HEPATOPROTECTIVE EFFECT OF AQUEOUS AND N-HEXANE EXTRACT OF *NIGELLA SATIVA* IN PARACETAMOL (ACETAMINOPHEN) INDUCED LIVER DISEASES OF RATS: A HISTOPATHOLOGICAL EVALUATION

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ABSTRACT

Acute over dose of paracetamol (acetaminophen) causes serious hepatic necrosis. So, this study was conducted to observe the hepatoprotective activity of aqueous and n-hexane extract of *Nigella sativa* in paracetamol induced hepatotoxicity in rats in Dhaka, Bangladesh from 2008 to 2010. Single dose of paracetamol was administered on day one and rats were sacrificed on day three. Liver damage was evaluated by hepatic histology. Aqueous and n-hexane extract of *Nigella sativa* was administered orally into two other rat groups through intra-gastric tube for 28 days in which paracetamol was administered orally on day 28 and were sacrificed on day 30. Liver of all rats were excised and processed for light microscopy with a view to histopathological evaluation. The histological examination of the liver tissues in vehicle treated group and paracetamol-control group of rats showed normal hepatic architecture, centrilobular necrosis, polymorph (neutrophils) infiltration and pyknosis of the hepatocytes respectively. The hepatic architecture of rats pre-treated with aqueous extract of *Nigella sativa* showed improvement of necrosis with very few pyknotic nuclei when compared to the paracetamol-control group. The hepatic architecture of rats pre-treated with aqueous extract as well as with the n-hexane extract of *Nigella sativa* did not show pyknotic nuclei and polymorph infiltration while apparently regenerating hepatocytes, visible under the microscope. Aqueous extract and n-hexane extract of *Nigella sativa* extended hepatoprotection by reducing oxidative stress in experimental liver damage in rats. Furthermore, the protection afforded by the n-hexane extract of *Nigella sativa* pre-treated group was superior to the aqueous extract of *Nigella sativa* pre-treated group.

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

INTRODUCTION

The liver is actively involved in different metabolic functions¹ including metabolism of toxins of natural origin and synthetic products and alsomedicine². Hepatic damage caused by chemicals or infectious agents are associated with the alteration of metabolic functions^{3,4,5} and is always related to the disturbance of hepatocyte biochemistry and generation of reactive oxygen species (ROS)^{6,7}. Paracetamol is one of the most commonly used analgesics and antipyretics⁸. It is considered as the safest for domestic analgesic in therapeutic dose. This drug can be very fatal when consumed at a very high-dose leading to centrilobular necrosis of liver⁹. In the UK, USA, Australia, Scandinavian and Mediterranean countries paracetamol overdose is considered as the major cause of acute liver failure^{10,11}. Paracetamol is extensively used as a model toxin to evaluate novel hepatoprotective agents^{12,13}. Dr. Gillete in 1970s first described paracetamol induced toxicity¹⁴. The injury was primarily due to the Cytochrome P-450 mediated oxidative metabolism of paracetamol to the highly reactive intermediate metabolite, N-acetyl-p-benzoquinone imine (NAPQI)^{10,11}. Although there are intracellular mechanisms to detoxify NAPQI, excessive production can deplete intrahepatic glutathione (GSH) stores and bind to intracellular proteins result in hepatocellular necrosis^{15,16}. Although the initial biochemical and metabolic

events that occur in the early stages of toxicity has been well described but the precise mechanism of hepatocyte death is still poorly understood. Necrosis is recognized as the mode of cell death and apoptosis has been ruled out^{17,18}. At present N-acetylcysteine and methionine are the drugs of choice for the management of paracetamol overdose^{19,21}. They provide protection primarily by replenishment of hepatic GSH stores and direct detoxification of NAPQI. Although these antidotes have been available for more than two decades, experiments are still being carried out in search of more effective, non-toxic, inexpensive agents²². Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in the Indian traditional system of medicine²³. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low costs²⁴. Moreover, herbal drugs also been used in folk medicine as a natural remedy with various objectives and several plants have been scientifically evaluated for hepatoprotective activity^{25,26}. *Nigella Sativa*, commonly known as ‘Kalonji’ in India and Pakistan and ‘Kalo Jeeray’ in Bangladesh, has been used traditionally for various ailments^{27,28}. *Nigella sativa* also

called as Black seed is a common spice that grows once a year belongs to the botanical family of Ranunculaceae²⁹. Commonly grows in Europe, Middle East and Western Asia³⁰. One of the active principles of *Nigella sativa* is Nigellone (dithymoquinone). Nigellone have an antioxidant activity that reduces the hepatotoxicity resulted from many chemical insults^{30,31}. It was reported as hepatoprotective agents against CCl₄-induced liver fibrosis³², also contain anti-cestode and anti-nematode action³³, prevent lipid peroxidation through the decrease in malondialdehyde (MDA), increase in antioxidants, prevent liver damage³⁴ and have anti-inflammatory activity^{28,35}. Many scientists reported that thymoquinone, the main ingredient of *Nigella sativa* oil exhibits hepatoprotective activity possibly by antioxidant and immune-stimulant effects³⁶. Another group of researchers noticed that *Nigella sativa* can prevent liver fibrosis and cirrhosis, suggestive of the protective role of *Nigella sativa* against hepatic fibrosis possibly through immune-modulation and antioxidant activities³⁷. Although in modern medicine, acute paracetamol toxicity has quite good treatment but the expense is still high. Henceforth, it puts an excessive burden both on common and financially deprived people in Bangladesh as well as in other third world countries. The current work was conducted with the intention to find cheaper herbal alternatives for the marginalised people and also assuring medical doctors' societal responsibility³⁸⁻⁴⁰. Thus, present study was designed to evaluate the role of the aqueous and the n-hexane extracts of *Nigella sativa* in improving liver histology in experimental hepatic injury in rats.

MATERIALS AND METHODS

The present study was carried out in the laboratory of the Department of Pharmacology, BSMMU, Dhaka, Bangladesh during the period from October 2008 to July 2010. Thirty adult rats of the Long-Evans Norwegian strain, aged between 3-4 months (weighing between 160-210 g) were selected for the study. 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: They received a single dose of vehicle for paracetamol (propylene glycol) at a dose of 1 mL intra-peritoneal (i.p.) on the day 1 and were sacrificed on the 3rd day (48 h 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: They 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 h 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 *Nigella 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 28 and were sacrificed on 30th day.

Group-V (H+P_I) or (N-Hexane Extract + Paracetamol)

Treated Group: Received n-hexane extract of *Nigella 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.

Paracetamol induced hepatotoxicity was assessed by doing hepatic histopathology.

Collected Liver from sacrificed Rat of all groups was prepared for histological examination by following steps⁴¹.

Fixation of Tissue: Liver tissue was taken and placed in the fixing fluid (10 % formalin) about 15-20 times the volume of the tissue. The tissue was kept in the fixative overnight.

Dehydration: Dehydration was carried out by keeping the tissue successively in ascending concentrations of ethanol (in 70 % ethanol 1 change for 1 h, in 80 % ethanol 1 change for 1 h, in 95 % ethanol 2 change for 1 h each and in 100 % ethanol 3 changes for 1 h each).

Clearing or Dealcobolisation: After dehydration, the liver sections were cleared in xylene (2 changes for 1 h each).

Paraffin Infiltration: There after tissue was kept in a paraffin bath and treated with melted paraffin at 70°C (2 change for 1 h each).

Paraffin Embedding: The tissue infiltrated with melted paraffin were finally embedded carefully in the proper plane in melted paraffin metallic moulds and allowed to harden at room temperature to form paraffin blocks. After hardening of paraffin, the mould were removed and the solidified bar was then cut into pieces to get tissue blocks. The blocks were kept in an ice chamber for sometimes before section cutting.

Section Cutting: Tissue in paraffin blocks was sectioned at 5 µm thickness with a rotary microtome.

Preliminary Mount: Sections were placed in a hot water bath at 45°C and allowed to float. The better section were selected and taken on glass slides previously smeared with adhesive (mixture of albumin and distilled water at a ratio of 50: 50) and then dried.

Staining: The dry slides were stained with hematoxylin and Eosin (H and E) stain.

Photography: Photographs were taken from the representative sections of each group using a camera fitted with Olympus (B 205) microscope.

OBSERVATIONS AND RESULTS

Histology of Liver Slides

Using the scoring method or counting the absolute number of necrotic or healthy cells in unit area from the statistically adequate number of samples and subsequently comparing the findings with the control group is the best option for the observation and estimation of toxicity of an organ or viscera in light microscopy. However, current study could not use any of the procedure mentioned above due to certain limitations. So, hepatic architecture and its gradual change were described by using photo micrograph. Observations of the rat hepatic architecture of the control and treatment groups of the present study were carried out as follows:

Vehicle Treated Group: Microscopic Examination

Representative sections of liver from the vehicle treated group (Group-I) suggested normal hepatic architecture. Hepatic lobules were normal and hepatocytes were arranged in regular fashion with well-defined nuclei situated inside the cell. [Figure 1 and 2]

Figure 1: Photomicrograph Showing Hepatic Architecture of Control Rat Group (H and E stain, x 200)

Figure 2: Photomicrograph Showing Hepatic Architecture of Control Rat Group (H and E stain, x 400)

Paracetamol-control group (sacrificed on 3rd day): Sections of livers from paracetamol-control group (Group-II) sacrificed on the 3rd day following paracetamol administration suggested centrilobular necrosis, polymorph (neutrophils) infiltration and pyknosis of the hepatocytes. [Figure 3]

Figure 3: Photomicrograph of Liver of Paracetamol Control Group Showing Centrilobular Necrosis and Pyknotic Nuclei (H and E stain, x 100)

Paracetamol-treated group (sacrificed on 30th day): Section of livers from paracetamol-treated group (Group-III) sacrificed on the 30th day following paracetamol administration showed regeneration of hepatocytes. Few pyknotic nuclei were observed (not shown in figure).

Aqueous extract treated group: The hepatic architecture of rats pre-treated with aqueous extract of *Nigella sativa* (Group-IV) showed improvement of necrosis with very few pyknotic nuclei when compared with those from the paracetamol-control group (Group-II). [Figure 4]

Figure 4: Photomicrograph of Liver Tissue of the Aqueous Extract Pre-treated Group Showing Improvement of Necrosis but Presence of Few Pyknotic Nuclei (H and E stain, x 100)

N-hexane extract treated group: The hepatic architecture of rats pre-treated with n-hexane extract of *Nigella sativa* (Group-V) showed no polymorph infiltration. Repopulation or emergences of the hepatocytes were apparent when compared with those from the paracetamol-control group (Group-II). [Figure 5]

Figure 5: Photomicrograph of Liver Tissue of the N-hexane Extract Pre-treated Group Showing Improvement of Necrosis and Repopulation of the Hepatocytes (H and E stain, x 100)

DISCUSSION

The present study suggests that the aqueous and n-hexane extract of *Nigella sativa* might protect the liver against paracetamol induced toxicity. The vehicle treated group shows normal hepatic architecture. Hepatocytes were arranged in regular fashion with well-defined nuclei. Paracetamol-treated group exhibits centrilobular necrosis, polymorph (neutrophils) infiltration and pyknosis of the hepatocytes.

The hepatic architecture showed improvement of necrosis with very few pyknotic nuclei in the pre-treatment of paracetamol-treated group with the aqueous extract of *Nigella sativa* when compared with those from the paracetamol-control group. These observations of the present study coincide with previous similar study³⁵ with the aqueous suspension of *Nigella sativa* reduced the CCl₄-induced liver damage demonstrating almost normal hepatic architecture. Paracetamol in large doses produces liver necrosis after undergoing bio-activation to a toxic electrophile, NAPQI by cytochrome P-450 monooxygenase. Another study also reported similar improvement in hepatic damage induced by CCl₄ in their experimental animals following *Nigella sativa* seed administration⁴². Researchers claimed that 6 % *Nigella sativa* seed diet was able to alleviate paracetamol-induced hepatotoxicity⁴³. The antioxidant effects of the *Nigella sativa* seed or its extracts were probably responsible for this alleviation. The histological observation of the pre-treated

paracetamol group with the n-hexane extract of *Nigella sativa* group did not show pyknotic nuclei while apparently regenerating hepatocytes were visible under the light microscope. The hepatic architecture showed no polymorph infiltration when compared with those from the paracetamol-control group. This finding is also in line with a very recent study⁴⁴ that demonstrated the extensive hepatic necrosis with mononuclear lymphoplasmocytic inflammatory infiltration in paracetamol treated animals which significantly decreased in animal's pre-treated with *Nigella sativa* extract. Multiple researchers observed that the essential oil of *Nigella sativa* possessed antioxidant activities and free radical scavenging activity^{45,46}. Multiple studies accounted the similar findings for thymoquinone and dithymoquinone (Nigellone) (the active ingredients of *Nigella sativa* oil) which exhibits hepatoprotective activity by its antioxidant effect^{36,47}. The results of the n-hexane extract pre-treated group of the present study thus supports the observations of the above mentioned studies and also suggesting that the Nigellone present in *Nigella sativa* oil might be responsible for the better alleviation of the n-hexane extract pre-treatment compared to the aqueous extract pre-treatment.

CONCLUSION

Further study is warranted to explore the individual effects of active components of *Nigella Sativa* in hepatocellular damage resulted from other drugs where oxidative damage or lymphocytic infiltration is evident. A limitation of this study might be included as using only two doses of extract for demonstrating a dose-response relationship, where three or more doses are usually required. *Nigella Sativa* is efficacious in protecting against paracetamol induced liver injury possibly via preventing depletion of GSH and cellular injury by Paracetamol.

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