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Der Pharmacia Lettre, 2016, 8 (10):1-10
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

The Anti-Diabetic and Anti-Lipidemic Effects of *Peganum harmala* Seeds in Diabetic Rats

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ABSTRACT

This study was performed to determine the influence of the Peganum harmala L. (Zygophyllaceae) on rats as the antidiabetic factor. Some biochemical parameters and histological were realizated in this study. Also antioxidant activities were performed. Hypoglycemic activity of the seeds oil extract was realizated at 100 mg/kg b.w. dose level in the treated rats with streptozotocin (STZ). In comparison between Glucophage as hypoglycemic drug and the seeds oil extract was studied. Oral administration of Peganum harmala seeds oil extract modulated the diabetic elevation in the level of blood glucose and alpha amylase activity revealing the anti-hyperglycemic. Seeds oil extract effectively decreased the total cholesterol, total lipids and triglycerides levels with consequence decrease in alpha amylase activity. Furthermore, it decreased lipid peroxidation product MD and increased the activities of catalase and superoxide dismutase enzymes. It could be concluded that the current P. harmala has many benefit procedures in controlling blood glucose and harmful results induced in pancreas and liver and may be select as a natural antidiabetic drug.

Key words: Diabetes Mellitus, *Peganum harmala*, Hyperglycemia, Diabetic rats, Streptozotocin.

INTRODUCTION

Diabetes mellitus was evaluated to increase in 1995 from 4% to 5.4% in 2025. In the devolved countries, the diabetes mellitus elevated from 51 million to 72 million (about 42 percent), then increased from 84 million to 228 million (about 70 percent) as reported by [1]. The world health organization (WHO) determined that diabetes was the 8th cause leading to death and in 2012 it was a reason for death 1.5 million people [2]. The diabetes was estimated in 2014 as a cause for death 4.9 million people as delivered by the International Diabetes Federation [3].

Peganum harmala was a member of Zygophyllaceae family and it was discovered in North Africa and the Middle East. The derivatives of beta carboline alkaloids as harmine and harmalol were isolated from *P. harmala*. Other compounds were extracted from *Peganum harmala* as deoxyvasicinone, L-vasicinone, vasicine ,vodiamin , and fagomine [4]. These compounds had significant pharmacological effects covering immunomodulatory, cardiovascular, nervous system, emmenagogue, gastrointestinal, osteogenic, antidiabetic, antimicrobial and antitumor activity among many other effects [5].

The alkaloids of *P. harmala* had a cytotoxic effect on the cancer cells as a result of the ability of harmine to display a cytotoxic effect on HL60 and K562 leukemic cell lines [6]. The antidiabetic effect of *P. harmala* seeds due to its alkaloids especially the harmine [7]. The antioxidant activity of *Peganum harmala* because of the presence of these compounds: ascorbic acid, tocopherol, cysteine, glutathione and polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.)[8].

MATERIALS AND METHODS

Plant material: The seeds of *P. harmala* were bought from a vendor seeds market in Bab El-Khala in the month of August 2014. The plant was identified and authenticated by plant biochemistry laboratory of the National Research Centre, Dokki, Giza.

Preparation of oil extract: The extraction was performed by using non-polar solvent. The powdered seed of *Peganum harmala* (50 g) was packed into a thimble made of Whatman filter paper No. 1 and extracted with 500 ml of hexane solvent using soxhlet extraction apparatus for 48 h until the solvent extracted no more color. The extract was concentrated by using rotary-vacuum evaporator [9].

Experimental animals: 20 male albino rats of Wistar / Sprague-Dawley strain of 6 to 7 weeks of age and 120 ± 10 g of body weight were procured from the animal house of the National Research Centre, Dokki, Giza. The animals were kept for two weeks to acclimatize under the laboratory conditions and were fed on Fodder as a standard commercial diet. The following norms for the animal room were: temperature 23 ± 2°C; humidity 50-60%; light 300 lux at floor level with regular 12 h light cycle; noise level 50 decibel; ventilation 10-15 air changes per hour. The animals had free access to the pellet diet and tap water unless stated otherwise.

Induction of diabetes: The animals fasted overnight and diabetes was induced by intraperitoneal injection administration of a freshly prepared streptozotocin (Sigma Co.) at the dose of 50 mg/kg body weight (b. w.), and the crystals of streptozotocin (STZ) were dissolved in slain (sodium chloride) 0.9%. Fasting blood glucose level was measured after 48 hours and animals showing blood glucose level above 180 mg/dl were considered as diabetic. The rats with marked hyperglycemia (high fasting blood glucose) were selected and used for the study [10].

Grouping of animals: The rats were divided to four groups in plastic cages, 5 rats in each group:
Group 1 (Normal control): Normal rats without any treatment.
Group 2 (Diabetic control): Diabetic rats without any treatment.
Group 3 (Diabetic + *P. harmala* oil): Diabetic rats treated with oil of *Peganum harmala* seeds.
Group 4 (Diabetic + Drug): Diabetic rats treated with Drug (Glucophage 500mg).

The diabetic treated groups with the oil of *P. harmala* and drug were orally by a stomach tube at a dose level of 100 mg /kg b.w. day after day for four weeks.

Record the rat's body weight: The rat's weights were recorded after four weeks from the beginning of the experiment to define the rat's health and compare the treated group's weight with the diabetic control rats and normal control rats.

Blood samples: After an overnight starvation, blood samples were collected after the administration of the experimental extracts at zero time and after one to four weeks. The blood samples were withdrawn from orbital sinus vein [11]. The portion was collected in clean tubes without anticoagulant and then centrifugated at 3500 r.p.m for 15 min to separate the serum. The serum was frozen at -20º C until biochemical assays as follows:-

Biochemical analysis:
Serum parameters
Serum glucose level was determined as described by [12], Total protein was estimated according to [13], Serum albumin was determined by using Stanbio direct albumin kit [14], Determination of serum total lipids [15], Serum triglyceride was determined as described by [16], The serum cholesterol level was determined using Stanbio enzymatic cholesterol kit [17], Determination of the high density of lipoprotein (HDL) in serum sample was carried out using method of [18], Low Density Lipoproteins (LDL) in serum sample was determined by using an equation as reported by [19], Serum bilirubin level was determined using Stanbio enzymatic bilirubin kit [20], Serum urea

[21], Determination of uric acid in serum sample was carried out using Stanbio uric acid (UV – Rate) kit [21], Serum creatinine [22], The α-amylase activity was determined using enzymatic α-amylase kit [23], Determination of ALT (GPT) activity according to [24], Determination of serum AST (GOT) activity [25].

Liver parameters

At the end of the biological experiment, the treated animals were scarified and record its organs weight (liver-kidney-lungs-spleen-tests-heart-pancreas), and then its livers were collected, quickly blotted with filter paper and kept at -20°C until analysis. The liver was homogenated according to [26] and subjected for Estimation of lipid peroxidation (Thiobarbituric acid) as reported by [27], Estimation of glycogen according to the method of [28], Estimation of superoxide dismutase (SOD) as described by [29], According to the method of [30] was used for the determination of CAT activity in homogenated liver samples and the catalase content (CAT) was measured spectrophotometrically

Histopathological examination: Liver of the sacrificed rats were taken and immersed in 10 % formalin solution. The specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Dehydrated specimens were cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Haematoxylin and Eosin for histopathological examination according to the method described by [31]. The histopathological examination was done in Department of Pathology, Faculty of Veterinary Medicine, Cairo University.

Statistical analysis: Data analysis was made by SPSS (version 16.0) statistical software. Non-parametric data were analyzed by LSD (the least significant difference). Results are expressed as mean ± standard deviation of mean (SD). Differences between groups were determined using one-way analysis of variance (ANOVA) with Scheffe multiple comparison tests. The minimal level of significance was identified at $P<0.05$ [32].

RESULTS AND DISCUSSION

Biological Evaluation of *P. harmala* seeds oil

A set of animals experiment was carried out to elucidate the influence of *P. harmala* seeds oil as an antidiabetic and antilipidemic agent to use it as a human food additive.

Effect of *P. harmala* seeds oil on body weight and organs weight of STZ-induced diabetic rats

From The results of body weight of all rats, groups are shown in Table (1). There was a slight decrease in the body weight of diabetic + drug rat group (was about 242 g) when compared with non-diabetic rats group, but this decrease was not significant ($p < 0.05$). The body weight was slightly increased in treated diabetic group (diabetic + *P. harmala* oil) compared to the normal control group was about 256 g. There was a significant decrease in the body weight of diabetic rat group (was about 184 g). These results in agreement with the results found by [33] whose showed the changes of body weight in diabetic and non-diabetic rats varied, and mentioned that the non-diabetic rats even show a slight weight gain but in rats, diabetes is accompanied by loss of weight.

A significant elevation in organs weight of treated diabetic rats (*P. harmala* seeds oil) compared to diabetic rats were concomitant with the results of [34] whose mentioned that the induced diabetic rats' organs weight were decreased in diabetic control rats than that in normal control rats. Data listed in Table (10) demonstrate the organs weight of treated diabetic rats after four weeks of administration of the oil of *Peganum harmala* seeds compared to the diabetic control normal. The liver weight was significantly increased in treated diabetic rats with *P. harmala* seeds oil than that in diabetic rats compared to normal control rats. The kidney weight of diabetic rats (was about 0.96 g) was significantly lower than that in normal rats (was about 1.92 g). A significant increase in kidney weight was observed in treated diabetic groups: the oil of *Peganum harmala* seeds was about 1.94 g and drug group was about 1.74 g compared with the diabetic group after four weeks from the beginning of the experiment. As well as, Lungs and spleen weight was significantly decreased in diabetic rats compared to normal rats. On other hand, diabetic treated rats with the oil of *Peganum harmala* seeds had larger weight of lungs and spleen than that in diabetic rats. Concerning the weights of lungs in treated rats with the oil of *P. harmala* were larger than that in diabetic rats and also treated rats with the drug, so the oil rats' lungs weights were semi-similar to normal control rat's lungs weight. The spleen weights of the treated diabetic rats with the oil of *Peganum harmala* seeds and drug group were significant; increased after four weeks. In addition, the weight of tests in diabetic rats (was about 1.6 g) compared to the control normal rats (was about 2.6 g). The treatments with the oil of *Peganum harmala* seeds had an effective factor on increasing the tests weight in diabetic rats (was about 2.28 g) after four weeks. This means that

the oil of *Peganum harmala* seeds had a significant effect in increasing tests weight in diabetic rats. Also, the hearts weight of the treated diabetic rats with the oil of *Peganum harmala* seeds and drug group were significantly increased (were about 0.82 g and 0.84 g, respectively) compared to the diabetic control group after four weeks from the beginning of the experiment. The results revealed that hearts weight of diabetic rats decreased (was about 0.54 g), while in normal rats was about 1.14 g. Concerning pancreas weight, it was noticed a significant increase in normal control rats (was about 1 g) compared to diabetic rats (was about 0.4 g). On another hand, diabetic treated groups with the oil of *Peganum harmala* seeds and drug (were about 0.46 & 0.46 g) had heavier pancreas weight than that in diabetic rats compared to normal control group.

Effect of *P. harmala* seeds oil on blood glucose levels and alpha amylase activity of STZ-induced diabetic rats

These results were in concomitant with the present study results and the effect of *Peganum harmala* seeds oil on blood glucose levels as shown in Table (2) and Figure (1). Blood glucose a level of diabetic rats (was about 257.8 mg/dl) was significantly higher than that in normal rats (was about 90.8 mg/dl). Significant decreases in blood glucose levels were observed in treated diabetic groups: the oil of *Peganum harmala* seeds and drug were about 112.4 mg/dl and 119.8 mg/dl, respectively after four weeks from the beginning of the experiment. Administration of *Peganum harmala* seeds oil to diabetic rats caused an anti-diabetic and antioxidant activities by the diminution in plasmatic glucose level [35].

A significant increase in blood glucose level, while the significant decrease in α-amylase enzyme activity ascertained the diabetic state. The results in Table (2) revealed that serum alpha-amylase activity of diabetic rats increased about 794.33 U/L; while in normal rats, it was about 736.06 U/L. After treatment with the oil of *Peganum harmala* seeds and drug the activity of alpha amylase was reduced about 766.26 and 774.9 U/L, respectively. Similar observations were obtained by [36], whose showed that alpha amylase activity reduced in treated diabetic rats and normal control rats compared to diabetic control rats.

Effect of *P. harmala* seeds oil on liver function and liver glycogen level of STZ-induced diabetic rats

Table (4) showed a significant reduction in liver function parameters associated with significant elevation in total protein and albumin content as compared to diabetic control group. Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase indicate liver function and their activities were determined in diabetic rats after four weeks of administration of the oil of *Peganum harmala* seeds compared to the diabetic control normal. The results revealed that serum GPT levels of diabetic rats increased about 66.1 U/ml; while in normal rats it was about 40.5 U/ml. After treatment with the oil of *Peganum harmala* seeds, the activity level of GPT was reduced after four weeks. The data for GOT activity indicate that the level of this enzyme in the normal control rats less than that in the diabetic control rats. The activity of GOT in diabetic rats was about 75.53 U/ml compared to the control normal rats which was about 58.05 U/ml. The treatments with the oil of *Peganum harmala* seeds had an effective factor on lowering the GOT activity in diabetic rats after four weeks. This means that the oil of *Peganum harmala* seeds had a significant effect in reducing GOT levels in the blood.

Considering albumin levels, its level in normal control rats was about 3.82 g/dl and it was about 3.16 g/dl in the diabetic rats and in treated diabetic group (*Peganum harmala* seeds oil) was about 3.56 g/dl. In other words, serum albumin levels were reduced in diabetic rats. A significant increase was noticed in serum albumin levels of treated rats with the oil of *Peganum harmala* seeds compared to diabetic rats.

It has been easily noticed that serum bilirubin value was significantly increased in diabetic rats compared to normal control rats. On another hand, diabetic treated rats with the oil of *Peganum harmala* seeds (was about 1.96 mg/dl) had lower values than that in diabetic rats (was about 2.64 mg/dl). Concerning the levels of serum bilirubin, diabetic rats had higher levels than that normal control and diabetic treated rats.

Regarding the serum total protein levels, the treated diabetic rats with the oil of *Peganum harmala* seeds was significantly increased compared to diabetic control group in its total protein level after four weeks from the beginning of the experiment. The results revealed that serum total protein level of diabetic rats decreased (about 5.36 g/dl) while in normal rats it was about 6.88 g/l. After treatment with the oil of *Peganum harmala* seeds, the levels were increased to about 6.15 g/dl after four weeks. Complementing the study, the liver glycogen level in normal control rats was about 7.58 µg/mg tissue and it was about 1.87 µg/mg tissue in the diabetic rats and in treated diabetic groups: (the oil of *Peganum harmala* seeds and drug) were about 2.46 and 3.83 µg/mg tissue, respectively. In other words, liver glycogen levels were reduced in diabetic rats compared to non-diabetic rats (normal control).

These data were in symmetry with [37] showed that the lowest activity of GOT and GPT enzymes were in the treated group with *P. harmala* extract while the highest values were in the infected group. The albumin and bilirubin levels were in accompaniment with [38] results who showed that there was a significant reduction ($P<0.05$) in the serum albumin levels of diabetic rats while the bilirubin levels were increased significantly ($P<0.05$) in diabetic rats as compared to the normal control rats. [39] whose mentioned that the serum total protein was increased due to the *P. harmala* administration. Treatment with the oil of *Peganum harmala* seeds significantly increased liver glycogen levels of diabetic rats after four weeks. Similar observations were showed about the liver glycogen levels, it was noticed that the liver tissue of the diabetic rats showed a significant decline in glycogen activity as compared to normal control rats and treated diabetic rats [40].

Effect of *P. harmala* seeds oil on kidney function of STZ-induced diabetic rats

The illustrated data in Table (4) represent kidney function parameters of treated diabetic rats compared with normal and diabetic rats. The serum urea is one of kidney function parameters, its level in the treated diabetic rats with the oil of *Peganum harmala* seeds was significantly reduced after four weeks compared to diabetic control rats, on other words diabetic treated rats with the oil of *Peganum harmala* seeds and drug (were about 49.74 and 42.16 mg/dl) had urea level nearly similar to normal rats (was about 37.33 mg/dl) than that diabetic rats (was about 72.68 mg/dl).

Concerning the serum uric acid level, its values was about 3.74 and 4.06 mg/dl in treated diabetic groups with *P. harmala* seeds oil and drug, respectively and about 4.46 mg/dl in diabetic group compared with normal control group (was about 3.28 mg/dl), the results showed significant decrease in uric acid of administrated diabetic rats with the oil of *Peganum harmala* seeds compared to diabetic rats.

In addition, serum creatinine level of treated diabetic rats (*P. harmala* seeds oil and drug) were significantly reduced compared with diabetic control group, this means that diabetic rats was about (3.73 mg/dl) had higher levels of creatinine than that normal control rats (was about 2.26 mg/dl) and diabetic treated rats with *P. harmala* seeds oil and drug (were about 2.96 and 3.18 mg/dl, respectively). Similar observations were obtained by [41] whose found that the serum urea, uric acid and creatinine were significantly increased in streptozotocin-induced diabetic rats as compared to normal control and treated diabetic rats.

Effect of *P. harmala* seeds oil on lipid profile of STZ-induced diabetic rats

Data obtained in Table (5) show the lipid profile in normal control, diabetic and diabetic treated rats with the oil of *Peganum harmala* seeds. Serum total lipids value was significantly increased in diabetic rats (was about 490.04 mg/dl) compared to normal rats (was about 400.52 mg/dl). On another hand, the diabetic treated group with the oil of *Peganum harmala* seeds (was about 428.28 mg/dl) had the lower level than that in diabetic rats. One of the possible actions of the oil of *Peganum harmala* seeds on serum total cholesterol were observed in lowering its levels in treated diabetic rats, the levels of serum total cholesterol in diabetic rats were higher than that in other rats. Diabetic rats treated with the oil of *Peganum harmala* seeds induced significant decrease in total cholesterol levels in serum (was about 136.4 mg/dl) compared to diabetic control rats (was about 144.2 mg/dl). Generally, the oil of *Peganum harmala* seeds had an effect in lowering total cholesterol in serum. In addition, blood triglycerides level of diabetic rats (was about 167.36 mg/dl) were significantly higher than that in normal rats (was about 116.12 mg/dl). Blood triglycerides value was significantly increased in diabetic rats compared to normal control rats. On another hand, diabetic treated groups with the oil of *Peganum harmala* seeds and drug (were about 137.22 and 160.1 mg/dl, respectively) had lower values of triglycerides than that in diabetic rats.

Oral administration of *P. harmala* seeds increased serum HDL value in treated rats compared to diabetic rats (was about 67.1 mg/dl), So the treated diabetic rats with the oil of *Peganum harmala* seeds and drug were significantly increased in serum HDL levels about 81.02 and 82.38 mg/dl, respectively compared to diabetic control group after four weeks from the beginning of the experiment. While LDL levels of untreated diabetic rats (was about 42.82 mg/dl) were significantly higher than that in normal control rats (was about 7.96 mg/dl), significant decreases in LDL levels were observed in treated diabetic groups: the oil of *Peganum harmala* seeds was about 28.32 mg/dl and the drug group which was about 25.36 mg/dl.

The abnormal high concentration of serum lipids of diabetic rats was mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits because insulin inhibits the hormone sensitive lipase production [42]. Administration of *Peganum harmala* to rats after hyperglycemia produced a marked decrease in blood cholesterol level. Similar observations were obtained by [43], whose found that the dietary administration of 120 mg ethanol

extract of *Peganum harmala* for 30 days to diabetic mice reversed the decreased blood cholesterol level. A significant decrease was influenced in serum triglycerides and LDL associated with significant increase in serum HDL of treated diabetic group compared with diabetic control group. These data were in accompaniment with [44] whose showed that the serum triglycerides and LDL levels were decreased significantly (>0.05) due to the *P. harmala* treatment, while the HDL levels were increased after the administration with *P. harmala*.

Effect of *P. harmala* seeds oil on the antioxidant enzymes activity and lipid peroxidation of STZ-induced diabetic rats

Table (6) demonstrated the activity of CAT and SOD in the liver tissue of different groups. Catalase value was significantly increased in diabetic rats (were about 0.184 U/mg tissue) compared to normal rats (was about 0.093 U/mg tissue). On the other side, diabetic treated rats with oil extract of *Peganum harmala* seeds and the drug had lower levels (were about 0.11 and 0.107 U/mg tissue, respectively) than those in diabetic rats. Diabetic-treated rats had CAT level nearly similar to that of control normal rats. This means that the oil of *Peganum harmala* seeds possessed an obvious reduction in CAT level in diabetic treated rats. Superoxide dismutase activity in normal rats was lower than that in diabetic rats. The treatment with the oil of *Peganum harmala* seeds and drug-induced an elevation of SOD activity compared with diabetic rats and its activity in normal rats was approximately little lower than that in diabetic treated rats. This indicates the remarkable effect of the oil of *Peganum harmala* seeds towards resuming SOD activity. The values of lipid peroxides in normal and diabetic treated rats were lower than that in diabetic rats. Malonaldehyde (MDA) level in normal control rats was about 0.42 µM/mg tissue and it was about 1.5 times as low as that in the diabetic rats (was about 0.68 µM/mg tissue). Treatment with the oil of *Peganum harmala* seeds and drug significantly decreased the lipid peroxide levels compared to diabetic rats after four weeks (were about 0.46 and 0.45 µM/mg tissue, respectively).

The present study revealed that the treatment of diabetic rats with the oil of *P. harmala* seeds caused a significant decrease in liver antioxidant enzymes (CAT and SOD) and lipid peroxidation (MDA) compared to diabetic control group. This diminution in the both liver antioxidant enzymes and lipid peroxidation was in symmetry with [45] who mentioned that the liver SOD and CAT activities significantly increased in diabetic control group and that indicated an increased oxidative stress in diabetic control group, as showed also a significant elevation in lipid peroxidation (MDA) in diabetic control rats while reduction in the treated diabetic rats.

Histopathological results of the oil of *Peganum harmala* seeds

Rats liver from normal control group: Figure (2) and (3) represent lives for two rats from the normal control group, it indicates that there weren't any histological changes but also it shows the normal structure of hepatic lobule in the liver of rats in normal control group.

Rats liver from diabetic control group: The two rats' livers from diabetic control groups were showed from figure (4) & (5), the lives in diabetic control group rats were found portal infiltration with massive inflammatory cells In addition to focal hepatic necrosis associated with inflammatory cells infiltration as well as congestion of hepatic sinusoids.

Rats liver from treated diabetic group with the oil of *P. harmala* (100 mg/Kg rats): The diabetic treated groups with the oil extract of *Peganum harmala* seeds there weren't any histological changes and that means the liver made recovery to its cells and other showed Kupffer cells activation as shown in Figure (6) & (7), that explains the magical effect of the oil of *Peganum harmala* seeds as hypoglycaemic, antioxidant and antitumor drug. Oral administration of *P. harmala* seeds oil improved structural changes induced by diabetes. This preservation by *P. harmala* seeds oil might be due to both inhibitions of metabolism and/or detoxification of cytotoxic radicals. Our findings were in agreement with [46, 4 7, 48].

Rats liver from treated diabetic group with drug: The Figure (8) & (9) show livers for two rats from diabetic group administrated with drug (Glucophage), it appeared more similar to the rats' livers in diabetic treated rats with the oil of *P. harmala* seeds which seemed no histopathological changes and other seemed Kupffer cells activation, so we could conclude that the oil of *P. harmala* seeds and drug had the same histopathological effect.

Table (1): Effect of the oil of *P. harmala* seeds on the body weight and organs weight of streptozotocin-induced diabetic rats

Parameter	Normal control	Diabetic control	Diabetic + *P. harmala* oil	Diabetic + Drug
Body weight (g)	244.00 ± 25.10 **	184.00 ± 18.16 ^{b}	256.00 ± 35.07 *	242.00 ± 26.83 **
Liver (g)	8.06 ± 0.54 *	4.80 ± 0.65 ^{a}	6.86 ± 1.17 ^{c**}	7.08 ± 0.86 **
Kidney (g)	1.92 ± 0.14 *	0.96 ± 0.32 ^{a}	1.94 ± 0.40 *	1.74 ± 0.23 *
Lungs (g)	2.60 ± 0.37 *	1.56 ± 0.40 ^{a}	2.14 ± 0.31 ***	1.68 ± 0.13 ^{b}
Spleen (g)	1.36 ± 0.45 **	0.70 ± 0.27 ^{b}	1.12 ± 0.21 ***	1.30 ± 0.15 ***
Tests (g)	2.60 ± 0.17 *	1.60 ± 0.46 ^{a}	2.28 ± 0.37 **	2.10 ± 0.22 ^{c***}
Heart (g)	1.14 ± 0.33 *	0.54 ± 0.18 ^{a}	0.82 ± 0.20 ^{c***}	0.84 ± 0.08 ^{c***}
Pancreas (g)	1.00 ± 0.28 *	0.40 ± 0.15 ^{a}	0.46 ± 0.15 ^{a}	0.46 ± 0.11 ^{a}

● *Data are presented as mean ± SD of five rats.*
● *a P ≤ 0.0005, b P ≤ 0.005 ,c P ≤ 0.05, compared with normal control group.*
● ** P ≤ 0.0005, ** P ≤ 0.005, *** P ≤ 0.05, compared with diabetic control group.*

Table (2) Effect of the oil of *P. harmala* seeds on the blood glucose levels and α–amylase activity of streptozotocin-induced diabetic rats

Parameter	Normal control	Diabetic control	Diabetic + *P. harmala* oil	Diabetic + Drug
Glucose level (mg/dl)	90.80 ± 6.83 *	257.80 ± 29.54 ^{a}	112.40 ± 10.41 ^{c*}	119.80 ± 2.49 ^{c**}
Alpha amylase (U/L)	736.06 ± 14.64 **	794.33 ± 17.81 ^{b}	766.26 ± 37.81	774.90 ± 17.50 ^{c}

● *Data are presented as mean ± SD of five rats.*
● *a P ≤ 0.0005, b P ≤ 0.005 ,c P ≤ 0.05, compared with normal control group.*
● ** P ≤ 0.0005, ** P ≤ 0.005, *** P ≤ 0.05, compared with diabetic control group.*

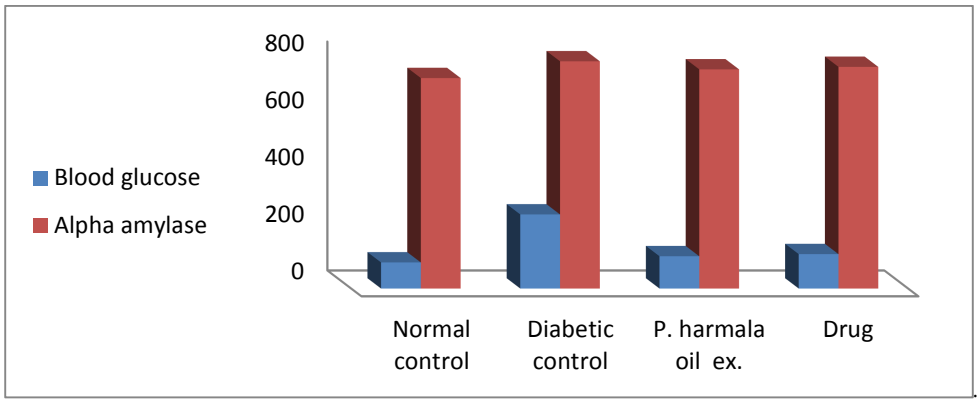

Figure (1): The blood glucose level and alpha amylase activity in groups treated with either *P. harmala* or drug compared to normal and diabetic control groups

Table (3): Effect of the oil of *P. harmala* seeds on the liver function and liver glycogen level of streptozotocin-induced diabetic rats

Parameter	Normal control	Diabetic control	Diabetic + *P. harmala* oil	Diabetic + Drug
GPT (U/ml)	40.50 ± 3.18 *	66.10 ± 12.08 ^{a}	45.88 ± 5.20 *	45.14 ± 1.38 *
GOT (U/ml)	58.05 ± 4.19 *	75.53 ± 3.56 ^{a}	63.46 ± 3.39 *	60.81 ± 6.59 *
Albumin (g/dl)	3.82 ± 0.11 *	3.16 ± 0.27 ^{a}	3.56 ± 0.15 ^{c**}	3.50 ± 0.15 ^{b**}
Bilirubin (mg/dl.)	1.95 ± 0.05 **	2.64 ± 0.46 ^{b}	1.96 ± 0.04 **	2.13 ± 0.22 ***
Total protein (g/dl)	6.88 ± 0.19 *	5.36 ± 0.32 ^{a}	6.15 ± 0.25 ^{b**}	6.42 ± 0.47 ^{c*}
Glycogen (µg/mg liver)	7.58 ± 0.18 *	1.87 ± 0.22 ^{a}	2.46 ± 0.08 ^{a}	3.83 ± 0.31 ^{a*}

● *Data are presented as mean ± SD of five rats.*
● *a P ≤ 0.0005, b P ≤ 0.005 ,c P ≤ 0.05, compared with normal control group.*
● ** P ≤ 0.0005, ** P ≤ 0.005, *** P ≤ 0.05, compared with diabetic control group.*

Table (4) Effect of the oil of *P. harmala* seeds on the kidney function of streptozotocin-induced diabetic rats

Parameter (mg/dl)	Normal control	Diabetic control	Diabetic + *P. harmala* oil	Diabetic + Drug
Urea	37.33 ± 2.22 *	72.68 ± 8.18 ^{a}	49.74 ± 4.64 ^{a*}	42.16 ± 3.18 *
Uric acid	3.28 ± 0.21 *	4.46 ± 0.24 ^{a}	3.74 ± 0.15 ^{b*}	4.06 ± 0.42 ^{a***}
Creatinine	2.26 ± 0.15 **	3.73 ± 0.25 ^{b}	2.96 ± 0.45 ***	3.18 ± 0.66 ^{c}

● *Data are presented as mean ± SD of five rats.*
● *a P ≤ 0.0005, b P ≤ 0.005 ,c P ≤ 0.05, compared with normal control group.*
● ** P ≤ 0.0005, ** P ≤ 0.005, *** P ≤ 0.05, compared with diabetic control group.*

Table (5) Effect of the oil of *P. harmala* seeds on the lipid profile of streptozotocin-induced diabetic rats

Parameter (mg/dl)	Normal control	Diabetic control	Diabetic + *P. harmala* oil	Diabetic + Drug
Total lipids	400.52 ± 15.30 *	490.04 ± 32.98 a	428.28 ± 31.77 **	424.88 ± 20.62 **
Total cholesterol	124.20 ± 3.56 *	144.20 ± 8.31 a	136.4 ± 2.30 b***	133.40 ± 3.78 c***
Triglycerides	116.12 ± 3.28 *	167.36 ± 7.53 a	137.22 ± 12.84 a*	160.10 ± 1.66 a
HDL	96.22 ± 2.19*	67.10 ± 5.74a	81.02 ± 6.57b**	82.38 ± 8.88b*
LDL	7.96 ± 0.84 *	42.82 ± 5.39 a	28.32 ± 5.87 a*	25.36 ± 3.88 a*

● *Data are presented as mean ±SD of five rats.*
● *a P ≤ 0.0005, b P ≤ 0.005 ,c P ≤ 0.05, compared with normal control group.*
● ** P ≤ 0.0005, ** P ≤ 0.005, *** P ≤ 0.05, compared with diabetic control group.*

Table (6) Effect of the oil of *P. harmala* seeds on the antioxidant enzymes activity and lipid peroxidation of streptozotocin-induced diabetic rats

Parameter	Normal control	Diabetic control	Diabetic + *P. harmala* oil	Diabetic + Drug
CAT (U/mg tissue)	0.09 ± 0.01 ***	0.18 ± 0.02 c	0.11 ± 0.02 ***	0.11 ± 0.06 ***
SOD (U/mg tissue)	36.15 ± 2.62 *	66.57 ± 1.86 a	48.34 ± 13.06 c**	56.84 ± 6.57 b
Malonaldehyde (MDA) (µM/mg tissue)	0.42 ± 0.05 *	0.68 ± 0.09 a	0.46 ± 0.05 **	0.45 ± 0.02 **

● *Data are presented as mean ±SD of five rats.*
● *a P ≤ 0.0005, b P ≤ 0.005 ,c P ≤ 0.05, compared with normal control group.*
● ** P ≤ 0.0005, ** P ≤ 0.005, *** P ≤ 0.05, compared with diabetic control group.*

Effect of *Peganum harmala* seeds oil on histopathological results of STZ-induced diabetic rats

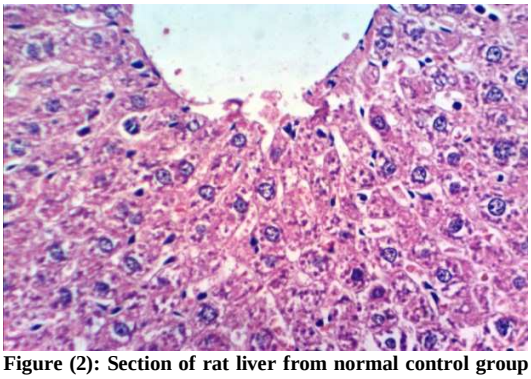

Figure (2): Section of rat liver from normal control group showing a normal appearance of liver cells (H & E X 400)

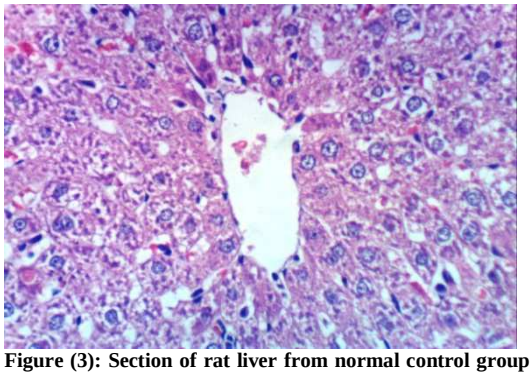

Figure (3): Section of rat liver from normal control group showing the normal histological structure of hepatic lobule (H & E X 400)

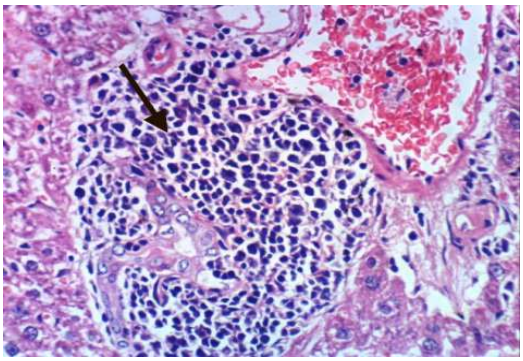

Figure (4): Section of rat liver from diabetic control group showing portal infiltration with massive inflammatory cells (black arrow) (H & E X 400)

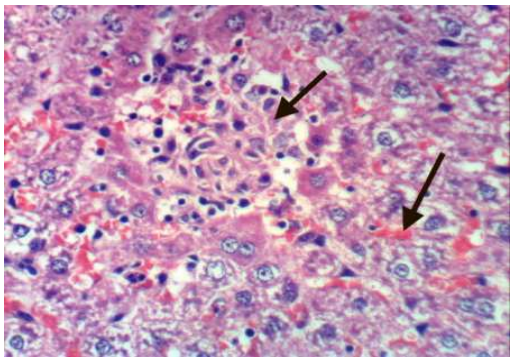

Figure (5): Section of rat liver from diabetic control group showing focal hepatic necrosis associated with inflammatory cells infiltration as well as congestion of hepatic sinusoids (H & E X 400)

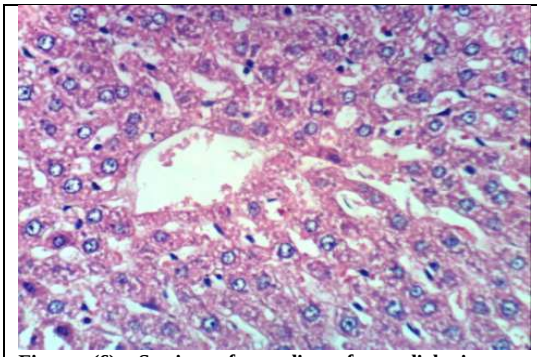

Figure (6): Section of rat liver from diabetic group administrated with the oil of *P. harmala* seeds showing no histopathological changes (H & E X 400)

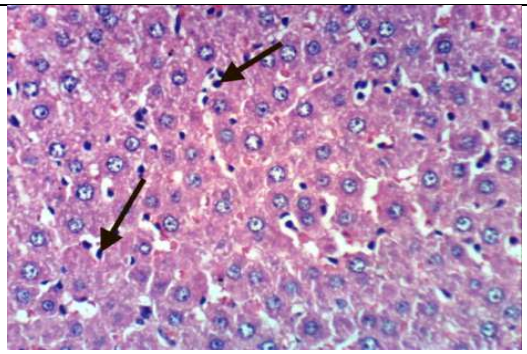

Figure (7): Section of rat liver from diabetic group administrated with the oil of *P. harmala* seeds showing Kupffer cells activation (H & E X 400)

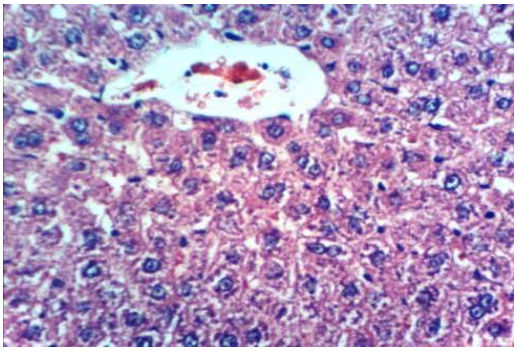

Figure (8): Section of rat liver from diabetic group administrated with drug showing no histopathological changes (H & E X 400)

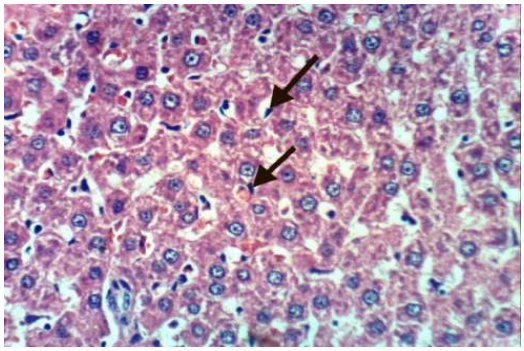

Figure (9): Section of rat liver from diabetic group administrated with drug showing Kupffer cells activation (H & E X 400)

CONCLUSION

This study aimed to investigate the antidiabetic effect of oil extract of *P. harmala* in streptozotocin-induced diabetic rats. The overall goal of this study can be said that the extract is effective in reducing blood glucose levels in diabetic rats, and of course, the most effective dose was 100 mg/ kg b.w., while in normal rats was increased blood sugar.

REFERENCES

[1] M. J. Bearse, T. Han, M. E. Schneck, S. Barez, C. Jacobsen, A. J. Adams, *Invest Ophthal Vis Sci*, **2004**, 45: 3259-3265.
[2] World Health Organization (WHO), **2013**.
[3] International Diabetes Federation (IDF), **2014**.
[4] A. Rudolph, J. Sainz, R. Hein, M. Hoffmeister, B. Frank, A. Forsti, H. Brenner, K. Hemminki, J. Chang-Claude, *Endocr Relat Cancer*, **2011**, 18(3):371-84.
[5] M. Moloudizargari, P. Mikaili, S. Aghajanshakeri, M. H. Asghari, J. Shayegh, *Pharmacogn Rev.* , **2013**, 7(14): 199–212.
[6] F. Jahaniani, S. A. Ebrahimi, N. Radar-Roshandel, M. Mahmoudian, *Phytochemistry*, **2005**, 66 (13): 1581–92.
[7] J. J. Fortunato, G. Z. Réus, T. R. Kirsch, R. B. Stringari, L. Stertz , F. Kapczinski , J. P. Pinto, J. E. Hallak, A, W. Zuardi, J. A. Crippa, J. Quevedo, *Prog Neuropsychopharmacol Biol Psychiatry*, **2009**, 33: 1425–30.
[8] A. Baghianil, M. Djarmouni, S. Boumerfeg, H. Trabsa, N. Charef1, S. Khennouf, L. Arrar, *European Journal of Medicinal Plants*, **2012**, 2(1): 42-56.
[9] I. E. Leger, Debaene, **1989**, 2: 360-860.
[10] R. Ahmadi, S. Pishghadam, F. Mollaamine, M. Z. Reza, Monfared, *Int J Endocrinol Metab*, **2013**, 11(4): e10266.
[11] S. H. Stone, *Science*, **1954**, 119: 100-103.
[12] P. Trinder, *Clin. Biochem.*, **1969**, 6 :24–27.

[13] H. Yatzidis, *Clin. Chem*., **1977**, 23(5): 908.
[14] D. S. Young, L. C. Pestaner, V. Gibberman, *Clin Chem*., **1975**, 21: 286D–288D.
[15] N. Zollner, K. Kirsch, *Z. ges. exp. Med*., **1962**, 135: 545.
[16] P. Fossati, Prencipe, *Clin. Chem*., **1982**, 28(10): 2077-80.
[17] N. W. Tietz, Philadelphia , **1976**, pp 622- 628.
[18] W. P. Castelli, J. T. Doyle, T. Gordon, C. G. Hames, M. C. Hjortland, S. B. Hulley, A. Kagan, W. J. Zukel, *Circulation*, **1977**, 55(5):767-72.
[19] G. R. Warnick, R. H. Knoop, V. F. Patrick, H. Branson, *Clin Chem.*, **1990**, 36(1): 15–19.
[20] M. I. Walters, H. W. Gerarde, *Microchem J*., **1970**, 15: 231–243.
[21] A. Vassault, D. Grafmeyer, C. Naudun, G. Dumont, M. Belly, J. Henny, *Ann Biol Clin*, **1986**, 44:686-745.
[22] M. Jaffe, *Physiol Chem*, **1886**, 10: 391–400.
[23] W. T. Caraway, *Am J Clin Pathol*., 1959, 32(1):97-9.
[24] S. Reitman, S. Frankel, *Amer. J. Clin. Pathol*., **1957**, 28: 56-63.
[25] H. U. Bergmeyer, *Verlag Chemic*, **1978**, 6: (4).
[26] S. L. Fowler, A. C. McLean, S. A. L.Bennett, *Cell Commun Adhes*, **2009**, 16: 117-130.
[27] H. Haraguchi, H. Ishikawa, I. Kubo., Planta Med, **1997**, 63: 213-215.
[28] S. Seifter, S. Dayton, B. Novic, E. Muntwyler, **1949**, PP. 193:194.
[29] M. Nishikimi, N. A. Roa, K. Yogi, *Biochem Biophys Res Commun*, **1972**, 46(2): 849–854.
[30] P. Fossati, L. Prencipe, G Berti, *Clinical Chemistry*, **1980**, 26 (2): 227-231.
[31] H. M. *Carleton*, **1980**, P. 520.
[32] M. C. Page, S. L. Braver, D. MacKinnon, D. P., **2003**.
[33] A. Akbarzadeh, D. Norouzian, M.R. Mehrabi, Sh. Jamshidi , A. Farhangi , A. Allah Verdi, S. M. A. Mofidian1, B. Lame Rad, *Indian Journal of Clinical Biochemistry*, **2007**, 22 (2): 60-64.
[34] C.O. Eleazu, M. Iroaganachi, P. N. Okafor, I. I. Ijeh,, K. C. Eleazuu, *Int J Biomed Sci*, **2013**, 9(2): 82–90.
[35] K. Hamden, S. Carreau, K. Jamoussi, F. Ayadi, F. Garmazi, A. Elfeki, *Food Sci. Biotechnol*., **2009**, 18(3): 739-744.
[36] H. F. Osman, M. G. Eshak, E. M. El-Sherbiny, M. M. Bayoumi, *Life Science Journal*, **2012**, 9(3).
[37] L. Adeeb, *International Journal of Science and Research*, **2013**, 4: 2319-7064.
[38] E. Mansouri, L. Khorsandi, H. A. Abedi, *Iran J Basic Med Sci*., **2014**, 17(6): 460–464.
[39] H. Ahmed, H. Abu El Zahab, G. Alswiai, *Turkish Journal of Biology*, **2013**, 37: 39-48.
[40] P. V. Rao, K. Madhavi, M. Dhananjaya Naidu, S. H. Gan, Evidence-Based Complementary and Alternative Medicine, **2013**, 2013(7).
[41] P. Arulselvan, G. P. Senthilkumar, D. S. Kumar, S. Subramanian, *Pharmazie*., **2006**, 61(10):874-7.
[42] R. Udayakumar, S. Kasthurirengan, T. S. Mariashibu, M. Rajesh, V. R. Anbazhagan, S.C. Kim, A. Ganapathi, C.W. Choi, *Int J Mol Sci.*, **2009**, 10(5): 2367–2382.
[43] E. Poorbarkhordaria, K. Fooladsazb, S. H. Hosseinic, H. Danafard, H. K. Manjilie, A. Ramazania, *Iranian Journal of Pharmaceutical Sciences*, **2014**, 10 (3): 47- 54.
[44] N. Kalhora, A. M. Eini, Y. Sharifia, H. Fazaelya, M. A. Fa, *The European Proceedings of Social & Behavioural Sciences*, **2015**, 2357-1330.
[45] Berkan M. and Eren M., *International Journal of Histology and Cytology*, **2014**, 1(2): 10-19.
[46] N. H. Al-Rawi, *Diab Vasc Dis Res*., **2011**, 8: 22–28.
[47] FK El Baz, GS El-Baroty, H H Abd El Baky, OI Abd El-Salam, EA Ibrahim ., Grasas y Aceites, **2013**, 64 (5): 561-571
[48] H. H. Abd El Baky; F.K. El Baz, and El-Baroty, G.S., *African Journal of Biotechnol*., **2009**, 8 (24): 7059-7067.