Study the Effect of *Lepidium sativum(*garden cress) and *Glycyrrhiza glabra* (Licorice)on Alloxan-Induced Diabetes in Male Rats

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Abstract:

Diabetes is a complex metabolic disorder affecting the glucose status of the human body. Chronic hyperglycaemia related to diabetes is associated with end organ failure. The aim of this study to estimate the effect of *Lepidium sativum(*garden cress) and *Glycyrrhiza glabra* (Licorice)on bodyweight, feed intake, lipid profiles, renal, hepatic function markersand the glucose levels in diabetic rats. Forty two (42) adult male albino rats (Sparague Dawley Strain), weighing 150 ± 5 g, were used. Rats of diabetic group were equally divided into 6 subgroups each one include 6 rats. The first group received basal diet as negative control group for 28days.Body weight, lipid profile & renal function (urea, uric acid creatinine) ALT & AST activities in addition to glucose and histological structure of liver and pancrease were estimated .Data showed that diabetic rats without treatments significantly increased liver enzymes and lipid profile concentration compared with negative control, while significantly decreasing final body weight and HDL; meanwhile treatment with *Lepidium sativum(*garden cress) , *Glycyrrhiza glabra* (Licorice) and their mixture significantly decreased the lipid profile , liver enzymes whereas administration of herbal powder and its mixture significantly ameliorated these hepatic and pancreatic structures.So, Treatment with *Glycyrrhiza glabra* (Licorice) followed by herbal mixture and *Lepidium sativum(*garden cress) improved diabetes and its associated metabolic problems in different degrees. Also they might be a safe combination on the organs whose functions were examined, as a way to surmount the diabetes state; and it has a distinct anti-diabetes effect.

Keywords: Diabetic rats, kidney functions, hepatic function ,lipid profile, *Lepidium sativum* (garden cress), Licorice(*Glycyrrhiza glabra)*

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by total or partial deficiencies in insulin secretion and/or insulin function associated with hyperglycemia and alteration in the metabolism of carbohydrate, protein, and fat (**ADA, 2008**). Diabetes mellitus (DM) is one of the most common chronic conditions affecting millions of people all over the world leading to morbidity and mortality worldwide particularly in developing countries **(Roden and Shulman, 2019).** Approximately 537 million adults between the ages of 20-79 years had diabetes mellitus worldwide and is anticipated to surge to 643 by 2030 and 783 million by 2045 (**Chan , 2020).** The uncontrolled diabetes mellitus may lead to severe clinical consequences such as retinopathy (eye disease), nephropathy (kidney disease), neuropathy (nerve disease), cardiovascular disease, and other diabetes-related health problems **(Magliano, 2021).**

Lifestyle modifications including diet and exercise, anti-hyperglycemic drugs, and insulin are the main modes of treatment for diabetes. The key objective of all diabetic treatment is to combat hyperglycemia and to maintain the blood glucose level as near to normal as possible **(Chatterjee *et al.*, 2017).** However, many of the used diabetes synthetic drugs have side effects and may develop drug resistance **(Gastaldelli *et al.*, 2017).** Plants play an essential role in medicines. Medicinal plants have cultural acceptance , compatible, adaptable and effective with fewer side effects **(Bahmani *et al*., 2015).**

Glycyrrihiza glabra, commonly known as licorice, belongs to the family of Leguminosae and has been used as a flavoring agent and medicinal remedies for years. Glycyrrghiza is derived from the ancient Greek term *glykos* meaning sweet , and *rihza*, meaning root. Medicinal parts used are roots and Rhizome. It contains active components such as glycyrrhizin, glycyrrhetic acid, flavonoids, isoflavonoids, and chalcones (**Sharma *et al.*, 2020).**

Licorice root is commonly used in traditional medicine, particularly for gastric and duodenal ulcers, dyspepsia and allergenic reactions. Licorice components also exhibit anti-arthritic, anti-arrhythmic, anti-bacterial, anti-viral and steroid like anti-inflammatory activity (**Kalaiarasi et al., 2009 and Batiha *et al*., 2020**).

Leptdium sativum L., commonly known as garden cress, is an annual herb belonging to Brassicaceae (Cruciferae) family that is native to Egypt and West Asia. Garden cress is also known as garden pepper cress, pepper grass, or pepperwort. Seeds, leaves, and roots are used in treating various human diseases. Garden cress seeds are a rich source for

proteins, dietary fibers, omega-3 fatty acids, iron, and other essential nutrients and phytochemicals (**Sharma *et al.*, 2012 and Mishra *et al.*, 2017).**

The aim of this work was to determine the effects of *Lepidium sativum L.* and *Glycyrrhiza glabra* on biological parameters, liver enzymes, kidney functions and histological structure of liver and pancreas of alloxan-induced diabetic rats.

Materials and Methods

Material

Source of garden cress and licorice roots

Garden cress (*Lepidium sativum*) and licorice roots (*Glycyrrhiza glabra*) were obtained from Harraz, Cairo, Egypt. Alloxan to induce diabetes in rats was purchased from El-Gomhoria Company for Drugs, Chemical and Medical Instruments, Giza, Egypt.

Alloxan

Alloxan, which is chemically known as 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione is an organic compound which was obtained from Al-Gomhoria Company for Drugs, Chemical and Medical Instruments, Giza, Egypt.

Animals

Forty two (42) adult male albino rats (Sparague Dawley Strain), weighing 150 ± 5 g, were used. The animals were obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt. The animals were housed in cages and received normal rat chow and tap water *ad-libitum* in a constant environment with a 12h light and 12h dark cycle. The animals were fed on the basal diet and were under observation for 7 days for adaption prior to the start of the experiments.

Diet and chemicals kits

The basal diet were prepared according to the following formula as mentioned by **AIN(1993)**. Every 100g consists of the following: 12g. casein, 10 g. corn oil, 0.3 g. methionine , 0.2 g. choline chloride, 4 g. minerals **(Hegested *et al.*, 1941)**., 1 g vitamin mixture **(Campbell, 1963)**, 5 g cellulose and 67.5 g corn starch . The basal diet was stored in a dry place away from direct sunlight. Casein, vitamins mixture, minerals mixture, cellulose, methionine and choline chloride were be obtained from El-Gomhoriya Company, Cairo, Egypt. Chemical kits used in this study (TC, TG, HDL-c, ALT, AST, ALP, bilirubin, urea, creatinine, albumin) were obtained from Al-Gomhoria Company for Drugs, Chemical and Medical Instruments, Cairo, Egypt.

Methods

Preparation of garden cress and licorice roots

To prepare garden cress and licorice roots powder were drying them at 45 °C for 3 hr., and ground to a fine powder using an air mill, high speed mixture (Molunix, Al-Araby company, Benha, Egypt) and then serving as powder .

Induction of diabetic rats

Thirty six rats was intravenously injected with 150 mg/kg body weight alloxan (50 mg/kg bw for three consecutive days) , after fasting for 12 hours to induce diabetes according to **Etuk and Muhammed(2010).** Rats induced diabetes mellitus when blood glucose was more than 200mg/dl in the fasting state.

Experimental design

Forty two (42) adult male white albino rats, "Sprague Dawley" Strain, 10 weeks age, weighing (150±10g) were used in this experiment. After adaptation period, rats were divided into 7 groups, six rats per each as follows: **Group (I):** Rats fed on basal diet as a negative control.

Group (2): Diabetic rats fed on basal as a positive control .

Groups(3): Diabetic rats were fed on basal diet with 5% *Lepidium sativum* **.**

Groups (4): Diabetic rats were fed on basal diet with 10% *Lepidium sativum* **.**

Groups(5): Diabetic rats were fed on basal diet with 5% *Glycyrrhiza glabra*.

Groups(6): Diabetic rats were fed on basal diet with 10% *Glycyrrhiza glabra*.

Group (7): Diabetic rats were fed on basal diet containing 10%mixture of *Glycyrrhiza glabra* and *Lepidium sativum.*

Biological Evaluation

The diet consumed was recorded every day, body weight was record every week. The body weight gain (BWG), feed efficiency ratio (FER), and relative organs weight were determined according to **Chapman *et al.*, (1959)**. The relative organ weight was calculated by dividing the organ weight on the total body weight of each rat and then multiplied by 100.

Blood Sampling

Blood samples were collected after 12 hours fasting at the end of experiment in which the rats were scarified under ether anaesthesia **(Schermer, 1967).** Blood samples were collected into dry clean centrifuge tubes and left to clot in water bath (37ºC) for half an hour. The blood was centrifuged for 10 minutes at 3000 r.p.m. to separate the serum. Serum was carefully aspirated into clean cuvetle tube ans stored at (-20ºC) until biochemical analysis.

Chemical Analysis:

Moisture, protein, fat, fiber, and ash contents of garden cress and licorice were determined according to methods described by the **A.O.A.C. (2010)**. Carbohydrate was calculated by differences as follow : % carbohydrate = 100 – (% moisture + % protein + % fat + % fiber + % Ash).

Biochemical Analysis

Serum glucose level was determined by enzymatic colorimetric method according to **Trinder (1969).** Triglycerides levels in serum were measured according to **Fassati and Prencipe (1982),** total cholesterol according to **Allain (1974),** HDL – cholesterol according to **Lopez (1997).** Calculations of VLDL and LDL – cholesterol according to the method of **Lee and Nieman (1996)** as follows: VLDL (mg/dl) = triglycerides/5 , LDL (mg/dl) = total cholesterol-HDL-c – VLDL-c. Determination of GPT (ALT) and GOT (AST): alanin amino transferase activity and asparate amino transferase activity was determined calorimetrically according to the method of **Henry(1974)** and **Reitman &Frankel(1957)**. Serum alkaline phosphatase (ALP) was determined according to **(IFCC methods, 1983),** and serum albumin was measured according to **(Doumas *et al.*, 1971).** Serum urea was measured according to the enzymatic method of (**Patton and Crouch ,1977),** uric acid was measured using the modified kinetic method according to **Baraham and Trinder (1972),** serum and urine creatinine was measured according to kinetic method of (**Henry, 1974).**

Histopathological Examinations

Liver and pancrease of the scarified rats were dissected, removed, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol. Specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope according to **Weibel and Paumgartner(1978).**

Statistical Analysis

The data was analyzed using the software SPSS version 20. Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one way analysis of variance (ANOVA), followed by student' t-test. The p-value less than 0.05 were considered statistically significant **(Snedecor and Cochran, 1980).**

Results and discussion

Data in **Table (1)**. shows the chemical composition of *L. sativum* and *G.glabra*. From the obtained results, *L. sativum* was significantly higher in mean values of protein, fat, fiber, ash, and energy than *G. glabra*, whereas *G. glabra* was significantly higher in carbohydrate than *L. sativum*. There was no statistical differences in moisture content. Proximate composition (%) of *L. sativum* seeds reported by **Eddouks and Maghrani, (2008**) indicated that it had appreciable amounts of protein (17.9 to 24.2), lipids (13.7 to 23.2), carbohydrates (34.7 to 40.11), fiber (11.9 to 19.76), ash (4.01 to 7.1), and moisture (2.9 to 7.09) and These data are in agreement with the obtained results. The results obtained by **Weidner *et al*. (2012) and Kataria *et al*.(2013)** are in agreement with the obtained results which revealed that licorice's fiber (10.98-20.41 g/100g) are the most abundant constituents that show potential health benefits. While ash and protein contents were represented with moderate amount 7.61to 10,76 and 3.12to 7.19 g/100g, respectively. Low moisture content (4.11 g/100g) was found in licorice root so remains an asset in storage attributes and preservation of the nutrients. Licorice root had a really low amount of fat (about 2.21 g/100g), so it might be helpful for weight loss. It has revealed that licorice roots has reducing sugars as a major component (40.47 g/100g) that corresponds to its high energy (210.53 to 360.43kcal/100g) .

Table (1). Chemical composition of *L. sativum* and *G. glabra* g/100g dry weight

Constituents (g/100g)	*Lepidium sativum L.*	*Glycyrrhiza glabra*
Moisture	$6.87^{a} \pm 1.07$	$6.32^{a} \pm 1.02$
Protein	$18.03^{a} \pm 1.07$	$9.03^{b} \pm 0.37$
Fat	$13.76^{a} \pm 0.87$	$2.07^{b} \pm 0.23$
Fiber	$18.08^{a} \pm 2.03$	$11.33^{b} \pm 1.32$
Ash	$4.42^{a} \pm 0.87$	$3.78^{b} \pm 0.09$
Carbohydrate	$38.84^{b} \pm 4.95$	$67.47^{a} \pm 10.97$
Energy	$351.32^{a} \pm 7.94$	$324.63^{b} \pm 8.76$

*The results are means of triplicate determination ± standard deviation

The effect of feeding *L. sativum* and *G. glabra* on feed intake, feed efficiency ratio and body weight gain are shown in **Table (2)**. Feed intake values decreased in diabetic rats fed on diet containing *L. sativum* or *G. glabra* or the mixure compared to positive control group. The decrease was significant ($P \leq 0.05$) in groups 10 % *L. sativum*, 10 % *G. glabra*, and 10 % (*L. sativum* + *G. glabra*). Concerning body weight gain, there was a significant increase in the above mentioned groups compared to positive

group ($P \leq 0.05$). FER increased in groups fed on diet containing *L. sativum* or *G. glabra* or the mixure compared to positive control group ($P \leq 0.05$). The increase in FER in group 10 % *G. glabra* and 10 % mixture was nonsignificant when compared to negative control group. The current results due to the effect of HMF, showed a significant decrease in body weight and food consumption in accordance with the findings of **York *et al.*** (2007). Several isoflavans constituents of licorice are unique phytoestrogens, which like estradiol, affect the serotonergic system, inhibiting serotonin re-uptake and thereby increasing the levels of serotonin in synaptic clefts. This enhances satiety and resembles the action of sibutramine, but naturally, Dietary phytoestrogens may also activate AMPK leading to a reduction in food intake (**Cederroth *et al.*,2018**). Our study is a novel trial for using a phytoestrogen in fennel, licorice and anise as a natural serotonin reuptake inhibitor instead of sibutramine.

The reduction in food intake produced by Glycyrrhiza glabra root extract, in this study, could be possibly attributed to the presence of either a glycoside glycyrrhizin, which is 50-fold sweeter than sugar or bitter principles that may reduce food intake. The increase in body weight gain by Glycyrrhiza glabra root extract is in accordance with that reported by **Ofir et al., (2013).** The authors of the previously mentioned study explained this finding on the basis that Glycyrrhiza glabra inhibits 11 β-hydroxysteroid dehydrogenase and that induces excess release of mineralocorticoids, which causes retention of sodium and water that leads to oedema and increase in body weight. However, the increased body weight gain that reported in this study could be possibly explained by the improvement in feed efficiency reported herein.

Table (2). The effect of feeding *L. sativum* and *G. glabra* on feed intake, body weight gain, and feed efficiency ratio in diabetic rats

Groups parameters	Negative Control (G1)	Positive control (G2)	5%*L. sativum* (G3)	10%*L. sativum* (G4)	5%*G. glabra* (G5)	10%*G. glabra* (G6)	10% Mixture (G7)
FI g/100g	12.9^{a} ±1.78	10.90^{b} ±1.02	9.73^{b} ±0.05	8.08^{c} ±0.91	9.78^{b} ±0.52	8.04^{c} ±0.49	7.67^{c} ±0.63
BWG g/28 days	42.27^{a} ±3.11	22.41^{c} ±5.13	22.58^{c} ±2.54	27.82^{b} ±0.76	23.27^{c} ±3.54	28.98^{b} ±4.70	29.92^{b} ±3.04
FER g/100g	0.117^{a} ±0.001	0.078^{c} ±0.002	0.086^{b} ±0.002	0.111^{a} ±0.006	0.085^{b} ±0.002	0.118^{a} ±0.01	0.119^{a} ±0.01

Means with different letters (a, b, c, d) in the same raw differ significantly at p≤0.05, while those with similar letters are non-significantly different.

As shown in **Table (3)**. There was a significant increase ($P \leq 0.05$) in relative organs weight (liver, kidney, and pancreas) in positive control group compared to the negative control group. Liver and pancreas weight were decreased in groups 10 % *L. sativum*, 10 % *G. glabra*, and 10 % mixture compared to positive control group ($P \leq 0.05$). Kidney weight decreased significantly ($P \leq 0.05$) in diabetic rats fed on diet supplemented with 10 % *L. sativum*, 5 % *G. glabra*, 10 % *G. glabra*, and 10 % mixture.

Table (3). The effect of feeding *L. sativum* and *G. glabra* on some relative organs weight (gram) of diabetic rats

Animal Groups Parameters	Negative Control (G1)	Positive control (G2)	*L. sativum* 5% (G3)	*L. sativum* 10% (G4)	*G. glabra* 5% (G5)	*G. glabra* 10% (G6)	10% Mixture (G7)
L w /Bw g/100g	2.13^{b} ±0.14	2.86^{a} ±0.77	2.66^{a} ±0.75	2.58^{b} ±0.98	2.73^{a} ±0.04	2.52^{b} ±0.74	2.34^{c} ±0.76
K w/Bw g/100g	0.59^{b} ±0.42	0.67^{a} ±0.33	0.64^{a} ±0.39	0.60^{b} ±0.29	0.61^{b} ±0.01	0.59^{b} ±0.21	0.59^{b} ±0.11
P w/Bw g/100g	0.34^{c} ±0.02	0.53^{a} ±0.61	0.53^{a} ±0.61	0.42^{b} ±2.03	0.51^{a} ±0.62	0.43^{b} ±0.57	0.40^{b} ±0.69

All results are expressed as mean ± SD. Values in each raw which have different letters are significantly different ($P \leq 0.05$).

Data in **Table (4)**. demonstrated that there was a significant increase in blood glucose level in the positive control group compared to the negative control group at the end of the study. There was a significant decrease ($P \leq 0.05$) in blood glucose level in diabetic rats fed on diet with different levels of *L. sativum* and *G. glabra* or the mixture. The best results was recorded for group 7
(10 % mixture).

Hypoglycemia induced by Ls is probably mediated through enhanced secretion of insulin from the beta-cells of the pancreatic islets or through an extra pancreatic mechanism. Moreover, LS may reduce the effect of inflammatory cytokine release during diabetes which may be one of the causative agents for the insulin resistance **(Frayn , 2002).** Also, its effect due to increased fat mobilization from adipose tissue and resistance to the antilipolytic actions of insulin. Impaired insulin action is associated with an oversupply of lipids. This increased availability led

to either elevated lipid stored in insulin target tissues (e.g. muscle, liver adipose) or increased plasma FFA or triglyceride (**Rao *et al*.,2016).**

Table (4). The effect of feeding *L. sativum* and *G. glabra* on fasting blood glucose in diabetic rats (mg/dl).

Intervals	Negative Control (G1)	Positive control (G2)	*L. sativum* 5% (G3)	*L. sativum* 10% (G4)	*G. glabra* 5% (G5)	*G. glabra* 10% (G6)	10 %Mixture (G7)
Blood glucose mg/dl	94.8^{e} ±6.87	302.1^{a} ±9.28	270.6^{b} ±4.79	213.2^{c} ±9.67	265.1^{b} ±4.53	209.9^{c} ±7.31	195.7^{d} ±6.22

All results are expressed as mean ± SD. Values in each raw which have different letters are significantly different ($P \leq 0.05$).

Data in **Table (5)**. demonstrated that parameters associated with lipid profile such as TC, TG, HDL-c, LDL-c, and VLDL-c were improved in diabetic rats fed on diets containing *L. sativum* or *G. glabra* or the mixture when compared with positive control group. These improvements were statistically significant ($P \leq 0.05$) in all parameters in groups 4 (10 % *L. sativum*), 5 (10 % *G. glabra*), and 7 (10 % mixture). T. chebula components of HMF have hypocholesterolemic activity that might be mediated through increased cholesterol excretion in the feces. In addition anthraquinone glycosides from rhubarb in the HMF have lipid-lowering effects, resulting in depression of lipid accumulation. It consequently has anti-atherosclerotic properties (**Liu *et al.*, 2008).** The results showed that oral administration of *Glycyrrhiza glabra* root extract to male rats for 4 weeks induced significant ($p < 0.05$) decreases in serum total cholesterol and triglycerides associated with non-significant decreases in concentrations of HDL-c, LDL-c and VLDL-c fractions . The decrease in serum total cholesterol and triglycerides reported in this study is similar to that reported by **Ofir *et al.*, (2013)**.. The authors of the previously mentioned studies attributed the hypocholesterolemic effect of *Glycyrrhiza glabra* to the presence of certain isoflavones, which act as antioxidants via inhibition of LDL-c oxidation and that inhibits the local mechanism of atherogenesis. Glycyrrhizin, the active constituent in licorice root, which is another plant in the mixture, exerts hypocholesterolemic action by stimulating the conversion of cholesterol into bile acids without effect on cholesterol synthesis **(Novikov *et al.*, 2009).** Glabridin, a natural polyphenolic isoflavone antioxidant from licorice root extract in HMF possesses potent free radical scavenging activity that could promote a decrease in lipid peroxidation and could protect LDL from oxidation. This would

occur via a direct interaction with the lipoprotein and/or an indirect effect through accumulation in arterial macrophages. Therefore licorice represents a potent nutrient, which can attenuate the development of atherosclerosis, secondary to its antioxidant properties against lipids peroxidation in arterial cells (**Vaya *et al.*, 2007 and Thornalley , 2008).**

Table (5). The effect of feeding *L. sativum* and *G. glabra* on lipid profile in diabetic rats

Serum lipids	Negative Control (G1)	Positive control (G2)	*L. sativum* 5% (G3)	*L. sativum* 10% (G4)	*G. glabra* 5% (G5)	*G. glabra* 10% (G6)	10% Mixture (G7)
Total cholesterol	95.26^{d} ±1.19	163.73^{a} ±2.15	156.87^{a} ±0.13	140.36^{b} ±0.12	160.8^{a} ±3.21	142.57^{b} ±1.51	131.44^{c} ±1.21
Triglycerides	106.48^{c} ±0.13	129.8^{a} 0.03±	127.4^{a} ±2.01	116.68^{b} ±0.63	124.36^{a} ±0.02	117.28^{b} ±0.45	114.88^{b} ±0.02
HDL-cholesterol	53.94^{a} ±0.12	40.87^{c} ±1.15	40.89^{c} ±0.04	45.92^{b} ±0.03	43.90^{b} ±0.97	43.93^{b} ±0.03	46.96^{b} ±0.14
LDL-cholesterol	20.2^{d} ±1.17	96.9^{a} ±4.34	90.5^{a} ±0.74	71.1^{b} ±0.91	73.8^{b} ±0.24	64.1^{c} ±3.41	61.5^{c} ±2.43
VLDL-cholesterol	21.29^{c} ±1.07	25.96^{a} ±2.18	25.48^{a} ±0.17	23.34^{b} ±4.13	24.87^{a} ±0.98	23.45 ^{b}±1.19	22.98 ^{b}±0.15

All results are expressed as mean ± SD. Values in each raw which have different letters are significantly different ($P \leq 0.05$).

Data in **Table (6)**. shows a significant increase ($P \leq 0.05$) in serum urea, uric acid, and creatinine levels in the positive group compared with the negative control group due to the diabetic nephropathy. There were improvements in renal function parameters upon feeding a basal diet with different levels of *L. sativum* and *G. glabra*. The decrease was statistically significant ($P \leq 0.05$) in group 4 (10 % *L. sativum*), 5 (10 % *G. glabra*), and 7 (10 % mixture).

Abnormal renal function is mainly associated with diabetic nephropathy. The pathophysiology involves glucose that binds irreversibly to proteins in the kidney circulation to form advanced glycosylation end products that can form complexes that contribute to renal damage by stimulation of fibrotic growth factors . The oral administration of LS extract clarified that serum concentration of urea; uric acid and creatinine were significantly decreased. The herbal drugs containing tannins have a uremic-toxin-decreasing action, whereas rhubarb's tannins significantly improved BUN creatinine, glomerular filtration rate and renal blood flow. In this respect rhubarb has proven effective as a diuretic in rabbit models, apparently by blocking sodium-potassium ATPase in the renal medulla **(Rao *et al.*, 2016).**

Based on previous recommendations, LS was advised for treatment of hypertension, diabetes, and renal disease . The protective and curative effect of LS extract on the renal function was observed as it significantly reduced the level of urea and creatinine, indicating increased glomerular filtration rate when administrated daily for 3 weeks **(Al Hamedan, 2010).** Glabridin, a natural polyphenolic isoflavone antioxidant from licorice root extract in HMF possesses potent free radical scavenging activity that could promote kidney functions(**Deng *et al*., 2020).**

Table (6). The effect of feeding *L. sativum* and *G. glabra* on kidney functions in diabetic rats.

Parameters	Negative Control (G1)	Positive control (G2)	*L. sativum* 5% (G3)	*L. sativum* 10% (G4)	*G. glabra* 5% (G5)	*G. glaba* 10% (G6)	10% Mixture (G7)
Creatinine mg/dl	0.69^{e} ±0.31	1.96^{a} ±0.21	1.82^{a} ±0.07	1.43^{c} ±0.14	1.78^{a} ±0.01	1.67^{b} ±0.02	1.21^{d} ±0.07
Uric Acid mg/dl	2.35^{c} ±0.15	2.95^{a} ±0.22	2.82^{a} ±1.00	2.56^{b} ±0.21	2.75^{a} ±0.11	2.67^{b} ±1.5	2.51^{b} ±0.81
Urea Nitrogen mg/dl	25.2^{d} ±1.15	48.46^{a} ±0.1	42.76^{b} ±1.3	37.18^{b} ±1.2	39.15^{b} ±0.5	40.21^{b} ±0.7	35.1^{c} ±0.2

All results are expressed as mean ± SD. Values in each raw which have different letters are significantly different ($P \leq 0.05$).

Table 7 shows the effect of administration of *L. sativum* and *G. glabra* powder for 4 weeks on liver functions in the serum of diabetic rats. The mean values of AST, ALT, and ALP in serum of the positive control group was significantly ($P \leq 0.05$) higher than that of the negative control. The mean values of serum albumin in the positive control were significantly ($P \leq 0.05$) lower than that of the negative control. Treating the diabetic rats with different levels of *L. sativum* and *G. glabra* decreased the mean values of AST, ALT, and ALP in the serum compared with that of the positive control and increased the mean values of serum albumin. The present results demonstrate that the LS showed a significant decrease in the activity of both AST and ALT agreeing with the resulted obtained by **(Al Hamedan, 2010).**The presence of flavanoids, triterpens, alkaloid, tannin, and coumarins in LS explains its role in hepatoprotection by inhibiting the toxic radicals mediated damage.

Moreover, **Woo *et al.* (2008)** reported that the glycosides of Glycyrrhiza glabra prevent accumulation of cholesterol in cells as well as human blood serum. Results obtained in this study revealed that oral administration of Glycyrrhiza glabra root extract to male rats for 4 weeks

produced a significant ($p < 0.05$) reduction in the levels of AST and ALT enzymes in the serum. The results revealed that Glycyrrhiza glabra root extract reduced the levels of hepatic enzymes (AST and ALT) in the serum of rats. This finding is consistent with the findings of who reported that glycyrrhizin of Glycyrrhiza glabra reduced the liver enzymes in rats and induced interferon production in patients with chronic hepatitis B and C(**Vaya *et al.*, 2007).**

Table (7). The effect of feeding *L. sativum* and *G. glabra* on liver functions in diabetic rats.

Parameters	Negative Control (G1)	Positive control (G2)	*L. sativum* 5% (G3)	*L. sativum* 10% (G4)	*G. glabra* 5% (G5)	*G. glabra* 10% (G6)	10% Mixture)G7)
AST(U/L)	26.1^{d} ±0.67	45.2^{a} ±4.91	38.5^{b} ±0.21	31.7^{c} ±0.75	39.1^{b} ±0.90	32.1^{c} ±9.3	27.7^{d} ±1.91
ALT(U/L)	19.8^{c} ±1.31	38.9^{a} 3.51±	38.4^{a} ±4.5	30.7^{b} ±0.25	36.4^{a} ±3.51	29.5^{b} ±6.82	20.9^{c} ±4.06
ALP (U/L)	80.1^{c} ±0.67	97.7^{a} ±2.71	86.7^{b} ±4.46	79.7^{c} ±1.55	84.1^{b} ±4.61	80.1^{c} ±2.31	78.5^{c} ±1.92
Albumin mg/dl	3.84^{a} ±0.152	2.81^{b} ±1.05	2.90^{b} ±0.05	3.22^{b} ±0.01	2.98^{b} ±0.03	3.09^{b} ±2.31	3.28^{a} ±1.65

All results are expressed as mean ± SD. Values in each raw which have different letters are significantly different ($P \leq 0.05$).

The histopathology of liver is shown in **photo (1)**. Liver tissues of rat from negative control group showing average central venule (yellow arrow) , average blood sinusoids (red arrow) and hepatocytes (blue). Liver section from Alloxan treated (diabetic control) group, sinusoidal congestion , dilated c.v central vein of liver lobule, Vascular degeneration of hepatocytes in zone 3. Increase vacualation in the cytoplasm of hepatocytes as indistinct clear vacuoles indicating glycogen infiltration in diabetes as shown in **photo** (2). Liver section of diabetic rat administered with 10% ***G. glabra*** showed normal hepatocytes and normal of hepatic architecture with mild vacuolation of hepatocytes arranged in normal sheets around central vein **photo (4)**. Liver section of diabetic rat administered with 5% ***G. glabra*** showed minimal portal inflammation. inflammatory cells limited to portal tracts the presence of mild lymphocytic inflammatory infiltrate with occasional eosinophils in the portal area are shown in **photo (3).** According to **Rizzato *et al.* (2017),** glycyrrhizin and glycyrrhetinic acids prevent drug-induced liver injury and ensure the disruption of bile acid metabolism in humans. Indeed glycyrrhetinic acid has been reported as anti-inflammatory and hepatoprotective compound whereas glycyrrhizin, when compared with the placebo, induced a significant reduction in the serum

aminotransferases and improved the liver histology . It has also been reported that the long-term use of glycyrrhizin prevents the development of hepatocellular carcinoma in chronic hepatitis C (**Yin *et al.*, 2017).**

Liver section of diabetic rat administered with 5% ***L. sativum*** showed minimal portal inflammation, inflammatory cells limited to portal tracts the presence of mild lymphocytic inflammatory infiltrate with occasional eosinophils in the portal area **photo (5)**. Liver section of diabetic rat administered with 10% ***L. sativum*** showed normal hepatocytes and normal of hepatic architecture with mild vacuolation of hepatocytes arranged in normal sheets around central vein **phoyo (6).** Liver section of diabetic rat administered with mixture showed few portal inflammation. low necrosis consist of inflammatory cell aggregates, mostly composed of lymphocytes and macrophages **photo (7).** The present results agreeing with the resulted obtained by **(Al Hamedan, 2010)** who reported that the presence of flavanoids, triterpens, alkaloid, tannin, and coumarins in LS explains its role in hepatoprotection by inhibiting the toxic radicals mediated damage.

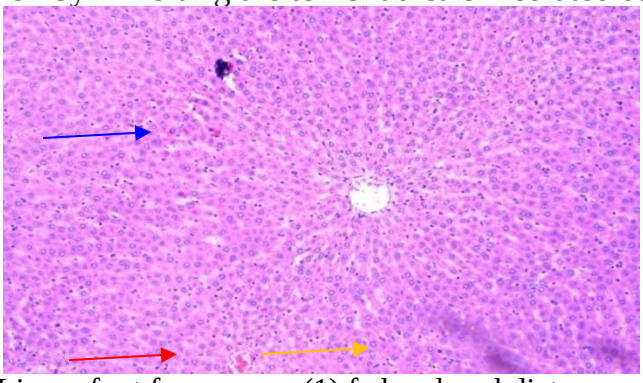

Photo (1): Liver of rat from group (1) fed on basal diet as control group (H&E X200)

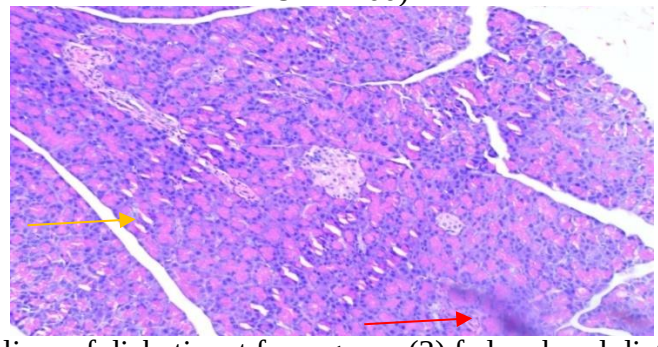

Photo (2): liver of diabetic rat from group (2) fed on basal diet as positive control group(H&E X200)

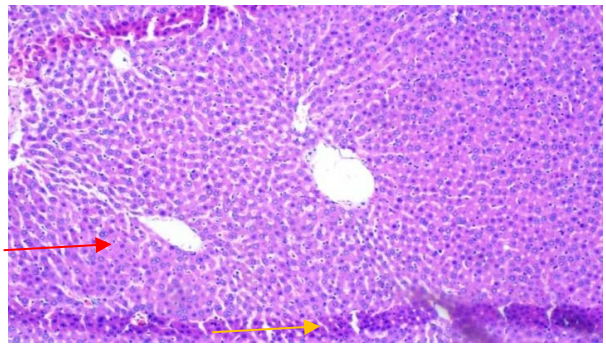

Photo (3) : Liver of diabetic rat fed on basal diet with 5% *G. glabra* (G3) (H&E X200)

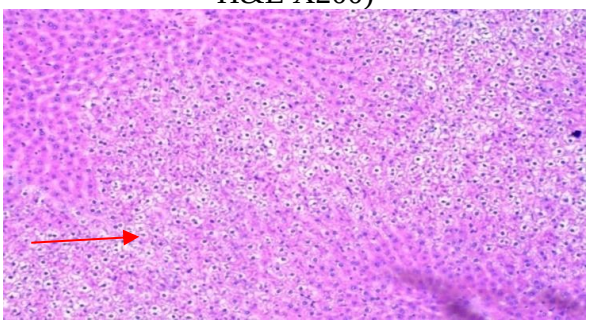

Photo (4): Liver of diabetic rat fed on basal diet with 10% *G. glabra* (G4) (H&E X200)

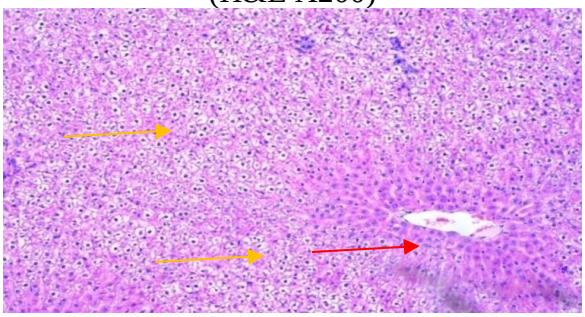

Photo (5): Liver of diabetic rat fed on basal diet with 5% *L. sativum* (G5) (H&E X200)

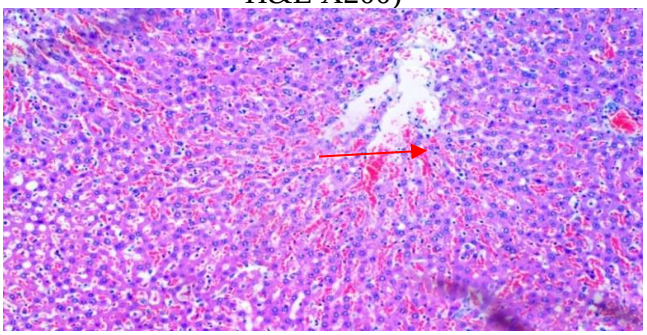

Photo (6): Liver of diabetic rat fed on basal diet with 10% *L. sativum* (G6) (H&E X200)

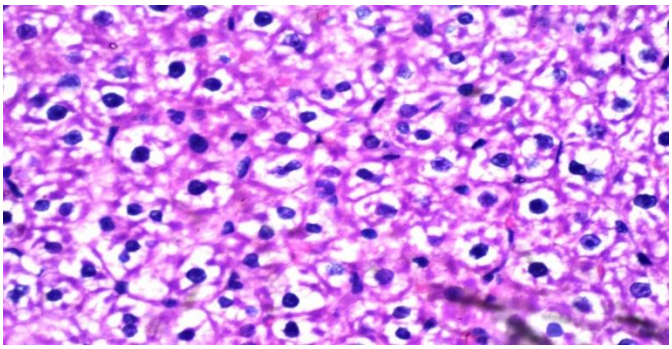

Photo (7): Liver of diabetic rat fed on basal diet with 10% mixture (G7) (H&E X200)

The histopathology of pancrease is shown in **photo (8)**. Normal average islets of Langerhnas (yellow arrow) , normal average secretory acini (red arrow) . For Pancreatic tissue of diabetic rats **Photo (9)**showed architecture of the islets is disrupted islets of Langerhans exhibited hydrophobic cells, necrotic cells, vacuolizations and irregular hyperchromic nuclei. Pancreas of diabetic rat treated with *G. glabra* 5% showing normal sized islets of langerhans some degeneration of the β-cell **Photo (10)**. Pancreas of diabetic rat treated with *G. glabra* 10% showing normal sized islets of langerhans some degeneration of the β -cell **Photo (11).**Pancreatic tissue of diabetic rats treated with *L. sativum* 5 % showing hypertrophy and vacuolations of β-cells of islets of Langerhans **Photo (12).** Pancreatic tissue of diabetic rats treated with *L. sativum* 10% showed no vacuolizations and appear highly divided β-cells in the islets of Langerhans **Photo (13)**. Rat fed on mixture of herbs , pancrease showed normal β-cells in the islets of Langerhans **Photo (14).** In the same line, **Brady *et al*.(2002)** showed a decrease in serum fasting glucose levels upon administration of 200 and 400 mg/kg *Glycyrrhiza glabra* root extract in diabetic rats. Likewise, *Glycyrrhiza glabra* contains glabridin, a polyphenolic flavonoid, which posess a hypoglycemic effect. Glycyrrhizic acid is another bioactive component in *Glycyrrhiza glabra* has shown to improve glucose metabolism in rats fed on a high-sucrose diet **(Kalaiarasi and Pugalendi, 2019).** The decrease in serum fasting glucose concentration seen in our study can be explained by the presence of benzyl isothiocyanate (BITC), a bioactive phytochemical found in *Lepidium sativum* **(Kholoud *et al*., 2019)**.

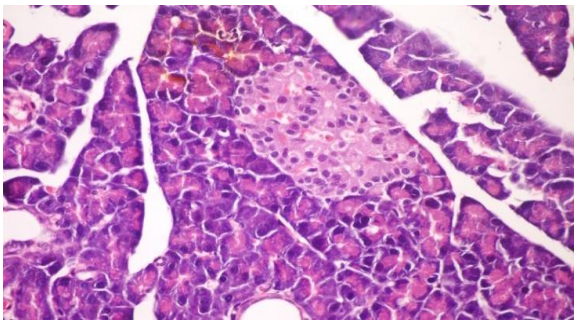

Photo (8):Pancrease of rat from group (1) fed on basal diet as control group(H&E X200)

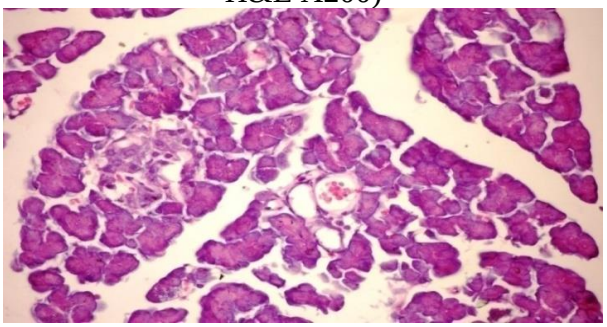

Photo (9): Pancrease of diabetic rat from group (2) fed on basal diet as positive control group(H&E X200)

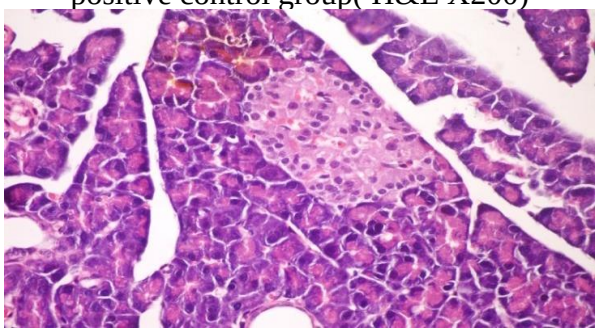

Photo (10): Pancrease of diabetic rat fed on basal diet with 5% *G. glabra* (G3) (H&E X200)

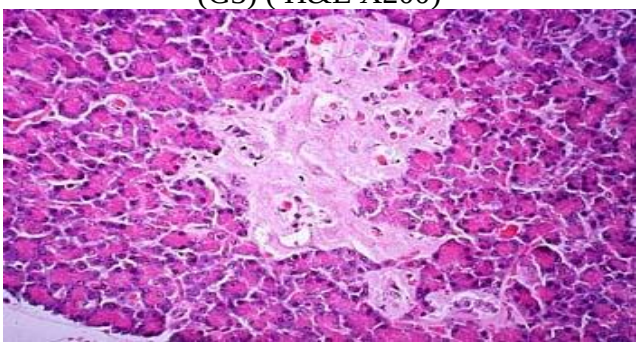

Photo (11): Pancrease of diabetic rat fed on basal diet with 10% *G. glabra* (G4) (H&E X200)

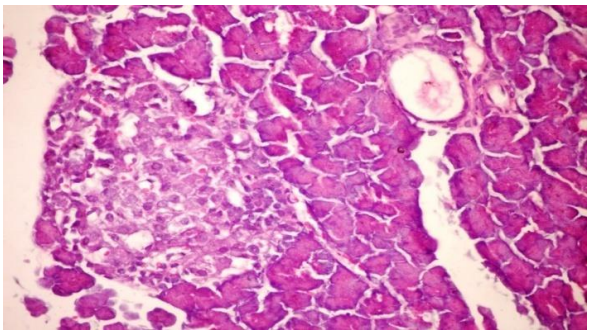

Photo (12) : Pancrease of diabetic rat fed on basal diet with 5% *L. sativum* (G5) (H&E X200)

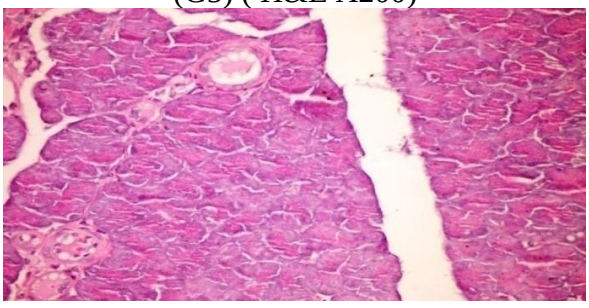

Photo (13): Pancrease of diabetic rat fed on basal diet with 10% *L. sativum* (G6) (H&E X200)

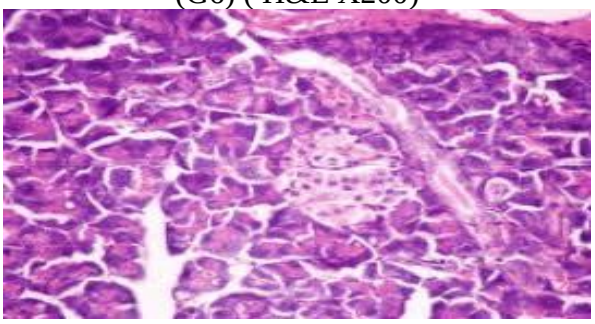

Photo (14) : Pancrease of diabetic rat fed on basal diet with 10% mixture (G7) (H&E X200)

Conclusion

Diabetes mellitus is a metabolic disease characterized by increased blood glucose levels, known as hyperglycemia. It poses a great challenge to the health care system in the world. Many of the used diabetes synthetic drugs have side effects and may develop drug resistance **.** Thus, the need to use medicinal plants as a mode of treatment for diabetes has been increased as *Glycyrrhiza glabra* root and *Lepidium sativum* powder. 10% *G. glabra* followed by 10% had higher effect on the biological and biochemical markers of diabetic rats. The histological studies showed altered pathological changes in the tissues of liver and pancreas as a result of diabetes in the positive control group. Treating diabetic rats with *G. glabra* root powder followed by the mixture and *L. sativum* seeds and restored the altered tissues.

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دراسة تأثير كل من حب الرشاد والعرقسوس على الفئران المصابة بالبول السكرى بواسطة الالوكسان

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نادية عبد المنعم مصطفى البنا

الملخص العربى:

مرض السكري هو اضطراب أيضي معقد يؤثر على مستوى الجلوكوز في جسم الإنسان. يرتبط ارتفاع السكر المزمن في الدم المصاحب لمرض البول السكري بفشل الأعضاء الطرفية. وتهدف هذه الدراسة قياس تاثير كل من حب الرشاد والعرقسوس على وزن الجسم والمتناول من الغذاء وتركيب الدهون ودلائل ظائف الكلى والكبد ومستويات الجلوكوز في الفئران المصابة بداء السكري. تم استخدام اثنين وأربعين (42) من ذكور الفئران البيضاء البالغة، بوزن 150 ± 5 جرام. تم تقسيم فئران المجموعة المصابة بالسكري بالتساوي إلى 6 مجموعات فرعية تضم كل مجموعة 6 فئران. تلقت المجموعة الأولى نظاما غذائيا ضابطا كمجموعة ضابطة سلبية لمدة 28 يوما. تم تقدير وزن الجسم، مستوى الدهون ووظيفة الكلى (اليوريا، حمض اليوريك والكرياتينين) ونشاط الانين امينوترانسفيريز واسبرتات امينوترانسفيريز بالإضافة إلى الجلوكوز والتركيب النسيجي للكبد والبنكرياس. أظهرت النتائج أن الفئران المصابة التي لم يتم علاجها إلى زيادة كبيرة في إنزيمات الكبد وتركيز الدهون مقارنة مع الفئران الغير مصابة، في حين انخفضت بشكل ملحوظ وزن الجسم النهائي والكولسترول العالى الكثافة. بينما اضافة حب الرشاد والعرقسوس وخليطهما إلى انخفاض ملحوظ في مستوى الدهون وإنزيمات الكبد، ايضا ادت إلى تحسن في التركيب النسيجى للكبد والبنكرياس بشكل ملحوظ. ووجد ان تركيزات العرقوس يليه الخليط من الاعشاب حسن من اثار البول السكري المرتبطة بالتغيرات الميتابلزمية بدرجات مختلفة. كما أنها تركيبة آمنة على الأعضاء التي تم فحص وظائفها، لذلك هى وسيلة للتغلب على مرض البول السكري؛ حيث له تأثير عالى لمرض السكر.

الكلمات الافتتاحية : فئران مصابة بالبول السكرى–وظائف الكلى– الوظائف الكبدية –الدهون– حب الرشاد – العرقسوس.