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Ameliorating Effect of Garden cress on Diabetic Rats

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ABSTRACT

The aim of this investigation was to determine histopathological changes and some biochemical activities of two concentrations of Garden cress (*Lepidium sativum* L.) on normal and diabetic rats for 30 successive days . Forty mature rats of body weight (150-180 gm) were divided into 4 equal groups 10 rats each.(G1) control ,(G2) diabetic control group injected intrapretonial by alloxan in a dose 150 mg/kg. b.wt ,(G3& G4) diabetic rats fed on basal diet mixed with 1% & 2% dried Garden cress (*Lepidium sativum* L.) respectively for 30 successive days. Blood samples were collected to separate serum to determine some biochemical parameters , some oxidative markers , relative organs weights and histopathology of kidney and pancreas were conducted at the end of the experiment .

Results revealed that there were a significant decrease of serum levels of AST, ALT, ALP, cholesterol, triglyceride ,urea and glucose as well as MDA than diabetic control groups, on the other hand result showed no effect on albumin ,globulin and A/G ratio. While catalase enzyme activity showed a significant increase than control diabetic rats . The relative organs weight in (G3)and (G4) returned toward normal values. Conclusively , feeding of Garden cress at both concentrations for diabetic rat had a beneficial practical tool to minimize the effect of diabetes without any adverse effect on metabolic parameters and organs weight of rats.

INTRODUCTION

Cazzola and Estaro, 2014, Ping, et al 2010 and Mosaad and Abd Allah 2004 mentioned that diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia associated with disturbances of carbohydrate, lipid and protein metabolism & deficiency in insulin secretion or function. In addition, various biochemical disorders associated with vascular complications such as hyperlipidemia and oxidative stress frequently coexist with diabetes mellitus (DM).. It is one of the most frequent chronic diseases affecting millions of people globally leading mortality worldwide particularly in developing countries of**.** It is considered a chronic metabolic disease that causes several complications **(Lu,et al ,2012 and Prabhakar and Doble,2011).** Traditional medicines play an important role in health such as Cinnamon and Garden cress seeds are members of a list containing 150 plants which are safer and more effective drugs in the treatment of DM **(Ranilla et al, 2010 , Eddouks et al ,2005 and El- demerdash et al., 2005)**

In spite of the fact that insulin has become one of the most important therapeutic agents and it has been making efforts to find insulin substitutes for synthetic or plant sources for the curing from diabetes. Many medicinal plants have

act as a therapy especially in poor areas where insulin is not readily available (**Sanchez et al., 1994**). Garden cress has been widely used to treat a number of diseases in traditional medicine. *L. sativum* is being cultivated as vegetable in some counries (**Nadkarni, 1976**) .It is grown in many regions of Saudi Arabia, such as Hijaz, AL-Qaseem, and the Eastern Province and is called "Habel Rashaad" or "Thufa" (**Rahman et al., 2004 , Ageel et al., 1987and Kloos, 1976 and Nadkarni, 1976**) and can serve as raw material for functional foods (**Snehal et al., 2012; Rehman et al., 2012).** Garden cress seeds contain Vit. C, carbohydrate, protein, fatty acid, some types of Vit.B ,glycoside, essential aromatic oils (**Nadakarni, 1976***)*and mucilage in its dry seed coat (**Prajapati et al, 2014**) .Garden cress seed have some medicinal uses such as(bronchodilator, anti-inflammatory, analgesic, anticoagulant , anti rheumatic , antihypertensive and diuretic activities **(Rehman et al., 2011, Patel et al., 2009,Mali et al., 2008**, **Paranjape and Mehta, 2006**, **Maghrani et al., 2005**,**Al-Yahya et al., 1994&Ahsan et al., 1989**). It also has anti hyperglycemic properties which help to control glucose level in diabetics (**Hassan et al,2015 ,Behrouzian et al ,2014 and Patole, 1998**). The seeds of *L. sativum* are, diuretic, tonic, demulcent, carminative, galactagogue, and emmenagogue, are used to induce an abortion,and also possess antibacterial and antifungal properties **(Bansal, et al 2012).**

The present study aimed to estimate the stimulation of the pancreas by the anti diabetic effect of 1 and 2% of garden cress seed (*Lepidium sativum*) and histopathological changes in male rats with alloxan induced diabetes.

MATERIALS AND METHODS

Materials:

Plants: Garden cress (*Lepidium sativum*) was obtained from Faculty of Agriculture farm. Plants were identified by Faculty of Pharmacy, Department of Pharmacognosy , Cairo University, Egypt. The plant was air dried then grinded and kept in a glass bottles till mixed with ration.

Chemicals: Alloxan tetrahydrate pure (99%) was obtained from Sigma Company (Germany)

Experimental animals:

Forty male Albino rats of an average body weight 150-180g were obtained from the laboratory of animal colony, Helwan , Cairo, Egypt. Rats were fed on standard ration and water supply was given *ad-libtum*.

Preparation of diabetic rats:

Alloxan tetrahydrate pure (99%) was obtained from Sigma Company (Germany) was dissolved in distilled water and injected to thirty rats intrapretoneally at a dose of 150 mg/Kg. b. wt according to (**Desai and Bhide,1985**).

Experimental design:

Forty mature rats were divided into 4 equal groups. All groups were fed the experimental basal diet with or without the tested plant for 30 successive days as follows:

Group (1) : Control group was fed on basal diet.

Group (2) : Diabetic rats were fed on basal diet.

Group (3): Diabetic rats were fed on basal diets mixed with *Lepidium sativum* in Concentration of 10g/kg ration (1%).

Group (4): Diabetic rats were fed on basal diets mixed with *Lepidium* sativum in concentration of 20 g/kg ration (2%).

I - Sampling:

A- Blood samples:

Blood samples were taken from retro-orbital venous plexus into clean, sterile tubes for separation of serum to determine some biochemical parameters as follows: Serum aspartate aminotransferase (AST) and serum alanine transferase (ALT) were determined according to **Reitman and Frankel (1957)** and alkaline phosphatase (ALP) were assayed according to **Roy (1970)**, Malondialdehyde (MDA) according to **(Ohkawa et al., 1979**) &**Tietz (1986**). Total cholesterol (T.C.) and triglycerides (T.G) described by **Watson** (**1960**) and **Whalerfeld (1974**) respectively. Urea and glucose were measured spectrophotometrically according to **Reises et al., (1965)** and **Trinder, (1969)** respectively. Total protein (TP) and albumin (ALb) were determined according to method described by **Weichselbaum** (**1946)** and **Doumas et al., (1971)** respectively while globulin (Glob) was calculated by substracting of albumin levels from total protein levels and A/G ratio was calculated mathematically. Activity of catalase enzyme was determined as reported by **Aebi, (1974**).

B-Relative organs weight:

Rats of all groups at the end of the experiment were weighed & sacrificed. Organs (liver, kidney and spleen) were taken and weighed to calculate the relative organs weights (the organ/body weight per 100g ratio)

II- Histopathological examination

The histopathological samples (kidney and pancreas) were fixed in 10% formalin. Fixed tissue samples were processed routinely by the paraffin embedding technique. Sections at 4 micron thickness were stained with Hematoxylin and Eosin (**Bancroft and Gamble,2002**)

III- Statistical analysis:

Parametric data were statistically analyzed by using ANOVA test and comparison between means were preformed using Duncan Multiple range test for comparative of means using **SPSS version 14 (2006**). Results were represented as (Mean $\pm$ S.E.).

RESULTS AND DISCUSSION

Diabetes mellitus is a chronic, systemic and metabolic disease manifested by hyperglycemia. The cumulative effects of these metabolic derangement lead to cell damage and circulatory changes. Other clinical symptoms of diabetes include nephropathy, retinopathy and liver dysfunction (**Wild et al., 2004**). Medicinal plants are the most common forms of complementary and alternative medicine (**Graham et**

al., 2005). They can used without medical prescription because they must be safe for human (**Ernst, 2006**). Many studies have reported that most plants contain glucoside, alkaloids, terpenoids, flavonoids, and carotenoid which may be implicated as having anti diabetic and antioxidant effect against many toxic materials (**Shati and Alamri 2010 and Loew and Kaszkin, 2002**). Anti hyperglycemic effects of these plants may be due to their ability to restore the function of pancreatic tissues by increase in insulin output or inhibit the intestinal absorption of glucose (**Oliver – Bever, 1986**).,

The present study was carried out to investigate the ameliorative effect of available herb as *Lepidium sativum* on some biochemical parameters ,antioxidant markers and histopathology in normal and diabetic rats. The obtained results in (**Table 1**) showed that rats feed on a ration mixed with *Lepidium sativum* 1% and 2% produced a significant decrease in AST, ALT and ALP .Our results are in agreement with (**Safaa et al 2016 and Datta et al. 2002)** who studied the hypoglycemic and antioxidant effects of *L.S.* extracts in diabetic rats and reported that the above mentioned biochemical parameters are restoring them nearly to the normal levels of control group. **Thnaian Althnaian**, **2014; Chauhan et al.,2012& Al Hamedan ,2010** reported that the activities of AST and ALT remained comparable to that of control groups. The increase in ALT activity of rats fed high cholesterol diet comparing to control . (**Table 1)** indicate that diabetic rats showed a significant elevation in T.C and T.G. levels comparing with control group while rats fed *L.S.* significantly decrease in T.C and T.G concentrations compared to diabetic control group. Our data in agreement with (**Thnaian Althnaian , 2014Jelodar et al**. **2007 and Khan** and **Balish 2001**) who found that Garden cress decreased serum levels of total cholesterol and triglyceride of diabetic rats . The effect of feeding 1% a n d 2 % *L.S.* to normal and diabetic rats are recorded in **(Table 1)** . Significant reduction in urea level in groups fed 1% *L.S.* than diabetic rats. These results are consistent with (**Safaa et al 2016 and Datta et al. 2002)** who recorded that *L.S.* are effective in reducing urea and uric acid levels and have the ability to decrease inflammation and oxidative stress they are also rich in vitamins B complex, A& C .

Glucose level **(Table 1**) it was reduced significantly in Garden cress treated rats as compared to diabetic group rats . This data was in agreement with **khalid et al. (2013**),who recorded that effect may be attributed to their phenolic content which scavenging of free radical and consequently the diseases. Also, it has an inhibitory effect on the reductase enzyme which played a role in catalyzing the reduction of glucose to sorbitol which cannot diffuse out of cell membrane. **(Bafeel and Ali, 2009 and Jain et al. 2009).**

Results in(**Table 2)** indicated that feeding *L.S* to diabetic rats at concentrations 1% & 2% for 30 successive days exhibited no marked changes in the level of total protein, albumin, globulin and A/G. Our finding are in agreement with (**Thnaian Althnaian ,2014 ; Al-Taee 2013 Kholif and El-shewy,2004)** who found that the administration of *L.S.* showed insignificant differences in serum total proteins,

albumin, globulins in rats and rabbits fed on cholesterol diet. However, the findings of our study disagreed with that of **Bafeel and Ali (2009)** who reported that the oral administration of *L.S.*. in different concentrations to rats showed an increase in serum total protein, while albumin increase only at high concentration group.

Table(3) showed the antioxidant parameters after feeding 1% *&2% Lepidium sativum* to diabetic rats . The results revealed significant decrease MDA levels while catalase activity was significant increased in groups fed the tested plants compared to diabetic control rats. Our results consistent with **(Safaa et al,2016 Ghosh et al ,2015, Yae et al., 2010 and Dugoua et al, 2007)** who said that *Lepidium sativum* contains high level of phenolic content that cause scavenging of free radicals which inhibit lipid peroxidation . In addition, our results showed elevation in MDA and decrease in antioxidants enzyme activity due to ameliorating afterof *L.sativum*. Antioxidant compounds like phenolic acids, terpenoids and flavenoids scavenge the free radicals and thus inhibit the oxidative stress **(Kintzios et al.2010). Table (4)** illustrated the effect of feeding tested plant *L.sativum* in concentration 1% & 2% for 30 successive days on relative organ weight (kidney, liver and spleen) of normal and diabetic rats. The total body weight (g) in G2 showed a significant decrease as a result of diabetes, whereas it increased with received *Lepidium sativum* .This result is consistent with that of **(Beejmohun et al,2014 and Safaa et al,2016).** In our study, weight of kidney, and liver in all groups showed a significant increase as a result of diabetes . Restoring the normal organs weight as a result of treating diabetic rats with cress seed is consistent with **(Elgawish and Abdelrazek 2014).**

The histology of the pancreas (**Figure 1**) reveals that the alloxan-induced diabetic rats (diabetic control rats) showed marked degenerative changes of its acini as well as sever cellular degeneration and necrosis of affecting islets of Langerhans and marked edema between pancreatic acini with marked degeneration and necrosis of some acini(**Figure 2**). Further improvements were observed in rats that had been treated with 2%of LS, which indicated an improvement in the pancreas as the concentration of the *Lepidium sativum* increased.

The histology of the kidneys in diabetic rats group (**Figure 3)**& **(Figure 4)** Renal medulla showed congestion of interstitial blood vessels and patches of mononuclear cell infiltration affect renal medulla, as well as marked degenerative changes affect epithelial lining of renal tubules and renal cortex with sever congestion of most interstitial blood vessels as well as congestion of glomerular tuft with segmentation, hyper cellularity and mesangeal expantium, renal tubules have degenerative changes. However, as the concentration of LS increased from 1% to 2%, noticeable improvements in the tissue were kidneys showing renal medulla with few eosinophillic structure less and epithelial lining renal tubules showing some degenerative changes(**Figure 5)**. The histological studies showed altered pathological changes in the tissues of kidney and pancreas as a result of diabetes in the positive control group similar (**Ping et al ,2010& Al-Malki and El Rabey, 2015**)

whereas treating the diabetic rats with cress seed directed the tissues nearly to the normal conditions. **Ullah et al. (2012)** stated that Garden cress and cinnamon significantly improving the urea, creatinine, uric acid, urinary protein levels, and histopathological changes of the kidneys. In addition, our result is in agreement with that of **Al-Malki and El Rabey** (**2015**).

Conclusion:

It could be concluded that both cress seed (*Lepidium sativum*) seeds succeeded in controlling hyperglycemia in rats with alloxan induced diabetes. These seeds also ameliorated all biochemical parameters and kidney and pancreas functions and tissues and restored them to the normal state. These effects may be due the antioxidant activity of both phenolic and flavonoids phytochemical constituents of these seeds

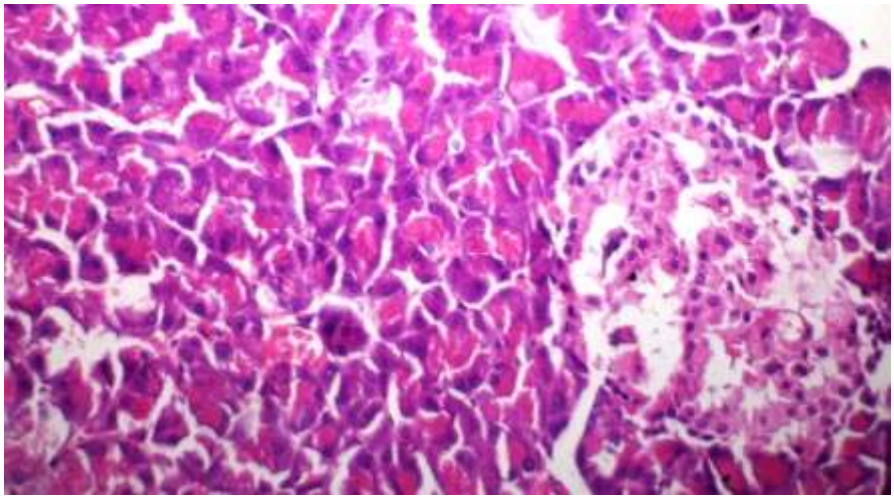

Fig 1:Pancreas showing marked degenerative changes of its acini as well as sever cellular degeneration and necrosis of affecting islets of Langerhans (H&E x400)

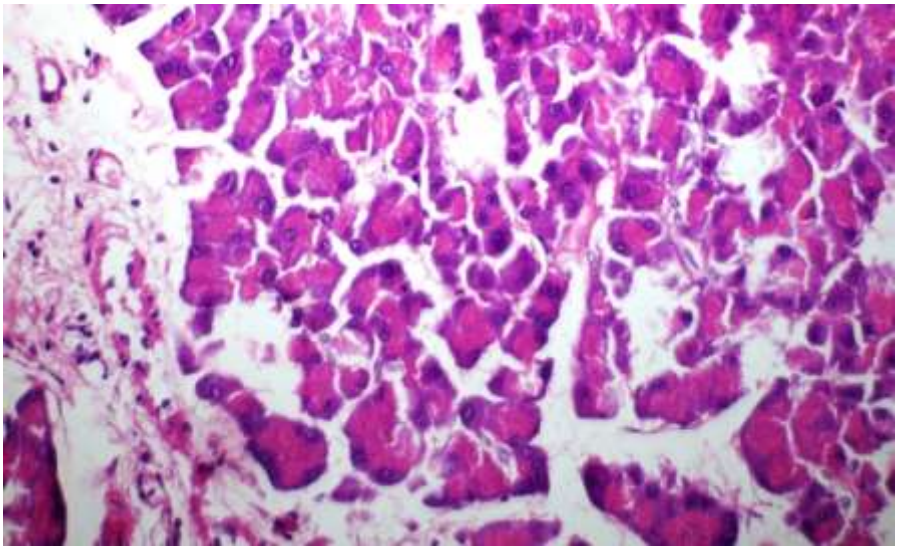

Fig 2: pancreas has marked edema between pancreatic acini with marked degeneration and necrosis of some acini (H &E x400)

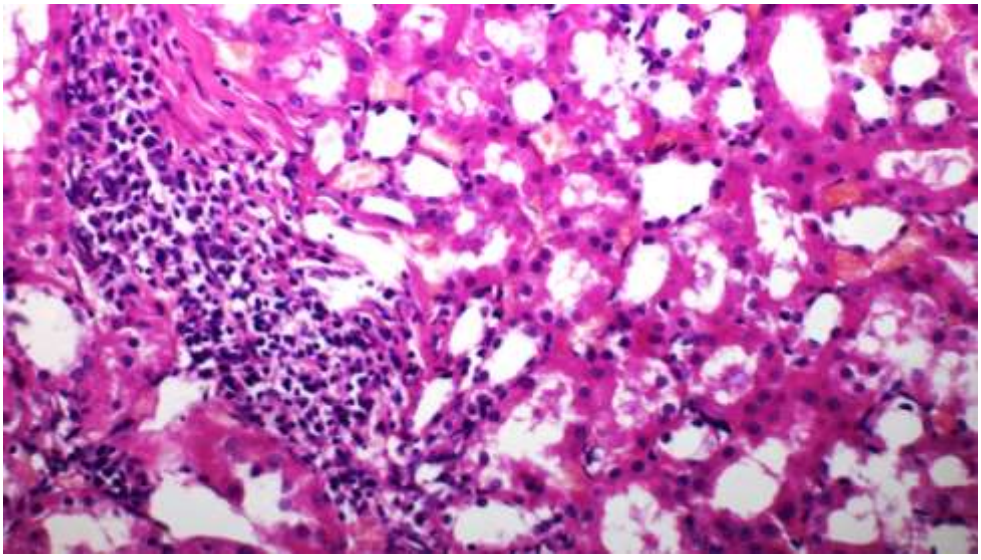

Fig 3: Renal medulla showed congestion of interstitial blood vessels and patches of mononuclear cell infiltration affect renal medulla, as well as marked degenerative changes (H &E x400)

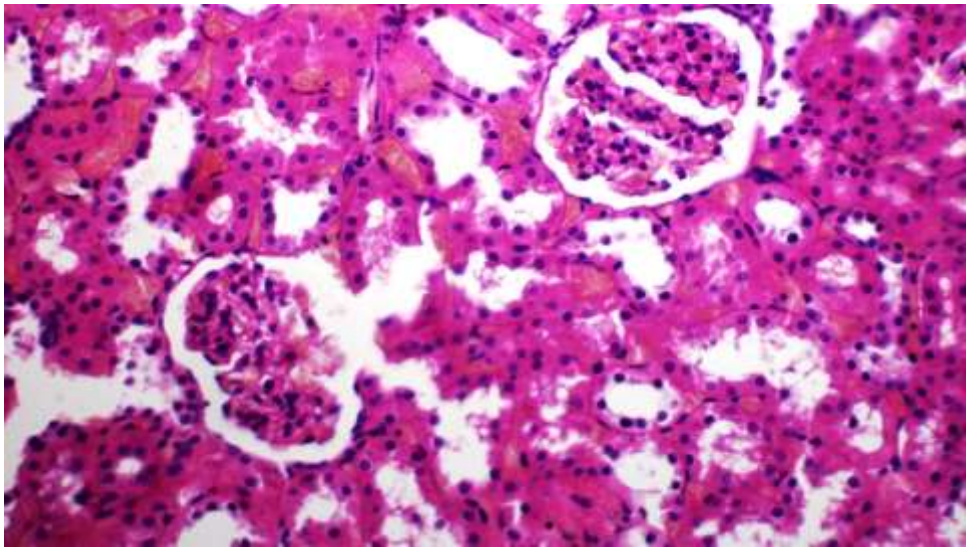

Fig 4: kidney showing renal cortex with sever congestion of most interstitial blood vessels as well as congestion of glomerular tuft with segmentation, hyper cellularity and mesangeal expantium. renal tubules have degenerative changes (H & E X400)

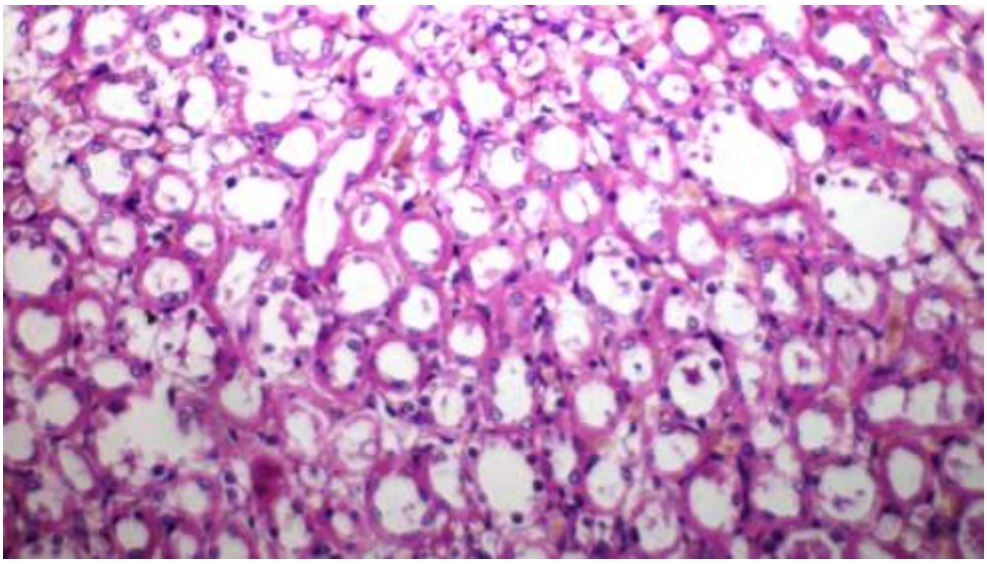

Fig 5: kidney showing renal medulla with few eosinophillic structure less and epithelial lining renal tubules showing some degenerative changes(H &EX400)

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Table (1): Effect of 1% and 2% of *Lepidium sativum* mixed with ration for 30 successive days on some biochemical parameters in normal and diabetic rats (n =10)

Groups	AST (U/L)	ALT (U/L)	ALP. (U/L)	T.C. (mg/dl)	T.G. (mg/dl)	Urea (mg/dl)	Glucose (mg/dl)
control	25.263 ±0.214^{a}	12.35 ± 0.594^{a}	41.28 ±0.608^{a}	61.98 ±2.71^{a}	76.72 ±0.52^{a}	36.23 ±0.043^{a}	104.7 ±2.922^{a}
Diabetic control	48.94 ±0.193^{b}	26.62 ±0.162^{b}	81.039 ±1.57^{b}	99.077 ±3.375^{b}	163.14 ±1.5^{b}	39.05 ±0.877^{b}	211.13 ±3.6^{b}
Diabetic rats Fed 1% *L.S*	24.31 ±0.2^{c}	9.5 ±0.37^{c}	34.28 ±0.29^{c}	92.34 ±0.18^{c}	65.442 ±2.2^{a}	29.213 ±0.15^{c}	110.32 ±0.17^{c}
Diabetic rats fed 2% *L.S*	43.268 ±0.219^{d}	15.4 ±0.416^{d}	45.518 ±0.163^{d}	87.63 ±2.55^{b}	52.3 ±0.21^{c}	27.212 ±0.189^{a}	130.6 ±0.134^{d}

Mean± SE . Means with different superscripts in the same column are significantly (P<0.05) different .

Table (2): Effect of 1% and 2% of *Lepidium sativum* mixed with for 30 successive days on protein profile in normal and diabetic rats (n = 10)

Groups	T.P (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G
Control	6.44 ± 0.13^{a}	3.57 ± 0.099^{a}	2.89 ± 0.125^{a}	1.224 ± 0.027^{a}
Diabetic Control	6.5 ± 0.245^{a}	4.679 ± 0.117^{b}	1.71 ± 0.146^{b}	2.956 ± 0.332^{b}
Diabetic rats Fed 1% *L.S*	6.21 ± 0.04^{ab}	3.19 ± 0.101^{cd}	1.99 ± 0.08^{b}	2.21 ± 0.133^{bc}
Diabetic rats Fed 2% *L.S*	$6.36\ 0.175^{a}$	3.812 ± 0.13^{bc}	1.922 ± 0.219^{b}	1.67 ± 0.12^{acd}

Mean±SE . Means with different superscripts in the same column are significantly ($P<0.05$) different.

Table (3): Effect of 1% and 2% of *Lepidium sativum* mixed with for 30 successive days on some oxidative markers in normal and diabetic rats (n =10)

Groups	MDA (nmol)	Catalase (mmol)
control	7.833 ± 0.28^{a}	196.12 ± 1.1^{a}
Diabetic control	11.4 ± 0.337^{b}	110.8 ± 3.2^{b}
Diabetic rats fed 1% L.S	8.716 ± 0.31^{b}	134.02 ± 3.834^{abc}
Diabetic rats fed 2% *L.S*	7.982 ± 0.32^{b}	124.8 ± 2.135^{c}

Mean±SE .Means with different superscripts in the same column are significantly ($P<0.05$) different.

Table (4): Effect of 1% and 2% of *Lepidium sativum* mixed with for 30 successive days on relative organs weight in normal and diabetic rats (n=10)

Groups	Body weight (g)	Kidney (g)	Liver (g)	Spleen (g)
control	206.6 ±3.06^{a}	0.721 ±0.006^{a}	2.77 ± 0.033^{a}	0.196 ±0.008^{a}
Diabetic control	194.3 ± 4.28^{b}	0.89 ±0.022^{b}	2.41 ±0.099^{a}	0.364 ±0.02^{b}
Diabetic rats fed 1% *L.S*	202.32±2.131^{a}	0.763±0.07^{c}	2.83±0.25^{a}	0.242±0.009^{c}
Diabetic rats fed 2% *L.S*	200.7±2.01^{ab}	0.799 ±0.021^{c}	2.79 ±0.16^{a}	0.252 ±0.019^{c}

Mean±SE , Means with different superscripts in the same column are significantly ($P<0.05$) different

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