

Full Length Research Paper

Anti-diabetic effects of aqueous fruits extract of *Diospyros lotus L.* on streptozotocin-induced diabetic rats and the possible morphologic changes in the liver, kidney and heart

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The aim of this study was to assess hypoglycemic effect of aqueous fruits extract of *Diospyros lotus L.* on streptozotocin-induced diabetic rats and the possible morphologic changes in the liver, kidney and heart. Diabetes mellitus was induced by a single intraperitoneal (IP) dose of 70 mg/kg of streptozotocin (STZ). Animals were post-treated with different doses of *D. lotus L.* (500, 750, 1000 and 1500 mg/kg) by oral administration (gavage) for 16 consecutive days after induction of diabetes. In the special days (before treatment and 1, 8 and 16 days after induction of diabetes), according to a pre-planned schedule, animal's weight and their Fasting Blood Sugar (FBS), were determined in different groups under treatments. Also at the end of the study, the animals were sacrificed and their livers, kidneys and hearts were removed for histopathological examination. Administration of different doses of *D. lotus L.* (500, 750, 1000 and 1500 mg/kg) to diabetic animals caused significant decrease in glucose level, since the maximum reduction was observed in the animals group with 1000 mg/kg after 16 days post-treatment. ($P < 0.001$) Aqueous fruits extract of *D. lotus L.* at dose of 1000 and 1500 recovered significantly the body weight towards the control level. Histological comparison has shown that *D. lotus L.* in parenchymal and portal inflammation and lymphocytes had also been replaced by few eosinophils in the liver. These results suggest that the product of *D. lotus L.* may provide a new therapeutic avenue against diabetes and diabetes-related complications-a global burden.

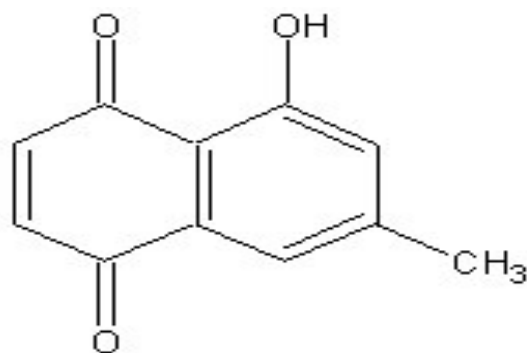
Key words: Anti-diabetic, *Diospyros lotus L.*, streptozotocin, morphologic changes.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves (Nagappa et al., 2003). It is known

that diabetes mellitus, characterized by hypoglycemia, is a genetically and clinically heterogeneous group of disorders with the common feature of glucose intolerance (World Health Organization, 1994). Based on WHO recommendation, diabetes mellitus is classified into three major subtypes: type I (insulin dependent diabetes mellitus, IDDM), type II (non-insulin dependent diabetes mellitus, NIDDM) and malnutrition-related diabetes mellitus. IDDM or Juvenile-onset diabetes results from a cellular mediated autoimmune destruction of the β -cells of the pancreas (Aikinson and Maclaren, 1994; Takeshi et al., 2002). However, NIDDM or adult onset diabetes

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7-Methyljuglone

Figure 1. Chemical structure of 7-methyljuglone.

results from the development of insulin resistance and the affected individuals usually have insulin deficiency (Fronzo et al., 1997). On the other hand, many oral hypoglycemic agents, such as biguanides and sulfonylurea are available along with insulin for the treatment of diabetes mellitus but they have significant side effects (Holmann and Turner, 1991; Williams and Pickup, 1991) and sometimes they are found to be ineffective in chronic diabetic patients (Nagarajan et al., 1987). Thus, there is an increasing demand of natural and synthetic products with high anti-diabetic potential and fewer side effects (Rao et al., 1999). Studies conducted over the last several decades have shown that plant and plant-based therapies have a high potential to treat and control diabetes (Ivorra et al., 1989; Bailey and Day, 1989; Marles and Fransworth, 1995) and its complications (Grover et al., 2001). Therefore, search for safe and more effective agents has continued to be an important area of active research. Since several years ago, diabetes has been treated orally with several medicinal plants or their extracts, based on folklore medicine. These herbal remedies are apparently effective, produce minimal or no side effects and are of relative low costs as compared to oral synthetic hypoglycemic agents. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation of hypoglycemic agents from medicinal plants have become more important (Alberti and Zimmet, 1998; Gupta et al., 2005; Kesari et al., 2005).

The genus, *Diospyros* (Syn: Persimmon, ebony) with more than 350 species is the most important, both numerically and economically. The characteristic features of the *Diospyros* species are: trees, rarely shrubs, Leaves alternate; flowers green, white or yellow, few to many; axillary cymes or the pistillate solitary; fruit is large juicy 1 - 10 seeded berry. The results of many studies indicate that *Diospyros* genus elaborate a great diversity of compounds ranging from hydrocarbons, steroids, terpenoids, naphthoquinones to naphthalene based aromatics. About

75% of the phytochemical reports of *Diospyros* are on the detection and isolation of 1, 4-naphthoquinones, which include several monomers, dimers, a few trimers and tetramers. Of these, Plumbagin and 7-methyljuglone are the most abundant ones and are found to accumulate in significant quantities in a number of *Diospyros* species (Mallavadhani et al., 1998) *Diospyros lotus* L. which is indigenous to temperate Asian forest, China and also seen in the North of Iran in parts of the coast of the Caspian Sea as high as 1100 m from sea level in Astara to Ramian, Gorgan. It can easily be reproduced through planting of seeds and can appear in different parts of the Northern forests of Iran. Its fruit is used to produce juice, and its leaves are extended oval shaped with pointed edge with dimensions varying from 5 - 18 by 3 - 7 cm. The surface of the leaves is dark green, their back is light, its flowers small, its fruit is round, and is as big as a hazel nut. When ripe in fall, the fruit turns brown, and it has 2 - 3 clear and big seeds (Sabeti, 1997). *D. lotus* L. such as other species of *Diospyros*, have a high amount of naphthoquinones special 7-methyljuglone (Figure 1). Many studies showed that it possesses many biological and pharmacological properties including: being anti-febrile, which is used to promote secretions, and being sedative for cough (Mallavadhani et al., 1998). It is commonly known as "Khormendi" in Mazandaran province, North of Iran. The fruits of Khormendi are used traditionally to treat diabetes in this area but not scientifically, therefore the present investigation was carried out to evaluate the traditional use of *D. lotus* L. scientifically.

The present study was performed to assess the anti-diabetic effects of aqueous fruits extract of *D. lotus* L. on streptozotocin-induced diabetic rats, and the possible morphologic changes in the liver, kidney and heart.

MATERIALS AND METHODS

Plant material

Fresh matured fruits of *D. lotus* L. were collected from mountains of Mazandaran province, Iran in the months of October- November, 2007. A voucher specimen of the herbarium has been deposited at the department of pharmacognosy, faculty of pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

Preparation of extracts

This method has been designed based on traditional extraction which is practiced in Mazandaran province, North of Iran. In this method, the pip becomes separated from the ripe fruits. Further, the extract can be obtained by pressing 500 g of fresh fruit. The extract is poured into multiple smooth glass containers so that a fine layer is produced. The glass container, containing extract is put in the oven and is dried at the temperature of 45°C. In the next step, the dried extract is removed from the container and is placed in the form of powder in a light proof container and kept in a cool and dry place. The weight of the dried extract is calculated against that of the fruit and is standardized according to the rate of naphthoquinones in the extract.

Chemicals

Streptozotocin (STZ) was purchased from sigma chemical Co. Biphasic isophan insulin (NPH) was obtained from Exir pharmaceutical Co. (Tehran, Iran). All other chemicals were obtained from Merck Company (Germany).

Animals

Male Wistar Albino rats (n = 35), 5 - 7 months old with a weight of 130 - 180 g, were purchased from the Pasteur Institute of Iran (North Branch, Amol, Iran and kept in a good condition at the university animal section and given standard food pellets and water *ad libitum*. All of the animals were maintained under controlled conditions of light (12/24 h) and temperature ($23 \pm 1^\circ\text{C}$). Their use and the experimental protocol used in this study was approved by the Ethical committee of the medical Sciences University of Mazandaran

Induction of diabetes

Diabetes mellitus was induced by single intraperitoneal (IP) dose of 70 mg/kg of streptozotocin (STZ) dissolved in 0.9% fresh cold Normal Saline into 12h-fasted rats. After one week of STZ injection, the rats were fasted for 6 h and blood was taken from tail artery of the rats. Rats with moderate diabetes having hyperglycemia (that is, with blood glucose level of higher than 250 mg/dl) were chosen for the experiment.

Experiment design

In the experiment, a total of 35 rats were used. The rats were randomly divided into the 7 groups (groups 1 - 7), with five animals in each group:

Group 1: normal control; received distilled water (10 ml/kg b.w.) by gavage for 16 consecutive days.

Group 2: Positive control; received a single dose of STZ (70 mg/kg b.w., ip) in Normal Saline (10 ml/kg b.w., 0.9%).

Group 3: treated with *D. lotus L.* extract (500 mg/kg b.w.) in distilled water (10 ml/kg b.w.) by gavage for 16 consecutive days after induction of diabetes.

Group 4: treated with *D. lotus L.* extract (750 mg/kg b.w.) in distilled water (10 ml/kg b.w.) by gavage for 16 consecutive days after induction of diabetes.

Group 5: treated with *D. lotus L.* extract (1000 mg/kg b.w.) in distilled water (10 ml/kg b.w.) by gavage for 16 consecutive days after induction of diabetes.

Group 6: treated with *D. lotus L.* extract (1500 mg/kg b.w.) in distilled water (10 ml/kg b.w.) by gavage for 16 consecutive days after induction of diabetes.

Group 7: Treated with Biphasic isophan insulin (6 iu/kg b.w., SC) for 16 consecutive days after induction of diabetes.

Sample collection

Animals from each group were deprived of food overnight but with free access of water before taking the samples of blood. Blood samples were collected from tail artery of the rats for evaluation of the blood glucose. Fasting blood glucose estimation and body weight measurement were performed before the treatment and on days 1, 8 and 16 of the study. Blood glucose levels were estimated using an electronic glucometer manufactured by (Infopia Co., Ltd. Korea).

Consequently, at the end of the study, on day 16, after induction of diabetes with STZ, animals were sacrificed and their liver, kidney and heart were removed for histopathological studies.

The Histological study

At the end of study, the animals were sacrificed and their livers, kidneys and hearts were removed, then small slices of them were fixed in 10% formalin solutions, and processed routinely. Sections of 5 μ thickness were cut and stained by hematoxylin and eosin (H&E) for histological examination.

Statistical analysis

The data are presented as means \pm SD. One-way analysis of variance (ANOVA) and Tukey's post test were used for multiple comparisons of data. The differences were considered significant, when $p < 0.05$

RESULTS

Body weight

Body weight was decreased significantly in streptozotocin-induced diabetic rats with respect to control. Average weight of animals in Control and STZ group before the beginning of this study was 142 and 156.6 while at the 16th day of the study they were found to be 185.2 and 121.6 respectively. Accordingly, significant differences in the reduction of weight were observed between the Control and the STZ groups ($P < 0.001$). However, aqueous fruits extract of *D. lotus L.* at the doses of 1000 and 1500 recovered significantly the body weight towards the control level. After the treatment period the average of body weight in this group was 166.3 and 165, respectively compared to the diabetic Control group (121.6) ($P < 0.01$; $P < 0.05$) The average body weight of animals before the beginning of the study, after induction of diabetes and also after treatment with *D. lotus L.* for 16 consecutive days are shown in Table 1.

Fasting blood glucose level (FBG)

One week after STZ injection, the diabetic rats with fasting blood glucose levels higher than 250 mg/dl, were selected for further studies. One week after STZ injection, Fasting Blood Glucose Level in control, STZ, *D. lotus L.* at doses of 500, 750, 1000, 1500 and also insulin was 126.5, 481.25, 394.5, 443.6, 439.3, 488.3 and 373.6, respectively. There was a significant elevation in fasting blood glucose level after a single dose of streptozotocin (70 mg/kg, ip) compared to the control group. ($P < 0.01$ - $P < 0.05$) The effect of *D. lotus L.* on FBG of the diabetic rats is shown in Table 2. All groups with different doses of *D. lotus L.* significantly decreased FBS after 16 days of treatment after induction of diabetes in rats except *D. lotus L.* at the dose of 500 mg/kg; however FBS of this

Table 1. Effect of aqueous fruits extract of *D. lotus* (DL) on body weigh in streptozotocin (STZ) - induced diabetic rats

Group	Treatment Groups*	Pretreatment	Day after induction of diabetes in rats		
			1	8	16
1	Control	142 ± 3.6	174 ± 4.3	179 ± 4.4	185.2 ± 3.7
2	STZ	154 ± 7.7	148.4 ± 4.9 ^f	134 ± 5.5 ^a	121 ± 4 ^a
3	STZ + DL 500 mg/kg b.w.	145 ± 2.3	151.6 ± 3.5 ^f	158.2 ± 5.7 ^b	142.7 ± 12.7 ^e
4	STZ + DL 750 mg/kg b.w.	150.6 ± 4.6	130.2 ± 3.3 ^a	136.4 ± 1.6 ^e	122 ± 5.1 ^e
5	STZ + DL 1000 mg/kg b.w.	143.2 ± 5.3	157.6 ± 3.2 ^e	163.6 ± 4.7 ^b	166.3 ± 3.8 ^b
6	STZ + DL 1500 mg/kg b.w.	140.4 ± 1.6	153.6 ± 3.7 ^e	165.3 ± 4.6 ^b	165 ± 5.1 ^c
7	STZ + insulin 6 iu/kg b.w.	155.3 ± 11.1	159 ± 3.5 ^e	173.6 ± 0.33 ^d	183.3 ± 4.4 ^d

*Values are means ± SD for each group of five rats. ^a P < 0.001 comparison to Control; ^f P < 0.01 comparison to Control; ^b P < 0.01 comparison to STZ; ^c P < 0.05 comparison to STZ; ^d P < 0.001 comparison to STZ; ^e no significant comparison to STZ. The data were analyzed using One-way ANOVA analysis and Tukey's post test.

Table 2. Effect of aqueous fruits extract of *D. lotus* (DL) on Blood glucose levels on streptozotocin (STZ) - induced diabetic rats.

Group	Treatment Groups*	Pretreatment	Blood glucose levels (mg/dl)		
			1	8	16
1	Control	104.5 ± 4.6	126.5 ± 0.5	120 ± 4.5	114 ± 6.1
2	STZ	117.25 ± 5.9	481.25 ± 77 ^b	692.25 ± 71.4 ^a	704.2 ± 65.4 ^a
3	STZ + DL 500 mg/kg b.w.	117.25 ± 2.8	394.5 ± 38.9 ^g	447.5 ± 50.8 ^e	539.5 ± 24.5 ^e
4	STZ + DL 750 mg/kg b.w.	109.2 ± 5.09	443.6 ± 54.5 ^b	397.25 ± 63.7 ^f	384.8 ± 14.6 ^c
5	STZ + DL 1000 mg/kg b.w.	97.8 ± 3.3	439.3 ± 69.8 ^b	337 ± 64 ^f	260.3 ± 40.8 ^c
6	STZ + DL 1500 mg/kg b.w.	98.6 ± 6.3	488.3 ± 86.8 ^b	482.3 ± 68.7 ^e	394.3 ± 92.1 ^d
7	STZ + insulin 6 iu/kg b.w.	115 ± 4.5	373.6 ± 59.4 ^g	388.6 ± 92.4 ^f	243 ± 13 ^c

* Values are means ± SD for each group of five rats. ^a P < 0.001 comparison to Control; ^b P < 0.01 comparison to Control; ^c P < 0.001 comparison to STZ; ^d P < 0.01 comparison to STZ; ^e no significant comparison to STZ; ^f P < 0.05 comparison to STZ; ^g P < 0.05 comparison to Control. The data were analyzed using One-way ANOVA analysis and Tukey's post test.

dose was lower than that of the STZ group. The fall observed in FBG after 16 days of treatment was 13.2, 40.7, 19.2 and 34.9% with *D. lotus L.* at the dose of 750, 1000 and 1500 mg/kg and insulin at the dose of 6 iu/kg, respectively, suggesting thereby that 1000 mg/kg is the most effective dose for assessing the anti-diabetic potential of fruits extract of *D. lotus L.* in diabetic animals. A dose dependent effect was observed in diabetic animals also up to the dose of 1000 mg/kg. However, higher doses up to 1500 mg/kg did not show any dose-dependent effect and a maximum decrease in FBG was observed at the 16th day of treatment after induction of diabetes at the dose of 1000 mg/kg of *D. lotus L.* that decreased the FBG to 260.3 ± 40.8 from an initial level of 439.3 ± 69.8 mg/dl towards the insulin group with FBS of 243 ± 13 mg/dl. In addition, there was a significant elevation in FBS level Between *D. lotus L.* at the dose of 1000 mg/kg and the STZ group (FBG of 704.2 ± 65.4 mg/dl) at the end of the study (P < 0.001). The average fasting blood glucose of animals before the beginning study, after induction of diabetes and also after treatment with *D. lotus L.* for 16 consecutive days is shown in Table 2.

Histological results

Liver: By light microscopy, liver of the STZ treated diabetic rats showed 2 - 3 foci of interlobular lymphocytes predominant inflammatory cells infiltration per ×100 magnifications as compared by necrosis and apoptosis of few hepatocytes. Mild lymphocytic infiltration and congested vessel intlemajority of portal spaces were noted. Histological examination of livers of the diabetic rats treated by increasing dosages of *D. lotus L.* fruits extract, showed gradual significant reduction in parenchymal and portal inflammation and lymphocytes were replaced by few eosinophils. By using 1000 mg/kg of *D. lotus L.* fruits extract, the hepatic tissue appeared somewhat like the control and the Insulin treated groups (Figure 2).

Kidney: Histological examination of the STZ-induced diabetic rats' renal tissue compared to the controls groups revealed mild increase in mesangial cells and matrix of glomeroli. Hyaline thickening of some arteriole wall was noted. By using increasing dosages of *D. lotus L.* fruits extract, these pathologic changes improved toward to the

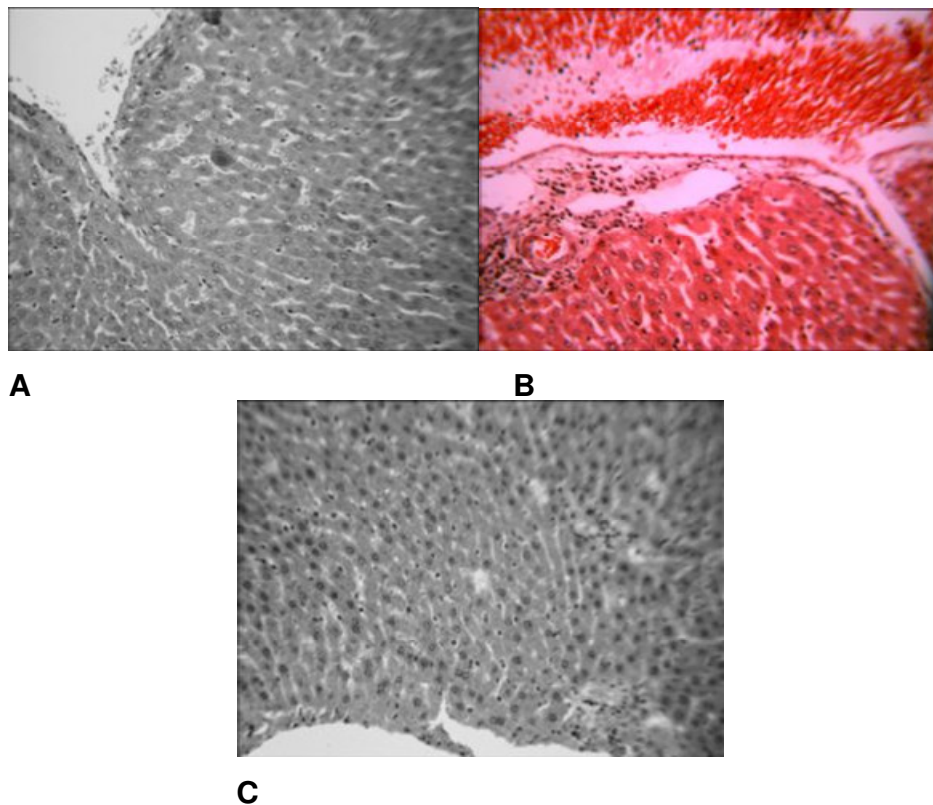


Figure 2. Photomicrograph of liver section of rats on the 16th day of control (A) and STZ (B), *D. lotus L.* 1000 mg/kg (C). The specimens were stained with Hematoxylin and Eosin.

Insulin treated groups. (Figure 3)

Heart: Histological examination of heart of the diabetic rats didn't show significant pathological changes as compared to the control groups.

DISCUSSION

Currently, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of biguanides, thiazolidinediones, sulphonylureas, Diphenylalanine derivatives, meglitinides and α -glucosidase inhibitors in addition to insulin. However, due to unwanted side effects the efficacies of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes (Jackson and Bressler, 1981; Thirunavukkarasu et al., 2003). Hence, plants have been suggested as a rich, as yet unexplored source of potentially useful anti diabetic drugs. However, only a few have been subjected to detailed scientific investigation due to a lack of mechanism-based available *in vitro* assays (Saxena and Vikram, 2004).

The present study for the first time reports the hypoglycemic and anti diabetic effect of an aqueous extract of *D. lotus L.* In light of the results, our study indicates that

D. lotus L. fruits extract possesses a good anti-diabetic activity. Aqueous extracts of *D. lotus L.* exhibited significant anti-hyperglycemic activities in the STZ induced hyperglycemic rats with a significant change in body weight. The findings of this study indicate that the graded doses of 750, 1000 and 1500 mg kg⁻¹ bw of the aqueous extract of *D. lotus L.* had a significant hypoglycemic effect of 13.2, 40.7 and 19.2% after 16 days of treatment in diabetic rats. The effect was dose-dependent up to 1000 mgkg⁻¹ bw. However, the response decreased at the higher dose of 1500 mg kg⁻¹ bw. Such a phenomenon of less hypoglycemic response at higher doses is common in indigenous plants and has already been observed in *Momordica cymbalaua* (Rao et al., 1995), *Aegle marmelose* (Sharma et al., 1996a) and *Vinca rosea* (Chattopadhyay et al., 1991).

Streptozotocin selectively destroys the pancreatic insulin secreting β -cells, leaving less active cells and resulting in a diabetic state (Kamtchoung et al., 1998). STZ-induced diabetes is characterized by severe loss in body weight (Chen and Ianuzzo, 1982), and this reduction is due to loss or degradation of structural proteins, as the structural proteins are known to contribute to body weight. In our study, a significant weight loss was observed in the diabetic group and significant improvement in weight was observed in the groups treated with

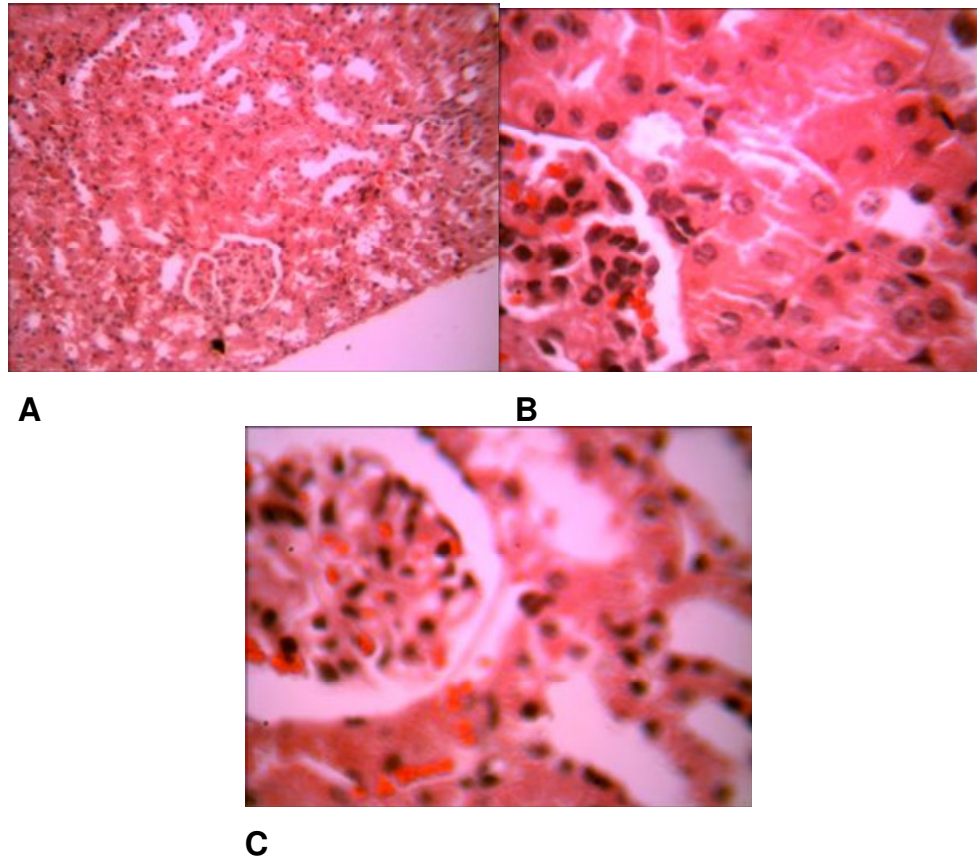


Figure 3. Photomicrograph of kidney section of rats on the 16th day of control (A) and STZ (B), *D. lotus L.* 1000 mg/kg (C). The specimens were stained with Hematoxylin and Eosin.

D. lotus L. This may be due to the ability of *D. lotus L.* to reduce hyperglycemia.

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves the overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues (Latner, 1985), and studies have shown that the level of blood glucose was elevated in STZ-induced diabetic rats. Hence, in the present study, we observed an increased level of blood glucose. Oral administration of *D. lotus L.* resulted in a significant reduction in blood glucose and a 1000 mg/kg body weight dose exhibited maximum reduction when compared to the other three doses.

Our results showed that oral administration of *D. lotus L.* fruits extract for 16 days effectively controlled hyperglycemia. Maintenance of normoglycemia and other reasons such as normalization of serum lipid profile and suppression of oxidative stress prevent the onset of microvascular complications and also delay progression of complications in diabetes. The *D. lotus L.* fruits extract maintains the blood glucose to normoglycemia during diabetes, which acts as an essential trigger for both liver and kidney to revert to their normal metabolic homeostasis. The liver and kidney exhibits numerous morphological and functional alterations during diabetes

(Sochar et al., 1985). In the present study, the histological examination of liver of diabetic rats showed 2 - 3 foci of interlobular lymphocytes predominant inflammatory cells infiltration per $\times 100$ magnifications as compared by necrosis and apoptosis of few hepatocytes. Light micro-scopy of kidney sections of diabetic rats showed a mild increase in mesangial cells and matrix; also Hyaline thickening of some arteriole wall was noted. Our results indicated that treatment of diabetic rats with *D. lotus L.* fruits extract significantly prevented the alteration in the liver and kidney pathology with the return to their normal texture. The best dosage of *D. lotus L.* fruits extract for improvement of morphologic change in the liver and kidney was found to be 1000 mg/kg, the same dose that possesses a maximum reduction in FBG and recovery in the body weight in diabetic rats.

Many studies have demonstrated the biological activity of genus of *Diospyros in vitro*. One of this studies showed that *Diospyros gaultheriifolia* has an antioxidant activity and low toxicity in vitro (David et al., 2007). It is possible that the radical scavenging of this specie is related to the presence of naphthoquinones. In another other study, Maiga showed that *Diospyros abyssinica* with high content of naphthoquinones has a radical scavenging activity and 15-Lipoxygenase inhibition (Maiga et al., 2006).

Also Ganapaty showed that anti-protozoal and cytotoxic effect of *Diospyros assimilis* are related to the presence of naphthalene derivatives and naphthoquinones (Ganapaty et al., 2006).

The exact mechanism of *D. lotus L.* fruits extract for anti diabetic effect was not clear, but plausible hypothesis that may be involved in the therapeutic action of *D. lotus L.* can be considered here. *D. lotus L.* may exert its therapeutic effect through its naphthoquinone content specially 7-methyljuglone via its antioxidant activity preventing oxidative damage and protecting β -cells of pancreas. Therefore, further investigation is warranted for elucidation of the exact mechanism of anti-diabetic effects of *Diospyros lotus L.* fruits extract.

Taken together, the present study demonstrates that aqueous fruits extracts of *D. lotus L.* possesses anti-diabetic properties suggesting the presence of biologically active components which may be worth further investigation and elucidation. The effective anti-diabetic dose was also found to be 1000 mg/Kg body weight. These results suggest that the product of *D. lotus L.* may provide a new therapeutic avenue against diabetes and diabetes-related complications a global burden.

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