Role of *Lepidium sativum* in Treating Diabetes and Managing Bodily Functions' Disorders in Rats.

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Recommended Citation

El-Emary, Gehan (2021) "Role of *Lepidium sativum* in Treating Diabetes and Managing Bodily Functions' Disorders in Rats.," *Karbala International Journal of Modern Science*: Vol. 7 : Iss. 2 , Article 4.

Available at: <https://doi.org/10.33640/2405-609X.2773>

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Role of *Lepidium sativum* in Treating Diabetes and Managing Bodily Functions' Disorders in Rats.

Abstract

: *Lepidium sativum* seed (*LS*) is a plant used globally for its medicinal effect in treating diabetes. This study examined the ability of an aqueous extract of *LS* to treat diabetes and manage disorders of bodily functions. It used 70 male Wistar rats, each weighing between 190 g and 200 g. Diabetes was established in the rats by injecting them with Alloxan Solution at 150 mg/kg of body weight. The rats were considered diabetic if, after 24 hours, their blood glucose levels were higher than 180 mg/dl. Diabetes caused severe alterations in their blood serum glucose (376.608 mg/dl); their kidney functions (84.512 mg/dl for urea, 4.716 mg/dl for uric acid, and 0.376 mg/dl for creatinine); their liver functions (46.150 U/L for ALT, 45.654 U/L for AST, 5.431 U/L for albumin, and 7.611 U/L for globulin); and their lipid profile (79.123 mg/dl for triglycerides, 121.113 mg/dl for total cholesterol, 62.776 mg/dl for LDL, and 27.453 mg/dl for HDL). The findings suggest that it is beneficial to use *LS* to treat and manage disorders of bodily functions in rats.

Keywords

Diabetes, Kidney Functions, Liver Functions, Alloxan, *Lepidium sativum* aqueous extract.

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1. Introduction

Lepidium sativum (LS), or garden cress, is a well-known medicinal herb belonging to a plant family called Brassicaceae [1,2]. LS seeds possess numerous benefits that make it a superb medicinal herb [3,4]. LS has been used as a valuable medicinal plant for hundreds of years. It is commonly used in treating various diseases, including abdominal problems like diarrhea and dysentery. LS has great beneficial effects as an anti-rheumatic, anti-flatulent, febrifuge, diuretic, and anti-hiccup medicine [5,6]. LS is also used in many countries across the globe for managing disorders like bronchitis and asthma [7], as well as diabetes [8]. Moreover, LS contains numerous precious compounds that make it even more useful than a majority of medicinal plants; these compounds are riboflavin, alkaloids, ascorbic acid, oleic acid, linoleic acid, α -tocopherol, stearic acid, carotene, β -sitosterol, and palmitic acid [9]. In addition, LS is characterized by its valuable amounts of L-arabinose and mono-unsaturated fatty acids. It has been used extensively in helping breastfeeding mothers to secrete more milk [10,11], and in the treatment of skin disease and diabetes [12,13].

Lepidium sativum extract shows efficacy in the prevention and management of diabetes mellitus and its related complications [8]. *Lepidium sativum* seeds can be used in food supplement preparations or as a food additive, both supporting caloric gain and protecting against the oxidation of nutrients in products [14]. The preliminary findings of [15] highlighted that lectin protein-loaded chitosan-TPP nanoparticles could be a promising anticancer agent.

In the same way, [16] appraised *Lepidium sativum* extract to have a potential therapeutic effect against liver toxicity and hepato-carcinoma. Diabetes mellitus (DM) is generally considered a dysfunction causing an increase in the amount of sugar in the blood. Experts repeatedly use the full title, “DM,” as a substitute for “diabetes” alone in order to distinguish this dysfunction from diabetes. Diabetes insipidus is a comparatively uncommon dysfunction with no effect on blood sugar levels, but it is a bit similar to DM in that it causes enhanced urination [17].

Diabetes affects most bodily functions, increasingly causing problems with kidney and liver functions, as well as the lipid profile. Therefore, seeking a quick treatment for diabetes is always of high priority in

order to prevent the onset of the more dangerous symptoms that could appear in later stages. [18–20] stated that these symptoms include high blood pressure, blindness, and other afflictions that could be fatal. Diabetes has almost spread throughout the entire world, leading to severe complications that can cause morbidity and mortality, especially in the poor and underdeveloped countries of Asia, South America, and Africa. Additionally, [21,22] illustrated that diabetics are forced to pay huge sums of money just to keep their blood sugar levels close to normal; the cost increases dramatically when other complications set in, which could negatively affect both the family's budget and the nation's economy as a whole. *Lepidium sativum* is a promising medicinal plant with a wide range of possible pharmacological activities and medical applications because of its effectiveness and safety [23]. *Lepidium sativum* is extensively used as a biostimulant in agriculture [24]. The results of [25] supported the use of *Lepidium sativum* as a bone healer; moreover, the study revealed the nature of the bio-actives responsible for the observed activity and extraction action mechanism. The integration of LS in doe rabbits increased reproductive hormones level and improved the antioxidant status of biochemical blood and their reproductive performance [26].

The present study was designed to investigate the use of LS in controlling diabetes and managing all the disorders that diabetes causes in Wistar rats.

2. Materials and methods

2.1. Plant material and collection

Top quality local *Lepidium sativum* dry seeds were obtained from a famous herbal company in Cairo. The percentage of yield was 9.46%; test was done according to [27] and moisture content was 6.35%; test was done according to [28]. LS seeds were kindly classified (collection No. 111) by Prof. Dr. A. A. Mohamed, Chief Researches, Flora Phytotaxonomy Researches Department, HRI, ARC, Agricultural Museum, Dokki, Giza.).

2.2. Chemicals and reagents

The following reagents and chemicals necessary for this study were obtained from the Agriculture Research Center: methanol, ethanol, gallic acid, sodium

carbonate, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Chloroform and Folin–Ciocalteu phenol (FCR).

2.3. Total carbohydrates and total amino acids

These two tests were conducted at the Faculty of Agriculture, Cairo University (CURP unit), according to guidance available from [29,30].

2.4. Scavenging activity on DPPH radicals

Antioxidant activities were carried out using the following method described by [31,32], where ethanol extract (50 μ L) with different quantities of powder ethanol extract (50, 100, 150, and 200 μ g/mL of distilled water in each reaction) was well blended with 450 μ L of 50 mM Tris-HCl buffer (pH 7.4) and 1.0 ml of 0.1 mM DPPH-ethanol solution. After incubation at room temperature for half an hour, the reduction of DPPH free radicals was measured through reading the absorbance at 517 nm. The positive control was L-ascorbic acid. The following equation was used to calculate inhibition percent: (% inhibition) [(absorbance of control-absorbance of the test sample)/absorbance of control] $\times$ 100.

2.5. Preparation of LS aqueous extract

Preparation of LS aqueous extract was done according to the following method described by [12]; LS seeds were washed carefully under tap water followed by distilled water, left to dry in the shade, then crushed into a fine powder. One gram of the dried fine powder was then boiled in 100 ml of distilled water for about 10 minutes or so, left for about 30 minutes to infuse, then cooled and filtered. The resultant filtrate was then lyophilized, and the required dose was poured into 10 ml of distilled water/kg of body weight immediately before being orally administered to the rats.

2.6. Quantification of total flavonoid content

A modified colorimetric method proposed by [33] was used for the quantification of the total flavonoid content of LS. In a mortar, about 25 mg of desiccated plant materials was well grounded with 10 ml of 80% methanol solution. The resultant mixture was left at room temperature for 20 minutes, and then filtered using a G4 filter. The produced filtrate (about 0.4 ml) was then well blended with 0.6 ml of distilled water and 0.06 ml of 5% NaNO₂; the mixture was left for about five minutes at room temperature, after which

10% of AlCl₃ solution was added, then 0.4 ml of 1N NaOH and 0.45 ml of distilled water were added, after which the mixture was then placed aside for about 30 minutes. The measurement of total flavonoid was used by (+) catechin, which acts as a standard compound, while determination of the absorbance was done using Spectrophotometer at 510 nm.

2.7. Quantification of total phenolic content

Quantification of total phenolic content of LS was carried out according to [34]; 0.25 ml Folin–Ciocalteu reagent and about 3 ml of distilled water were carefully added to 0.5 ml of extracts or fractions. The combination was then kept at room temperature for about two minutes, after which exactly 0.75 ml of sodium carbonate (20%) was added, and, by using distilled water, volume was increased to 5.0 ml. Then the whole combination allowed to stand for about two hours prior to being measured at 765 nm.

2.8. LS aqueous extract doses

LS aqueous extract dose of 20 mg/kg. body weight suggested by [35]. We expanded the range of action from 10 mg/kg body weight to 40 mg/kg. body weight to fully study the effect of the LS aqueous extract in controlling diabetes and Management of functional disorders.

2.9. Experimental protocol

This study was conducted on 70 male Wistar rats, each weighing between 190 g and 200 g. The rats were left for two weeks under normal conditions, fed with commercial food and pure tap water for acclimatization. Twenty rats were randomly selected and classified into two groups. The first group was the control group, which was fed with commercial food pellets. The second group was administered commercial food pellets and LS aqueous extract at 10 mg/kg of body weight. The remaining 50 rats were then injected with alloxan solution at 150 mg/kg of body weight to establish diabetes in them [36]. Diabetes was confirmed after 24 hours, as their blood glucose levels were higher than 180 mg/dl. Of these, 10 rats were then selected to be the third group (control + diabetes group). These rats were left for one week without any treatment to establish diabetes. The remaining 40 rats were then classified into four equal groups as follows: 10 rats fed with commercial food and administered LS aqueous extract at 10 mg/kg of body weight (fourth

group); 10 rats were fed with commercial food and *LS* aqueous extract at 20 mg/kg of body weight (fifth group); 10 rats were fed with commercial food and administered *LS* aqueous extract at 30 mg/kg of body weight (sixth group); and 10 rats were fed with commercial food and administered *LS* aqueous extract at 40 mg/kg of body weight (seventh group).

2.10. Blood collection

After completing all experimental tests, blood was collected and subjected to different biochemical analyses. The serum obtained from the blood was refrigerated until it was used. The blood glucose levels were determined according to [37].

2.11. Kidney functions determination

Methods described by [38–40] were used to determine uric acid, urea, and creatinine, respectively.

2.12. Determination of lipid profile

Triglyceride was analyzed according to [41], total cholesterol was determined according to [42], and serum LDL-cholesterol and serum high-density lipoprotein cholesterol tests were done according to [43].

2.13. Determination of liver functions

ALT and AST analyses were done according to the methods described by [44], protein and albumin were determined according to [45], and globulins were determined according to [46].

2.14. Statistical analysis

The collected data were subjected to the analysis of variance (ANOVA) for a completely randomized design. Statistically significant differences between means were compared at $p \leq 0.05$ using Least Significant Difference (LSD) test. The statistical analysis was carried out using GenStat 17th Edition (VSN International Ltd., Hemel Hempstead, UK) [47].

3. Results and discussion

Tables 1–3 show the total carbohydrate and amino acid contents, the total flavonoid and phenol contents, and the antioxidant activity of *LS*. The data confirmed that the total carbohydrate content was 58.78 g/100 g of dry weight of *LS*; the total amino acid content of *LS*

Table 1

Total amino acids & total carbohydrates contents of *LS*.

Total Carbohydrates (g/100g) Dry weight	Total amino acids (g/100g) Dry weight
58.78	12.35

Table 2

Total flavonoids and total phenols contents of *LS*.

<i>LS</i>	Flavonoid content mg/g (Dry weight)	Phenol content mg/g (Dry weight)
Ethanol Extract	57.384	185.6
Chloroform Extract	2.004	7.960

Table 3

Antioxidant activity of *LS*.

<i>LS</i>	Sample concentration (µg/ml)	DPPH %
Ethanol extract	50	66.941
	100	81.490
	150	89.529
	200	92.196
Chloroform extract	50	50.857
	100	51.184
	150	51.347
	200	51.673

was 12.35 g/100 g of dry weight; the total flavonoid content was 57.384 mg/g of dry weight of *LS* for ethanol extract and 2.004 mg/g of dry weight of *LS* for chloroform extract. The total phenol content was 185.6 mg/g and 7.960 mg/g of dry *LS* weight for ethanol extract and chloroform extract, respectively. In terms of antioxidant activities, the data showed that the ethanol extract of *LS* was 66.941%, 81.490%, 89.529%, and 92.196% DPPH, with sample concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml, respectively. The chloroform extract of *LS* was 50.857%, 51.184%, 51.347%, and 51.673% DPPH, with sample concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml, respectively. As a whole, the data from the three tables clearly reveals that *LS* contains a considerable amount of nutritional and beneficial constituents, confirming the importance of *LS* in both nutritional and medical uses. These findings were in an agreement with data obtained by [48], who stated that the mixing of *Lepidium sativum* seed oil with n-6 PUFA consumable vegetable oils significantly increased natural antioxidant content, antioxidant enzymes, and radical scavenging activity.

Table 4 and Fig. 1 show the effect of oral consumption of *LS* aqueous extract on the blood serum

Table 4
Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on blood serum glucose of tested rats.

Tested Groups	Blood serum glucose mg/dl
Control (–)	89.643
Control (–) + LS extract (10 mg/kg b. wt.)	65.296
Diabetic control group: control (+)	376.608
Control (+) + LS extract (10 mg/kg b. wt.)	212.824
Control (+) + LS extract (20 mg/kg b. wt.)	125.416
Control (+) + LS extract (30 mg/kg b. wt.)	115.616
Control (+) + LS extract (40 mg/kg b. wt.)	107.865
LSD (0.05)	3.0127
Sd	52.6

glucose of the tested rats. The blood serum glucose for the control group was 89.643 mg/dl, while the blood serum glucose for the second group (fed a basal diet and administered LS aqueous extract at 10 mg/kg of body weight) decreased to 65.296 mg/dl, due to the effect of LS aqueous extract. The diabetic control

group (the third group) showed a wild increase in blood serum glucose, which reached 376.608 mg/dl because of their diabetes induced via alloxan. The increased blood serum glucose in these four groups decreased significantly when the administration of LS aqueous extract increased from 10 mg/kg to 40 mg/kg of the rats' body weight. Thus, these results revealed that the administration of LS could be helpful in treating and managing diabetes. Such finding have been previously reported [49] who indicated that the administration of *Lepidium sativum* fostered a better lipid profile as well as decreases in the sugar level of hypercholesterolemic rats. Similarly, one more study has concluded that the reduction of serum blood glucose after the administration of LS is due to the LS seeds containing a considerable amount of linolenic acids [50].

Table 5 and Fig. 2 show the effect of oral consumption of LS on the weights of the tested rats' organs. The control group (which was fed a basal diet) had organs weighing 12.115 g (liver), 1.765 g (kidney),

Fig. 1. Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on blood serum glucose of tested rats.

Table 5
Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on organ weights (g) of tested rats.

Tested Groups	Liver (g)	Kidney (g)	Spleen (g)	Heart (g)
Control (–)	12.115	1.765	0.769	0.724
Control (–) + LS extract (10 mg/kg b. wt.)	12.035	1.723	0.813	0.742
Diabetic control group: control (+)	7.255	1.386	0.234	0.415
Control (+) + LS extract (10 mg/kg b. wt.)	7.754	1.468	0.271	0.439
Control (+) + LS extract (20 mg/kg b. wt.)	7.910	1.798	0.291	0.523
Control (+) + LS extract (30 mg/kg b. wt.)	10.368	1.932	0.346	0.598
Control (+) + LS extract (40 mg/kg b. wt.)	11.635	2.324	0.439	0.661
LSD_{0.05}	0.4861	0.1672	0.0254	0.0181
Sd	0.892	0.384	0.006	0.011

Fig. 2. Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on organ weights (g) of tested rats.

0.769 g (spleen), and 0.724 g (heart). Thus, it became clear that alloxan induction caused great reduction in these organs' weights; the liver reduced to 7.255 g, the kidney to 1.386 g, the spleen to 0.234 g, and the heart at 0.415 g. These weights were significantly increased with as LS administration increased from 10 mg/kg to 40 mg/kg of body weight. Thus, it can be concluded that LS helped restore of the proper weights of these organs, which were almost the same as their weights in non-diabetic rats. The results were similar to those indicated by [51], who reported that the exposure to AlCl_3 caused a group of structural and functional alterations in the kidneys and livers of rats. The antioxidant activity of *Lepidium sativum* seeds supported a protective and curative effect of LS against such changes.

Table 6 and Fig. 3 show the effect of oral consumption of LS aqueous extract on the kidney functions of the experimental rats. The control group that was fed with commercial food showed measurements of 2.652 mg/dl (urea), 51.413 mg/dl, (uric acid) and

0.264 mg/dl (creatinine). The diabetic group showed 4.716 mg/dl (urea), 84.512 mg/dl (uric acid), and 0.376 mg/dl (creatinine). Due to the consumption of LS aqueous extract, kidney functions improved significantly when the LS aqueous extract was increased from 10 mg/kg to 40 mg/kg of body weight. [52] stated that the natural extracts of *Lepidium sativum* are more suitable for usage, as they considered the safety and economy of products; as a result, these extracts can be additives for foodstuffs and therapeutic drugs owing to their multi-medicinal benefits.

The data shown in Table 7 and Fig. 4 indicates that diabetes caused a significant increase in the lipid profile, as the diabetic group was measured at 79.123 mg/dl (triglycerides), 121.113 mg/dl (total cholesterol), 62.776 mg/dl (LDL), and 27.453 mg/dl (HDL), compared to control group that was fed with a basal diet, which was measured at 62.613 mg/dl, 104.312 mg/dl, 45.326 mg/dl, and 46.245 mg/dl for the same respective factors. These data revealed that LS aqueous extract administration resulted in a significant

Table 6

Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on kidney functions (mg/dl) of the tested rats.

Tested Groups	Uric acid mg/dl	Urea mg/dl	Creatinine mg/dl
Control (-)	2.652	51.413	0.264
Control (-) + LS extract (10 mg/kg b. wt.)	2.413	46.854	0.233
Diabetic control group: control (+)	4.716	84.512	0.376
Control (+) + LS extract (10 mg/kg b. wt.)	4.451	82.876	0.334
Control (+) + LS extract (20 mg/kg b. wt.)	4.237	77.243	0.296
Control (+) + LS extract (30 mg/kg b. wt.)	3.826	68.652	0.275
Control (+) + LS extract (40 mg/kg b. wt.)	3.563	66.453	0.242
LSD (0.05)	0.3111	1.5723	0.0421
Sd	14.742	0.883	0.051

Fig. 3. Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on kidney functions (mg/dl) of the tested rats.

Table 7

Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on lipid profile (mg/dl) of tested rats.

Tested Groups	Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Control (-)	62.613	104.312	45.326	46.245
Control (-) + LS extract (10 mg/kg b. wt.)	53.432	79.543	38.457	44.170
Diabetic control group: control (+)	79.123	121.113	62.776	27.453
Control (+) + LS extract (10 mg/kg b. wt.)	75.763	117.653	55.122	38.248
Control (+) + LS extract (20 mg/kg b. wt.)	74.712	114.733	51.672	37.04
Control (+) + LS extract (30 mg/kg b. wt.)	72.874	112.651	49.330	34.652
Control (+) + LS extract (40 mg/kg b. wt.)	70.245	109.549	47.896	32.641
LSD_{0.05}	1.7679	2.7134	2.9621	1.6527
Sd	5.294	13.415	3.758	1.764

reduction of triglycerides, total cholesterol, and LDL, while it caused a significant increase in HDL. Similar findings were also reported by [53], who concluded that *Lepidium sativum* seeds caused a significant reduction in total cholesterol, triglycerides, and LDL,

while it caused an increase in HDL. He also stated that Apelin and VLDL almost returned to normal after the addition of *Lepidium sativum* powder to rats' diets.

The effect of oral consumption of LS aqueous extract on the liver functions of the experimental rats is

Fig. 4. Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on lipid profile (mg/dl) of tested rats.

Table 8

Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on liver functions (mg/dl) of tested rats.

Tested Group	ALT (U/L)	AST (U/L)	Albumin (U/L)	Globulin (U/L)
Control (–)	37.852	36.152	2.691	4.872
Control (–) + LS extract (10 mg/kg b. wt.)	34.541	32.341	2.283	4.452
Diabetic control group: control (+)	46.150	45.654	5.431	7.611
Control (+) + LS extract (10 mg/kg b. wt.)	44.233	43.563	5.013	7.312
Control (+) + LS extract (20 mg/kg b. wt.)	42.235	41.134	4.652	6.893
Control (+) + LS extract (30 mg/kg b. wt.)	40.675	39.603	4.238	6.254
Control (+) + LS extract (40 mg/kg b. wt.)	38.225	37.892	3.796	5.765
LSD_{0.05}	1.001	0.9651	0.061	0.2375
Sd	4.511	4.783	1.281	1.205

Fig. 5. Table 8: Effect of oral consumption of *Lepidium sativum* (LS) aqueous extract on liver functions (mg/dl) of tested rats.

tabulated in Table 8 and graphically presented in Fig. 5. These results indicate that the control group recorded scores of 37.852 U/L (ALT), 36.152 U/L (AST), 2.691 U/L (albumin), and 4.872 U/L (globulin). When alloxan was induced, the diabetic control group showed a big increase for all the liver functions examined, i.e., 46.150 U/L (ALT), 45.654 U/L (AST), 5.431 U/L (albumin), and 7.611 U/L (globulin). Increasing the administration of LS aqueous extract from 10 mg/kg to 40 mg/kg of body weight caused a significant reduction for all the liver functions that were examined. A similar observation has also been reported by [54], who concluded that *Lepidium sativum* has significant hepatoprotective activity.

4. Conclusion

This study focused on the treatment of diabetic Wistar rats by administering an aqueous extract of *LS*. The study's findings reveal that *LS* is one of the medicinal plants that has the potential to cure diabetes and manage disorders of bodily functions. A plan for a future study to explore the effects of *Lepidium sativum* on diabetic human patients is now being prepared.

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