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Protective Role of *Nigella sativa* Seed Meal and Its Alcohol Extract in Hepatorenal Syndrome Model in Rats.

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ABSTRACT

Hepatorenal syndrome (HRS) is a major health problem where the functions of both liver and kidney are affected. The aim of the present research was to evaluate the protective effect of *Nigella sativa* meal and its ethanol extract in hepatorenal syndrome model in rats. Proximate composition and amino acid contents of *Nigella sativa* meal were analyzed. Total phenolic contents and phenolic compounds were determined in the ethanol extract. HRS was induced in rats via intraperitoneal injection of galactosamine. Three weeks prior to HRS induction, one group of rats was given daily oral dose of *Nigella sativa* meal alcohol extract, another group was fed on diet containing *Nigella sativa* meal, the third group served as control with HRS. A control normal group was run without any treatments. Different biochemical and nutritional parameters were followed. Gene expression of Interlukin 6 (IL-6) in both liver and kidney was determined. Safety of ethanol extract was assessed through acute toxicity test. Results showed that *Nigella sativa* meal contain 27.1% protein and a Fischer ratio of 3.303. Total phenolic content was 42.64 ± 0.929 mg gallic acid equivalent/g dry ethanol extract, identified phenolic compounds were protochatechuic, chlorogenic, vanillic, sinapic, coumaric and cinnamic acid and chrysin. HRS control group showed liver and kidney dysfunction reflected in the elevated plasma creatinine, urea, transaminases activity, total and direct bilirubin and endothelin 1 and urinary N-acetyl-β-D-Glucosaminidase together with reduction in plasma albumin and creatinine clearance. Treatment with either forms of *Nigella sativa* significantly improved all determined parameters except for creatinine and creatinine clearance. Reduction in hemoglobin, plasma total antioxidant capacity and calcium along with elevation of plasma malondialdehyde and tumor necrosis factor-α were noticed in HRS control. All these parameters were significantly improved on treatment with *Nigella sativa* meals and extract except for calcium in case of the extract. Total cholesterol and high density lipoprotein cholesterol showed dyslipidemia in HRS control which were significantly ameliorated on consumption of *Nigella sativa* meals and extract. Liver and kidney Interlukin 6 gene expression was significantly enhanced in HRS control and significantly down regulated on treatment with *Nigella sativa* meals and extract. Ethanol extract showed complete safety on the highest tested dose.

Keywords: *Hepatorenal syndrome- rats- Nigella sativa meal- ethanol extract- Interlukin 6- gene expression- biochemical changes.*

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INTRODUCTION

Black cumin or black seed (*Nigella sativa*) is an important spice, condiment, flavoring agent and medical herb that used in both food products and medical purpose in different regions of the world. It is popular in Egypt where it is commonly used in bakery products and Folk medicine. Earliest cultivation of *Nigella sativa* was reported in several sites in ancient Egypt [1]. The oil yield of the seed expressed by commercial scale screw press machine was 22.5 ± 3.0% according to Al-Okbi et al. [2] while it was 31.4–38.7% when extracted by organic solvent [3]. The major fatty acid in the oil is linoleic acid, an omega 6 fatty acid. The oil extracted on cold is rich in volatile oils that range from 0.1 to 2.6 % ± 0.3 of the crude oil's weight. The major bioactive volatile components are thymoquinone and p-cymene [2, 4]. Different biological activities of *Nigella sativa* oil were reported including hepato-protective, protection from nephrotoxicity and hepatorenal syndrome, anti-inflammatory, antioxidant, improving dyslipidemia, relieving asthma and reduction of postprandial blood sugar [2, 4-7]. Only scarce literature studied the therapeutic potential of the meal and its crude ethanol extract. After extracting the oil from the seed by pressing; the remaining black cumin meal is still containing residual bioactive oils in addition to the main constituents of the meal in concentrated form from the original seed. The meal is used as animal feed due to its high protein content [8-10]. To the best of our knowledge the Fischer ratio (Branched chain amino acids/ aromatic amino acids) of black cumin protein did not studied well. High Fischer ratio could indicate health benefit of the protein towards liver cirrhosis [11]. The ethanol extract of *Nigella sativa* seed prepared by soxhlet was reported to contain total phenolics as 31.15± 0.29 mg gallic acid equivalent/g, total flavonoids as 16.34± 0.71 mg gallic acid equivalent /g, total flavonol as 6.86 ±2.34 mg quercetin equivalent/g and total flavonones as 3.64±0.46 mg naringenin equivalent/g and to possess high antioxidant activity [12]. The aforementioned bioactive constituents of ethanol extract pointed to the expected anti-inflammatory activity of the alcohol extract as reported previously [7] as well as the meal of black cumin. Black seed contains vitamin B1, B2, B6, niacin, folic acid, phosphorus, potassium, sodium, iron, zinc, calcium, magnesium, manganese and cupper [13, 14] reflecting its importance as food and medicine.

Hepatorenal syndrome (HRS) is known as development of acute renal failure in a patient who has advanced liver disease [15]. Patients with severe liver failure have a reduction in renal blood flow, indicative of renal vasoconstriction, and that this is most marked in those patients who develop HRS [16]. However, studies have demonstrated that other factors must be involved as the decrease in glomerular filtration rate (GFR) is not always proportional to renal blood flow [17]. This suggests that events independent of renal vasoconstriction may be involved in the pathogenesis of this syndrome. Inflammation and oxidative stress are among the different factors for progression of HRS. Renal dysfunction in patients with cirrhosis with superimposed inflammation is associated with significant tubular injury and apoptosis [18]. Inhibition of nitric oxide synthase prevents the development of renal failure in animal model of HRS which means that nitric oxide (an inflammatory and oxidative stress biomarker) plays an important role in development of HRS [19]. It is hypothesized that both *Nigella sativa* meal and its alcohol extract could prevent induction of hepatorenal syndrome due to the presence of the aforementioned functional food ingredients and their strong bioactivity as antioxidant and anti-inflammatory agents. In a previous work *Nigella sativa* crude oil proved to reduce the progression of modified HRS in rats [4]. So, the aim of the present study was to extend our search and to evaluate the potential protection of HRS on treatment with either *Nigella sativa* meal or its crude ethanol extract in rats. The aim includes determination of proximate and amino acid composition of *Nigella sativa* meal and calculation of Fischer ratio. Total phenolic and individual phenolic compounds of the ethanol extract were also assessed in the meal.

MATERIALS AND METHODS

Materials

Plant materials: *Nigella sativa* L (black cumin) seeds, family Ranunculaceae were purchased from local markets, Cairo, Egypt.

Chemicals: D-(+)-Galactosamine hydrochloride was supplemented by Sigma, USA. The different chemicals used in the study were of analytical grade.

Animals: Male Sprague Dawley rats of average body weight of 140 ± 18.1 as Mean ± SD were obtained from Animal house of National Research Centre, Cairo, Egypt for HRS experiment. Rats were kept individually in

stainless steel metabolic cages; water and food were given *ad-libtium*. Mice of 23-25 g body weight were purchased from the same previous place for acute toxicity test. Animal experiments was carried out according to the Medical Research Ethics Committee, National Research Centre; Cairo, Egypt.

Methods

Preparation of plant materials. *Nigella sativa* seeds were dried in an air-circulated oven at 40 °C and crushed twice using grinder model (MF10 microfine grinder drives). The crushed samples were pressed with laboratory type of Carver hydraulic press under 10.000 Ib/in (pic) pressure for 1h at room temperature according to Üstun *et al*. [20]. The meal (defatted part) was kept in deepfreeze till used.

Preparation of crude ethanol extract of *Nigella sativa* seed meal: The dried powder of *Nigella sativa* seed meal was subjected to extraction by ethanol in soxhlet. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40°C. The extract was kept in deepfreeze till used.

Chemical analysis of *Nigella sativa* meal: Protein, fat, ash, and crude fiber contents were determined in *Nigella sativa* meal according to A.O.A.C. [21]. Carbohydrates were calculated by difference. Amino acid analysis was carried out [22, 23] using LC3000 amino acid analyzer (Eppendorf-Biotronik, Germany). Fischer ratio (Branched chain amino acids/Aromatic amino acids) was calculated

Determination of total phenolic content in the extract: Total phenolics were determined colorimetric in the crude ethanol extract according to Singleton and Rossi [24]. Phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry extract.

HPLC analysis of phenolic compounds in *Nigella sativa* extract: Alcohol extract of *Nigella sativa* meal was prepared for HPLC analysis [25] using Agilent Technologies 1100 series liquid chromatography. Peaks of phenolic compounds were identified by retention times and UV spectra and compared with those of standards.

Diets: Balanced and *Nigella sativa* meal diets (Diet1 and 2, respectively) were prepared as in table (1). It was taking into consideration that both diets must contain the same percentage of protein, fat, crude fibers and carbohydrates through deducting these nutrients from the balanced diets according to their content in *Nigella sativa* meal concerning diet 2.

Table 1: Composition of the diets (g per 100 g)

Ingredients	Balanced diet Diet 1	*Nigella sativa* meal diet 2
Casein	12*	11.6
Corn oil	10	9.8
***Nigella sativa* meal**	-	1.21**
Starch	47	46.66
Sucrose	23.5	23.33
Salt mixture	3.5	3.5
Vitamin mixture	1	1
Cellulose	3	2.9

* 12 g casein equals to 10 g. protein as determined according to A.O.A.C. [21].
**The quantity of the meal that would be consumed by rat was calculated to be equivalent to the quantity that gives the same alcohol extract dose (250mg/kg rat).

HRS experiment: Twenty four rats were fed on balanced diet (diet 1) for two weeks as an adaptation period, and divided into four groups; each of six rats. The 1st group served as normal healthy control and fed on diet 1 without any treatments for 3 weeks. Rats of group two consumed diet 1 and given daily oral dose of 250 mg crude ethanol extract of *Nigella sativa* meal /kg rat body weight for three weeks. Rats of group three were fed on balanced diet containing *Nigella sativa* meal (diet2) for three weeks. Rats of the fourth group consumed diet1 for three weeks. At the 34th day all rats except those of the 1st group were given 1.1 g/kg body weight of D-(+)-galactosamine hydrochloride via intraperitoneal injection as a 200 mg/ml solution in saline for induction

of hepatorenal syndrome as reported previously [19]. Group 2 and 3 were considered as test groups while group 4 was control with hepatorenal syndrome. The experiment continued for 5 weeks. Body weight and food intake were followed weekly. Total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated at the end of the experiment. Twenty-four-hours urine samples were collected after 24 h from the galactosamine hydrochloride injection for assesment of creatinine and N-acetyl-β-D-Glucosaminidase (NAG) according to Houot [26] and Price and Whiting [27], respectively. Blood samples were obtained from fasted rats after 24 hours from the galactosamine hydrochloride injection. Blood hemoglobin was determined according to Vankampen & Zijlstra [28]. Plasma was separated from heparinized blood for assessing plasma total cholesterol (T-Ch) and high density lipoprotein cholesterol (HDL-Ch) by colorimetric methods according to Watson [29] and Burstein *et al*. [30], respectively. Malondialdehyde (MDA), a measure of lipid peroxidation, was estimated in plasma according to Satoh [31] while total antioxidant capacity (TAC), an indicator of antioxidant state, was determined by the method of Koracevic *et al*. [32]. The inflammatory biomarker, tumor necrosis factor-α (TNF-α), was assessed by ELISA technique according to Stepaniak *et al*. [33]. Liver function was followed through determination of the activity of plasma aspartate transaminase (AST) and alanine transaminase (ALT) [34], plasma albumin [35] and total and direct bilirubin [36]. Plasma creatinine [26] and urea [37] were determined as indicator of kidney function. Plasma endothelin 1 (ET-1) was estimated using ELISA [38] which was proposed previously as it could be a biomarker of hepatorenal syndrome. Plasma calcium and phosphorus were estimated according to Gindler *et al*. [39] and Taussky and Shorr [40], respectively. Kidneys and livers were separated and weighed and a weight of 40 mg was taken from each organ for assessing gene expression of Interlukin 6 using PureLink® RNA Mini Kit and reverse transcription kit (ambion® Life technologies™) and according to Mabrok et al. [41], Khan et al.[42], Livak and Schmittgen [43] and AL-Okbi et al [44]. Percentage Liver weight/body weight and kidney weight/body weight were calculated.

Acute toxicity test: Acute lethal toxicity test of crude ethanol extract of *Nigella sativa* meal was assessed [45]. The 24 h mortality counts among equal sized groups of mice (8 animals/group) receiving progressively increasing oral dose levels of the extract were followed.

Statistical analysis: Data of animal experiments were designated as the mean±SE. One-way analysis of variance ANOVA followed by Duncan's test were applied for statistical analysis. Pearson correlation test was applied between different biochemical parameters of HRS control group. Results from gene expression were analyzed by one-way ANOVA followed by the Dunnett´s multiple comparison test using the SPSS statistical program. In all cases $p<0.05$ was used as the criterion of statistical significance. Total phenolic content was expressed as mean ±SD.

RESULTS

Chemical analysis of *Nigella sativa* meal clarified that *Nigella sativa* meal contains high percentage of protein (27.1%). Residual fat content was 12.6% while carbohydrate was 40.5%. Crude fibers were present as 8.1%. The ash content was 7.3 /100g *Nigella sativa* meal. Moisture was 4.4% of the meal. Amino acids analysis (table, 2) showed *Nigella sativa* meal to have high Fischer ratio calculated as 3.303.

The yield of crude ethanol extract that obtained from *Nigella sativa* meal was 27.6%. Total phenolic content of the crude alcohol extract of *Nigella sativa* meal as mean± SD was 42.64 ± 0.929 mgGAE/g dry extract. HPLC analysis of phenolic compounds showed the presence of Protochatechuic (5.1 mg/100g), chlorogenic (10.6), vanillic (9.3), sinapic (16.9), coumaric (9.3) and cinnamic (2.8) acid and Chrysin (49.1).

Table 3 showed the different analyzed biochemical parameters of the experimental groups. Plasma levels of total and direct bilirubin and plasma activities of AST and ALT were increased significantly in hepatorenal control (HRS), indicating liver dysfunction. Also plasma albumin and total protein was reduced significantly in HRS control rats compared with normal healthy rats. Plasma levels of creatinine, urea and endotheline-1 (ET-1) and urinary NAG increased significantly in hepatorenal control rats compared with normal rats, with significant reduction in creatinine clearance, indicating kidney dysfunction. Concerning plasma lipids; HRS control rats exhibited a significant increase in plasma total cholesterol and significant decrease in HDL-Ch compared to normal healthy control. Blood hemoglobin and plasma calcium were reduced significantly in HRS control rats with no change in plasma phosphorus compared with normal control**.** Plasma TAC showed

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significant reduction while MDA and TNF-α (as indicator of lipid peroxidation and inflammation, respectively) increased significantly in HRS control group compared with normal control.

Table 2: Amino acids content of *Nigella sativa* meal:

Amino acids	Amino acid content in *Nigella sativa* meal (mg/100g)
Essential amino acids	
Valine	434.4
Methionine	60.5
Isoleucine	119.5
Leucine	721
Phenylalanine	306.3
Histidine	777.3
Lysine	475.8
Arginine	1308.4
Threonine	432.6
Non- Essential amino acids	
Aspartic	1277.2
Serine	926.7
Glutamic	9620
Glycine	893.2
Cystine	0.0
Tyrosine	79.7
Proline	234.2
Alanine	2233.9
Total essential amino acids	4635.8
Total non- essential amino acids	15264.9
Total amino acids	19900.7
Fisher ratio	3.303

Table 3: Biochemical parameters of different experimental groups.(Mean+SE).

Parameters	Normal control	Hepatorenal control	Nigella meal extract	Nigella meal powder
Blood Hb (g/dl)	13.9^{a}±0.309	9.9^{b}±0.399	11.9^{c} ±0.487	12.7^{c}±0.662
Plasma ET-1 (ng/ml)	22.9^{a}±1.589	41.1^{b}±1.798	30.2^{c}±1.973	31.3^{c}±1.475
Plasma creatinine (mg/dl)	0.712^{a}±0.039	1.008^{b}±0.019	0.923^{b} ±0.041	0.938^{b}±1.475
Plasma urea (mg/dl)	26.9^{a}±1.885	43.8^{b}±2.198	31.4^{a}±1.492	35.4^{c}±1.628
Creatinine clearance (ml/min)	0.930^{a}±0.039	0.550^{b}±0.049	0.561^{b} ±0.029	0.601^{b}±0.022
Urinary NAG (IU/l)	37.1^{a}±0.498	47.8^{b}±1.297	40.8^{c} ±0.42	41.2^{c}±0.753
Plasma albumin (g/dl)	4.4^{a}±0.120	2.70^{b}±0.158	3.7^{a}±0.321	3.8^{a}±0.289
Plasma total protein (g/dl)	6.9^{a} ±0.142	5.7^{b} ± 0.198	6.5^{a} ± 0.179	6.4^{a} ± 0.051
Plasma ALT (U/l)	55.9^{a}±0.899	89.3^{b}±1.099	69.8^{c} ±1.276	71.2^{c}±0.909
Plasma AST (U/l)	40.9^{a}±0.689	83.1^{b}±0.698	64.2^{c}±1.815	65.7^{c}±2.059
Plasma T. Bilirubin (mg/dl)	0.373^{a}±0.009	0.514^{b}±0.014	0.393^{c}±0.006	0.408^{c}±0.015
Plasma D. Bilirubin (mg/dl)	0.160^{a}±0.003	0.252^{b}±0.006	0.163^{c}±0.004	0.173^{c}±0.005
Plasma T-Cholesterol (mg/dl)	49.2^{a}±1.979	116.5^{b}±7.998	73.3^{c} ±5.351	81.2^{c}±3.469
Plasma HDL-Ch (mg/dl)	30.1^{a}±1.698	5.6^{d}±0.352	14.3^{c}±1.111	15.0^{c}±1.539
PlasmaTAC (mm/l)	1.7^{a}±0.03	0.875^{b}±0.032	1.3^{c}±0.037	1.32^{c}±0.045
Plasma MDA (nmol/ml)	6.2^{a}±0.496	22.6^{b}±1.710	16.4^{c}±2.023	16.0^{c}±1.874
PlasmaTNF-α (pg/ml)	20.1^{a}±0.498	32.3^{b}±0.587	28.8^{c}±1.352	28.5^{c}±1.284
Plasma Ca (mg/dl)	9.32^{a}±0.215	7.3^{b}±0.214	8.0^{b}±0.618	10.3^{c}±0.257
Plasma P (mg/dl)	4.4^{a}±0.300	3.6^{a}±0.422	4.5^{a}±0.352	5.1^{a}±0.434

In each row same letters means non-significant difference; different letter means significance difference at 0.05 probabilities.

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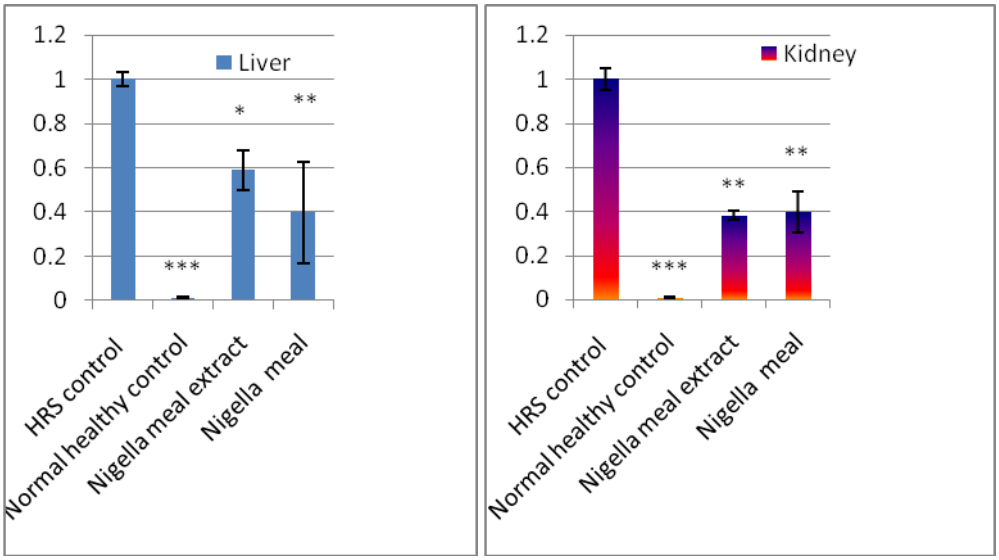
In HRS control rats; Pearson correlation test showed that plasma ET-1 had significant negative correlation with plasma TAC (r =-0.616, p<0.05) and significant positive correlation with plasma activity of AST (r= 0.736, p<0.05). Urinary NAG showed significant positive correlation with plasma creatinine (r= 0.816, p<0.05), ET-1 (r= 0.560, p<0.05) and TNF-α (r= 0.706, p<0.05). Plasma TNF-α as inflammatory biomarker had significant positive correlation with plasma creatinine (r= 0.778, p<0.05) and urea (r= 0.544, p<0.05). Plasma MDA as lipid peroxidation marker correlated significantly positive with total bilirubin (r= 0.779, p<0.05) and direct bilirubin (r= 0.806, p<0.05). Plasma TAC as a measure of antioxidant status showed negative correlation with the activity of AST (r= -0.923, p<0.05).

Treatment of HRS rats by *Nigella sativa* meal and its alcohol extract produced significant improvement of the majority of biochemical parameters except for plasma creatinine and creatinine clearance that only improved insignificantly. Also, plasma calcium level was significantly improved on treatment with *Nigella sativa* meal but not the ethanol extract. It can also be noticed that plasma albumin and total protein of HRS rats treated by *Nigella sativa* meal and its alcohol extract were normalized where they match those of the control normal rats.

Figure 1: The relative expression of IL-6 gene in liver and kidney of different experimental groups (mean± SE).



Significant values compared to HRS control: *: *P*<0·05; **: *P*<0·001; ***: *P*<0·0001

Figure1 represents the mRNA expression of IL-6 in liver and kidney. HRS control showed significant fold increase in IL-6 gene expression in liver and kidney compared to control normal rats. The mRNA expression of IL-6 was significantly lower in liver of rats consumed Nigella extract and Nigella powder by 41% and 60%, respectively, compared to HRS control rats. Nigella powder down-regulated the expression of IL-6 in rats' kidney by 61% compared to HRS control rats. IL-6 was reduced by 52% in kidney of rats consumed Nigella extract compared to HRS control rats. The mRNA expression of IL-6 was normalized with housekeeping gene (GAPDH).

Nutritional parameters (body weight gain, total food intake and food efficiency ratio), liver weight/body weight% and kidney weight/body weight% showed non-significant changes among all the studied groups (data are not shown).

The acute lethal toxicity test revealed that the ethanol *Nigella sativa* meal extract was very safe up to the highest tested dose (12g/kg mice body weight) which reflects the highest safety of the extract.

DISCUSSION

In the present study the protective effect of *Nigella sativa* meal and its alcohol extract was studied towards HRS in rats. One of HRS group was provided with *Nigella sativa* meal, a quantity that could provide the rat with the same dose of *Nigella sativa* meal extract (250 mg/kg) that was given to the other HRS group.

Previously, *Nigella sativa* seeds alcohol extract (100mg/kg rat body weight) did not improve biochemical parameters reflecting kidney dysfunction, while histopathology of the kidneys relatively recovered after administration of the extract [46]. However it is to be expected that elevating the dose of the extract to > 100mg/ kg or using the meal (concentrated form of hydrophilic constituents of the seed) or its alcohol extract could afford improvement in kidney dysfunction which was relatively achieved in the present study. In the current work; dose of *Nigella sativa* meal alcohol extract of 250 mg/kg and the meal itself produced improvement of kidney function represented by plasma urea, ET-1 and urinary NAG but not plasma creatinine and creatinine clearance. Previously, *Nigella sativa* seeds and its waste aqueous extracts were shown to have hepatoprotective effect against CCL4-induced hepatotoxicity and damage [47, 48]. These results are in accordance with the present study that showed improvement in liver function of HRS rats treated with *Nigella* meal and its alcohol extract. The antioxidant and anti-inflammatory activity of *Nigella sativa* meal and its alcohol extract could be the mechanism underlying the improvement of kidney and liver function seen in the present research. Hosseini et al [49] showed that hydro-alcohol extract of *Nigella* reduced oxidative stress and MDA level in scopolamine treated rats. Alcohol extract of *Nigella sativa* seed also was reported to possess antioxidant activity [50]. These results agreed with the present study that showed reduction in plasma MDA and elevation of TAC that indicate reduction in oxidative stress on treating HRS rats with either *Nigella sativa* meal or its alcohol extract. The antioxidant activity of both *Nigella sativa* meal and its alcohol extract was supported by reduction of inflammatory cytokines reflected in down regulation of liver and kidney Interlukin 6, and inhibition of plasma TNF-α in HRS rats. Michel et al. [48] reported that the aqueous extract of *Nigella* and its waste attenuated the CCl4 -induced liver damage likely due to the decrease of proinflammatory cytokines and T-cell proliferation. This was noticed in Michel et al. study by a significant decrease in both serum and tissue cytokines; tumor necrosis factor-alpha, interferon-gamma and interlukin-beta (IL-1β), in the markers of liver functions; bilirubin and glutamic pyruvic transaminase and in the oxidative stress markers; malondialdehyde and glutathione content. Improvement of liver function in HRS rats on different treatment could lead to reduction in total cholesterol and elevation of HDL-Ch which was observed in the present study. *Nigella sativa* meal was shown previously to be a rich source of protein and fat 33.13%, and 12.72, respectively [10] which is somehow similar to that in the present study. In the current work chemical analysis of *Nigella sativa* meals showed the presence of residual oil content of 12.6%, crude fibers as 8.1 % and protein as 27.1% of high Fischer ratio that could have great impact in improving liver and kidney function in HRS rats in the present study. This is because residual oil of *Nigella sativa* contains omega-3 fatty acid and volatile active constituents like thymoquinone and p-cymene that possess variable bioactivities including antioxidant, anti-inflammatory, hypocholesterolemic and hepatoprotective effect [2, 4]. In a previous work proximate analysis of black cumin seeds showed a composition of 20.85% protein, 38.20% fat, 4.64% moisture, 4.37% ash, 7.94% crude fibre and 31.94% total carbohydrates. Potassium, phosphorus, sodium and iron were the predominant elements while zinc, calcium, magnesium, manganese and copper were found at lower levels. Linoleic and oleic acids were the major unsaturated fatty acids. Glutamic acid, arginine and aspartic acid were the main amino acids while cystine and methionine were the minor amino acids according to Al-Jassir [14]. The amino acid results in the present study showed glutamic acid, arginine, alanine and aspartic acid were the main amino acids while cystine and methionine were the minor amino acids which are similar to that of Al-Jassir study.

The alcohol extract showed to contain high total phenolic content and variable individual phenolic compounds to which the antioxidant and anti-inflammatory activity might be ascribed [51, 52] and participate in protection from HRS. *In vitro* study showed that the phenolic compound chrysin that present in *Nigella* inhibits COX-2 expression and IL-6 signaling [53], which together with the other identified phenolics may contribute to the anti-inflammatory effects shown in the present study.

Previously, the crude protein of the seed waste were reported by Michel et al. [48] to reach 36.85% while protein fingerprint showed four bands ranging from 91.97 KD to 29.00 KD, in addition of high content of polyphenols. The protein fraction of *Nigella sativa* seed waste induced a significant reduction in both serum and tissue cytokines, in the biomarkers of liver function and in the oxidative stress markers. Liver

histopathology showed that the protein fraction reduced the incidence of liver lesions, hepatic necrosis and fibrous connective tissue proliferation induced by CCl4 in mice. Therefore protein fraction of the aqueous extract of *Nigella sativa* seed waste exhibited a promising hepatoprotective effect in the management of different liver disorders according to Michel et al. [48]. Improvement of the level of plasma albumin in HRS rats shown in the present study is very important for the treatment of HRS. Garcia-Martinez et al. [54] and Hinz et al. [55] reported that albumin concentration and albumin function is reduced in liver failure and that albumin infusion reduces serum creatinine with consequent reduction in mortality and is useful in the management of patients with hepatorenal syndrome. Also improvement of Ed-1 reflected protective effect of *Nigella sativa* meal and its alcohol extract. This is because plasma endothelin 1 was considered as biomarker of HRS as reported previously [56]. The correlation study in the present work showed that endothelin 1 correlated positively with NAG and AST and negatively with TAC indicating that it could serve as biomarker of both liver and kidney dysfunction and that reduced antioxidant status might be one of the factors that contribute in induction of HRS. Branched chain amino acids supplementation is important to prevent encephalopathy in liver cirrhotic patients as reported by Laviano *et al*. [57] and which represented in the present study by the high Fischer ratio of *Nigella* meal.

In HRS control rats, it could be noticed that plasma creatinine, urea and urinary NAG showed positive correlation with the inflammatory biomarker, TNF-α, indicating that inflammation is involved in induction of renal dysfunction in HRS. Elevated MDA and reduced TAC in HRS control rats were correlated with liver dysfunction represented by elevation of bilirubins and AST, respectively; indicated that elevated oxidative stress contribute in liver dysfunction.

CONCLUSION

HRS induced in rats by intraperitoneal injection of galactosamine hydrochloride produced liver and kidney dysfunction, reduced hemoglobin, elevated oxidative stress and increased inflammatory biomarker. It also elevated ET-1, elevated plasma cholesterol, reduced plasma HDL-Ch, reduced plasma albumin, reduced plasma Ca and elevated urinary NAG with no change in plasma P and nutritional parameters. Pretreatment with *Nigella sativa* meal or its crude alcohol extract, afforded great protection from HRS.

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