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Possible Effects of Lotus (Petals & Seeds) in Streptozotocin-Induced Diabetic Rats

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Abstract:

The present study was designed to compare the effect of different parts of louts (*Nelumbo nucifera*) (petals and seeds) on diabetic rats. Forty adult male albino rats were used in this study, weighting (140±10g) were divided into eight groups, five rats each. Louts petals, seeds and their mixture as powder were added at percent of 2.5 % and 5% from the basal diet. Rats injected with streptozotocin dose of 40 mg/kg to infect diabetes. Evaluation the chemical composition and nutrient contents of louts such as total phenol, total flavonoids and antioxidant activity. Initial weight, final weight, feed intake (FI), Feed Efficiency Ratio (FER), (BWG %) and relative organs weight, serum glucose, serum lipid profiles (TG, TC, LDL-c, VLDL-c, HDL-c and AI), serum liver enzymes activities (AST, ALT and ALP) and kidney function (creatinine, uric acid and urea levels) were determined. From the obtained results it was shown that feeding on lotus petals, seeds and their mixture as powder caused significant ($P \leq 0.05$) increases in HDL-c, compared with control (+ve) group, and enhanced the kidney and liver functions with the decrease of ALT, AST, ALP, serum glucose, creatinine, uric acid, urea which reflects the powerful nutraceutical therapeutic effect for feeding on lotus petals, seeds and their mixture powders for treatment diabetic in rats. The best result was that of 5% lotus mixture powder diet.

Key words: Louts flower, Diabetic, Rats and Biochemical analysis.

Introduction:

Diabetes mellitus (DM) is a chronic metabolic disorder which affects a significant portion of the population worldwide (**Wild et al., 2004**).

Diabetes is caused by impaired insulin production and or decreased tissue response to the insulin. The numbers of people with diabetes are increasing worldwide; about 366 million people have diabetes, and 552 million people are expected to have diabetes in 2030 (**Scully, 2012**).

Diabetes mellitus is group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.1, 2 the chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, like the eyes, kidney, nerves, heart and blood vessels (**Rana and Ray, 2017**).

As previously mentioned, type 1 diabetes is an autoimmune disorder where the beta cells of the pancreas are destroyed by the body. The exact cause of type 1 diabetes is unknown. It is suggested that it is as a result of a complex interaction between genetic and environmental factors. As of yet, there is no known specific environmental risk factor identified, that causes or increases the risk of onset of type 1 diabetes(**Global, 2016**).

Ragheb and Medhat (2011) reported that disturbances of lipids in the body lead to development of insulin resistance and metabolic diseases. The nature of type 2 diabetes is very different from type 1 diabetes as, in type 2 diabetes, it may be several years before glucose levels become elevated and uncontrolled by the body. Type 2 diabetes may go unnoticed between the time of onset and the time of diagnosis.

T1DM is mainly triggered by environmental factors. The main factors that contribute to the development of insulin resistance (T2DM) include obesity, physical inactivity, and smoking (**Kablan et al., 2010**).

In ancient times, lotus was common along the banks of the River Nile with closely related species known as 'sacred blue lotus' (*Nymphaea louts*). The Pharoic Egyptians worshipped the lotus flowers, fruit and sepals, which were widely depicted as architectural motifs. From Egypt, it was carried to Assyria and widely planted throughout Persia, India and China. It was first brought into horticulture in Western Europe during 1787 as a stove-house water lilly under the patronage of

Sir Joseph Banks and nowadays it can be seen almost everywhere in modern botanical garden collections. Lotus plants are common in Australia, China, India, Iran and Japan (**Anonymous, 1966**).

Lotus (*Nelumbo nucifera*) is native to India, but was widely spread through Persia, Egypt, and Asia in the ancient times. It was introduced in Europe as a type of water-lily in the 18th century, and nowadays it can be found in modern botanical gardens all over the world. Lotus plants are commonly cultivated in Australia, China, India, Iran, and Japan (**Anonymous, 1966**).

N.nucifera is an important aquatic economic plant, not only as a dainty and ornamental flower but also as a source of herbal medicine with strong antipyretic, cooling, astringent, and demulcent properties. The species is of religious significance in South East Asia (hence, the name sacred lotus) and the seeds and leaves are also eaten in this region. Virtually, all parts of the lotus plant are used: The rhizome is used as food, seed as medicine, thalamus as fruit, leaves as plate (thali), stalks as pickle, petals for colour extraction, and tender leaves as food after being blended with vegetables (**Mandal and Bar, 2013**).

Lotus (*Nelumbo nucifera*, L.) commonly known as lotus or sacred lotus is an aquatic perennial plant belonging to family Nelumbonaceae (**Sheikh, 2014**).

Petal blackening and the lack of flower opening are related to water stress and carbohydrates after harvesting accompanied by an increase in ethylene production (**Netlak and Imsabai, 2016**).

Mineral composition of 100 g of lotus seeds consists of sodium (7.86 mg), potassium (48.5 mg), calcium (313 mg), phosphorus (6.25 mg), magnesium (43.9 mg), copper (2.51 mg), zinc (7.72 mg), manganese (16.6 mg), iron (16.4 mg), and selenium (1.04 mg). Lotus seeds have been found to contain a variety of minerals such as chromium (0.0042%), sodium (1.00%), potassium (28.5%), calcium (22.10%), magnesium (9.20%), copper (0.0463%), zinc (0.0840%), manganese (0.356%) and iron (0.1990%). Normally, lotus seeds are rich in protein, amino acids, unsaturated fatty acids and minerals (**Wu, 2007**).

Seeds and roots of lotus are regarded as popular health food and the alkaloid (liensinine) extracted from them is effective to treat arrhythmia (**Ling et al., 2005**).

Lotus leaves are used in traditional medicine to treat hypertension, diarrhea, fever, weakness, infection, skin inflammation, and body heat imbalance (**Sridhar and Rajeev, 2007**).

All parts of the lotus plant have yielded significant amounts of phenolic and flavonoid compounds (**Huang et al., 2011**).

Material and Methods:

Analytical methods:

The moisture was determined according to the method recommended by **AOAC (2005)** using air oven at 100 – 102° C for about 3 hours. The total nitrogen was determined using Marco Kjeldahl methods, according to **AOAC (2005)**, crude protein then calculated as T.N. X 6.25. The fat content was determined following the method given by the **AOAC (2005)**. Soxhlet apparatus was used. The extraction continued for 16 hours with n-hexane as the extraction solvent. Ash content was estimated according to the method described by the **AOAC (2005)** after charring. The samples were placed in a muffle furnace at 525°C until white or light grey ash was obtained. Crude fiber was determined according the method of **Pearson (1971)**. Sample was digested in boiling 0.128 M sulphuric acid for 45 minutes, washed with distilled water three times, digested with boiling 0.223 M potassium hydroxide, washed with distilled water three times, followed by washing with acetone (cold extraction) three times, then dried at 150°C for one hour and finally weighted. The carbohydrate was calculated by the difference as follows:

$\% \text{ Carbohydrates} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ fiber})$. Total calories were calculated as the sum of multiplying 1g protein and carbohydrates by 0.4 ug and 1g fat by 9.0 according to **FAO (1982)**. The method used for the determination of total phenols using Folin-Ciocalteu reagent was adapted from **Mc Donald et al., (2001)**. Dried samples and standards were prepared in distilled water. Test solutions (samples and standards) of 0.5 ml were added to 4.0 ml of 1 M Na₂CO₃. Five milliliters of Folin-Ciocalteu reagent (1:10, v=v) were added and the solutions allowed to stand at 45 C for 15 min. Absorbance was measured at 750 nm. The blank consisted of all reagents and solvents without test compounds or standard. The standard was gallic acid prepared in concentrations of 50 to 200 mg/L. This is commonly used as a reference compound. The phenolic concentrations

were determined by comparison with the standard calibration curve. Total phenol values were expressed as gallic acid equivalents (mg g⁻¹ dry mass). The total flavonoid content was determined using **Heimler et al., (2005)** method. 250 µl of extractor rutin standard solution or 80% methanol (blank solution) was mixed with 1.25 ml of distilled water and 75 µl of 5% NaNO₃ solution. The mixture was vortexed for 15 sec and standard for 6 min at room temperature. 10% AlCl₃.6H₂O (150 µl) was added on the mixture and then, it was incubated for 5 min at room temperature. 0.5 ml of 0.1 M NaOH solutions and 275 µl of distilled water were added and the mixture was vortexed for 20 sec. The absorbance was measured at 510 nm immediately. Total flavonoid content was expressed as rutin equivalents (mg of RE/100 g dry matter) through the calibration curve of rutin that its linearity range was 25-350 µg/ml (R²>0.99). DPPH radical scavenging capacity of lotus seeds and petals extracts was performed in terms of the methods of **Sun et al., (2007)** and **Kirca & Özkan (2007)**. 15, 30, 45 µl of sample and 30 µl of Trolox were completed to 2 ml with 0.1mM DPPH. The mixture was vortexed for 20 sec. The absorbance was measured at 515 nm after 20min incubation at room temperature and dark area. 2 ml of 80% methanol was used as a blank solution. The absorbance of DPPH (2 ml) was a control. The inhibition percentage of the absorbance was calculated as follows: Inhibition % = (A control– A sample)/A control. The antioxidant activity was expressed as Trolox equivalent (mg Trolox/100 g dry matter). It was the ratio between the slope of the inhibition % versus amount of sample and that of Trolox. Linearity range of the calibration curve of Trolox was 0.1 to 1.0 mm (R²>0.99).

Materials:

Plant materials:

Lotus (*Nelumbo nucifera*) petals and seeds were obtained from herbalist where dried at 40°C in a vacuum oven, then milled. The powders stored in dark glass jars and kept at less than 30°C till use (**Parry et al., 2006**).

Chemical materials:

Streptozotocin powder obtained from El-Gomhoria Company, Cairo, Egypt.

Experimental animals:

The experimental was done in the Faculty of Medicine Ain Shams Research Institute. A total of 40 adult normal male albino rats Sprague Dawley strain weighing 140 ± 10 g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Biological investigations:

Rats were housed in individual stainless steel cages under controlled environmental conditions, and fed for one week on basal diet prior to start feeding on experimental diet for acclimatization. Diets were introduced to rats in a special non – scattering feeding cup to avoid loss of feed and contamination. Tap water was provided to rats by means of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage.

Basal diet composition of tested rats:

The basal diet in the experiment was prepared according to (Reeves *et al.*, 1993). It was consisted of 20% protein (casein), 10% sucrose, 4.7% corn oil, 0.20% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fiber (cellulose).

The induction of experimental diabetes:

Diabetes was induced in normal healthy male albino rats by intraperitoneal injection of streptozotocin 40 mg/kg body weight, according to the method described by Ramesh and Pugalendi (2006).

One week after the injection of streptozotocin, fasting blood samples were obtained to estimate fasting serum glucose 200 mg/dl rats which were considered diabetes (NDDG, 1994).

Experimental design:

Forty (40) rats were fed on basal diet for 7 days for adaptation. All rats were divided into 8 groups (5rats each), all groups were fed for 4 weeks according to the following groups: Group (1): Used as a negative control group and fed on the basal diet throughout the experiment. Group (2): Diabetic rats (streptozotocin at dose of 40 mg/kg) fed on basal diet only without any treatment as positive control group. Group (3): Diabetic rats fed on basal diet with 2.5% louts petals powder. Group (4): Diabetic rats fed on basal diet with 5% louts petals powder. Group (5): Diabetic rats fed on basal diet with 2.5% louts seeds powder. Group (6): Diabetic rats fed on basal diet with 5% louts seeds powder.

Group (7): Diabetic rats fed on basal diet with 2.5% lotus mixture (petals+ seeds) powder. Group (8): Diabetic rats fed on basal diet with 5% lotus mixture (petals+ seeds) powder.

After fasting for 12 hours, blood samples in initial times were obtained from retro orbital vein, while it obtained from hepatic portal vein at the end of each experiment. Blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 30 minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean cuvette tube and stored frozen in deep freezer till analysis according to method described by **Schermer (1967)**.

Biological evaluation:

During the experimental period (28 days) the net feed intake was daily recorded, while body weight was weekly recorded. The net feed intake and gained body weight were used for the calculation of feed efficiency ratios (FER) according to **Chapman *et al.*, (1959)** as follow:

$$\text{BWG \%} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$\text{FER \%} = \frac{\text{Body weight gain (g)}}{\text{Food intake (g)}} \times 100$$

Organs weight:

The different organs of rats (liver, heart, kidney and spleen) were carefully removed, washed in saline solution, dried between filter papers and immediately weighted. The relative organ weight was calculated as following:

$$\text{Relative organs weight \%} = \frac{\text{Organs weight (g)}}{\text{Total body weight (g)}} \times 100$$

Biochemical analysis:

Different tested parameters in serum were determined using specific methods as follows:

Serum glucose was measured using the modified kinetic method according to (**Kaplan, 1984**). Serum cholesterol (TC) was measured using the modified kinetic according to (**Richmond, 1973**). Serum triglycerides (TG) were measured using the modified kinetic method according to the method described by (**Fossati and Prencipe, 1982**).

Serum high density lipoprotein cholesterol (HDL-c) was measured using the modified kinetic method according to (Allain, 1974). Serum very low density lipoprotein cholesterol (VLDL-c) was calculated as mg/dl according to (Lee and Nieman, 1996) equation:

VLDL-c Concentration mg/dl = $\frac{T.G}{5}$. Serum low density lipoprotein cholesterol (LDL-c) was calculated as mg/dl according to (Castelli *et al.*, 1977) equation:

LDL Concentration mg/dl = Total Cholesterol – HDL-c – VLDL-c

The VLDL + LDL / HDL ratio: Atherogenic index (AI) was calculated according to the formula of Kikuchi *et al.*, (1998). AST activities were measured in serum using the modified kinetic method of (Henary, 1974). ALT activities were measured in serum using the modified kinetic method of (Tiez, 1976). Urea was determination in serum using the modified kinetic method or liquicolor of (Patton and crouch, 1977). Serum creatinine was measured using the modified kinetic method according to (Schirmeister, 1964). Serum uric acid was measured using the modified kinetic method according to (While *et al.*, 1970).

Statistical analysis:

Data were analyzed using a completely randomized factorial design (SAS, 1988) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

Results and discussion:

Table (1): Chemical composition of Petals:

Data given in table (1) showed that the proximate chemical composition of louts Petals. The result indicated that, the Protein, Fat, Ash, Fiber, Carbohydrates and Energy value content of lotus patels as weight dry (D/W) were 23.99, 5.62, 9.24, 14.72 & 46.43% and total calories were 332.26 Kcal/100g, respectively.

Table (2): Chemical composition of louts seeds:

Data given in table (2) show the proximate chemical composition of louts seeds. The result indicated that the Protein, Fat, Ash, Fiber, Carbohydrates and Energy value content of lotus seeds as weight dry (D/W) were 18.84, 2.38, 4.71, 9.22 & 64.85 %. While total calories were 356.18 Kcal/100g.

Table (3): Total phenolic, total flavonoid, antioxidant activity and anthocyanin of lotus:

Total phenolic, total flavonoid, antioxidant activity and anthocyanin of louts are show in table (3). Data indicated that plant have a high percentage of photochemical. Chemical analysis was performed to determine the content of louts petals and seeds of photochemical, the results indicated that louts petals contained a lower percentage of TPC (19.20mg/100g) than Lotus seeds which were (62.91mg/100g) respectively. The results indicated also (Table 3) that louts petals contained a higher values of TF, DPPH (102.44mg/100g, 144.27%) than lotus seeds which were (36.48 mg/100g, 22.87%) respectively.

Table (4): Effect of lotus petals, seeds and their mixture as a powder on initial weight and final weight of diabetic rats:

Data presented in table (4) show the effect of lotus petals, seeds and their mixture as a powder on initial weight and final weight of diabetic rats. As for the initial weight, it could be noticed that the mean value of groups 3, 4, 5, 6, 7 and 8 showed non-significant differences between them, which was 142.66, 143.33,144, 144.33, 145 and 147(%), respectively. The results indicated also that the mean value of final weight of positive control group was significantly lower than negative control group, which was 179 and 224.33 (%), respectively. the mean values of groups 3, 4, 5, 6, 7 and 8 showed significant differences when compared with positive control group; it was 190.33, 198.33, 205.33, 181.66, 188.66, and 211.66(%), respectively. 5% lotus mixture powder diet was considered as the nearest mean value for negative control group.

Table (5): Effect of lotus petals, seeds and their mixture as a powder on feed intake (FI), Feed Efficiency Ratio (FER) and Relative Body Weight Gain (BWG %) of diabetic rats:

Data presented in table (5) show the effect of lotus petals, seeds and their mixture as a powder on initial FI, FER and BWG % of diabetic rats. The mean value of FI (g) of positive control group was significantly lower than negative control group, which was 17.9 and 22.43 (g), respectively. Also the mean values of groups 3, 4, 5, 6, 7 and 8 differed significantly, it was 19.03, 19.83, 20.5, 18.16, 18.87, 21.166 (g), respectively, compared to that of positive control. Results indicated the

effect of lotus petals, seeds and their mixture as a powder on FER in diabetic rats. Result indicated that the mean value of FER of positive control group was significantly lower than negative control group, which was 0.068 and 0.126 (g), respectively. Also the mean value of groups 3, 4, 5, 6, 7 and 8 differed significantly in comparison with positive control, it was 0.089, 0.099, 0.106, 0.074, 0.082 and 0.109 (g), respectively. Results show also the effect of lotus petals, seeds and their mixture as a powder on BWG % in diabetic rats. Result indicated that the mean value of BWG % of positive control group was significantly lower than negative control group, which was 23.45 and 54.36 (g), respectively. Also the mean value of groups 3, 4, 5, 6, 7 and 8 differed significantly compared to positive control, it was 33.42, 38.37, 42.59, 25.86, 30.11 and 43.99 (%), respectively. Best group seem to be that of group (8).

Table (6): Effect of lotus petals, seeds and their mixture as a powder on relative weight of liver and heart of diabetic rats:

Data presented in table (6) Show the mean value of relative weight of liver and heart of diabetic rats. As for relative weight of liver, it could be noticed that the mean value of positive control group as signification higher than that of control group, which was 3.08 and 1.11 (g), respectively. As compared to positive control group, relative weight of liver of group 3, 4, 5, 6, 7 and 8 was 2.62, 2.39, 2.11, 3.04, 2.85 and 1.37 (g), respectively. It could be noticed that there is non-significant between negative control groups 8. The best result was recorded for group 8. Concerning relative weight of heart, results showed the mean value of relative weight of heart of positive control group was higher than negative control group, which was 0.81 and 0.26 (g), respectively. All treatment showed a significant difference when compared to positive control group. the mean value groups 3, 4, 5, 6, 7 and 8 was significant lower than positive control group the mean of 0.53, 0.45, 0.38, 0.71, 0.62 and 0.3 (g), respectively. It could be noticed that there is non-significant between negative control groups 8. The best result was recorded for group 8.

Table (7): Effect of lotus petals, seeds and their mixture as a powder on relative weight of kidney and spleen of diabetic rats:

Data presented in table (7) Show the mean value of relative weight of kidney and spleen of diabetic rats. As for relative weight of kidney, results indicated that the mean value of positive control group was significantly higher than negative control group, which was 0.83 and 0.22 (g), respectively. Not that there is an insignificant difference between positive control group and group 6 which was 0.83 and 0.8 (g), respectively. As compared to positive control group was 0.63, 0.52, 0.40, 0.73 and 0.29 (g) , respectively. The best result was recorded for group 8. Concerning relative weight of spleen, results showed the mean value of relative weight of spleen of positive control group was significantly higher than negative control group, which was 0.33 and 0.18 (g), respectively. When compared to positive control group and other groups the result showed a non-significant difference between group 4 and group 5. The result showed a non-significant difference between positive control group, group 6 and group 7. It could be noticed that there is non-significant between negative control groups 8. The best result was recorded for group 8.

Table (8): Effect of lotus petals, seeds and their mixture as a powder on glucose levels of diabetic rats:

Data presented in table (8) show the effect of lotus petals, seeds and their mixture on glucose levels of diabetic rats for negative control, positive control and other different groups of diabetic rats fed on lotus petals, seeds and their mixture as a powder. It's clear to notice that the highest glucose levels recorded for positive control group, while negative control group recorded the lower value with significant differences. The mean values were 285.00 and 101.00 mg /dl, respectively. On the other hand, the highest glucose levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lowest value recorded for 5% lotus mixed powder with significant differences. The mean values were 152.00 and 95.00 mg / dl, respectively. These results are in agreement with **Szkudelski (2001)** who repeated that administration of the methanolic extract (100-200 mg/kg b.w.), nuciferin and norcoclaurine (10 mg/kg) significantly reduced the

fasting glucose level of blood in alloxan induced diabetic rats as compared to normal control.

Table (9): Effect of lotus petals, seeds and their mixture as powder on serum total cholesterol levels (TC) and serum triglycerides (TG) of diabetic rats:

Data presented in table (9) show the effect of lotus petals, seeds and their mixtured powders on serum total cholesterol levels (TC) and serum triglycerides (TG) of diabetic rats for negative control, positive control and other different groups of diabetic rats fed on lotus petals, seeds and their mixture as powder. The obtained results indicated that the highest serum total cholesterol levels recorded for positive control group, while negative control group recorded the lower value with significant differences. The mean values were 115.55 and 77.85 mg/dl, respectively. On the other hand, the highest serum cholesterol levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lowest value recorded for 5% lotus mixture powder with significant differences. The mean values were 87.40 and 63.9 mg/ dl, respectively. In case of serum triglycerides levels, it could be concluded that the highest serum triglycerides levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 80.50mg/dl and 44.50mg /dl, respectively. On the other hand, the highest serum triglycerides levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lowest value recorded for 5% lotus mixture powder with significant differences. The mean values were 76.10 and 51.30 mg/ dl, respectively. These results are in agreement with **Onishi (1984)** where aqueous extract of *N.nucifera* leaves was studied for its effects on serum lipids in a rat model. The rats were fed a high-fat diet containing .5% cholesterol and 1% cholic acid. Subsequent oral treatment with a crude aqueous extract of lotus leaves resulted in sharp decreases in serum total cholesterol, free cholesterol and phospholipids compared with the high fat- loaded control group. **Wang (2009)** indicated that flavonoid-enriched lotus leaf extract (FLL) supplement may significantly improve the high fat diet-induced abnormal blood lipids and liver damage as significantly as the common drugs. **De Sereday et al., (2004)** reported that, the levels of TC and TG have been decreased significantly in diabetic mice after the FLL supplementation. **Sharma et**

al., (2003) showed that these effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin. The FLL supplementation also resulted in the significant increases in the levels of serum HDL-c toward the control level which again strengthens the hypolipidemic effect of this extract.

Table (10): Effect of lotus petals, seeds and their mixture as a powder on serum lipid profile levels of diabetic rats:

Data presented in table (10) show the effect of lotus petals, seeds and their mixture as a powder on high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) levels of diabetic rats for negative control, positive control and other different groups of diabetic rats fed on lotus petals, seeds and their mixture as a powder. The obtained results indicated that the highest high density lipoprotein cholesterol levels recorded for negative control group, while positive control group recorded the lowest value with significant differences. The mean values were 59.00 and 27.25 mg/dl, respectively. On the other hand, the highest high density lipoprotein cholesterol levels of treated groups (diabetic groups) recorded for 5% lotus mixture powder, while the west value recorded for 2.5% lotus Petals powder with significant differences. The mean values were 57.0 and 38.25 mg/ dl, respectively. Data also indicated that the highest low density lipoprotein cholesterol levels recorded for positive control group, while negative control group recorded the lower value with significant differences. The mean values were 72.2 and 9.95 mg/dl, respectively. On the other hand, the highest low density lipoprotein cholesterol levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lowest value recorded for 5% lotus mixture powder with significant differences. The mean values were 33.93mg/ dl and 8.64mg/ dl, respectively. In case of very low density lipoprotein cholesterol levels, it could be concluded that the highest VLDL- c levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 16.10mg /dl and 8.90mg /dl, respectively. On the other hand, the highest VLDL- c levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lowest value recorded for 5% lotus mixture powder with significant differences. The mean values were 15.22 and 10.26 mg/ dl, respectively. These results are in agreement with **Guérin *et al.*, (2001)** who reported

that increased exchange between the cholesterol esters of HDL and TG of TG-rich lipoproteins lowers blood HDL-CHOL. Thus, insulin resistance could induce hypertriglyceridemia and reduction in HDL-CHOL in mice. However, the results of this study showed that the lotus leaf extract effectively alleviated hypertriglyceridemia and hypercholesterolemia and elevated HDL-CHOL levels in db/db mice. **Stratton et al., (2000)** therefore, improved insulin sensitivity due to administration of a lotus leaf extract could contribute to controlling dyslipidemia, which is important in reducing the risk of micro and macrovascular complications in patients with diabetes. **Latha and Pari (2003)** reported that alloxan induced diabetic rats showed significantly increased serum lipid profiles except HDL compared with the control normal rats. Oral administration of the aqueous extracts of *Nelumbo nucifera* plant parts to the diabetic rats significantly reduced the level of TG, TC, and LDL and increased the level of HDL. The results suggest that aqueous extract of the plant parts possesses potential therapeutic value in combating atherosclerosis, which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level. **Venkateswaran and Pari (2002)**, repeated that oral administration of aqueous extracts of *Nelumbo nucifera* plant parts decreased TC, TG and LDL-C but increased HDL-C in treated diabetic rats. These results indicated that *N. Nucifera* plant parts possess hypoglycemic and hypolipidemic activities. In this context numbers of other plants have also been reported to have antihyperglycemic and insulin-release stimulatory effect. The oral administration of aqueous extract of rhizomes and leave of *Curcuma longa* and *Passiflora edulis* (*P.edulis*) Sims respectively in diabetic rats for 30 days reduced the total cholesterol, triglycerides (TG) and low density lipoprotein (LDL-c) but increased high density lipoprotein (HDL-c) in the serum of treated diabetic rats when compared to diabetic controls only. Likewise, **Sakuljaitrong et al., (2012)** observed that *Nelumbo nucifera* flower extract decreased the levels of total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL-c) but increased high density lipoprotein (HDL-c) in the serum of treated diabetic rats when compared to diabetic controls. All these studies underscore the importance of diabetes mellitus. It is with to mention that AI was also best for group (8).

Table (11): Effect of lotus petals, seeds and their mixture as a powder on liver function of diabetic rats:

Data presented in table (11) show the effect of lotus petals, seeds and their mixture as a powder on liver functions (AST, ALT and ALP) levels of diabetic rats for negative control, positive control and other different groups of diabetic rats fed on lotus petals, seeds and their mixture as powders. The obtained results indicated that the highest AST levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 49.25 and 19.65 U/L, respectively. On the other hand, the highest high AST levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lowest value recorded for positive control group with significant differences. The mean values were 41.8 and 29.3 U/L, respectively. Data also indicated that the highest ALT levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 41.45 and 20.80 U/L, respectively. On the other hand, the highest ALT levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lowest value recorded for 5% lotus mixture powder with significant differences. The mean values were 40.00 and 26.60 U/L, respectively. In case of ALP levels, it could be concluded that the highest serum levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 46.35 and 24.2 U/L, respectively. On the other hand, the highest serum ALP levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while the lower value recorded for 5% lotus mixture powder with significant differences. The mean values were 42.25 and 28.05 U/L, respectively. These results are confirmed by another study carried out by **Yuan *et al.*, (2014)** where ethyl acetate (NUEA) and n-butanol (NUBU) extracts of *N. nucifera* leaves were evaluated for hepatoprotective effect on CCl₄ induced acute liver injury in mice. Except for NUEA group, the levels of aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) in each treatment group significantly decreased. Moreover, the contents of malondialdehyde (MDA) and the level of SOD in liver of each group were significantly decreased.

Table (12): Effect of lotus petals, seeds and their mixture as a powder on kidney functions levels of diabetic rats:

Data presented in table (12) show the effect of lotus petals, seeds and their mixture as a powder on kidney functions (serum urea, serum uric acid and serum creatinine) levels of diabetic rats for negative control, positive control and other different groups. The obtained results indicated that the highest serum urea levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 36.20 and 12.18 mg/dl, respectively. On the other hand, the highest high serum urea levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while a lower value recorded for 5% lotus mixture powder with significant differences. The mean values were 30.80 and 16.40 mg/ dl, respectively. Data also indicated that the highest serum uric acid levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 9.85 and 3.60 mg/dl, respectively. On the other hand, the highest serum uric acid levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while a lower value recorded for 5% lotus mixture powder with significant differences. The mean values were 6.80 and 3.80 mg/ dl, respectively. In case of serum creatinine levels, it could be concluded that the highest serum creatinine levels recorded for positive control group, while negative control group recorded the lowest value with significant differences. The mean values were 1.45 and 0.77 mg/dl, respectively. On the other hand, the highest serum creatinine levels of treated groups (diabetic groups) recorded for 2.5% lotus Petals powder, while a lower value recorded for 5% lotus mixture powder with significant differences. The mean values were 1.27mg/ dl and 0.81mg/ dl, respectively.

Table (1): Chemical composition of lotus petals.

Constitutes %	D/W
Moisture %	----
Protein%	23.99±0.12
Fat%	5.62±0.12
Ash%	9.24±0.10
Fiber%	14.72±0.03
Carbohydrates%	46.43±0.20
Energy value (Kcal/100g)	332.26±0.04

DW= Dry weight

All values are expressed as mean ± standard deviation of duplicate results.

Table (2): Chemical composition of lotus seeds.

Constitutes %	D/W
Moisture %	----
Protein%	18.84±0.12
Fat%	2.38±0.12
Ash%	4.71±0.10
Fiber%	9.22±0.03
Carbohydrates%	64.85±0.20
Energy value (Kcal/100g)	356.18±0.04

DW= Dry weight

All values are expressed as mean ± standard deviation of duplicate results

Table (3): Total phenols, total flavonoids and DPPH contents of the lotus seeds and petals.

Material	Total phenols (mg GAE/100g DM)	Total flavonoids (mg RE/100 g DM)	Antioxidant activity (DPPH) (%)
Lotus petals	19.20	102.44	144.27
Lotus seeds	62.91	36.48	22.87

D.M= Dry matter

G.A.E= Gallic acid

R.E = Rutin

Table (4): Effect of lotus petals, seeds and their mixture as a powder on Initial weight and Final weight of diabetic rats.

Groups	Parameter	Initial weight (g)	Final weight (g)
		M ± SD	M ± SD
Group 1: control (-ve)		145.33 ^a ±3.51	224.33 ^a ±4.04
Group 2: control (+ve)		145 ^a ±2.0	179 ^f ±3.61
Group 3: Lotus Petals powder (2.5%)		142.66 ^a ±2.52	190.33 ^c ±2.52
Group 4: Lotus Petals powder (5%)		143.33 ^a ±1.15	198.33 ^d ±4.04
Group 5: Lotus seeds powder (2.5%)		144 ^a ±3.61	205.33 ^c ±2.52
Group 6: Lotus seeds powder (5%)		144.33 ^a ±1.52	181.66 ^f ±2.52
Group 7: Lotus mixture powder (2.5%)		145 ^a ±1.0	188.66 ^e ±1.53
Group 8: Lotus mixture powder (5%)		147 ^a ±2.0	211.66 ^b ±3.05
LSD (P ≤ 0.05)		4.07	5.345

Values with different column indicated significant differences between the group (p < 0.05), and vice versa.

Table (5): Effect of lotus petals, seeds and their mixture as a powder on FI, FER and BWG % of diabetic rats.

Groups	Parameter	Feed intake (g)	FER (g)	BWG %
		M ± SD	M ± SD	M ± SD
Group 1: control (-ve)		22.43 ^a ±0.404	0.126 ^a ±0.001	54.36 ^a ±1.78
Group 2: control (+ve)		17.9 ^f ±0.3	0.068 ^e ±0.002	23.45 ^e ±0.93
Group 3: Lotus Petals powder (2.5%)		19.03 ^e ±0.25	0.089 ^d ±0.004	33.42 ^d ±2.14
Group 4: Lotus Petals powder (5%)		19.83 ^d ±0.4	0.099 ^c ±0.006	38.37 ^c ±3.17
Group 5: Lotus seeds powder (2.5%)		20.5 ^c ±0.25	0.106 ^b ±0.008	42.59 ^b ±5.36
Group 6: Lotus seeds powder (5%)		18.16 ^f ±0.25	0.074 ^e ±0.001	25.86 ^e ±0.62
Group 7: Lotus mixture powder (2.5%)		18.87 ^e ±0.15	0.082 ^d ±0.003	30.11 ^d ±1.6
Group 8: Lotus mixture powder (5%)		21.166 ^b ±0.31	0.109 ^b ±0.004	43.99 ^b ±2.33
LSD (P ≤ 0.05)		0.536	0.008	4.495

FI=feed intake.

FER=Feed Efficiency Ratio.

BWG=body weight gain.

Values with different column indicated significant differences between the group (p < 0.05), and vice versa.

Table (6): Effect of lotus petals, seeds and their mixture as a powder on relative weight of liver and heart of diabetic rats.

Groups	Parameter	RWL	RWH
		M ± SD	M ± SD
Group 1: control (-ve)		1.11 ^e ±0.20	0.26 ^g ±0.04
Group 2: control (+ve)		3.08 ^a ±0.1	0.81 ^a ±0.02
Group 3: Lotus Petals powder (2.5%)		2.62 ^{ab} ±0.09	0.53 ^d ±0.03
Group 4: Lotus Petals powder (5%)		2.39 ^{cd} ±0.03	0.45 ^e ±0.02
Group 5: Lotus seeds powder (2.5%)		2.11 ^d ±0.17	0.38 ^f ±0.03
Group 6: Lotus seeds powder (5%)		3.04 ^a ±0.07	0.71 ^b ±0.02
Group 7: Lotus mixture powder (2.5%)		2.85 ^{ab} ±0.04	0.62 ^c ±0.02
Group 8: Lotus mixture powder (5%)		1.37 ^e ±0.37	0.3 ^g ±0.02
LSD (P ≤ 0.05)		0.293	0.045

RWL= relative weight of liver

RWH= relative weight of heart

Values with different column indicated significant differences between the groups (p ≤ 0.05), and vice versa.

Table (7): Effect of lotus petals, seeds and their mixture as a powder on relative weight of kidney and spleen of diabetic rats.

Groups	Parameter	RWK	RWS
		M ± SD	M ± SD
Group 1: control (-ve)		0.22 ^g ±0.04	0.18 ^d ±0.04
Group 2: control (+ve)		0.83 ^a ±0.04	0.33 ^a ±0.05
Group 3: Lotus Petals powder (2.5%)		0.63 ^c ±0.03	0.27 ^b ±0.01
Group 4: Lotus Petals powder (5%)		0.52 ^d ±0.03	0.24 ^c ±0.01
Group 5: Lotus seeds powder (2.5%)		0.40 ^e ±0.03	0.24 ^c ±0.01
Group 6: Lotus seeds powder (5%)		0.80 ^a ±0.02	0.35 ^a ± 0.01
Group 7: Lotus mixture powder (2.5%)		0.73 ^b ±0.03	0.31 ^a ±0.01
Group 8: Lotus mixture powder (5%)		0.29 ^f ±0.03	0.19 ^d ±0.01
LSD (P ≤ 0.05)		0.052	0.039

RWK= relative weight of kidney

RWS= relative weight of spleen

Values with different column indicated significant differences between the groups ($p \leq 0.05$), and vice versa.

Table (8): Effect of lotus petals, seeds and their mixture as powder on glucose (mg/dl) levels of diabetic rats.

Groups	Parameter	Glucose (mg/dl)
		M ± SD
Group 1: control (-ve)		101.00 ^f ± 0.20
Group 2: control (+ve)		285.00 ^a ± 0.65
Group 3: Lotus Petals powder (2.5%)		152.00 ^b ± 0.24
Group 4: Lotus Petals powder (5%)		141.25 ^c ± 0.88
Group 5: Lotus seeds powder (2.5%)		144.25 ^c ± 0.18
Group 6: Lotus seeds powder (5%)		125.00 ^d ± 1.14
Group 7: Lotus mixture powder (2.5%)		119.25 ^e ± 0.18
Group 8: Lotus mixture powder (5%)		95.00 ^g ± 0.80
LSD (P ≤ 0.05)		5.38

Mean under the same column bearing different superscript letters are different significantly ($p \leq 0.05$).

Table (9): Effect of lotus petals, seeds and their mixture as powder on serum total cholesterol levels (TC) and serum triglycerides (TG) (mg/dl) of diabetic rats.

Groups	Parameter	Triglycerides (mg/dl)	Total cholesterol (mg/dl)
		M ± SD	M ± SD
Group 1: control (-ve)		44.50 ^f ± 0.12	51.85 ^h ± 0.04
Group 2: control (+ve)		80.50 ^a ± 0.67	115.55 ^a ± 0.19
Group 3: Lotus Petals powder (2.5%)		76.10 ^b ± 0.10	93.55 ^b ± 0.69
Group 4: Lotus Petals powder (5%)		68.65 ^c ± 1.80	78.50 ^d ± 0.82
Group 5: Lotus seeds powder (2.5%)		73.45 ^b ± 0.60	89.15 ^c ± 0.909
Group 6: Lotus seeds powder (5%)		71.75 ^c ± 0.59	73.15 ^e ± 0.43
Group 7: Lotus mixture powder (2.5%)		58.80 ^d ± 0.99	60.10 ^f ± 0.60
Group 8: Lotus mixture powder (5%)		51.30 ^e ± 1.70	55.9 ^g ± 1.95
LSD (P ≤ 0.05)		3.54	4.28

Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Table (10): Effect of lotus petals, seeds and their mixture as powder on serum lipid profile (mg/dl) levels of diabetic rats.

Groups	Parameter	HDL- c (mg/dl)	LDL- c (mg/dl)	VLDL- c (mg/dl)	AI mg/dl
		M ± SD	M ± SD	M ± SD	M ± SD
Group 1: control (-ve)		59.00 ^a ± 0.65	9.95 ^c ± 0.04	8.90 ^b ± 0.42	0.32 ^f ± 0.13
Group 2: control (+ve)		27.25 ^c ± 0.30	72.3 ^a ± 1.04	16.10 ^a ± 0.73	3.24 ^a ± 0.52
Group 3: Lotus Petals powder (2.5%)		38.25 ^{bc} ± 0.18	33.93 ^b ± 1.05	15.22 ^a ± 0.82	1.29 ^b ± 0.16
Group 4: Lotus Petals powder (5%)		41.40 ^b ± 0.45	26.52 ^d ± 0.93	13.73 ^{ab} ± 0.36	0.97 ^d ± 0.31
Group 5: Lotus seeds powder (2.5%)		38.50 ^{bc} ± 0.636	30.51 ^c ± 0.18	14.69 ^a ± 0.7	1.17 ^c ± 0.18
Group 6: Lotus seeds powder (5%)		44.05 ^b ± 0.53	26.15 ^d ± 0.45	13.35 ^{ab} ± 0.92	0.90 ^d ± 0.20
Group 7: Lotus mixture powder (2.5%)		41.35 ^b ± 0.353	10.99 ^e ± 0.6	11.76 ^{ab} ± 0.19	0.55 ^e ± 0.15
Group 8: Lotus mixture powder (5%)		45.0 ^b ± 0.485	8.64 ^f ± 0.49	10.26 ^{bc} ± 0.94	0.42 ^f ± 0.13
LSD (P ≤ 0.05)		3.78	1.25	1.99	0.11

Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Table (11): Effect of lotus petals, seeds and their mixture as powder on AST, ALT and ATP (U/L) levels of diabetic rats.

Groups	Parameter	AST (U/L)	ALT (U/L)	ALP (U/L)
		M ± SD	M ± SD	M ± SD
Group 1: control (-ve)		21.65 ^f ± 0.04	20.80 ^e ± 0.404	24.2 ^f ± 0.66
Group 2: control (+ve)		49.25 ^a ± 1.08	41.45 ^a ± 0.71	46.35 ^a ± 0.60
Group 3: Lotus Petals powder (2.5%)		41.8 ^b ± 0.56	40.00 ^a ± 0.89	42.25 ^b ± 0.18
Group 4: Lotus Petals powder (5%)		37.65 ^c ± 0.25	36.90 ^b ± 0.83	36.9 ^c ± 0.94
Group 5: Lotus seeds powder (2.5%)		38.25 ^c ± 0.61	37.60 ^a ± 0.09	40.65 ^b ± 0.47
Group 6: Lotus seeds powder (5%)		36.26 ^c ± 0.63	34.20 ^c ± 0.778	32.5 ^d ± 0.51
Group 7: Lotus mixture powder (2.5%)		34.4 ^d ± 0.38	32.25 ^c ± 0.77	31.25 ^d ± 0.44
Group 8: Lotus mixture powder (5%)		29.3 ^e ± 0.95	26.60 ^d ± 0.80	28.05 ^e ± 0.58
LSD (P ≤ 0.05)		2.14	2.47	2.59

Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

Table (12): Effect of lotus petals, seeds and their mixture as powder on Kidney functions (serum urea, serum uric acid and serum creatinine) (mg/dl) levels of diabetic rats.

Groups	Parameter	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
		M ± SD	M ± SD	M ± SD
Group 1: control (-ve)		12.18 ^g ± 0.44	3.60 ^d ± 0.49	0.77 ^b ± 0.18
Group 2: control (+ve)		36.20 ^a ± 0.80	9.85 ^a ± 0.49	1.45 ^a ± 0.26
Group 3: Lotus Petals powder (2.5%)		30.80 ^b ± 0.07	6.80 ^b ± 0.49	1.27 ^a ± 0.04
Group 4: Lotus Petals powder (5%)		26.85 ^c ± 0.62	5.75 ^b ± 0.77	1.09 ^a ± 0.21
Group 5: Lotus seeds powder (2.5%)		28.0 ^c ± 0.11	6.50 ^b ± 0.28	1.15 ^a ± 0.05
Group 6: Lotus seeds powder (5%)		22.15 ^d ± 0.05	5.10 ^{bc} ± 0.68	1.00 ^b ± 0.11
Group 7: Lotus mixture powder (2.5%)		20.15 ^e ± 0.88	4.95 ^c ± 0.83	0.96 ^b ± 0.04
Group 8: Lotus mixture powder (5%)		16.40 ^f ± 0.54	3.80 ^d ± 0.84	0.81 ^b ± 0.31
LSD (P ≤ 0.05)		1.38	1.13	0.41

Mean under the same column bearing different superscript letters are different significantly (p ≤ 0.05).

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التأثيرات المحتملة لبتلات وبذور زهور اللوتس على الفئران المصابة بالسكري المستحث بالأستربتوزوتوسين

سهام عزيز خضر – فتحية شبل جندية – مني بلال محمد منير
كلية الاقتصاد المنزلي – جامعة المنوفية – قسم التغذية وعلوم الأطعمة

الملخص العربي

تم إجراء هذه الدراسة لمقارنة تأثير أجزاء مختلفة من زهرة اللوتس (البتلات والبذور) على الفئران المصابة بالسكري. تم استخدام أربعين من ذكور فئران الألبينو في هذه الدراسة ، وكان وزن الفئران (140 ± 10 جم) وتم تقسيمهم إلى ثماني مجموعات كل مجموعها بها خمسة فئران منهم مجموعة ضابطة سالبة ومجموعة ضابطة موجبة ، وتم إصابة الفئران بمرض السكري بالحقن بمادة الأستربتوزوتوسين بتركيز ٤٠ ملجم/كجم . تمت إضافة البتلات و البذور ومخلوطهما (نسبة ١:١) بنسبة ٢,٥ % ، ٥ % في الغذاء الاساسي. تم تقييم الفينولات والفلافونويدات والنشاط المضاد للأكسدة في اللوتس. تم تقدير الوزن الأولي ، والوزن النهائي ، والوزن النسبي للأعضاء وكمية التغذية (FI) ، ونسبة كفاءة التغذية (FER) ، (%BWG) و الجلوكوز في الدم ، (TG TC ، VLDL-c ، LDL-c ، HDL-c ، AI) ، إنزيمات الكبد في السيرم (ALT ، AST ، ALP) ، وظائف الكلى (الكرياتينين وحمض اليوريك ومستويات اليوريا). من النتائج التي تم الحصول عليها تبين أن التغذية على بتلات اللوتس والبذور ومخلوطهما تسبب في زيادة كبيرة ($P \leq 0.05$) في نسبة HDL-c ، بينما انخفضت مستويات LDL-c ، VLDL-c بنسبة عالية مع وجود فرق معنوي. كذلك حدث انخفاض معنوي في كلا من وظائف الكلى (الكرياتينين ، حمض اليوريك ، اليوريا) ووظائف الكبد (ALT ، AST ، ALP) وانخفاض معنوي في مستوى الجلوكوز في الدم ، الذي يعكس تأثير علاجي جيد للتغذية على بتلات اللوتس والبذور وخليطها كمسحوق لعلاج مرض السكري في الفئران. وكانت أفضل نتيجة لتركيز مسحوق خليط اللوتس ٥%.

الكلمات المفتاحية: زهور اللوتس ، مرض السكري، الفئران، التحاليل الكيميائية الحيوية.

