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Dietary *Nigella sativa* and *Peganum harmala* Oils Reverses Hyperglycaemia, Hepatotoxicity, and Metabolism in Rats

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Abstract This study aims to evaluate the therapeutic action of administration of *Nigella sativa* (*NS*) and *Peganum harmala* (*PH*) oils in diabetes and hepatic toxicity. Results show that treatment of diabetic rats with *NS* oil or *PH* oil ameliorate hyperglycaemia induced stress oxidative and hepatic dysfunction in diabetic rats. Administration of *NS* or *PH* oil to diabetic rats caused an anti-diabetic and antioxidant activities by the decrease in plasmatic glucose level and increase in hepatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities, reduced glutathione (GSH) and glycogen contents compared to untreated diabetic rats. Besides, *NS* and *PH* oils protect the hepatic function observed by decrease of triglyceride (TG), total cholesterol (TCh), and increase of high density lipoprotein-cholesterol (HDL-Ch) levels in serum and hepatic tissues. Moreover, a diminution in the bilirubin, transaminase glutanic pyruvic (TGP), and transaminase pyruvic oxaloacetic (TPO) contents in serum and the thiobarbituric acid-reactive substances levels (TBARs) in hepatic tissues are also detected.

Keywords: diabetes, *Nigella sativa* oil, *Peganum harmala* oil, antioxidant, heapatoprotective, metabolism

Introduction

Currently, there are 150 million diabetics worldwide, and this number is likely to increase to 330 million or more by the year 2025 (1). Chronic elevation of blood glucose will eventually lead to tissue damage, with consequent often serious disease. Whilst evidence of tissue damage can be found in many organ and systems (1,2). In modern medicine, there is still no satisfactory effective therapy available to cure diabetes (2). Therefore, it has become necessary to search for an economically and therapeutically effective treatment, especially for usage in developing and under-developed countries. Many indigenous medicinal plants have been found to be useful to successfully manage diabetes and immune system (3,4). *Nigella sativa* and *Peganum harmala* or theirs constituents prepared by various means have diverse biological activities, including antioxidant, anticarcinogenic, hepato-protective, antidiabetic, and various other biological actions (5-7). Besides, dietary *N. sativa* and *P. harmala* oils have recently attracted significant attention, as their beneficial effects are shown in a number of disease states such as aldose reductase inhibitor and immunomodulating action (8-10). The benefits derived from an oil-containing diet are explained by their antioxidant action (9). Recently, it was reported that *N. sativa* oil and β-carboline alkaloids reversed hyperglycemia, and alleviated oxidative stress and damage in liver and kidney in alloxan-induced diabetic rats (8-12). In fact studies also showed that supplementation of the diet with *N. sativa* oil and β-carboline alkaloids regulated cytokine production, interact with the β-cell imidazoline I3 site and are involved in the physiological control of insulin secretion and anti-inflammatory activity (12-15).

In this experimental study, we aimed to investigate the therapeutic effect of *N. sativa* and *P. harmala* oils on diabetes, hepatotoxicity, and metabolism in rats.

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Materials and Methods

Plant material *Nigella sativa* (*NS*) and *Peganum harmala* (*PH*) oils were extracted by the methods described by Fararh *et al.* (5). *NS* and *PH* seeds were authenticated by Botanical professor Mohamed Chaieb in the Department of Life Sciences. Then the seeds were washed, dried, and crushed to a powder with an electric microniser. Twenty g of the powdered seeds were added to 400 mL of distilled water and the extraction was carried out by steam distillation. The process of distillation was continued until about 200 mL of the distillate were collected. The distillate was extracted 3 times with chloroform. Moisture was removed by anhydrous sodium sulphate and the resultant extract was evaporated using a water bath (40°C); this led to the appearance of the volatile oil. The products of various extractions were pooled together giving an average yield of 0.3 %. A 500 mg of the volatile oil was dissolved by the initial addition of 1 mL of dimethyl sulfoxide (DMSO), followed by the addition of 9 mL of normal saline to yield a concentration of 50 mg volatile oil/mL solution.

Experimental induction of diabetes Adult male Wistar rats, weighing 179±10 g, and obtained from the Central Pharmacy, Tunisia, were employed in the study. The animals

were kept in an environmentally controlled breeding room (temperature: 20±2°C, humidity: 60±5%, 12-hr dark/light cycle). All rats had free access to tap water and fasted overnight before blood and tissue collection. Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared alloxan solution in normal saline at a dose of 150 mg/kg body weight. The rats were then kept for the next 24 hr on 5% glucose solution bottles in their cages to prevent hypoglycemia. After 2 weeks, rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with blood glucose levels of 2 g/L) were chosen for the experiment. The handling of the animals was approved by the Tunisian Ethical Committee for the care and use of laboratory animals.

Experimental procedure A total of 40 rats (32 diabetic surviving rats and 8 control animals) were used. For diabetic rats, 1 month after alloxan injection and diabetes apparition, the day of beginning of experiments, 8 diabetic were sacrificed and referred as a diabetic rats before treatment [(Diab(BT)] (glycemia 2 g/L). The other diabetic rats were divided into 4 groups: group 1, diabetic control rats named diabetic rats after treatment [Diab(AT)]; group 2, diabetic rats treated with *NS* oil (5% in food); group 3, diabetic treated with *PS* oil at a dose of 5% in food. Eight normal rats were used as controls. Four weeks after the beginning of oils administration to diabetic rats, the animals were sacrificed by decapitation, and the trunk blood collected. The serum was prepared by centrifugation (1,500×g, 15 min, 4°C) the liver was removed, cleaned of fat; all these samples were stored at −80°C until used.

Analytical methods The lipid peroxidation in the liver of controls and treated groups of animals was measured by the quantification of thiobarbituric acid reactive substances (TBARS) determined by the method of Buege and Aust (16). The activity of superoxide dismutase (SOD) was assayed by the spectrophotometric method of Marklund

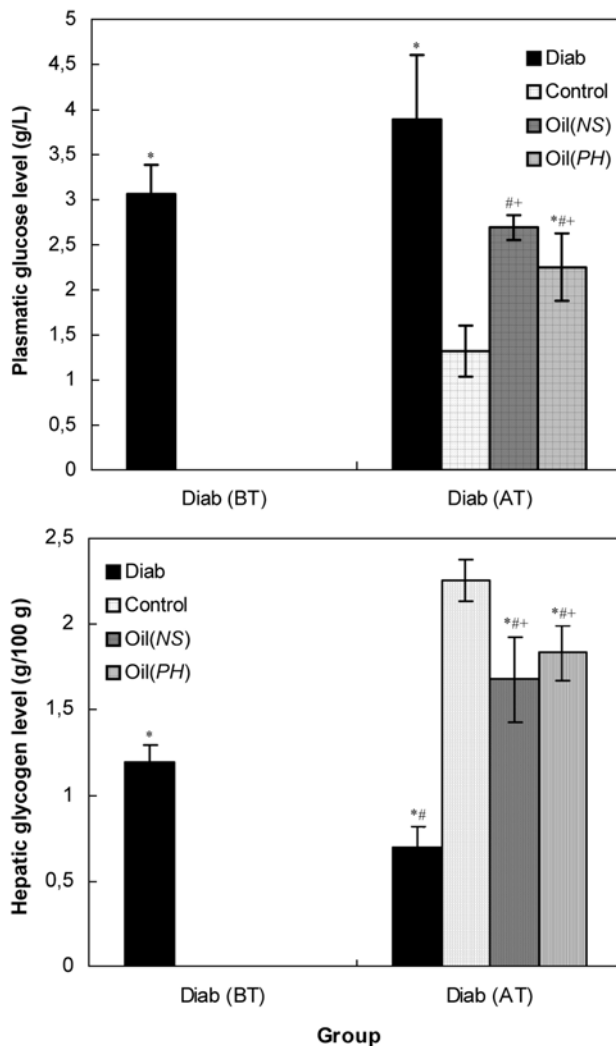

Fig. 1. The plasmatic glucose and glycogen hepatic levels in diabetic rats before (BT) and after (AT) *N. sativa and P. harmala* oil administration. Values are given as mean±SD for group of 8 animals each. *$p<0.05$ as control, #$p<0.05$ as control as diab (BT), +$p<0.05$ as diab (AT).

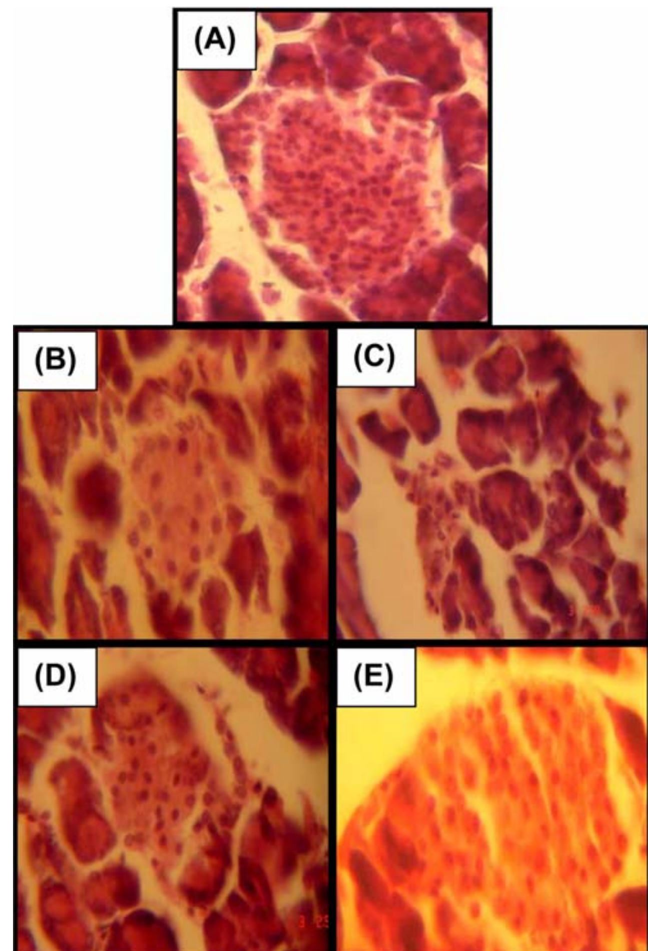

Fig. 2. Effect of diabetes and *N. sativa* and *P. harmala* oils on the histological changes of rats' pancreas by HE staining (100×). A, Normal control rats β-cells; B, severe injury in β-cells in the pancreas of maile rats given alloxan for 4 weeks; C, injury in the β-cells aggraved by more of damage and necrosis in pancreas of male rats after 2 months of alloxan administration; in diabetic rats treated with the oil *N. sativa* (D) or and *P. harmala* (E), pancreatic β-cells showing protective action.

and Marklund (17). The gluthation peroxidase (GPX) activity was measured by the method described by Pagila and Valentine (18). Catalase (CAT) was assayed by a colorimetric method at 240 nm and expressed as mol of H_2O_2 consumed/min/mg of protein described by Aebi (19). The reduced glutathione (GSH) level in plasma and liver was measured using the colorimetric method of Ellman (20). The activity of transaminase glutanic pyruvic (TGP) and transaminase pyruvic oxaloacetic (TPO) and the levels of blood and hepatic glucose, bilirubin, total cholesterol (TCh), triglyceride (TG), high density lipoprotein-cholesterol (HDL-Ch) in serum and liver were measured using commercial kits from Biomagreb (Tunis, Tunisia) and Biomerieux (Lyon, France). The assay described by Ohinishi *et al.* (21) was used to determine 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging. For histological studies, pieces of pancreas were fixed in a Bouin's solution for 24 hr, and then embedded in paraffin. Sections of 5 μm thickness were stained with hematoxylin-eosin and examined under the Olympus CX41 light microscope (Tokyo, Japan).

Statistical analysis Data are presented as mean±standard deviation (SD). The determinations were performed from 10 animals/group and the differences were examined by the one-way analysis of variance (ANOVA) followed by the Fisher test (Stat View) and the significance was accepted at $p<0.05$.

Results and Discussion

Blood glucose and liver glycogens contents The results of this study clearly demonstrate that oral administration of oil extracted from *NS* or *PH* seeds to diabetic rats produced a significant decrease in blood glucose by 30 and 42%, respectively (Fig. 1). The hepatic glycogen level is lower in diabetic rat's liver compared to this in normal rats; however, after *NS* or *PH* oils administration, this level increased by 140 and 160% respectively compared to untreated diabetic rats (Fig. 1). This finding might explain the use of *NS* and *PH* seeds, in addition to other plants, in preparations widely used as anti-diabetic remedies in

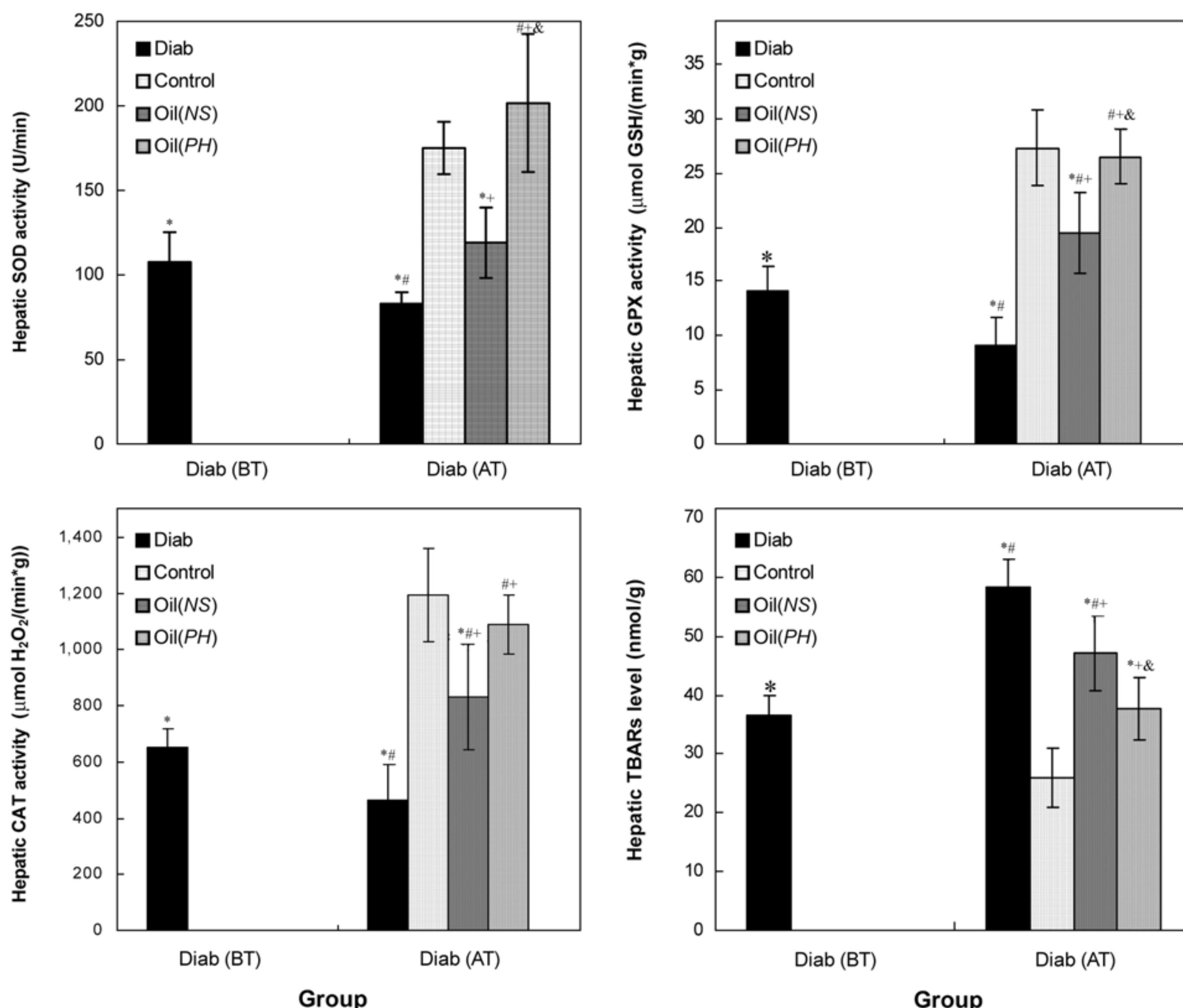

Fig. 3. Hepatic enzyme activities of SOD (U/g tissue), CAT (U/g tissue/min), GPX (U/g tissue/min) activities and lipid peroxidation levels (TBARS) (nM/g tissue) in diabetic rats before (BT) and after (AT) *N. sativa* and *P. harmala* oils administration. Values are given as mean±SD for group of 8 animals each. *p<0.05 as control, $^{\#}p$<0.05 as control as diab (BT), ^{+}p<0.05 as diab (AT), $^{\&}p$<0.05 as Oil(*NS*).

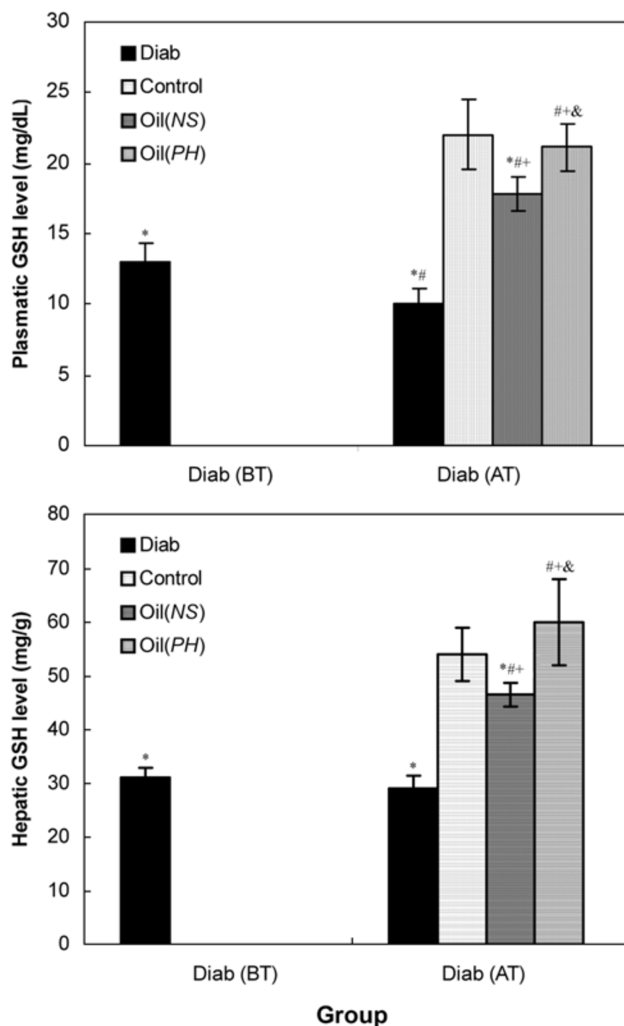

Fig. 4. Reduced glutathione level (GSH) in serum and hepatic tissues after supplementation of *N. sativa* and *P. harmala* oils in diabetic rats. Values are given as mean±SD for group of 8 animals each. *$p<0.05$ as control, #$p<0.05$ as control as diab (BT), +$p<0.05$ as diab (AT), &$p<0.05$ as Oil (*NS*).

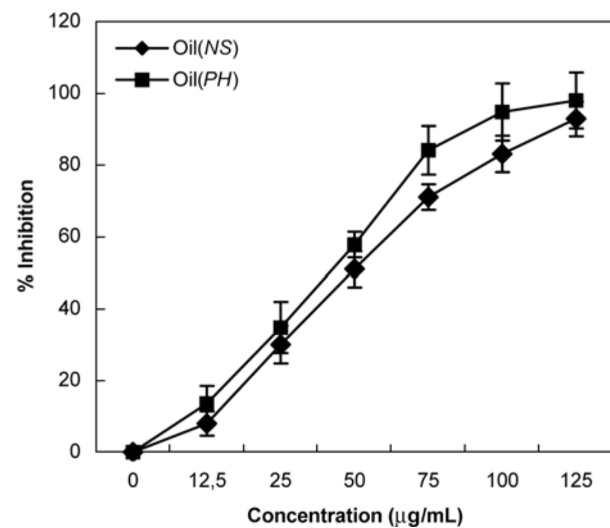

Fig. 5. Free radical activity of *N. sativa* and *P. harmala* oils measured using DPPH assay (*n*=3).

Middle East folk medicine. The anti-diabetic action of theses oils was explained by many mechanisms. Firstly, the active constituent of *NS* oil which is thymoquinone (22) would affect the production of inflammatory cytokines, suppress the synthesis of nitric oxide via the inhibition of iNOS expression in macrophages (23); consequently the immuno-regulation of immune system (24) lead to the inhibition of the auto-immunity reaction in pancreatic β-cells like protection of these cells against death and damage. Also, β-carbolines which are the major compound in *PH* oil (25) decrease the white blood cell (WBC), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and lymphocytes count (unpublished results). This decrease inhibits auto-immunity reaction and protects against pancreatic β-cells death. Figure 2A shows that the pancreatic β-cells have normal histology in control group. However, in diabetic rats, the histological examination reveals extensive alterations in pancreas. In this group of rats (i.e., diabetic without treatment), degenerative and necrotic changes in pancreatic β-cells were detected. *NS* and *PH* oils treatment protects the majority of cells of Langerhans islet (Fig. 2D and 2E). This protection allows to a high level of insulin, which is in agreement with our results (unpublished) and those of Altan *et al.* (26) and Kim and Kim (27). Consequently a decrease in blood glucose and an increase in hepatic glycogen levels observed in this study and in agreement with others (28). Also the increase in hepatic glycogen level resulted from the increase in the insulin sensitivity in agreement with Hamden *et al*. (29) and Ryan *et al*. (30). Besides *NS* or *PH* oils can reduce gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase and fructose-1,6-bisphosphatase (31). This hypoglycaemic prevent from oxidative stress by decrease the production of free radicals especially reactive oxygen species (ROS), the glucose autoxidation and protein glycosylation. Secondly, an antioxidant action of *NS* or *PH* oils was observed.

Other mechanisms were proposed by which *NS* and *PH* oils exert the anti-diabetic effect that polyphenols substances exist in these oils which able to i) enhanced the detoxifying phase II enzyme including by increasing the quinine reductase consequently protection of hepatic function (32); ii) free enhanced the antioxidant capacity lead to the protection against pancreatic β-cells from damage and death (33-36); and iii) that oils induced the antioxidant enzyme activities such as SOD, CAT, and GPx (37).

SOD, CAT, and GPX activities in liver Figure 3 and 4 show a decrease of the hepatic SOD, CAT, and GPx activities by −56, −77, and −78% respectively in diabetic rats. Moreover, our results show a significant reduces in GSH rate by −46 and −121% respectively in diabetic rat liver and plasma. In diabetic rats treated with *NS* or *PH* oils a clear ameliorative action was observed and a significant increase in all theses parameters was measured in agreement with other studies (38-44).

DPPH radical scavenging The antioxidant activity of *NS*

Table 1. Hepatic indices toxicity in blood of diabetic rats treated with *N. sativa* or *P. harmala* oils[1)]

Group	TPO (U/L)	TGP (U/L)	Total bilirubin (U/L)
Control	69.75±5	46.06±3.6	0.87±0.03
Diab (BT)	88.8±4*	78.8±1.2*	1.58±0.19*
Diab (AT)	116.6±9*#	84.6±4.6*#	2.28±0.23*#
Diab+Oil (*NS*)	79.1±3*#+	57.9±2.4*#+	1.25±0.13*#+
Diab+Oil (*PH*)	77.1±2.4*#+	59.6±1.6*#+	1.30±0.10*#+

[1)]TPO, transaminase pyruvic oxaloacetic; TGP, transaminase glutanic pyruvic.

Table 2. Total cholesterol (TCh), triglycerides (TG), and HDL-cholesterol in serum (HDL-Ch) and liver of diabetic rats treated with *N. sativa* or *P. harmala* oils

Groups	TCh	TG	HDL-Ch
***Serum* (g/L)**			
Control	1.51±0.13	0.58±0.05[1)]	0.49±0.08[1)]
Diab (BT)	2.3±0.11*	1.09±0.08*	0.31±0.04*
Diab (AT)	2.77±0.2*#	1.31±0.21*#	0.29±0.02*#
Diab+Oil (*NS*)	1.73±0.12*#+	0.84±0.06*#+	0.63±0.07*#+
Diab+Oil (*PH*)	1.67±0.1*#+	0.73±0.07*#+&	0.85±0.04*#+&
***Liver* (mg/g)**			
Control	0.97±0.17	0.78±0.08[1)]	0.53±0.07
Diab (BT)	2.41±0.17*	1.28±0.11*	0.37±0.03*
Diab (AT)	2.95±0.38*#	1.61±0.07*#	0.26±0.02*#
Diab+Oil (*NS*)	2.23±0.18*#+	0.91±0.03*#+	0.71±0.06*#+
Diab+Oil (*PH*)	2.18±0.14*#+	0.78±0.05*#+&	0.76±0.07*#+

[1)]Values with different letters within a column are significantly different at $p<0.05$ by the Fisher test.

or *PH* oils *in vitro* was evaluated by its ability to scavenge DPPH free radicals *in vitro*. In this study, Fig. 5 shows that *NS* or *PH* oils have a scavenging free radicals activity with a percentage decrease, versus the absorbance of DPPH standard solution of 92.8 and 97.9% respectively at a concentration of 125 μL/mL. All the beneficiates actions of these oils protect the hepatic function.

Liver function and lipid peroxidation The results in Table 1 show that administration of *NS* and *PH* oils in diabetic rats during 4 weeks caused an ameliorative activity in hepatic function. In fact a significant decrease in plasmatic level of hepatic dysfunction as TGP and TPO activities and bilirubin respectively by −31, −32, and −45% after *NS* oil administration. Also a decrease in TGP, TPO, and bilirubin rates respectively by −33, −29, and −42% after *PH* compared to untreated diabetic rats was showed. The TBARs contents in liver increases in diabetic rats, conversely, administration of *NS* or *PH* in diabetic rats return the TBARs level near the normal.

Lipid profile The anti-diabetic and antioxidant effects of *NS* and *PH* oils protect the hepatic metabolism and calm the increase in TCh and TG and the decrease in HDL-Ch observed in diabetic rats. Table 2 shows in diabetic rats a significant increase in TCh and TG levels by +53 and +87% in plasma and by +148 and +64 in hepatic tissues respectively compared to non-diabetic rats. However the HDL-Ch in diabetic rats decreased by −36 and −30% in plasma and liver, respectively. Diabetic rats treated with *NS* or *PH* oils show a therapeutic action. In fact, a significant decrease in TCh and TG content and increase in HDL-Ch rate were observed in both plasma and hepatic tissues. This hypolipemic and hypercholesterolemia effect of *NS* and *PH* oils probably the result of theirs role in the control of peroxisome proliferator-activated receptor α (PPAR-α) that controls the expression of genes involved in hepatic fatty acid oxidation and the transcription factor SREBP1c that is required for suppression of *de novo* lipogenesis and monounsaturated fatty acids synthesis (45,46).

In conclusion, this study demonstrates that dietary *NS* or *PH* oils reduces the availability of plasma lipid flux and normalizes glucose homeostasis which is able to reverse hepato-toxicity and lipid metabolism. *NS* or *PH* oils restore the activities of key enzymes involved in antioxidant defence. Furthermore, our findings suggest that the manipulation of dietary fats may play a key role in the management of lipid disorders, thus protecting against the development of hepatic diseases.

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