Effect of lipid extracts of *Nigella sativa* L. seeds on the liver ATP reduction and alpha-glucosidase inhibition

Widad Sobhi[1], Caroline Stevigny[2], Pierre Duez[2], Bedro Buc Calderon[3], Djebbar Atmani[4] and Mustapha Benboubetra[1]*

[1]Laboratory of Applied Biochemistry, Faculty of Life and Nature Sciences, University of Ferhat Abbas Setif 1, Algeria.
[2]Laboratory of Pharmacognosy, Bromatology and Human Nutrition, Institute of Pharmacy, Université Libre de Bruxelles, Belgium.
[3]Metabolism and Nutrition group (MNut), Louain Drug Research Institute, Catholic university of Louvain , 1200 Belgium.
[4]Department of Physical and Chemical Biology, Faculty of Life and Nature Sciences, University of Bejaia; Algeria.

Abstract: Various extracts from the seeds of *Nigella sativa* have been used in traditional folk medicine to treat inflammation, liver disorders and arthritis. These seeds have been experimentally shown to possess antioxidant and hepatoprotective properties. Beside the hypoglycaemic and hypolipidemic effects, this study was carried out to evaluate, *in vitro*, toxicological effect of lipid extracts from the *Nigella sativa* seeds. The tested fractions were: (i) defatted methanolic extract, *(ii)* total lipid extract obtained by hexane extraction from methanolic extract and (iii) neutral and polar lipid fractions. The fractions were assessed, *in vitro*, for their inhibitory activity potential on the enzyme alpha-glucosidase as suppressing the enzyme activity is one among the therapeutic approaches to attenuate postprandial hyperglycemia. High inhibition of alpha-glucosidase by the two polar lipid fractions (F6 and F7) was reflected by their IC50 (0.51±0.04mg/ml and 0.55±0.09mg/ml, respectively), compared to acarbose (0.53±0.06mg/ml) and thymoquinone (0.65±0.05mg/ml). The hypoglycaemic effect of the polar lipid fraction of *Nigella sativa* could be explained by the inhibition of alpha-glucosidase, which is one of early steps of carbohydrate metabolism. Toxicological evaluation was investigated on precision-cut rat liver slices (PCLS). On PCLS, lipid extracts reduced ATP levels by 27 to 35%. Results indicate suggest that *Nigella sativa* extracts don't show a hepatoprotective effect against acetaminophen, but don't exhibit a major hepatotoxicity when tested alone.

Keywords: Alpha-glucosidase, hepatotoxicity, induced-diabetes, *Nigella sativa*, polar lipids.

INTRODUCTION

Plants and herbs represent about a quarter of currently used drugs (De Smet, 1997). Medicinal plants, including *Nigella saliva* L, *Aloes sp* dried juice (aloe), and *Commiphora sp* gum-resin (myrrh), with hypoglycaemic activity are found in plants used in traditional medicine of numerous cultures worldwide (Yeh *et al*., 2003; Otoom *et al*., 2006). To meet their primary health needs, an important fraction of people in developing countries use traditional medicines (WHO, 2002).

With its medicinal amazing virtues, *Nigella sativa L*. (Ranunculaceae) has a rich historical and religious background. The plant in general and its seeds, known as black seeds, in particular have been used for more than two millennia as natural remedy in many Middle Eastern countries (Swamy and Tan, 2000) for diabetes and other ailments (Haddad *et al*., 2001). Black seed components possess a great number of pharmacological and biochemical properties such as bronchodilatory (Gilani *et al*., 2001, Keyhanmanesh *et al*., 2009), anti-inflammatory and analgesic (Houghton *et al*., 1995), antihepato and nephrotoxic action (Boulos *et al*., 1983), anti-carcinogenic (El Gazzar *et al*., 2006) and hypoglycaemic activities (Benhaddou-Andaloussi *et al*., 2008, 2010).

**Corresponding author:* e-mail: benboubetra@yahoo.co.uk

Anti-hyperglycaemic activity of *Nigella sativa* seed extracts in streptozotocin (STZ) or alloxan-induced diabetes (type 1) in rats (Houcher *et al*., 2007; Kanter *et al*., 2003; Kanter, 2008), hamsters (Fararh *et al*., 2004) and rabbits (Meral *et al*., 2001) have been reported. *Nigella sativa* seed oils have been used for the treatment of experimentally induced diabetic animals. Fixed and essential oils, as well as the thymoquinone compound, were heavily investigated for their anti-hyperglycaemic activity (Fararh *et al*., 2005; Atlan *et al*., 2007; Kanter, 2008). Oral administration of fixed oils to STZ-induced diabetic rats, reduced hepatic gluconeogenesis, increased phagocytic activity of peritoneal macrophages (Fararh *et al*., 2004), decreased significantly the production of nitric oxide (El-Mahmoudy *et al*., 2005), reduced the production of liver endogenous glucose (Fararh *et al*., 2005) and showed a protective effect of both pancreatic and hepatic cells (Kanter *et al*., 2004, 2010).

The present study was carried out to further investigate the anti-hyperglycaemic activity of the lipids of *Nigella sativa* seed on nicotinamide/streptozotocin (N/STZ)-induced diabetic (type 2diabetes but without obesity as pancreas is partially protected by nicotinamide). As the reduction in blood glucose level could be done through remodeling of the hepatic metabolism (Al-Awadi *et al*., 1985), we evaluated the hepatotoxicity of *Nigella sativa*

seed lipids using a precision-cut rat liver slices (PCLS) model (Krumdieck *et al* 1980; Dogterom, 1993). In this model, the tissue organization of the organ is preserved and hence, representing an *in vitro* model closer to *in vivo* conditions than cultures of hepatocytes (Vickers and Fisher, 2004). To elucidate a possibly new mechanism of anti-diabetic activity of *Nigella sativa* seed extracts, we looked at the inhibition of a key enzyme, alpha-glucosidase, in carbohydrate digestion, located in the brush-border surface membrane of intestinal cells.

MATERIALS AND METHODS

Plant materials and extraction procedure

Nigella sativa seeds were obtained from an herbalist in Batna, Algeria in June and properly authenticated [Prof. H. Laouar, botanist, laboratory of plant biology (University of Sétif, Algeria] where specimens are deposited. Seeds were washed, dried and then powdered with an electric micronizer. Nigella sativa oil was extracted according to Ramadan and Morsel (2003) and Sobhi and collaborators (2011) with slight modifications. Briefly, seed material, finely ground in a mill, was Soxhlet-extracted using methanol/hexane (2:1, v/v). The whole was thoroughly mixed without shaking, the layers were allowed to separate and the hexane layer was recovered. The extract was taken to dryness on a rotary evaporator at 40°C. The extracted oil fraction was then applied on a silica gel 60G (70-230 mesh) column (30cm x 3cm) where neutral lipids are eluted by chloroform (3 times, 100ml) whereas polar lipids were eluted by a gradient of acetone-methanol (from 40% to 0% acetone; 300 ml) following the protocol of Ramadan and Mörsel (2002). Based on thin layer chromatography profiles, using chloroform-ethyl acetate-methanol (2v/1v/1v) as mobile phase, seven lipid fractions (four neutral and three polar) were recovered.

Nicotinamide Streptozotocin (N/STZ)-induction of diabetes in rats

a) Animals

Female healthy albinos rats of Wistar strain, aged 7 weeks and weighing between 180 and 220g, were obtained from the Centre for Research and Development (CRD) at the Algerian National Pharmaceutical Company (SAIDAL) in Algiers (Algeria). Animals were maintained in controlled environment (12 hours light/dark cycles) at 22-24°C with 50 % humidity. Rats were fed on pellet standard diet [constituted mainly on sweet corn, soybean, amino acids, vitamins (A, D3, B1, B2, B3, B6, B12, E, K3, PP, folic acid and biotin), minerals (Ca and P), trace elements (Fe, Zn, Co, Se, I, Mg, Mn and S) and the antioxidant Butylhydroxytoluene BHT] and water *ad libitum*. All experiments were in compliance with the guidelines for the care and use of laboratory animals published by the US National Institute of Health (NIH publication No 85-23, revised 1985) with approval of SAIDAL ethic committee.

b) Experimental protocol

The animals were randomly divided into five groups of ten rats in each group and kept for three weeks on pellet standard diet. Diabetes was induced in overnight fasted experimental groups by a single intra-peritoneal (ip) injection of freshly prepared nicotinamide (NA) (230 mg/kg b.w) followed, 15 minutes later, by ip injection of freshly prepared streptozotocin (STZ) (65mg/kg b.w) according to Masiello and co-workers (1998) for all groups except group 5. This model is known to induce, after two days, an experimental non-insulino dependent diabetes mellitus, similar to human type2 diabetes in that it has a significant response to glucose and sulfonylurea and hence provides a particularly advantageous tool for pharmacological investigations of new insulino-tropic agents.

Treatment with Nigella sativa extracts

Two days after N/STZ induction, animals of the first group received orally 2ml of water-oil emulsion containing 400mg/kg bw/day of *Nigella sativa* seed extracted oil, the second group received 400mg/kg bw/day of *Nigella sativa* seed fat-free methanolic extract, dissolved in 2ml of water, the third group received 65 mg/kg bw/day of metformin dissolved in 2ml of water, according to the CRD recommendations. The fourth group, used as positive control (diabetic rats) and the fifth group as negative control (normal rats). Animals of these latter two groups received daily a volume of tap water equivalent to that of the *Nigella sativa* extracts by intragastric gavage. The experiment lasted thirty days.

Plasma glucose, triglycerides and cholesterol determination

Plasma glucose levels were determined using a commercial (Elitech Diagnostics, France) based on glucose oxidase method at days 2, 15 and 30. On day 30, cholesterol, triglycerides (cholesterol oxidase /peroxidase (CHOD-POD) and glycerol-3-oxidase/peroxidase (GPO-POD) were measured using commercial kits (Spinreact, Spain) and urea levels by the kit from Randox, UK. Body weights of the various groups were weekly recorded.

Evaluation of the hepatotoxicity of extracts from Nigella sativa seeds using PCLS method

a) Preparation and incubation of precision-cut liver slices

This test was carried out according to the method described by Evdokimova *et al.* (2001), based on the initial method developed by of Krumdieck and his team in 1980. This method allows to evaluate the hepatotoxicity extracts *in vitro* on liver slices. Hepatotoxicity was evaluated by measuring the change in ATP levels. Rat surgical procedures were carried out under pentobarbital (60 mg/kg) anaesthesia. PCLS (200µm thickness) were prepared using a Krumdieck tissue slicer following procedures described by Evdokimova *et al*. (2001), and incubated for 30 min at 4°C in Krebs-Henseleit buffer. PCLS were transferred to vials containing Krebs-

Henseleit buffer (2 slices/4ml) solutions of *Nigella sativa* seed extracts, to obtain a final concentration of 1mg extract/ml. PCLS were incubated in shaking water-bath at 37°C under a continuous flow of O_2/CO_2 (95%/5%) for 24 hours then rinsed with saline buffer and used to determine protein and ATP contents.

b) ATP and proteins contents

Liver slices were washed twice in saline and sonicated immediately in 1ml of 2% perchloric acid. The intracellular ATP content was measured on neutralized perchloric acid extracts using the ATP Bioluminescence Assay Kit CLS II from Bochringer-Mannheim (Germany) (Evdokimova *et al*., 2001). Protein content was determined according to the method of Lowry. Results are expressed in nmol ATP/mg protein.

Alpha-glucosidase inhibitory assay

Alpha-glucosidase assay was performed according to the chromogenic method described by Schäfer and Högger (2007), with slight modifications. Briefly, 3mM of p-nitrophenyl α-D-glucopyranoside (*p*NPG) was prepared in 0.1M phosphate buffer, adjusted to pH 6.9, to simulate a model of intestinal fluid. Yeast α-glucosidase was dissolved in 0.1M phosphate buffer, pH 6.7, to yield a final stock-solution of 1 IU/ml. For each assay, 0.075 IU of enzyme solution was premixed with *Nigella sativa* seed extracts at various concentrations and pre-incubated for 10 min at 37°C. The enzymatic reaction was initiated by adding 0.95mM *p*NPG and the reaction mixture was incubated for 10 min at 37°C. The activity of α-glucosidase was determined by measuring the product ρ-nitrophenol released from ρNPG at 405nm using a microplate reader and compared to that of the control which had buffer solution in place of the extract. The *α*-glucosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

% inhibition = [($\Delta Abs_{control}$ – Δabs_{sample}) / $\Delta Abs_{control}$] x 100

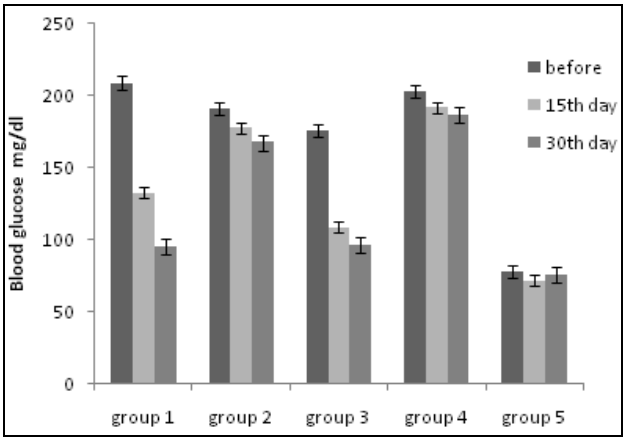
The inhibitory results were expressed as the half maximal inhibitory concentration (IC_{50}), which is a measure of the effectiveness of a compound in inhibiting biological or biochemical function.

STATISTICAL ANALYSIS

All experiments were performed three times and analysis for each experiment was carried out in triplicate. Two lots of samples from the same harvest were subjected to extraction. All data are expressed as mean ± SD. The statistical analysis was done by one-way analysis of variance (ANOVA). $p \leq 0.05$ were considered statistically significant.

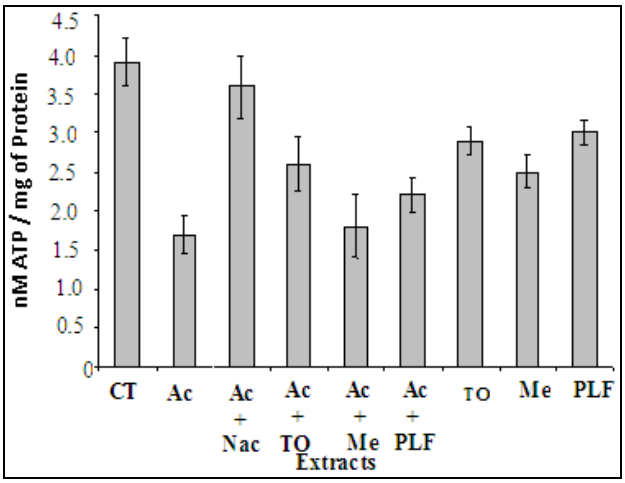
RESULTS

Treatment of diabetic rats (n=7) with *Nigella sativa* total lipid extract (400mg/Kg bw) resulted in a significant ((p< 0.05) reduction of plasma glucose (from 208mg/dL at day 2 to 132mg/dL at day 15 and 96mg/dL day 30), triglycerides (16.3±4mg/dL at day 30) and cholesterol (61.2±4 at day 30) levels and with a significant weight loss (data not shown). The data were similar to those obtained in the group (n=8) treated with metformin at 65 mg/kg bw). However, the group of rats treated with lipid free extract (group 2), did not show any significant reduction in plasma sugar (fig. 1).



Group 1; diabetic rats (n=6) treated total oil extract of *Nigella sativa*, 400mg/Kg bw, group 2; diabetic rats (n=8) treated with methanolic extract of *Nigella sativa*, group 3; diabetic rats (n=6) treated with 65mg/kg of metformin, group 4; control diabetic rats (untreated) (n=7), group 5; normal rats group (n=5). P≤0.05 compared to values at day 2 for groups 1 to 4.

Fig. 1: Effect of *Nigella sativa* extracts on glycaemia of N/STZ-induced diabetic rats



CT; control slices (without treatment), AC; slices treated with acetaminophen (positive control), Ac+NAC; slices treated with acetaminophen + N-acetyl-cysteine, Ac+TO; slices treated with acetaminophen and total oil, Ac+Me; slices treated with acetaminophen + methanolic extract, Ac+PLF: slices treated with acetaminophen + polar fraction of total oil, TO: slices treated with total oil, Me: slices treated with methanolic extract, PLF: slices treated with polar lipid fraction of total oil. PCLS were incubated for 24h at 37°C. Concentrations used are as flowing: PC; acetaminophen (10mM), NAC; N-acetyl-cystein (10mM), TO; total oil (1mg/ml), Me: methanolic extract (1

mg/ml), PLF: polar fraction of total oil (1mg/ml). $p \leq 0.05$ as compared to values of control group (except for PNAC). Mean of duplicate ATP measurements on 2 PCLS obtained from the same rat.

Fig. 2: Evaluation of the hepatotoxicity of *Nigella sativa* seed extracts.

Blood sugar, urea, triglycerides and cholesterol levels were monitored at days 2, 15 and 30 after N/STZ induction of diabetes and in control groups. Animals from treated groups 1 to 4 developed, at day 2, hyperglycaemia consistent with a diabetes status (random glycaemia >195 mg/dl). At day 15, glycaemia significantly decreased in groups 1, 2 and 3 while that of groups 4 and 5 remained steady. At the end of the experiment period, day 30, plasma glucose level of the first three groups continued to decrease, reaching levels of 96.5±3.1mg/dL, 168.3±5.6 mg/dL and 97.3±5.2 respectively (table 1).

Total plasma triglycerides, cholesterol and urea were determined at the end of the experiment (table 2). Results showed clearly the positive effect of *Nigella sativa* seed extracts in rats. Levels of urea in rats treated with total oil and the fat-free methanolic extract were significantly ($p \leq 0.05$) lower than those of untreated diabetic rats, effect similar to metformin. Triglycerides levels in rats treated with total oil were lower than in those treated with fat-free methanolic extract, metformin and normal control rats ($p \leq 0.05$). Compared to normal rats, untreated diabetic rats had the highest levels of triglycerides (67.5±6mg/dL vs 35.2±7mg/dL). Both extracts showed a cholesterol lowering potential in contrast to metformin, which had no action on cholesterol level (table 2).

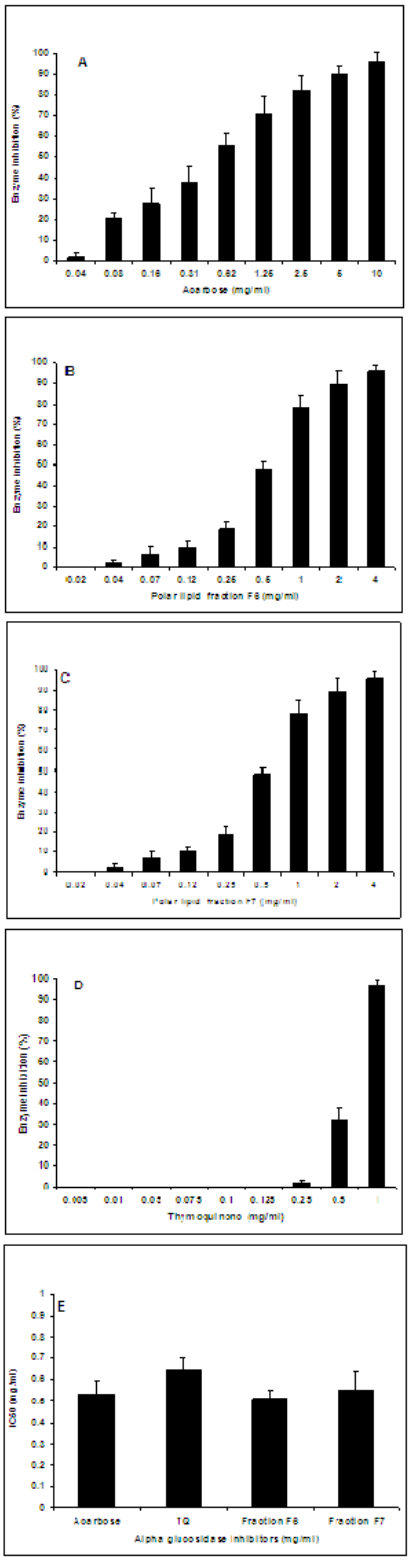
Precision-cut rat liver slices (PCLS) were used to evaluate possible protective potential of *Nigella sativa* seed extracts on rat liver cells. Three *Nigella sativa* seed extracts: total lipid extract (TO), fat-free methanolic (Me) extract and polar lipid fraction (PLF) were tested alone and in combination with the hepatotoxic compound acetaminophen (Ac). Data were compared to control hepatoprotective agent, N-acetyl-cysteine (NAC) (fig. 2). Ac (10mM) induced an important drop in cellular ATP level, which indicates a hepatotoxic effect. Such effect was abolished in the presence of NAC. When applied alone, without acetaminophen, Me reduces the level of ATP to 2.4±0.2nmol/mg compared to 3.88nmol/mg of control. In the presence of acetaminophen (Ac+ME), the ATP level is 1.8±0.3nmol/mg. The total oil used alone (TO) allows generation of 2.9±0.2nmol/mg of ATP and in the presence of acetaminophen (AC+TO), the level of ATP is 2.7nmol /mg. Finally only polar lipids fraction (PLF) produces a level of ATP 3.0±0.15nmol / mg and in the presence of acetaminophen (AC+PLF), ATP decreases to 2.25±0.2nmol / mg. from the these results, we can conclude that All three extracts do not have a protective effect against the action of the AC; improved ATP levels are not significant. The treatment of slices with only (TL, Me and PLF) improves the level of ATP, compared to treatment AC ($p \leq 0.05$). However, this increase is not as great as that observed in the treatment AC + NAC. Only fraction PLF significantly improves ATP levels ($p \leq 0.05$). Consequently, these three extracts may be considered non hepato-protective but not or low toxicity compared to AC.

The inhibitory potential of fat-free methanolic extract on α-glucosidase activity was very weak (less than 10%) even at high concentration (3mg/ml). At 2 mg/ml, total lipid fraction gave an inhibition, varying from 6 to 13% (8.7+/-3.4%), of the enzyme activity. To use cleaner fraction, total lipid extract was partitioned on silica gel into seven lipid fractions [five neutral fractions (F1 to F5) and 2 polar fractions (F6 and F7)]. All the five neutral lipid fractions up to 1mg/ml had a very weak inhibitory effect on the enzyme (less than 8 %). However, polar lipid fractions 6 and 7 (F6 and F7) had significant ($p<0.05$) inhibitory potential effect on α-glucosidase enzymatic activity and hence they were investigated in details in comparison to thymoquinone (fig. 3B-D). Acarbose, a synthetic inhibitor of α-glucosidase, was used as positive control (fig. 3A).

Kinetic slope measurements, at different doses, generated dose-response curves from which the inhibition was computerised as IC_{50} values (fig. 3E). Although *Nigella sativa* seed total lipid extract and the fat-free methanolic extract had no significant effect on α-glucosidase activity, at least at the tested doses, the enzyme was strongly inhibited by polar lipid fractions (F6 and F7) (IC_{50} were 0.51±0.04mg/ml and 0.55±0.09mg/ml, respectively) comparable to those of acarbose (0.53±0.06mg/ml) and thymoquinone (0.65±0.05 mg/ml).

DISCUSSION

Over the years an increasing and renewed interest to alternative medicine and natural therapies has stimulated research into more efficacious plant compounds with lesser side effects (Kim *et al*., 2006). Initially we started investigating the hypoglycaemic potential of total lipid extract from *Nigella sativa* seeds on experimentally N/STZ induced diabetic rats. Oral administration of total lipids (400mg/ kg bw/ day) significantly reduced blood glucose, triglycerides and cholesterol levels after 15 days of treatment. The hypoglycaemic effect of *Nigella sativa* seed oil fraction was observed in STZ-induced diabetic hamsters, where it was found that the oil fraction lowered significantly blood glucose and hepatic glucose production, from gluconeogenesis (Fararh *et al*., 2004). Such findings are in agreement with our present results. It has been reported that the significant reduction of plasma glucose level, in STZ-induced diabetic hamsters, by thymoquinone, was partially mediated through a decrease in hepatic gluconeogenesis (Fararh *et al*., 2005).



Inhibition of α-glucosidase rate by acarbose (A), thymoquinone (B) and polar lipid fractions F6 (C) and F7 (D) with a respective comparison of their IC_{50} (E).

Fig. 3: Inhibitory effects of *Nigella sativa* seed oil fractions and thymoquinone on α- glucosidase activity.

In previous study we have shown that oral administration of *Nigella sativa* seed ethanolic extract (810 mg/kg bw per day) and commercial oil of *Nigella sativa* seed (2.5 ml/kg bw per day) for 25 days led to a significant decrease in plasma glucose and lipids (triglycerides and total cholesterol) in alloxan-induced diabetic rats (Houcher *et al*., 2007). In this study, we showed, in N/STZ-induced diabetic rat model, that the administration of *Nigella sativa* seed total lipid extract lowered significantly plasma glucose and lipid (triglycerides and total cholesterol) levels with a significant weight loss, probably by its polar lipid fraction as the neutral fraction had no significant effect. Our data are in agreement with previous findings of Le and his research team (Le *et al*., 2004) using either petroleum extract or volatile oil of *Nigella sativa* seeds. The mechanism of action of *Nigella sativa* seeds extracts on decreasing blood glucose and lipid levels is still unclear; although, it is likely that the hypoglycaemic effect is by remodeling hepatic sugar and lipid metabolisms (Meral *et al*., 2001; Le *et al*., 2004; Houcher *et al*., 2007).

The use of isolated cells raises several constraints: damages to cellular membranes, disruption of cell-to-cell contact by the use of proteolytic enzymes and loss of normal polarity of hepatocytes as well as the architecture of the whole liver (Evdokimova *et al*., 2001; Vickers and Fisher, 2004). A PCLS model was selected to further evaluate the effect of *Nigella sativa* oil extracts on hepatocytes. In our hands, the ATP content in PCLS decreased after incubation with all *Nigella sativa* seed extracts, dropping by about 30%. Whether this indicates toxicity or reduction in hepatocytes metabolism remains to be determined. It has been previously reported that TQ exhibits a hepatoprotective activity by inhibiting the increase of of cell damage and enzyme leakage induced by tert-butyl hydroperoxide (TBHP) in isolated hepatocytes (Daba and Abdel-Rahman, 1998). It is well known that TQ preserves intracellular glutathione (GSH), which could explain its hepatoprotective effect. It was found that the volatile oil fraction of *Nigella sativa* seeds can prevent lipid peroxidation which induces liver damage in diabetic rats (Kanter *et al*., 2005; Kaleem *et al*., 2006). The protective effect is a result of an increase in the activity of the antioxidant defense systems, such as GSH and ceruloplasmin concentrations, and a decrease in the malondialdehyde (MDA) concentrations (Meral *et al*., 2001). Further investigation is needed to demonstrate the mechanism of liver damage protection in diabetes.

In the last part of the present study, we tested *Nigella sativa* seed total lipid extract and its chromatographic fractions as well as the *Nigella sativa* seed fat-free methanolic extract on alpha-glucosidase activity. Suppressing glucose production by alpha glucosidase is among the therapeutic approaches for reducing postprandial hyperglycaemia and a strategy for evaluating anti-diabetic activity (Fred-Jaiyesimi *et al*., 2010). The

Table 1: Reduction in plasma glucose level of diabetic rats after treatment with *Nigella sativa* seed extracts

Groups	Blood glucose mg/dL			% of reduction in blood glucose	
	Before treatment	After treatment			
		Day 15	Day 30	Day 15	Day 30
Group 1	208.3±4.7	132.7±6.3	96.5±3.1	36.5	53.5
Group 2	191.3±3.8	178.4±4.7	168.3±5.6	6.5	11.8
Group3	176.3±7.2	109.1±3.2	97.3±5.2	37.9	44.6
Group 4	203.2±4.7	192.3±3.6	187.1±8.3	-	-
Group 5	78.4±3.1	72.3±4.3	75.5±3.6	-	-

Group 1 (diabetic rats, n=6, treated with total oil of at 400mg/Kg bw), group 2 (diabetic rats, n=9 treated with defatted methanolic extract at 400 mg/Kg bw), group 3 (diabetic rats, n=8, treated with metformin at 65mg/kg bw) and group 5 (normal rats, n=5). $p \leq 0.05$ compared to values of diabetic non-treated control group (group 4, n=9).

Table 2: Effect of *Nigella sativa* seed extracts on urea, triglycerides and cholesterol levels in diabetic rats.

Animal Groups	Urea level (mg/dL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)
Group 1	90±40*	16±3*	61±4
Group 2	125±65*	24±3*	59±4
Group 3	78±28*	38±10*	72±8
Group 4	213±13	67±2	73±11
Group 5	65±12	35±4	45±8

Group 1 (diabetic rats, n=6, treated total oil extract at 400 mg/Kg bw), group 2 (diabetic rats, n=9 treated with defatted methanolic extract at 400mg/Kg bw), group 3 (diabetic rats, n=8, treated with metformin at 65mg/kg bw) and group 5 (normal rats, n=5). $p \leq 0.05$ compared to values of diabetic non-treated control group (group 4, n=9). Results are expressed as mean ± SE and compared to non treated rats (group4) and control (group 5); * $p<0.05$, ** $p<0.01$

methanolic extract and the neutral lipid fractions (F1 to F5) showed a weak or no effect on the enzyme activity. However, TQ and polar fractions (F6 and F7) exhibited a significant inhibitory activity on alpha-glucosidase with IC_{50} comparable to the potential inhibitor acarbose. These findings could well explain the observed hypoglycaemic effect of polar lipid fractions. In fact, the inhibition of alpha-glucosidase is well-known to induce a decrease in post-prandial blood glucose level and reduces the body weight of rats (Schäfer and Högger, 2007).

CONCLUSION

In this study we have demonstrated that *Nigella sativa* seed extracts reduced significantly blood glucose and lipid levels. Only the polar lipid fraction showed an inhibitory effect on alpha-glucosidase activity, suggesting that their blood glucose lowering effect is a result of the inhibition of the early steps of carbohydrate metabolism. The reduction in blood lipids and urea levels suggests that *Nigella sativa* extracts could probably modulate the lipid and protein metabolisms, possibly at hepatic level. Moreover, we present indications that the polar lipids of *Nigella sativa* may be an interesting candidate for the anti-hyperglycaemic effect of this plant. To clarify the mechanism of *Nigella sativa* seed activities, further work is required on the hepatic metabolism, the post-prandial activity and on characterisation of polar lipid factions of total oil.

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