysis of serum

The blood tests were centrifuged and serum was isolated to gauge some biochemical parameters.The following parameters were analysed according to the methods described as follow:

- Serum glucose as portrayed by Trinder, (1959).
- Lipid profil
- Serum cholesterol was resolved as portrayed by Allain *et al.,* (1974).
- Triglycerides was resolved as portrayed by Foster and Dumns, (1973).
- High density lipoprotein HDL-c was resolved as portrayed by Lopes-Virella *et al.*, (1977).
- Low density lipoprotein LDL-c and VLDL-c was by resolved as portrayed Friedwald *et al.*, (1972).
- kidney functions
- Uric acid was resolved as portrayed by Fossati *et al.*, (1980).
- Urea nitrogen was resolved as portrayed by Patton and Crouch, (1977).
- Creatinine was resolved as portrayed by Bohmer, (1971).
- Liver enzymes
- Aspartate Amine Transaminase (AST) and Alanine Amine Transaminase (ALT) were resolved as portrayed by Reitman and Frankel, (1957).
- Alkaline Phosphatase (ALP) was resolved as portrayed by Belfield and Goldberg, (1971).
- Superoxide dismutase (SOD) activity was resolved by the strategy portrayed by Kakkar *et al.*, (1984).
- Glutathione peroxidase (GPx) activity was resolved by the strategy portrayed by Ellman, (1959).

Statistical analysis

Consequences of organic assessment of each gathering were factually investigated (mean ± standard deviation and one way ANOVA test) utilizing SAS bundle and contrasted and each other utilizing the reasonable test (minimum huge contrasts at P< 0.05 (SAS, 1996).

RESULTS AND DISCUSSION

Fractianation and identification of phenolic compounds of dried olive leaves methanolic extract :

Table (1) shows the main phenolic compounds identified in methanolic dried olive leaves. Rutin was found to be the predominant compound and amounted 217.77 mg/100g, while the mediated compounds were; Quercetin and Oleuropein which amounted in 97.00 and 86.00 mg/100g, respectively. Meanwhile, Catechin, Apigenin, Hydroxytyrosol and Caffeic acid were

amounted (23.50, 20.33, 16.26 and 13.97 mg/100g), respectively.

The additional virgin olive oil is created from the product of the olive tree, naturally known as Olea europaea L, rich in polyphenols and known for its cancer prevention agent limit (Soni *et al.,* 2006). In any case, the olive leaves contain higher measure of polyphenols than olive oil. For instance, the measure of oleuropein, which is the most plentiful phenolic compound extents from 0.005% and 0.12% in olive oil, while in olive abandons it goes in the vicinity of 1 and 14% (Japon-Lujan *et al.*, 2006).

Table 1. Main phenolic compounds identified in methanolic extract of dried olive leaves (mg /100 g).

Compound	mg/100g
Rutin	217.77
Quercetin	97.00
Oleuropein	86.00
Catechin	23.50
Apigenin	20.33
Hydroxytyrosol	16.26
Caffeic acid	13.97

Biological Assay:

Animals and experimental design

Forty two male Albino rats (150 ± 10g) were kept in individual stainless steel confines under clean conditions and sustained one week on basal eating routine adlibitum for adjustment as indicated by (Reeves *et al.,* 1993). After a time of adjustment on basal eating routine (7 days), the rats were isolated into seven gatherings. The main gathering (6 rats) bolstered on basal eating routine, as a negative control amass. The six gatherings (36 rats) was infused with alloxan (150 mg alloxan/kg body weight) to incite diabetes as indicated by the technique portrayed by (Kumar *et al.,* 2010). Following four days, blood tests were gathered from the eye of all rats to decide the levels of glucose to guarantee the enlistment of diabetes. At that point, prompted rats were isolated into six subgroups (6 rats) as per the accompanying: Group (2) bolstered on basal eating regimen, as a positive control (diabetic gathering). Assembles (3 and 4) were nourished on basal eating routine and treated with 2 and 4 ml decoction of olive leaves, separately. Bunches (5 and 6) were bolstered on basal eating routine and treated with 2 and 4 ml olive leaves fluid concentrate , individually. Assemble (7) was nourished on basal eating regimen containing 10% dried olive leaves powder. Amid the trial time frame (6 week), the eating regimens ate up and body weights were recorded every week. At the complete of the examination, the rats were fasted overnight, then the rats were anesthetized and surrendered, the blood tests were accumulated from the aorta of all rats.

Effect of olive leaves and its extracts on growth parameters of diabetic rats.

The effect of basal diet containing 10% of olive leaves powder or two dosage (2 and 4ml of decoction and watery concentrate of olive leaves /rat/day) on food intake (g/day/each rat), body weight gain% and (liver and kidney) weights/body weight% of diabetic rats

presented in table (2). The mean value of food intake (g/day/each rat) of the negative control group showed non-significant deference, as compared to the positive control group. Food intake of all treated groups with olive leaves powder or the tow dosage of decoction and aqueous extracts of olive leaves (2 and 4 ml /each rat/day) recorded non-significant changes, as compared to the positive control group.

The mean value of body weight gain (%) of diabetic group decreased significantly p<0.05, as compared to the negative control group. The data presented in this table revealed that, treating rats which were suffering from diabetes with 2ml (decoction or aqueous extracts of olive leaves) showed non-significant difference in body weight gain %, as compared to the positive control group, while treating diabetic rats on the decoction or aqueous extracts with 4ml recorded significant decrease p<0.05 in body weight gain (%). On the other hand feeding diabetic rats on diet containing 10% olive leave decreased the mean value of body weight gain%, as compared to the positive control group. Non-significant difference in body weight gain (%) was observed between the groups treated with the high dosage of decoction, aqueous extract and olive leaves powder .

Liver and kidney weights / body weights (%) of diabetic rats increased significantly p< 0.05, as compared to the negative control group. Treating diabetic groups with high dosage (4ml/rat/day) of decoction or aqueous extracts of olive leaves decreased the mean values of liver and kidney weight/body weight %, as compared to the rats which treated with the low dosage (2ml/rat/day) . On the other hand, treating diabetic group with diet containing 10% olive leaves powder showed non-significant change in liver and kidney weight/body weight %, as compared to the groups which treated with high doses of decoction and aqueous extracts.

An investigation of Svobodova *et al.,* (2014) demonstrated that the oleuropein has a hostile to adipogenic impact through the hindrance and movement and Peroxisome proliferator actuated receptor PPAR which is basic for the arrangement and capacity of the adipocytes. The PPAR is likewise required in the control of insulin affectability.

Shen *et al.,* (2014) propose that olive leafs extricate applies advantageous impacts against heftiness by controlling the outflow of qualities required in adipogenesis and thermogenesis in the instinctive fat tissue of high fat eating routine sustained mice.

Table 2. Effect of olive leaves and its extracts on growth parameters of diabetic rats.

Parameters / Groups	Food Intake g/day/rat	Body weight gain%	Organs weight / body weight%	
			Liver	Kidney
Group(1) Negative control group	12.400 [a] ± 0.821	36.902 [a] ± 2.612	2.686 [e] ± 0.139	0.566 [e] ± 0.063
Group(2) Positive control group (Diabetic Group)	11.700 [ab] ± 0.758	14.126 [b] ± 1.445	3.476 [a] ± 0.063	0.781 [a] ± 0.054
Group(3) 2ml decoction extract of olive leaves	11.400 [b] ± 0.547	13.581 [bc] ± 1.547	3.286 [b] ± 0.048	0.709 [b] ± 0.014
Group(4) 4ml decoction extract of olive leaves	11.640 [ab] ± 0.482	11.418 [cde] ± 1.435	3.075 [cd] ± 0.101	0.646 [c d] ± 0.027
Group(5) 2ml aqueous extract of olive leaves	11.168 [b] ± 0.435	12.173 [bcd] ± 1.776	3.204 [bc] ± 0.132	0.691 [b c] ± 0.023
Group(6) 4ml aqueous extract of olive leaves	11.400 [b] ± 0.547	10.601 [de] ± 1.462	2.972 [d] ± 0.067	0.609 [d] ± 0.009
Group(7) 10% olive leaves powder	11.422 [b] ± 0.548	9.692 [e] ± 1.080	3.072 [cd] ± 0.106	0.621 [d] ± 0.006

All results are expressed as mean ± SD.
Values in each column which have different letters are significant different (p<0.05).

Effect of olive leaves and its extracts on serum glucose of diabetic rats.

The effect of olive leaves powder and its extractions by two methods on serum glucose of rats suffering from diabetes presented in table (3). The mean value of serum glucose increased significantly p<0.05 in the positive control group, as compared to the negative control group (164.00 ± 5.099 vs. 75.800 ± 4.381mg/dl). Serum glucose increased in the positive control group by about 116.358%, than that of the negative control group. In this respect, Kurup and Bhonde, (2000) and Szkudelski, (2001) reported that, hypo-secretion of insulin by pancreatic β-cells resulted in Alloxan-treated rats and led to reduction of serum insulin and increased of serum glucose. Alloxan selectively destroys the pancreatic insulin secreting β-cells and induces hyperglycemia. Stanely *et al.*, (2004) reported also, Alloxan prompts diabetes by harming the insulin emitting cells of the pancreas prompting to hyperglycemia.

All treated groups with olive leaves extractions and olive leaves powder showed significant decrease p<0.05 in serum glucose, as compared to the positive control group. Serum glucose decreased gradually with increasing the dosage of decoction or aqueous extract of olive leaves. The highest decrease in serum glucose recorded for the group treated with 4 ml aqueous extract/rat/day, followed by the group which treated with 10% olive leaves powder, these treatment decreased serum glucose by about (21.585% and 19.512%), respectively. In this regard, Komaki *et al.,* (2003) revealed likewise, the dynamic part of olive leaves, for example, luteolin and oleano¬lic corrosive constituents have been appeared to inhibitorily affect postprandial glucose increment in diabetic rats.

Table 3. Effect of olive leaves and its extracts on serum glucose of diabetic rats.

Parameters Groups	Glucose mg/dl
Group(1) Negative control group	75.800 ± 4.381 [e]
Group(2) Positive control group (Diabetic Group)	164.00 ± 5.099 [a]
Group(3) 2ml decoction extract of olive leaves	152.200 ± 2.863 [b]
Group(4) 4ml decoction extract of olive leaves	140.400 ± 2.073 [c]
Group(5) 2ml aqueous extract of olive leaves	144.400 ± 2.701 [c]
Group(6) 4ml aqueous extract of olive leaves	128.600 ± 3.974 [d]
Group(7) 10% olive leaves powder	132.000 ± 3.535 [d]

All results are expressed as mean ± SD.
Values in each column which have different letters are significant different (p<0.05).

Oral administration of decoction produced hypoglycemia in normal animals and diabetes associated oxidative stress. The method of activity of the dynamic elements of olive leaves is most likely interceded by an improved discharge of insulin, as sulphonylureas (Al-Azzawie and Alhamdani, 2006).

Effect of olive leaves and its extracts on lipid profile of diabetic rats.

Data presented in table (4) show the effect of olive leaves powder and its extracts by two methods on total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c), low and very low density lipoprotein-cholesterol (LDL-c) and (VLDL-c). Injected rats with 150mg alloxan /kg b.w. induced significant increase p<0.05 in serum cholesterol, triglycerides, low

and very low density lipoprotein-cholesterol, while high density lipoprotein-cholesterol decreased significantly, as compared to the negative control group. Ravi *et al.,* (2005) demonstrated that, all lipid profile, aside from HDL-c expanded in diabetic rats which are notable as hazard elements for cardiovascular illnesses.

All treated diabetic groups recorded significant decrease p<0.05 in all parameters, except HDL-c, which increased significantly p<0.05, as compared to the positive control group. The mean values of serum cholesterol, triglycerides, LDL-c and VLDL-c decreased gradually with increasing the dosage of decoction or aqueous extract of olive leaves, while HDL-c increased.

The outcomes in this table uncovered that, diabetic rats which treated with the two measurements of watery concentrate of olive leaves recorded more change in lipid divisions, than that of rats treated with the two dose of decoction of olive clears out. The outcomes uncovered likewise, non-huge changes in all lipid parameters, aside from LDL-c was seen between the gatherings treated with 10% powdered of olive leafs and 4ml fluid concentrate/rodent/day. In this way, the most astounding change of lipid profile recorded for the gathering treated with 4ml watery concentrate/n rodent/ day, trailed by the gathering which treated with 10% olive leaves powder.

Table 4. Effect of olive leaves and its extracts on lipid profile of diabetic rats.

Parameters Groups	Ch	TG	HDL-c mg/dl	LDL-c	VLDL-c
Group(1) Negative control group	80.717 [e] ± 3.817	42.062 [d] ± 2.782	43.585 [a] ± 2.512	28.719 [f] ± 1.901	8.412 [d] ± 0.556
Group(2) Positive control group (Diabetic Group)	128.068 [a] ± 6.684	63.440 [a] ± 4.223	23.208 [d] ± 1.846	92.170 [a] ± 4.161	12.689 [a] ± 0.844
Group(3) 2ml decoction extract of olive leaves	119.057 [b] ± 6.591	56.784 [b] ± 3.882	25.790 [d] ± 1.871	81.910 [b] ± 5.204	11.356 [b] ± 0.776
Group(4) 4ml decoction extract of olive leaves	108.516 [c] ± 6.627	50.752 [c] ± 2.958	29.793 [c] ± 1.961	68.573 [c] ± 5.151	10.150 [c] ± 0.591
Group(5) 2ml aqueous extract of olive leaves	110.991 [c] ± 6.214	53.048 [b c] ± 3.197	29.178 [c] ± 2.008	71.203 [c] ± 4.627	10.609 [b c] ± 0.639
Group(6) 4ml aqueous extract of olive leaves	98.127 [d] ± 5.190	45.593 [d] ± 2.413	35.092 [b] ± 2.251	53.917 [e] ± 3.133	9.118 [d] ± 0.482
Group(7) 10% olive leaves powder	104.199 [c d] ± 5.315	45.502 [d] ± 1.827	35.847 [b] ± 2.185	60.953 [d] ± 5.037	9.100 [d] ± 0.365

Ch: Cholesterol   TG: Triglycerides   HDL-c: High density lipoprotein-cholesterol
LDL-c: Low density lipoprotein-cholesterol   VLDL-c: Very low density lipoprotein-cholesterol
All results are expressed as mean ± SD.
Values in each column which have different letters are significant different (p<0.05).

The obtained results show that, olive leaves separate altogether enhanced sera lipid profiles by diminishing the estimations of aggregate cholesterol, triglycerides, low thickness lipoprotein and low thickness lipoprotein. This shows, olive leaves separate has a potential part in counteracting arrangement of atherosclerosis and coronary illness in diabetic patients (Zoair , 2014).

Oral organization of olive leaves concentrate of hypercholesterolemic mice lessened the levels of blood aggregate cholesterol, triglyceride and low thickness lipoprotein-cholesterol. Furthermore , the mice that got the phenolic extricates their levels of high-thickness lipoprotein cholesterol (HDL-c) restored (p<0.05). The review likewise computed the atherosclerotic file (AI),

characterized as the proportion of LDL-c and HDL-c, which was essentially lower in the gatherings that were directed phenolic mixes display in the olive leaf removes (p<0.05) and demonstrated that there was a noteworthy diminishment in the hepatic action of catalase (CAT) and superoxide dismutase (SOD) in mice nourished with a cholesterol-rich eating regimen contrasted and the control bunch. Notwithstanding, the levels were reestablished within the sight of phenolic mixes exhibit in the olive leaf removes (p<0.05) (Jemai *et al.,* 2008a).

In connection to the impacts of polyphenols of the olive leaf in hypocholesterolemic related with hyperglycemia, a review that surveyed the impacts of the organization of concentrates of oleuropein and

hydroxytyrosol in diabetic mice at centralizations of 16 and 8 mg/kg of body weight demonstrated altogether bring down groupings of TC in diabetic rats that got oleuropein and hydroxytyrosol contrasted and the control assemble. The organization of concentrates rich in phenolic mixes could reestablish the lipid profile, particularly in the gatherings that got oleuropein and hydroxytyrosol in a convergence of 16 mg/kg of body weight, demonstrating that oleuropein and hydroxytyrosol olive leaf concentrate can adjust fundamentally the hypercholesterolemia combined with hyperglycemia (Jemai *et al.,* 2009).

The counter atherosclerotic impact of olive leaves extract was additionally exhibited in rabbits on a high-fat eating routine. Twenty-four rabbits were allocated to control, high-fat eating regimen, or high-fat eating routine supplemented with hydroxytyrosol-improved OLE for a month and a half. Creatures in the high-fat eating regimen bunch had larger amounts of cho¬lesterol, triglycerides, and LDL cholesterol, and additionally a thick layer of lipid aura in the aortic intima com¬pared to those in the OLE amass. These outcomes bolster olive leaf's hostile to atherosclerotic impact, in all likelihood identified with concealment of aggravation (Wang *et al.,* 2008).

Effect of olive leaves and its extractions on kidney functions of diabetic rats.

The results in table (5) show the effect of diet containing 10% olive leaves powder and olive leaves extract by the two methods on serum uric acid, urea nitrogen and creatinine mg/dl of diabetic rats. Injected rats with 150mg alloxan /kg b.w. to induce diabetes, led to significant increase $p<0.05$ in all kidney parameters, as compared to the negative control group. In this respect, rise of the renal capacities might be because of metabolic unsettling influence in diabetic creatures reflected in high exercises of xanthine oxidase, lipid peroxidation, and expanded triacylglycerol and cholesterol levels (Madinov *et al.*, 2000).

Feeding diabetic group on diet containing 10% olive leaves powder, or feeding on basal diet and treating with decoction or aqueous extract) of olive leaves 2ml and 4ml/rat/day caused significant decrease in serum uric acid, urea nitrogen and creatinine, as compared to the positive control group. On the other hand, kidney parameters decreased gradually with increasing the dosage of (decoction or aqueous extract) of olive leaves.

Feeding diabetic rats on diet containing 10% olive leaves powder recorded the best results in kidney functions, followed by the groups treated with 4ml aqueous extract and decoction extract of olive leaves, respectively.

Table 5. Effect of olive leaves and its extracts on kidney functions of diabetic rats.

Parameters Groups	Uric acid	Urea nitrogen mg/dl	Creatinine
Group(1) Negative control group	1.272 [e] ± 0.043	26.662 [e] ± 1.015	0.518 [f] ± 0.024
Group(2) Positive control group (Diabetic Group)	2.201 [a] ± 0.087	55.215 [a] ± 1.721	1.141 [a] ± 0.098
Group(3) 2ml decoction extract of olive leaves	1.922 [b] ± 0.080	49.673 [b] ± 2.455	0.936 [b] ± 0.045
Group(4) 4ml decoction extract of olive leaves	1.708 [c] ± 0.063	43.484 [c] ± 2.751	0.811 [c] ± 0.025
Group(5) 2ml aqueous extract of olive leaves	1.863 [b] ± 0.072	45.881 [c] ± 3.421	0.867 [c] ± 0.033
Group(6) 4ml aqueous extract of olive leaves	1.501 [d] ± 0.065	35.833 [d] ± 2.121	0.689 [d] ± 0.041
Group(7) 10% olive leaves powder	1.410 [d] ± 0.098	33.691 [d] ± 2.166	0.614 [e] ± 0.018

All results are expressed as mean ± SD.
Values in each column which have different letters are significant different ($p<0.05$).

Treatment of diabetic rats with olive leaves extract reversed these parameters (uric acid, urea nitrogen and creatinine) towards normalcy which could be due to decreased metabolic disturbances of other pathways such as protein and nucleic acid metabolism as evidenced by improved glucose level (Zoair, 2014).

Abd El-Rahman, (2016) inferred that olive leaf acts in the kidney as a strong scrounger of free radicals to keep the poisonous impacts of gentamicin (GS) both in the biochemical and histopathological parameters. Al-Sowayan and Mousa, (2014) closed likewise, olive leaf separate OLE might be a remedial and nephroprotectiveagent against kidney disappointment incited by chemicals, for example, CCl4.

Al-Attar and Abu Zeid, (2013) assessed the impact of olive leaves extricate against diazinon poisonous quality in male mice. They showed that the concentrate of olive leaves can be considered as a promising remedial specialist against hepatotoxicity, cardiotoxicity, nephrotoxicity and metabolic issue instigated by diazinon treatment. Additionally, they recommended that the impact of olive leaves remove against diazinon was conceivably because of cell reinforcement properties of its normal synthetic constituents.

Effect of olive leaves and its extracts on liver enzymes of diabetic rats.

The mean values of liver enzymes including Aspartate Amine Transaminase (AST), Alanine Amine Transaminase (ALT) and Alkaline Phosphatase (ALP) of diabetic rats which treated with olive leaves powder and (decoction or aqueous extracts) of olive leaves presented in table (6).

Injected rats with alloxan to induce diabetes led to significant increase in serum AST, ALT and ALP $p<0.05$, as compared to the negative control group. All

treated experimental groups showed significant decrease in liver enzymes, as compared to the positive control group. The mean values of AST, ALT and ALP decreased gradually with increasing the dosage of (decoction or aqueous extracts) of olive leafs. Treating diabetic rats with diet containing 10% olive leaves powder recorded the best results in liver enzymes, this treatment decreased the mean values of serum AST, ALT and ALP by about (24.825%, 36.463% and

22.855%) than that of the positive control group, respectively.

Khalil, (2004) explored the hepatoprotective action of a fluid concentrate of olive leaves against overdose paracetamol in male pale skinned person rats. He inferred that a fluid concentrate of olive leaves has cell reinforcement property which can secure liver harm happened by overdose paracetamol in male pale skinned person rats.

Table 6. Effect of olive leaves and its extracts on liver enzymes of diabetic rats.

Parameters Groups	AST	ALT IU/l	ALP
Group(1) Negative control group	56.770 [f] ± 3.099	20.736 [e] ± 1.959	83.263 [e] ± 2.536
Group(2) Positive control group (Diabetic Group)	99.757 [a] ± 5.345	55.538 [a] ± 1.978	168.040 [a] ± 5.811
Group(3) 2ml decoction extract of olive leaves	92.972 [b] ± 5.204	49.588 [b] ± 2.371	157.610 [b] ± 6.266
Group(4) 4ml decoction extract of olive leaves	84.488 [cd] ± 4.095	43.316 [c] ± 2.246	146.396 [c] ± 6.530
Group(5) 2ml aqueous extract of olive leaves	89.503 [bc] ± 4.420	47.170 [b] ± 2.226	150.492 [c] ± 4.763
Group(6) 4ml aqueous extract of olive leaves	79.456 [de] ± 3.509	37.787 [d] ± 1.674	135.656 [d] ± 3.254
Group(7) 10% olive leaves powder	74.992 [e] ± 3.059	35.287 [d] ± 1.317	129.634 [d] ± 3.513

AST: Aspartate Amine Transaminase   ALT: Alanine Amine Transaminase
ALP: Alkaline Phosphatase   All results are expressed as mean ± SD.
Values in each column which have different letters are significant different ($p<0.05$).

Briante *et al.,* (2002) inferred that the phenolic structure of olive leaf separate lessens the free radicals which, came about because of hepatotoxin paracetamol

Olive leaves extricate at a measurement of 400 mg/kg b.w. to profenofos bunch reestablished the plasma chemicals close to control, This might be because of the capacity of the cancer prevention agent to ensure against oxidative harm to the liver by profenofos (Ambali *et al.,* 2007).

Effect of olive leaves and its extracts on superoxide dismutase SOD and glutathione peroxidase GPx activities in alloxan induced diabetic rats.

Data presented in table (7) showed the mean values of superoxide dismutase (SOD) and glutathione

peroxidase (GPx) of negative control group, positive group and tested groups. The mean values of SOD and GPx in the positive control group revealed significant decrease $p<0.05$, as compared to the negative control group. Treating diabetic rats with 2 and 4ml (decoction extract or aqueous extract of olive leafs) and or 10% olive leaves powder showed significant increase $p<0.05$ in SOD and GPx when compared them with the positive control group. The highest increase in SOD and GPx recorded for the groups treated with 4ml aqueous extract of olive leafs and 10% olive leaves powder, these groups significant increase in these parameters, as compared to the other treated groups.

Table 7. Effect of olive leaves and its extracts on SOD and GPx, activities in alloxan induced diabetic rats.

Parameters Groups	SOD u/l	GPx ng/ml
Group(1) Negative control group	0.537 [a] ± 0.005	0.420 [a] ± 0.025
Group(2) Positive control group (Diabetic Group)	0.191 [f] ± 0.005	0.094 [e] ± 0.003
Group(3) 2ml decoction extract of olive leaves	0.291 [e] ± 0.004	0.132 [d] ± 0.021
Group(4) 4ml decoction extract of olive leaves	0.336 [c] ± 0.016	0.224 [c] ± 0.016
Group(5) 2ml aqueous extract of olive leaves	0.314 [d] ± 0.013	0.224 [c] ± 0.032
Group(6) 4ml aqueous extract of olive leaves	0.377 [b] ± 0.004	0.370 [b] ± 0.036
Group(7) 10% olive leaves powder	0.367 [b] ± 0.004	0.340 [b] ± 0.033

All results are expressed as mean ± SD.
Values in each column which have different letters are significant different ($p<0.05$).

The levels of the antioxidant enzymes SOD and GPx were lowered significantly in the diabetics ($p<0.05$) than that of the control subjects (Briggs *et al.,* 2013). Diabetes with Type 2 related with abatement in cancer prevention agent protein levels coming about

because of expanded oxidative anxiety and lessened systemic hostile to oxidative guard in patients with Type 2 diabetes mellitus. (Djordjevic *et al.,* 2011).

Abd El-Rahman, (2016) reported that, treatment with olive leaves extricate keeps the lipid peroxidation

by upgrading the renal SOD, CAT, and GPx exercises. Lei *et al.*, (2008) presumed that, rats treated with STZ to actuate diabetes demonstrated a noteworthy increment in lipid peroxidation and critical lessening in the exercises of CAT, SOD and GPx in liver and kidneys contrasted and controls.

The olive leaf separate known to be effective cancer prevention agents in vivo (Jemai *et al.,* 2008b) and additionally invitro (Bouaziz and Sayadi, 2005). The organization of oleuropein and hydroxytyrosol-rich concentrates enhanced the cell reinforcement status in liver (Jemai *et al.,* 2009).

OLE supplementation to matured males rabbits, altogether expanded blood plasma of glutathione s-transferase (GST) action and Superoxide dismutase (SOD) action and diminished blood plasma of Thiobarbituric corrosive responsive substances (El-Damrawy, 2011).

CONCLUSION

Finally, it could be concluded that olive leaves powder and olive leaves extracts by two methods had improved the nutritional and biological status of diabetic rats.

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تأثير أوراق الزيتون ومستخلصاتها بطريقتين علي الفئران المصابة بالسكر.
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أجريت هذه الدراسة لبحث تأثير أوراق الزيتون ومستخلصاتها بطريقتين علي الفئران المصابة بمرض السكر ، هذا بالإضافة الي تقدير أهم المركبات الفينولية الموجودة في أوراق الزيتون المجففة. وقد أظهرت النتائج أن أعلي تركيز للمركبات الفينولية في أوراق الزيتون المجففة سجلت لكل من اوليوروبين وكيورستين و ريتن. وقد ازداد كل من وزن (الكبد والكلي) ومستويات الجلوكوزو دهنيات الدم بإستثناء كولسترول الليبوبروتينات عالية الكثافة ووظائف الكلي، وانزيمات الكبد في المجموعة المصابة بالسكر مقارنة بالمجموعة السليمة. ومن ناحية أخري تناقص كل من المأخوذ من الغذاء و النسبة المئوية للزيادة في الوزن و كولسترول الليبوبروتينات عالية الكثافة و سوبر أكسيد ديسميوتيز و الجلوتاثيون بيروكسيداز في المجموعة المصابة بالسكر عن المجموعة السليمة . كل المعاملات سواء بجرعتين من المستخلصات (مستخلص بالغليان والمستخلص المائي) و أوراق الزيتون المجففة قد أوضحت تحسناً في كل التقديرات وخاصة المجموعات التي عوملت بجرعة ٤ مم من المستخلص المائي لأوراق الزيتون و المجموعة المعاملة بنسبة ١٠% من أوراق الزيتون المجففة. ويستخلص من هذه الدراسة أن أوراق الزيتون المجففة و مستخلصات أوراق الزيتون أدت الي تحسن الحالة الغذائية والحيوية للفئران المصابة بالسكر.

الكلمات المفتاحية: أوراق الزيتون – المركبات الفينولية – استخلاص – فئران – مرض السكر.