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Research Article

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

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**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

Liver diseases are always dealt very seriously by the graduate physicians 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.

Ample researches have been carried out to obtain appropriate therapy for paracetamol-induced hepatotoxicity as well as different approaches of preventing and treating liver diseases. Antioxidant therapy used in different liver diseases is GSH [13], l-ascorbic acid [15, 16], *Andrographis paniculata (kalamegh*) [17], Spirulina [18], *Cajunus 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 flower, 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 $CCl_4$-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 $CCl_4$-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-Evans 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 $^0$ 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 16, using Bonferroni t-test and one way analysis of variance (ANOVA) followed post-hoc analysis. 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*** |
| III ($P_{II}$) | IV (A+$P_I$) | 0.005** |
| | V (H+ $P_I$) | 0.000*** |
| 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 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 ($P_I$) | III ($P_{II}$) | 0.011* |
| | IV (A+$P_I$) | 0.007** |
| | 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*** |
| | III($P_{II}$) | 0.000*** |
| | IV(A+$P_I$) | 0 .000*** |
| | V(H+$P_I$) | |
| 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-treated with the aqueous extract of *N. sativa* of paracetamol-treated group shows 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 $CCl_4$ exposed rats with *N. Saliva* 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 $CCl_4$-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 $CCl_4$ 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 pre-treatment 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 $CCl_4$ 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 sulfhydryl 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 pre-treatment compared to the aqueous extract pre-treatment.

## 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.

## DESCLAMER

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