

HEPATO-PROTECTIVE ROLE OF THE AQUEOUS AND N-HEXANE EXTRACTS OF *NIGELLA SATIVA* LINN. IN EXPERIMENTAL LIVER DAMAGE IN RATS

FARIDA YESMIN ¹, ZAIDA RAHMAN ², JESMIN FOUZIA DEWAN ³, ASADUL MAZID HELALI ⁴, NOR IZA A RAHMAN ⁵, AHMED G. ALATTRAQCHI ⁶, AREFUDDIN AHMED ⁷, RABEYA YOUSUF ⁸, ABDUS SALAM ⁹, MAINUL HAQUE ¹⁰

¹Assistant Professor, Department of Pharmacology & Therapeutics, Gonoshashthaya Samajvittik Medical College (GSSVMC), Savar, Dhaka, Bangladesh; ²Associate Professor, Department of Pharmacology & Therapeutics, Enam Medical College & Hospital, Savar, Dhaka, Bangladesh; ³Professor, Department of Pharmacology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag Dhaka, Bangladesh; ⁴Assistant Professor, Department of Pharmacology & Therapeutics, GSSVMC, Dhaka, Bangladesh; ⁵Medical Lecturer, Faculty of Medicine and Health Sciences (FPSK), Universiti Sultan Zainal Abidin (UniSZA), Terengganu, Malaysia; ⁶Medical Lecturer, FPSK, UniSZA, Terengganu, Malaysia; ⁷Senior Lecturer, Medical Radiation Programme, School of Health Sciences, Universiti Sains Malaysia, Malaysia; ⁸Medical Officer, Blood Bank Unit, Department of Pathology, Universiti Kebangsaan Malaysia (UKM) Medical Centre, Kuala Lumpur, Malaysia; ⁹Associate Professor, Department of Medical Education, UKM Medical Centre, Kuala Lumpur, Malaysia; ¹⁰Professor, FPSK, UniSZA, Terengganu, Malaysia. ¹⁰ Email: runurono@gmail.com

Received: 13 June 2013, Revised and Accepted: 3 July 2013

ABSTRACT

Objective: Liver disease is associated with the formation of oxygen derived free radicals. Reactive oxygen species (ROS) as well as nitrogen species are responsible for nuclear DNA fragmentation and cell death. The active principle of thymoquinone (TQ) of *Nigella sativa* acts as a scavenger of superoxide anion. The current study was conducted to evaluate the hepatoprotective effect of *Nigella sativa* on rats.

Methods: The study was carried out at prime postgraduate medical University of Bangladesh. Liver damage and oxidative stress were evaluated by measuring serum alanine amino transferase (ALT), hepatic malondialdehyde (MDA) and hepatic Glutathione (GSH) levels. Aqueous extract of *Nigella sativa* and n-hexane extract of *Nigella sativa* were administered orally into two groups of rat through intra-gastric tube for 28 days. Both the groups received paracetamol intra-peritoneally on day 28th and were sacrificed on day 30th. Subsequently, the following parameters were studied: Serum ALT, hepatic MDA, and hepatic GSH.

Results: Hepatic damage was evaluated by significant increases in serum ALT ($p < 0.001$) and hepatic MDA ($p < 0.001$) concentration with depleted hepatic GSH ($p < 0.001$) in paracetamol treated group. Pre-treated with aqueous extract of *Nigella sativa* significantly reduced serum ALT ($p < 0.001$) and hepatic MDA ($p < 0.001$) levels and also significantly associated with the increase in hepatic GSH ($p < 0.01$). Pretreatment with n-hexane extracts of *Nigella sativa* decreased serum ALT ($p < 0.001$), hepatic MDA ($p < 0.001$) and increased hepatic GSH ($p < 0.001$).

Conclusion: Hepatoprotective properties of *Nigella sativa* in liver damage of experimental rats by reducing oxidative stress are evident. The protection afforded by the n-hexane extract of *Nigella Sativa* in pre-treated group has also been validated.

Keywords: Hepatoprotective, Liver-damage, *Nigella sativa* Linn.

INTRODUCTION

In the current world, liver diseases are dealt with seriously by the physicians both in the urban and the rural areas due to their potentiality to cause morbidity and mortality. The prevalence rate of liver disease in Bangladesh is the highest in the world [1]. Liver is the main organ involved in the metabolism of biological toxins and medicinal agents [2]. Hence; metabolism is always associated with the disturbance of hepatocyte biochemistry and generation of ROS [3, 4, 5]. ROS are involved in liver damage induced by several conditions such as viral hepatitis [6], alcohol abuse [3], cirrhosis of liver [7], hepatocellular carcinoma [8] and paracetamol-induced liver damage [9].

Paracetamol (acetaminophen) is a safe and effective analgesic and antipyretic drug when used at therapeutic dose [10]. However, an overdose can produce fatal hepatic necrosis in man [11] and other animals [10]. It has been stated that paracetamol overdose is one of the most frequent causes of drug induced liver failure in the United States and in the Great Britain [12].

Previously, researchers studying the toxic mechanism of paracetamol focused on the metabolic activation of the drug by cytochrome P450 enzymes to a reactive metabolite that depleted GSH and covalently bound to protein. Reduced amount of GSH leads covalent binding of the reactive metabolite N-acetyl-*p*-benzoquinone imine (NAPQI) with cellular protein resulting in hepatic cell death [13, 10]. Current drug for the management of high-dose paracetamol-induced toxicity includes N-acetylcysteine and methionine. They provide protection after paracetamol overdose

primarily by replenishment of hepatic GSH stores and direct detoxification of NAPQI. Although these antidotes have been available for more than two decades, they possess certain limitations and hepatic damage and deaths are still frequently seen, largely because of late presentation [14]. Therefore, experiments are being carried out in search of more effective, non-toxic, inexpensive agents.

Abundant researches have been carried out to obtain appropriate therapy for paracetamol-induced hepatotoxicity as well as ways of preventing and treating liver diseases. Antioxidant therapy used in different liver diseases is GSH [13], l-ascorbic acid [15, 16], *Andrographis paniculata* (*kalamagh*) [17], *Spirulina* [18], *Cajanus indica* (*arhar*) [19], *Phyllanthus niruri* (*bhuiamla*) [20, 21, 22], *Silymarin* [23], vitamin E [24] and selenium (Se) [25].

The black cumin is an important spice, also known as black seed, fennel flower, nutmeg glower, Roman coriander, or black caraway. *N. sativa* is a common spice that grows once a year and a member of the Ranunculaceae family [26]. The seeds have traditionally been used in South Asia and Middle Eastern folk medicine as a natural remedy for various diseases as well as spice for over 2000 years.

The seeds of *N. sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations. Human studies and laboratory studies on the seeds and oil have been subjected to scientific experiments and have been reported to be effective for immune stimulation and treatment of rheumatism, [27], diabetes [28,29], cancer and inflammatory diseases [30]. The extracts of the black seeds have many therapeutic effects such as

antibacterial [31], antifungal [32], anthelmintic [33], analgesic [34], antiulcer [35], diuretic and antihypertensive [36, 37, 38], bronchodilator [39], antioxidant and hepatoprotective activities [23, 40, 41, 42,].

The black seed is composed of fixed oil, volatile oil, alkaloid, saponins, sterols and quinines [40]. The n-hexane extract of *N. sativa* contains TQ, tocopherols and carotenoids [43]. TQ has an antioxidant potential [44] and possesses superoxide anion radical-scavenging ability in vitro and vivo [45]. It was reported that TQ protects isolated rat hepatocytes against CCl₄-induced hepatotoxicity by preventing the depletion of intracellular GSH and thus maintain the integrity of cell membrane [40, 46]. The aqueous extract of *N. sativa* has been suggested to possess antioxidant property and reduces the hepatotoxicity against CCl₄-induced liver damage [47].

Therefore, the present study was designed to evaluate the hepatoprotective promises of the aqueous and the n-hexane extracts of *N. sativa*, in experimental liver injury in rats.

MATERIALS AND METHODS

This experimental study was carried out in the laboratory of the Department of Pharmacology, BSMMU, Dhaka, Bangladesh during the period from October, 2008 to July, 2010.

The study was carried out upon 30 adult rats of the Long-Evens Norwegian strain, aged between 3-4 months (weighing between 160-210 gm). They were obtained from the animal house of BSMMU. The rats were divided into five groups with six animals in each. Group I (V) or vehicle treated group received a single dose of vehicle for paracetamol (propylene glycol) (1 mL intra-peritoneal (i.p) on the day 1 and were sacrificed on 3rd day (48 hours after a single dose). This propylene glycol treated group was designated as the control group of the present study. Group II (P_I) or paracetamol-control group received a single dose of paracetamol solution in propylene glycol at a dose of 800 mg/Kg body weight (b.w). Paracetamol was given i.p on the day 1 and were sacrificed on the 3rd day (48 hours after a single dose). Group III (P_{II}) or paracetamol treated group received a single dose of paracetamol solution (in propylene glycol) at a dose of 800 mg/Kg b.w. Paracetamol was given i.p on the day 1 and were sacrificed on 30th day. Group IV (A +P_I) or [aqueous extract + paracetamol] treated group received aqueous extract of *N. sativa* at a dose of 500 mg/kg b.w, orally through a Ryle's tube from day 1-28 and paracetamol was administered on day 28th and were sacrificed on 30th day. Group V (H+P_I) or (n-hexane extract + paracetamol) treated group received n-hexane extract of *N. sativa* at a dose of 5 mL/kg b.w, orally through Ryle's tube from day 1-28 and paracetamol was administered on day 28 and were sacrificed on 30th day. Animals were sacrificed under anesthesia by cutting the carotid artery with the blade and blood samples were taken for investigation. Liver damage and oxidative stress were evaluated by measuring serum ALT, MDA and hepatic GSH levels.

Estimation of serum ALT concentration

Principle

ALT or glutamate pyruvate transaminase (GPT) catalyses the reversible transfer of an amino group from L-alanine to 2-oxoglutarate forming L-glutamate and pyruvate. The pyruvate produced was reduced to lactate by LDH and NADH. The Serum ALT level was estimated by kinetic method [48] according to the recommendation of the expert panel of the International Federation of Clinical Chemistry and Laboratory Medicine. Absorbance and the concentration of enzyme were measured in a spectrophotometer.

Estimation of serum GSH Concentration

Principle

The simple spectrophotometric procedure for GSH estimation in tissue is based on the method of Ellman, who reported that 5, 5-dithiobis-2-nitrobenzoic acids reduced by SH group to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The nitromercaptobenzoic acid anion has an intense yellow color and can be used to measure SH groups. The optimal condition for colour

development and preparation of homogeneous has been studied with respect to precision, reproducibility and specificity of the estimation [49]. This color intensity was measured by the spectrophotometer (UV-VIS spectrophotometer) at 412 nm wavelength.

Method for estimation of MDA level [50]

Principle: MDA is formed as a result of lipid peroxidation and reacts with thiobarbituric acid (TBA) at 90- 100 ° C temperature and in acidic condition. The reaction yields a pink MDA-TBA adduct the product of two moles of TBA plus 1 mole of MDA. The colored complex can be measured by spectrophotometer using wave length 532 NM. The extent of lipid peroxidation was estimated by using the TBA method to determine the level of MDA, which served as the index of lipid peroxidation.

Statistical analysis

Data obtained from the findings of the above experiments have been expressed as mean ± Standard deviation (mean ± SD). Statistical analysis was done by SPSS version 21. Post-hoc analysis of differences was done with Bonferroni't 't' test, by using one way analysis of variance (ANOVA) followed by Bonferroni't 't' test. The differences between groups were considered highly significant at P< 0.001, moderately significant at p< 0.01 and significant at P <0.05.

RESULTS

Serum ALT levels (U/L) (mean ± SD)

The mean values of serum ALT level in group I (V), group II (P_I), group III (P_{II}), group IV (A +P_I), group V (H+P_I) were 22.34± 4.69 U/L, 67.21± 5.39 U/L, 46.91± 5.99 U/L, 33.97± 4.38 U/L, 30.59± 4.52 U/L respectively (Table 1). The mean ± SD of serum ALT in paracetamol treated group (group II) was significantly higher (p<0.001) when compared to those of control (group I). So, pre-treatment with aqueous extract and n-hexane extract of *N. sativa* decreased the serum ALT concentration significantly and percentages of reduction were 49.46%, 54.49% respectively (Table 1). Serum ALT level among these groups were compared and significant difference was found [p<0.001] (Table 2). There were highly significant difference (p<0.001) observed between group I and II, group II and IV, group II and V respectively.

Table 1: Serum ALT levels (U/L) (mean ± SD) in pre-treatment groups

Group (n = 6)	ALT (U/L) (mean ± SD)	Reduction (%)	p-value
I (V)	22.34 ± 4.69	49.46%	<0.001***
II (P _I)	67.21±5.39	54.49%	
III (P _{II})	46.91± 5.99	35.23%	
IV (A+P _I)	33.97 ±4.38	26.69%	
V (H+P _I)	30.59 ± 4.52	54.49%	

*** Significant difference (p<0.001) between group I and II

Table 2: Comparison of serum ALT levels (U/L) (mean ± SD) between groups using Bonferroni't' test

Comparing groups	Compared groups	Level of significance
I (V)	II (P _I)	0.000***
	III (P _{II})	0.000***
	IV (A+P _I)	0.015*
	V (H+P _I)	0.266 ^{NS}
	III (P _{II})	0.000***
II (P _I)	IV (A+P _I)	0.000***
	V (H+P _I)	0.000***
	IV (A+P _I)	0.005**
III (P _{II})	V (H+P _I)	0.000***
	V (H+P _I)	1.000 ^{NS}

P<0.001= ***, P<0.01= **, P<0.05= *NS = no significant difference (p>0.05) between groups.

Hepatic GSH concentrations (mg/gm) (mean ± SD)

The mean GSH concentrations in liver in group I (V), group II (P_I), group III (P_{II}), group IV (A +P_I), group V (H+P_I) were 5.24±0.42 mg/gm, 2.20±0.56 mg/gm, 3.05±0.18 mg/gm, 3.08 ±0.27 mg/gm, 3.51±0.61 mg/gm respectively (Table 3). The mean ± SD of GSH in liver in paracetamol-treated group (group II) was significantly lower (p<0.001) when compared to those of control (group I). So, pre-treatment with aqueous extract and n-hexane extract of *N. Sativa* significantly increased the GSH concentration in the liver and by 40%, 59.54% respectively (Table 3). GSH concentrations among these groups were compared and significant difference was found [p<0.001] (Table 4).

There were significant difference (p<0.001) between group I and II, group I and IV, group I and V respectively. The significant difference (p<0.001) was also observed between group II and V, Significant difference (p<0.01) was observed between–group II and IV. No significant difference (P>0.05) between group IV and V.

Table 3: Hepatic GSH concentrations (mg/gm) (mean ± SD) in pre-treatment groups

Group (n = 6)	Hepatic GSH (mg/gm)	Increased (%)	p-value
I (V)	5.24±0.42		<0.001***
II (P _I)	2.20±0.56		
III (P _{II})	3.05±0.19	40%	
IV (A+P _I)	3.08±0.27	59.54%	
V (H+P _I)	3.51± 0.61		

Data were expressed as mean ± SD. The statistical significance of difference among the groups was evaluated by using one way ANOVA test between group I and II

Table 4: Comparison of hepatic GSH concentrations (mg/gm) between groups using Bonferroni't' test

Comparing groups	Compared groups	Level of significance
I (V)	II (P _I)	0.000***
	III (P _{II})	0.000***
	IV (A+P _I)	0.000***
	V (H + P _I)	0.000***
	II (P _I)	0.011*
II (P _I)	III (P _{II})	0.007**
	IV (A+P _I)	0.000***
	V (H + P _I)	0.000***
III (P _{II})	IV (A+P _I)	1.000 ^{NS}
	V (H + P _I)	0.930 ^{NS}
IV (A+P _I)	V (H+P _I)	1.000 ^{NS}

P<0.001= ***, P<0.01= **, P<0.05= *NS = no significant difference (p>0.05) between groups

Hepatic MDA concentrations (nmol/mg of protein) (mean ±SD)

The mean MDA concentrations in liver in group I (V), group II (P_I), group III (P_{II}), group IV (A +P_I), group V (H+P_I) (S+P_I) were 70.90 ±16.72, 208.95 ±14.30, 143.23 ± 8.19, 134.62 ± 7.80, 131.38 ± 6.02 (Table 5). MDA concentrations among these groups were compared and significant difference was found [p<0.001] (Table 4). The mean ± SD of MDA in the liver in paracetamol-treated group (group II) was significantly increased (p<0.001) when compared to those of control (group I). So, pre-treatment with aqueous extract and n-hexane extract of *N. sativa* significantly decreased the MDA concentration in liver and percentages of reduction were 35.57%, 37.12% (Table 5).

There were significant difference (p<0.001) between group I and II, group II and IV, group II and V. The study could not detect significant differences (P>0.05) between group IV and V.

Table 5: Hepatic MDA concentrations (nmol/mg of protein) (mean ± SD) in pre-treatment groups

Group (n = 6)	MDA (nmol/mg)	Reduction (%)	p-value
I (V)	70.89 ±16.72		<0.001***
II (P _I)	208.95 ± 14.30		
III (P _{II})	143.23 ± 8.19	35.57%	
IV (A+P _I)	134.62 ±7.80	37.12%	
V(H +P _I)	131.38 ± 6.08		

*** indicates significant difference (p<0.001) between group I and II

Table 6: Comparison of hepatic MDA concentrations (nmol/mg) between groups using Bonferroni't' test

Comparing groups	Compared groups	Level of significance
I(V)	II(P _I)	0.000***
	III(P _{II})	0.000***
	IV(A+P _I)	0.000***
	V(H+P _I)	0.000***
	II(P _I)	0.000***
II(P _I)	III(P _{II})	0.000***
	IV(A+P _I)	0.000***
	V(H+P _I)	0.000***
III(P _{II})	V(A+P _I)	1.000 ^{NS}
	V(H+P _I)	1.000 ^{NS}
IV(A+P _I)	V(H+P _I)	1.000 ^{NS}

P<0.001 = ***, NS = no significant difference (p>0.05) between groups.

DISCUSSION

The pre-treatment of paracetamol-treated group with the aqueous extract of *N. sativa* decreased the elevated levels of serum ALT and hepatic MDA and hepatic GSH concentrations were significantly higher. Another study [51] have reported that the treatment of CCl₄ exposed rats with *N. Sativa* was able to protect the liver from damage by decreased MDA and increased GSH (which indicates less lipid peroxidation and less oxidative stress) levels in their study. These findings are in accordance with the finding of the present study where the aqueous extract of *N. sativa* administration suggests less lipid peroxidation or less oxidative stress. Similar work was reported [47] that pretreatment with the aqueous suspension of *N. sativa* reduced the CCl₄-induced liver damage by decreasing elevated levels of serum enzymes (ALT, AST) and demonstrating almost normal hepatic architecture. Similar improvement also reported in hepatic damage [42] induced by CCl₄ in their experimental animals following *N. sativa* seed administration. One more research [52] claimed that 6% *N. sativa* seed diet was able to alleviate paracetamol-induced hepatotoxicity. The antioxidant effects of the *N. sativa* seed or its extracts were probably responsible for this alleviation.

The pretreatment of paracetamol-treated group with the n-hexane extract of *N. sativa* decreased the elevated levels of serum ALT and hepatic MDA while hepatic GSH concentrations were increased. Another work [41] observed that the essential oil of *N. sativa* possessed antioxidant activities and free radical scavenging activity. A number of studies [46, 53] have in a similar way, reported that TQ, (an ingredient of *N. sativa* oil) exhibits hepatoprotective activity (possibly by its antioxidant effect). It also reported that pretreatment of mice with TQ ameliorate the CCl₄ induced hepatotoxicity, that is further evidenced by a significant change in the elevated levels of serum ALT, AST, ALP and hepatic MDA concentration along with a significant rise in hepatic sulphydryl concentration [53]. To sum up, all these findings in the n-hexane

extract pre-treated group suggesting the TQ present in *N. sativa* oil was probably responsible for the better alleviation of the n-hexane extract pretreatment compared to the aqueous extract pretreatment.

CONCLUSION

This study concludes that n-hexane extract and the aqueous extract of *N. sativa* has a worthy hepatoprotective outcome. The protective effect was higher in the n-hexane extract of *N. sativa* pre-treated group than the aqueous extract pre-treated group. Well-designed prospective study is suggested to formulate more cheaper and indigenous treatment to ensure improved health care for common Bangladeshi people.

DECLAMER

This study has no funding obtained. This publication is supported by Universiti Sains Malaysia Incentive Grant No 2012/0712. Authors do not have any conflict of interest.

REFERENCES

- Hasan M, Khan AA. Development of gastroenterology in Bangladesh. *J Gastroenterol Hepatol*. 1997; 12: S13-S14.
- Satyanarayana U, Chakrapani U. *Biochemistry*. 3rd ed. Calcutta: Books and Allied (P) Ltd; 2008.
- Di-Luzio NR. A mechanism of the acute ethanol-induced fatty liver and the modification of liver injury by antioxidants. *Lab Invest*. 1966; 15: 50-63.
- Fernandez-Checa JC, Kaplowitz N. Hepatic mitochondrial glutathione: transport and role in disease and toxicity. *Toxicol App Pharmacol*. 2005; 204: 263-73.
- Harsha SN, Latha BV. In vitro antioxidant and in vitro anti-inflammatory activity of ruta graveolens methanol extract. *Asian J Pharm Clin Res*. 2011; 5(1): 32-35.
- Kageyama F, Kobayashi Y, Kawasaki T, Toyokuni S, Uchida K, Nakamura H. Successful interferon therapy reverses enhanced hepatic iron accumulation and lipid peroxidation in chronic hepatitis C. *Am J Gastroenterol*. 2000; 95: 1041-50.
- Wu J, Zern MA. Hepatic stellate cells: a target for the treatment of liver fibrosis. *J Gastroenterol* 2000; 35: 665-72.
- Troll W, Wiesner R. The role of oxygen radicals as a possible mechanism of tumor promotion. *Ann Rev Pharmacol Toxicol* 1985; 25: 509-28.
- Gerson RJ, Casini A, Gilfor D, Serroni A, Farber JL. Oxygen-mediated cell injury in the killing of cultured hepatocytes by acetaminophen. *Biochem Biophys Res Commun* 1985; 126: 1129-37.
- Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis, I. Role of drug metabolism. *J Pharmacol Exp Ther* 1973; 187:185-94.
- Prescott LF, Wright N, Roscoe P, Brown SS. Plasma paracetamol half-life and hepatic necrosis in patients with paracetamol overdose. *Lancet* 1971; 1: 519-22.
- Lee WM. Acetaminophen and the U.S. acute liver failure study group: lowering the risks of hepatic failure. *Hepatology* 2004; 40: 6-9.
- Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis, II. Role of covalent binding in vivo. *J Pharmacol Exp Ther* 1973; 187: 195-02.
- Brahams D. Paracetamol over dose: timing the antidote. *Lancet* 1989; 1: 567-68.
- Lake BH, Harris RA, Phillips JC, Gangoli SD. Studies on the effects of l-ascorbic acid on acetaminophen-induced hepatotoxicity. *Toxicol Appl Pharmacol* 1981; 60: 229-40.
- Krishnamoorthy P, Sangeetha M. Hepatoprotective effect of vitamin C on sodium nitrite-induced lipid peroxidation in albino rats. *Indian J Biochem Biophys* 2008; 45: 206-08.
- Chowdhury M. Studies of the effects of *Andrographis paniculata* (Kalmegh) and cimetidine on paracetamol-induced hepatotoxicity. University of Dhaka: M.Phil thesis; 1991. p. 1-76.
- Momtaz S. Study of the effect of Spirulina on serum GPT and Got levels of normal and paracetamol-induced hepatotoxic rats. University of Dhaka: M.Phil thesis; 1991.p. 1-67.
- Khan MAL. Study of effect of l-ascorbic acid and *Cajanus indicus* (Arhar) on paracetamol-induced hepatotoxicity in rats. University of Dhaka: M.Phil thesis; 1991. p. 1-59.
- Saha DR. Effects of *Phyllanthus niruri* on paracetamol-induced hepatotoxicity in rat. Bangabandhu Sheikh Mujib Medical University, Dhaka: M.Phil thesis; 1999. p. 1-74.
- Iqbal JM. Evaluation of hepatoprotective potential of four different extract of *Phyllanthus niruri* on paracetamol-induced hepatotoxicity in rat. Bangabandhu Sheikh Mujib Medical University, Dhaka: M.Phil thesis; 2001. p. 1-87.
- Chowdhury RT. Study of the hepatoprotective fraction (s) of *Phyllanthus niruri* Linn. (Bhuiamla) and their comparison with l-ascorbic acid in hepatotoxic rats. Bangabandhu Sheikh Mujib Medical University, Dhaka: M.Phil thesis; 2008. p. 1-82.
- Khadr ME, Mahdy KA, El-Shamy KA, Morsy FA, El-Zayat SR, Abd-Allah AA. Antioxidant activity and hepatoprotective potential of black seed, honey and silymarin on experimental liver injuries induced by CCl₄ in rats, *J Applied Sci* 2007; 7: 3909-17.
- Martinez-Calva I, Campos-Apaez A, Rosales-Vega E, Mourelle M. Vitamin E improve membrane lipid alterations induced by CCl₄ intoxication. *J Appl Toxicol* 1984; 4: 270-72.
- Fraga CG, Arias RF, Llesuy SF, Koch OR, Boveris A. Effect of vitamin E and selenium deficiency on rat liver chemiluminescence. *Biochem J* 1987; 242: 383-86.
- Nadkarni KM. *Indian Materia Medica*. 3rd ed. Bombay: Popular prakashan; 1976.
- Haq A, Lobo PI, Al-Tufail M, Rama NR, Al-Sedairy ST. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. *Int J Immunopharmacol*. 1999; 21: 283-95.
- Uddin N. Effects of *Nigella sativa* Linn (Kalajira) on serum glucose concentration in streptozotocin-induced diabetic rats. M.Phil thesis. Bangabandhu Sheikh Mujib Medical University: 2002. Bangabandhu Sheikh Mujib Medical University, Dhaka: M.Phil thesis; 2002. p. 1-72.
- Khanam M. Effects of n-hexane extract of *Nigella sativa* Linn. (kalajira) upon serum glucose concentration of streptozotocin-induced diabetic rats. Bangabandhu Sheikh Mujib Medical University, Dhaka: M.Phil thesis; 2007. p. 1-68.
- Ramadan MF, Morsed JT. Characterization of phospholipid composition of black cumin (*Nigella sativa*) seed oil. *Nahrung/ Food* 2002; 46: 240-44.
- Hanafy MS, Hatem ME. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *J Ethnopharmacol* 1991; 34: 275-78.
- Khan MAU, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH. The in vivo antifungal activity of the aqueous extract from *Nigella sativa* seeds. *Phytother Res* 2003; 17: 183-86.
- Akhtar MS, Riffat S. Field trial of saussurea lappa roots against nematodes and *Nigella sativa* seeds against cestodes in children. *J Pak Med Assoc* 1991; 41: 185-87.
- Abdel-Fattah AM, Matsumoto K, Watanabe H. Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice. *Eur J Pharmacol* 2000; 400: 89-97.
- Akhtar AH, Ahmed KO, Gilani SN. Antiulcer effect of aqueous extracts of *Nigella sativa* and pongamia pinnata in rats. *Fitoterapia* 1996; 67: 195-99.
- El-Tahir KE, Ashour MM, Al-Harbi MM. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: elucidation of the mechanism of action. *Gen Pharmacol*. 1993; 24: 1123-31.
- Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, Hassar M. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. *Therapie* 2000; 55: 379-82.
- Nanjmi A, Nasiruddin M, Khan RA, Haque SF. Indigenous herbal product *Nigella sativa* proved effective as an antihypertensive in metabolic syndrome. *Asian J Pharm Clin Res* 2013; 6(1): 61-64.

39. Gilani AH, Aziz N, Khurram IM, Chaudhary KS, Iqbal A. Bronchodilator, spasmolytic and calcium antagonist activities of *Nigella sativa* seeds (Kalonji): a traditional herbal product with multiple medicinal uses. *J Pak Med Assoc* 2001; 51: 115-20.
40. Daba MH, Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicol Lett* 1998; 95: 23-29.
41. Burits M, Bucar F. Antioxident activity of *Nigella sativa* essential oil. *Phytother Res* 2000; 14: 323-28.
42. Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of *Nigella sativa L* and *Urtica dioica L* on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World J Gastroenterol* 2005; 11: 6684-88.
43. Sultan MT, Butt MS, Anjum FM, Jamil A, Akhtar S, Nasir M. Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. *Pak J Bot* 2009; 41: 1321-30.
44. Houghton PJ, Zarka R, Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes a membrane lipid peroxidation. *Planta Med* 1995; 61: 33-36.
45. Nagi M, Mansour M. Protective effect of thymoquinone against doxorubicin-induced cardio toxicity in rat. A Possible mechanism of protection. *Pharm Res* 2000; 41: 283-89.
46. Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, Al-Bekairi AM. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. *Biochem Mol Biol Int* 1999; 47: 53-59.
47. Al-Ghamdi MS. Protective effect of *Nigella sativa* seeds against carbon tetrachloride-induced liver damage. *Am J Chin Med* 2003; 31: 721-28.
48. Schumann G, Klauke R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: Preliminary upper reference limits obtained in hospitalized subjects. *Clinica Chimica Acta* 2003; 327: 69-79.
49. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70-77.
50. Plaser ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malondialdehyde) in biochemical systems. *Anal Biochem* 1966; 16: 359-64.
51. Meral I, Yener Z, Kahraman T, Mert N. Effect of *Nigella sativa* on glucose concentration lipid peroxidation, antioxidant defense system and liver damage in experimentally-induced diabetic rabbits. *J Vet Med* 2001; 48: 593-99.
52. Elhabib EM, Homeida MMA, Adam SEI. Effect of combined paracetamol and *Cuminum cyminum* or *Nigella sativa* use in Wister rats. *J Pharmacol Toxicol* 2007; 2: 653-59.
53. Al-Kubaisy K, Al-Noaemi M.A Protective role of *Nigella sativa* oil against the harmful effect of CCl₄ on the liver cells. *The internet journal of nutrition and wellness* 2007; 3: 1-12.