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BIOCHEMICAL EFFECTS OF ADDING GRIND DRY OLIVE LEAVES IN DIETS OF ALLOXAN-DIABETIC MALE RABBITS

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Abstract

Current study was aimed to estimate the effects and protective effects of grind dry olive leaves on the glucose, triglycerides, cholesterol, total protein, globulin, Albumin, ALT enzyme activity and Glucagon hormone levels in the blood of local male rabbits, induced diabetic by Alloxan injected, experiment was done in the Animal House, College of Veterinary Medicine, University of Baghdad, from 15 November to 30 January, including 15 days as an adaptation period, animals were divided randomly and body weights were consider, into four groups 7 to each, the first group was treated with a 0.9% physiologic solution and kept as control negative group while and provide with basal diets, The other three groups and supplemented their diets by grind dry olive leaves by (0%, 4%, 8%) respectively and treated with150 mg/kg BW of Alloxan I/P, blood sample were taken directly from heart every two weeks and the blood serum kept for biochemical tests. The results showed that there was a significant increase ($P \leq 0.05$) in the concentration of glucose, cholesterol, triglycerides, total protein albumin, globulin, the ALT enzyme activities and glucagon hormone of positive control (diabetic) group in comparative with normal animals (control negative group), while there is improved and significant ($P \leq 0.05$) decrease in both groups that received 4% and 8% olive leaves (diabetic-injected with Alloxan) compared to (diabetic) positive control group.

Key words: glucagon hormone, improved, male rabbits, ALT enzyme, randomly.

Introduction

Many pathological alterations are associated with the conclusion and development of diabetes ranging from the autoimmune destruction of beta cells of the pancreas to the insulin resistance accompanying with obesity and fatness (American Diabetes Association, 2013). Leaves have been widely used in traditional medications in many countries of the world and among many leaves types, the olive leave favorable effects has recently being increasing, it was described that Earliest Egyptians charity olive leaf for mummification and as a therapy against various illnesses. The British used it to treat Malaria in the 1800s (Leila Abaza *et al.,* 2007). Olive leaves derived from olive tree (*Olea europaea*), have been used in the human and animal diets in different methods such as an extract, herbal tea and a powder, due to its contains of a lot of potentially bioactive composites that may have hypoglycemic effect (Sato *et al.*, 2007; Hedya Jemai *et al.,* 2009) anti-ischemic and hypolipidemic effects (Andreadou *et al.,* 2006), antioxidant, antihypertensive. (Sedef, Sibel Karakaya, 2009) anti-inflammatory effects (Tuck and IIayball, 2002; Bitler *et al.,* 2005; Miles, 2005), inhibition of LDL oxidation. (Visioli and Galli, 1994), furthermore, many researcher recently reported that olive leaves have an anti-carcinogenic effects by inhibition the initiation and progression of multistage of carcinogenesis due to the effects of oleuropein aglycone which is the most potent phenolic compound in *Olea europaea* as well as neuroprotective activity by preventing oxidative damage to mitochondria lead to preventing cellular dysfunction (German and Walzem, 2000). Alloxan mainly prompt fatal hypoglycemia as a result of massive pancreatic insulin release and to avoid this hypoglycemic effect, the animals were provided with 5% dextrose solution after 6 h. of alloxan treatment for next 24 h. Induction of diabetes was tested after 72 h. and the animals were allowed one week for the stabilization of blood glucose level (Despande *et al*., 1984; Hedya Jemai *et al.,* 2009S) So, The main aims of this research is to determine the biological effects of the grind of dry olive leaves against diabetes mellitus induced by Alloxan and its complications in domestic male rabbits.

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Material and Methods

A total of 28 healthy local male rabbits, 2-3 month of age and 1200-1500 gm weight was used obtained from different local markets. Animals were divided randomly into to four groups of seven animals each; body weight was considered and housed individually in cages of (50cm × 50cm × 40cm) along the study period of 70 days (from 15 November to January 25 of 2019) including 14 days as an adaptation period.

Experimetnal Design

Animals were divided into 4 groups 7 rabbits to each, Group 1: that getting basal diet free from feed additives and kept as negative control, Group 2: Control group, Diabetic rabbits (treated with Alloxan) fed with Basal diet free from an kept as positive controls. Group 3: Diabetic rabbits (treated with 150mg /km by Alloxan) fed basal diet supplemented with 4% grinding dry olive leaves, Group 4: Diabetic rabbits (treated with 150mg / km by Alloxan) fed basal diet supplemented with 8% of grinding dry olive leaves all group were provided with clean Tap water and feed ad libitum. Along the experimental period the photoperiods was (from 06:00AM to 06:00PM as 12D:12.

Olive Leaves Processing

The olive leaf will collect from different areas of local olive trees clean and dried by electric oven at 80°C for 24 hours, then Ground by use a milled electric grinding machine, the powder mixed with wet pellets (macerated in water) to form pasty material which pass from electric meat warp machine to form a new pellets included Olive leaf constitution. The new feed pellets were mixed with powder of Olive leaves as following formula: 40 gm of Ground Olive leaves plus 960 gm of basal diet (pellets)/ group of (8% Olive leaves /diet) and 80 gm of Grind olive leaves plus 920 gm of basal diet (Pellets) /group of (8% Olive leaves /diet). Animals were weighted at each twice week end in the morning before diet supplement.

Induce Diabetic

Diabetes was induced in overnight fasted rabbits by injection of Alloxan monohydrate dissolved in normal saline (0.9% NaCl) at a dose of 150 mg/kg body weight. Whereas the animals of control group received normal saline only. Alloxan can prompt fatal hypoglycemia as a result of massive pancreatic insulin release and to avoid this hypoglycemic effect, the animals were supplied with 5% dextrose solution after 6 h. of Alloxan treatment for next 24 h. Induction of diabetes was tested after 72 h. and the animals were permitted one week for the stabilization of blood glucose level. At seventh day, animals having a blood glucose level higher than 210 mg/dL were considered diabetic and used for the study Keys, (Al-Bert *et al*., 2014).

The Study Sample and Parameters

Body weights of all animals was taken biweekly to find out the total gain and the changes in body weight along the period of experiment. Blood samples were taken biweekly to study some blood parameters and included Total serum protein concentration, albumin globulin concentrations. Glucagon hormones, glucose concentrations. Triglycerides, Cholesterol Liver enzymes: AST, ALT ALP).

Blood Sampling

Blood samples of all groups were collected directly from the heart by sterile syringe (by heart-puncture) while the animals at alive into sterile vials every two week after starting of experiment period. The region of puncture was sterilized. 3ml of blood samples were taken and kept in sterilized gel tube free of anticoagulant substances, to isolated the serum for measuring liver enzymes (AST, ALT and ALP), albumin, serum total proteins, globulin, glucose, triglyceride,cholesterol and glucagon hormone. Immediately after collection the Sera separation samples of all animals were done by using centrifuge (3000 rpm) for 10 minutes and preserved in freezing at (-16°C -20°C) for later use. The most biochemical tests and blood testing were done at the Public Health Laboratory, College of Veterinary Medicine (Şahin *et al*., 2001).

Chemecal Compostion of Basal Diet

Items	% as fed
Corn	56.19
Soybean meal, CP 44%	34.6
Dicalcium phosphate	1.18
Soybean oil	5.75
Calcium carbonate	1.35
Salt	0.35
Mineral premix2	0.25
Vitamin premix1	0.25
DL-Methionine	0.08
Calculated of composition Metabolize	3200
able energy (Kcal/ Kg) Calcium (%)	0.9
Crude protein (%) Available	20
phosphorus (%) Lysine (%)	0.35
Methionine + Cystine (%)	1.077
Chromium analyzed (ppb)	0.72
	3.96

Table 1: Effect of grind dry olive leaves on body weight in male rabbits induced by Alloxan.

Body weight (gm)					
Period		**After two week**	**After four week**	**After six weeks**	**After eight weeks**
Groups	**n**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Con.Gr	7	1262.14±54.43 ^{a}	1357.28±48. 67 ^{ab} ab±4.34 ^{d}	1468.28 ±37.38 ^{a*d*}	1609.85±41.39 ^{*a*}
0% Gr.	7	1267.85 ±42.11 ^{aa*}	1098.57 ±164.88 ^{b}	1371.42 ±28.00 b ^{b}	1368.42 ±25.32 ^{b*}
4%Gr.	7	1252.85±36.52 ^{a}	1383.14 ±28.95 ^{a**}	1461..42 ±29.84 ^{a*****b*}	1570.71 ±35.95 ^{a*}
8%Gr.	7	1203.57 ±22.24 ^{a}	1355.57 ±37.17 ^{ab}	1455.14 ±35.59 ^{a*}	1583.42 ±24.48 ^{a*}

a,b,c,d, differences are statistically significant within groups marked with different letters on the same line ($p \leq 0.05$) (horizontal comparison).
* point to significant different compared to Control group ($p \leq 0.05$) (vertical comparison).

Data Analysis

The data was analyzed using one-way ANOVA and means were compared using Duncan multiple range test. according to statistical SPSS version 24.

Results and Discussion

The data in table 1. shows that body weight of normal rabbits or,control negative group had record significantly improvement values with age progress through all the experimental periods, while control positive (diabetic) group had revealed significantly ($p \leq 0.05$) lower body weights values compared to normal animals (control negative) as well as the both 4% and 8% treated groups that injected with Alloxan, started from second test after four weeks tend to the end of experiment, However, grind dry olive leaves-treated diabetic rabbits maintained their initial weights during the last 4 week treatment period although at the end of the experiment their body weights were significantly equal to those of normal rabbits. In contrast, with the control positive diabetic rabbits which showed significant ($p \leq 0.05$) loss in body weight when compared to both the normal rabbits and both the 4% and 8% diabetic rabbits at the end of the 4-week experiment despite the deleterious effects of free radicals and oxygen species promoted by induced of diabetic mellitus on the animals that treated by Alloxan this might be due to the protected effects of richness of valuable phenolics compounds in olive leaves and assigned their antioxidant capacity which was not so high, while the antioxidant capacity which was higher than vitamin C and E or pure hydroxyl tyrosol, which is a strong antioxidant (Keys, 1995) and compounds sharing an orthodiphenolic (catecholic) structure possess antioxidant activity (Tuck and Hayball, 2002)

The results of blood serum glucose in table 2, revealed that the control positive (Alloxan) group recorded significant ($p \leq 0.05$) higher mean values (346.75 mg/dl, 398.75, 363.00mg/dl 358.75) in first, second, third and fourth tests respectively in, comparative with normal rabbits in control negative as well as in both olive leaves treated groups 4%, 8% OL groups-injected by Alloxan-especially the 8% olive leaves group which were produced better lowering effect than 4% OL group started from second up to the end of the study these results of current study is closely agree with (Gonzalez M 3) reported that hypoglycaemic effects was results from samples collected of the compounds responsible for this activity was oleuropeoside, which revealed its effects at a dose of 0.16 g kg^{-1}. This compound also established antidiabetic effects in animals with alloxan-induced diabetes. The hypoglycaemic action of this compound might be result from one of di-mechanism: the potentiation of glucose-induced insulin release, or by increased peripheral uptake of glucose (Sahin Selin and Mehmet 2017).

From data illustrated in table 3, there was a significance increase in blood serum glucagon concentration ($p \leq 0.05$) of male rabbits of Alloxan induced diabetic group through all tests of the study period (91.51 ±2.73, 93.51 ±2.70, 97.04 ±2.24, 98.33 ±1.94 g/mL) respectively as compared with control negative group as well as the both receiving 4% and 8% olive leaves group and last group recorded

Table 2: Effect of grind dry olive leaves on glucose levels in male rabbits induced by Alloxan.

Blood glucose enzyme Levels (mg/dL)					
Period		**After two week**	**After four week**	**After six weeks**	**After eight weeks**
Groups	**n**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Con.Gr	7	89.25±2.95 ^{c}	94.50±3. 27 a ******* ab±4.34 ^{d}	87.75 ±3.90 ^{c}	94.00±2.82 ^{c*}
0% Gr.	7	346.75 ±21.72 ^{a*}	398.75 ±20.86 ^{a*}	363.00 ±15.14 ^{b*a*}	358.75 ±15.42 ^{a*}
4% Gr.	7	105.25±2.28 ^{b}	167.25 ±62.25 ^{b*}	176..42 ±62.25 ^{b*}	161.50 ±64.83 ^{b*}
8% Gr.	7	100.00 ±2.61 ^{b*}	97.50 ±0.64 ^{b}	93.50 ±3.10 ^{c*}	95.75 ±1.70 ^{c*}

a,b,c,d, differences are statistically significant within groups marked with different letters on the same line ($p \leq 0.05$) (horizontal comparison).
* point to significant different compared to Control group ($p \leq 0.05$) (vertical comparison).

Table 3: Effect of grind dry olive leaves on Glucagon hormone levels in male rabbits induced by Alloxan.

Blood glucagon hormone Levels pico-gram/mL					
Period		**After two week**	**After four week**	**After six weeks**	**After eight weeks**
Groups	**n**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Con. Gr	7	48.14±2.80 ^{d}	42. 78 ±1.11 ^{d}	54.44 ±1.01 ^{c*}	47.03±1.49 ^{d}
0% Gr.	7	91.51 ±2.73 ^{a*}	93.51 ±2.70 ^{a*}	97.04 ±2.24 ^{a*}	98.33 ±1.94 ^{a*}
4% Gr.	7	77.75±1.55 ^{b*}	75.48 ±2.10 ^{b*}	76..23 ±0.51 ^{bb*}	76.85 ±2.08 ^{b*}
8% Gr.	7	64.74 ±1.02 ^{c*}	63.03 ±1.01 ^{c*}	57.14 ±1.21 ^{d}	65.08 ±1.59 ^{c*}

a,b,c,d, differences are statistically significant within groups marked with different letters on the same line ($p \leq 0.05$) (horizontal comparison).
* point to significant different compared to Control group ($p \leq 0.05$) (vertical comparison).

the better reducing comparative with receiving 4% olive leaves group, It worthy to mention that the Glucagon is created by enzymatic cleavage of the proglucagon precursor by prohormone-convertase to yield fully processed glucagon of 29 amino acid (Solloway *et al.,* 2015), donates to keep of euglycemia by elevate hepatic glucose production via fasting by prompt of glycogenolysis and glucogenesis (Ramnanan *et al.*, 2011). Dipeptidyl peptidase (Dpp4) persists as a cell surface membrane-bound peptidase which is expressed in many tissues including the gastro-intestinal system, liver, kidney, the vascular epithelium and the exocrine pancreas, then transfer intracellular signals to transduction pathways (Röhrbon *et al*., 2014). Based on the obtained results which was closely agreement with Albert *et al.,* 2014, who revealed that there was a significance rise in blood serum glucagon values ($p \leq 0.05$) of male rabbits in Alloxan prompted diabetic group during the experiment time, On the same context some investigators found that there was a significant elevation in blood serum glucagon concentration in type 2 diabetic patient, (Ceriello *et al.,* 2016) and in accordance with this results with of (Lee *et al*. 2011), who showed a significant rise in mice streptozotocin induced diabetic That,can cause the prompt of production of new glucose process glucogenolysis proliferation of glucose values circulatory plasma (Moran and Dailey, 2011).

Table 4, data in this table declined that the cholesterol values in Alloxan group was recorded significant ($p \leq 0.05$) higher increasing in mean values in cholesterol levels started from beginning till to the end of experiment, in comparative with negative control and both traded group that dealing by Alloxan and treated with grind dry olive leaves particularly 8% OL group that recorded beter reduction than 4% OL group This finding is consistent with (Tadoa, 2005), who found that diabetes-induced by Alloxan, in rabbits caused lack of insulin in, Led to a decrease in the level of ApoE mRNA and increase the level of blood cholesterol (A. Lenich, 2010), such trend may suggested the increasing of cholesterol as a result of increase the activity of Cholesterol Transferaseacyl which responsible for the absorption of cholesterol from the intestine and its activity increase with the full deficiency of insulin (Hori, M., 2004) Acyl) current study was also agreed with (Kaleem, 2005). Who explain that olive leaves affects the manufacturing process, led to prevent cholesterol metabolize and thus reducing its level in the body.

The data shown in table 5, that the Triglyceride values of Alloxan group recorded significantly ($p \leq 0.05$) higher increasing in comparative with all groups during all periods of study. Furthermore the 4% and 8% olive leaves treated groups show numerically and significantly ($p \leq 0.05$) higher values in compared with normal rabbits in control negative group started from first to final tests after eight weeks of experiment, this finding is consistent with (Bilbis, 2012), who reported that absence or low insulin values through this diabetic will leads decrease in the lipoprotein lipase values that in charge for transforming the triglycerides into fatty acids and glycerol in same trend (Tadoa, 2005). Deficiency described that low level of insulin leads to stimulating hydrolysis of fats in the adipose tissues, leading to increased broadcast of fatty acids from the fatty tissue

Table 4: Effect of grind dry olive leaves on Cholesterol levels in male rabbits induced by Alloxan.

Blood cholesterol Levels (mg/dL)					
Period		**After two week**	**After four week**	**After six weeks**	**After eight weeks**
Groups	**n**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Con. Gr	7	103.45±16.83 ^{b}	81.90 ±5.61 ^{c}	85.06 ±4.22 ^{c}	75.98±2.08 ^{c}
0% Gr.	7	169.75 ±4.53 ^{a*}	178.91 ±2.83 ^{a*}	177.61 ±2.89 ^{a*}	165.39 ±13.88 ^{a*}
4% Gr.	7	112.09±6.29 ^{b*}	105.88 ±11.37 ^{b}	112.40 ±8.217 ^{bb*}	94.24 ±1.76 ^{c}
8% Gr.	7	97.25 ±1.05 ^{b}	100.79 ±4.32 ^{bc*}	100.56 ±2.59 ^{b*}	113.02 ±1.76 ^{d*}

a,b,c,d, differences are statistically significant within groups marked with different letters on the same line ($p \leq 0.05$) (horizontal comparison).
* point to significant different compared to Control group ($p \leq 0.05$) (vertical comparison).

Table 5: Effect of grind dry olive leaves on Triglycerides levels in male rabbits induced by Alloxan.

Blood Triglycerids Levels (mg/dL)					
Period		**After two week**	**After four week**	**After six weeks**	**After eight weeks**
Groups	**n**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Con. Gr	7	57.80±1.64 [b]	61. 39 ±0.98 [c]	64.46 ±1.87 [c]	66.74±1.45 [c]
0% Gr.	7	92.48 ±2.50 [a]	94.30 ±1.91 [a*]	93.26 ±1.42 [a*]	92.26 ±2.01 [a]
4% Gr.	7	61.95±7.89 [b]	71.64 ±0.94 [b*]	64.45 ±0.037 [bb*]	76.66 ±2.13 [b*]
8% Gr.	7	64.93 ±2.09 [b*]	63.57 ±1.51 [c]	62.96±2.42 [b]	65.06 ±1.59 [c]

a,b,c,d, differences are statistically significant within groups marked with different letters on the same line ($p \leq 0.05$) (horizontal comparison).
* point to significant different compared to Control group ($p \leq 0.05$) (vertical comparison).

Table 6: Effect of grind dry olive leaves on AST enzyme activity in male rabbits induced by Alloxan.

Serum total Ast enzyme (Unit/L)					
Period		**After two week**	**After four week**	**After six weeks**	**After eight weeks**
Groups	**n**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Con. Gr	7	81.83±4.60 [d*]	89. 18 ±1.61 [d]	87.89 ±5.96 [c*]	88.23±4.23 [c]
0% Gr.	7	186.17 ±3.12 [a*]	184.31 ±4.08 [a]	186.44 ±3.87 [a*]	195.80 ±1.09 [a]
4% Gr.	7	110.47±7.81 [b*]	127.96 ±7.43 [b]	115.20 ±1.62b [***b*]	111.73 ±1.42 [b*]
8% Gr.	7	104.39 ±3.67 [c*]	103.97 ±5.47 [c*]	106.60±2.56 [c]	102.80±2.42 [c*]

a,b,c,d, differences are statistically significant within groups marked with different letters on the same line ($p \leq 0.05$) (horizontal comparison).
* point to significant different compared to Control group ($p \leq 0.05$) (vertical comparison).

and Triglycerides in the liver.

The results obtained in table 6, showed that there was significant increasing ($p \leq 0.05$) of AST enzyme activities in blood serum of local male rabbits injected with Alloxan (0%) (OL) group in comparative with normal rabbits of negative group which recorded the lowest values,as well as the both receiving of 4% OL and 8% OL groups that injected with 150m/gm BW of Alloxan and the animals received 8% OL shown better decreasing than 4% OL group. The reduction in the means of the AST enzyme activities at the last test was (102.80±2.42 U/l) in compared with the positive control 195.80 ±1.09 U/l started from first to last test of experiment. This result is in agreement with some investigators whom reveled (Moran *et al*., 2011; Nannipieri, 2005; Ramnanan *et al*., 2011) that Increased activities of liver enzymes such as AST, ALT and ALP are associated with hepatocellular injury accompanied with insulin resistance, metabolic syndrome and type II diabetes A significant decrease in AST levels occurred from the start to the end of the month in the diabetic rabbits treated with olive leaves. The decrease in the liver enzymes may be due to the presence of some active constituent like flavonoids and terpenoids in the OL which have hepatoprotective effect against hepatotoxins (Miles *et al*., 2005**)**.

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