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Protective Effects of Ethanolic Extract of *Nigella sativa* Seed in Paracetamol Induced Acute Hepatotoxicity *In vivo*

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Abstract: Paracetamol overdose causes serious liver necrosis. Hepatoprotective activity of ethanolic extract of *Nigella sativa* in Paracetamol induced acute hepatotoxicity was investigated in rats. Fasted male Wistar rats were orally treated with *Nigella sativa* extract in graded doses for 5 days followed by *Nigella sativa* extract and paracetamol 3 g kg^{-1} on 6 and 7th day. Circulatory liver markers and reduced glutathione (GSH) levels were estimated and histopathological study of liver performed. Paracetamol caused a significant increase in serum alkaline phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and total Bilirubin and a significant decrease in GSH compared to control. *Nigella sativa* pretreatment significantly prevented the increase in liver enzymes and total bilirubin and decrease in GSH level as compared to paracetamol group. Liver histopathology showed marked reduction in sinusoidal dilatation, midzonal necrosis, portal triaditis and occasional apoptosis in *Nigella sativa* extract treated groups as compared to group receiving only paracetamol. *Nigella sativa* extract possesses hepatoprotective action against paracetamol induced acute hepatoxicity. Further research is needed to advocate its prophylactic use for drug induced hepatotoxicity.

Key words: Antioxidant, black cumin, drug induced hepatotoxicity, glutathione, hepatocellular injury, hepatoprotective

INTRODUCTION

Traditional NSAIDs like paracetamol (PCM) are one of the most commonly used drugs throughout the world (Al-Turki *et al*., 2010). PCM, safe at therapeutic doses, can lead to centrilobular hepatic necrosis in overdose which can be fatal (Najafzadeh *et al*., 2011). The US Food and Drug Administration (FDA) asked drug manufacturers to limit the strength of PCM in prescription drug products and a Boxed Warning was added to the label of all prescription products containing PCM highlighting the potential for severe liver injury (FDA, 2011).

In absence of reliable hepatoprotective agents there is an urgent need for searching an agent for prevention of PCM induced hepatotoxicity. Herbal drugs play a role in folk medicine due to their hepatoprotective activity and several plants have been scientifically evaluated for hepatoprotective activity (Iweala *et al*., 2011; Zamani-Moghaddam *et al*., 2012). Among the promising medicinal plants, *Nigella sativa*, commonly known as Kalonji in India and Pakistan, has been used traditionally for various ailments (Qidwai *et al*., 2009). It is one of the important medicines of Tibbe Nabawi, i.e., Prophetic Medicine (Ahmad *et al*., 2009) in which it is considered as a healing for all diseases except death. Al-Bukhari (1976a, b) Nigella sativa has been thoroughly studied scientifically and has been shown to have analgesic (Abdel-Fattah *et al*., 2000), reproductive efficiency improving (Bashandy, 2007), nephroprotective (Abdelaziz and Kandeel, 2011) diuretic and antihypertensive (Zaoui *et al*., 2000), bronchodilator and calcium antagonist (Gilani *et al*., 2001), hepatoprotective (Kushwah *et al*., 2012), anthelmintic (Akhtar and Riffat, 1991), antimicrobial (Abu-Al-Basal, 2011; Salman *et al*., 2009) and anticancer activities (Worthen *et al*., 1998).

Since, oxidative stress plays an important role in Paracetamol induced hepatotoxicity (Jaeschke *et al*., 2003; James *et al*., 2003) and *Nigella sativa* possesses strong anti-oxidative activity (Farrag *et al*., 2007), we hypothesized that *Nigella sativa* Extract (NSE) could protect against Paracetamol induced hepatotoxicity by improving Glutathione levels. This study has investigated whether or not pretreatment of oral NSE ameliorates Paracetamol induced acute hepatotoxicity.

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MATERIALS AND METHODS

Plant material: *Nigella sativa* (Black Cumin) seeds were purchased from the local market and authenticated by a botanist at National Botanical Research Institute, Lucknow. A voucher specimen of the seeds was kept in the museum of Department of Pharmacology, Era's Lucknow Medical College, Lucknow. Seeds were grounded to powder with the help of mortar and pestle and 150 mg of powder was soaked in 250 mL of 99% ethanol in a closed container at room temperature for 7 days with periodic stirring with a sterile glass rod. After 7 days it was filtered with Wattman's filter paper No.1 and extract was concentrated by rotary vacuum evaporator and kept in a vacuum desiccator for complete removal of solvent. The extract so obtained was stored at 4°C till further use.

Animals: Male Wistar rats, weighing 225-250 g, were purchased from Central Drug And Research Institute (CDRI), Lucknow, India, housed in a temperature controlled room (21±1°C) with a 12 h light-12 h dark cycle and allowed free access to a standard rat chow and filtered tap water for at least 5 days for acclimatization. Solid food but not the water was removed 12 h prior to an experiment. The study received the approval of the Institutional Animal ethics Committee of Era's Lucknow medical college and hospital. Animals were cared for in accordance with the internationally accepted principles for laboratory animal use and care and the procedures followed were in accordance with the standards set forth in the Guide for the Care and Use of Laboratory Animals (published by the National Academy of Science, National Academy Press and Washington, D.C).

Treatment and samples: Rats were randomly divided into groups of 8 each and treated as follows:

- Control group received normal saline for 7 days
- PCM group received normal saline for 7 days followed by PCM 3 g kg^{-1} on day 6 and 7
- NSE groups received NSE 250 or 500 mg kg^{-1} for 7 days followed by PCM 3 g kg^{-1} on day 6 and 7
- NSE alone group received NSE 500 mg kg^{-1} for 7 days

Administration of all doses was done with the help of oral feeding tube. At the end of the study (on day 8), the rats were sacrificed and dissected. Blood and liver tissue samples were taken for biochemical and histopathological investigations.

Biochemical study: Serum Alkaline phosphatase (ALP), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) and total bilirubin was determined using semi auto analyzer.

Biochemical assay: Serum was de-protonized by adding 1.0 mL of 10% TCA and centrifuged at 6000×g for 5 min. A 0.5 mL aliquot from clear supernatant was mixed with 0.5 mL double distilled water. Thereafter, 2 mL of 0.4 M tris buffer and 0.1 mL DTNB were added to it with proper stirring. The absorbance was read at 412 nm within 5 min of the addition of GSH (200-1600 μmoles). GSH reduced in the sample were calculated using the standard curve and the results were expressed as μmoles g^{-1} tissue.

Histopathological study: Liver tissues collected from the distal portion of the left lateral lobe of each rats were stored in 10% formalin solution for 48 h or until processing. For block preparation, livers were processed using a graded ethanol series and embedded in paraffin. Four micrometer paraffin sections (5 μm) were cut and stained with haematoxylin and eosin for light microscopic examination.

Statistical analysis: The different groups were compared using ANOVA followed by *post hoc* dunnett's test. All tests were performed using SPSS (17.0 versions). The $p<0.05$ was considered significant.

RESULTS

Administration of PCM caused a significant increase in ALP, SGOT, SGPT and total bilirubin and a significant decrease in GSH as compared to control. The rise in liver enzymes due to paracetamol was highest in case of SGOT (144.6 IU L^{-1} serum, 95% CI-106.25-182.94) and SGPT (170.85 IU L^{-1} serum, 95% CI-123.3-218.3) Pre-treatment with NSE significantly prevented the increase in liver enzymes and total bilirubin and the decrease in GSH level as compared to PCM group ($p>0.05$ as compared to control in all parameters) in a dose dependent manner. Most significant difference from paracetamol group was seen in SGOT (49.47 IU L^{-1} serum, 95% CI-30.77-68.92), SGPT (49.75 IU L^{-1} serum, 95% CI-31.50-68) and GSH (31.51 μmol g^{-1} tissue, 95% CI-27.13-35.88) levels in group pretreated with 500 mg kg^{-1} NSE.

All the parameters were found to be not significantly different from control group in NSE pre-treated groups ($p>0.05$ as compared to control in all parameters). Level of liver enzymes, total bilirubin and GSH was not significantly different from control group in animals who received NSE alone (Table 1).

Table 1: Effect of *Nigella sativa* extract on liver enzymes level in Paracetamol induced acute hepatotoxicity in rats

Groups	Dose (mg kg^{-1})	ALP (IU L^{-1} serum)	SGOT (IU L^{-1} serum)	SGPT (IU L^{-1} serum)	Tot.BIL (μmol g^{-1} tissue)	GSH (μmol g^{-1} tissue)
Control	-	70.88 (56.98-84.77)$^{\#}$	44.34(30.87-57.82)$^{\#\#}$	48.71(28.48-68.94)$^{\#\#}$	0.18(0.309-0.344)$^{\#}$	33.01(28.81-37.20)$^{\#\#}$
PCM toxicity	3000	202.63 (137.84-267.41)*	144.6(106.25-182.94)**	170.85(123.3-218.3)**	2.04(1.11-2.96)*	19.03(15.02-23.04)**
NSE1+PCM	250+3000	77.38 (52.84-101.91)$^{\#}$	55.35(31.52-79.18)$^{\#\#}$	56.99(39.81-74.17)$^{\#\#}$	0.26(0.714-0.448)$^{\#}$	28.32(24.43-32.21)$^{\#}$
NSE2+PCM	500+3000	71.63 (49.14-94.11)$^{\#}$	49.47(30.77-68.92)$^{\#\#}$	49.75(31.50-68)$^{\#\#}$	0.20(0.416-0.348)$^{\#}$	31.51(27.13-35.88)$^{\#\#}$
NSE2	500	72.18 (65.12-76.31)$^{\#}$	42.11(37.01-44.56)$^{\#\#}$	50.23(46.21-52.17)$^{\#\#}$	0.18(0.318-0.234)$^{\#}$	34.59(31.12-36.27)$^{\#\#}$

N = 8, Values are expressed as mean (C.I.); NSE 1-*N. sativa* extract (250 mg kg^{-1}); NSE 2-*N. sativa* extract (500 mg kg^{-1}); PCM-Paracetamol, *significantly different from control, *p<0.05, **p<0.01, ***p<0.001, $^{\#}$significantly different from Paracetamol treatment group, $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001

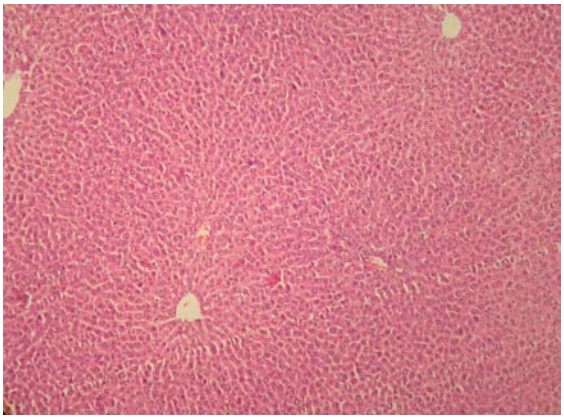

Fig. 1: Photomicrograph from control rat (H and E, 100x), showing normal liver parenchyma and normal architecture of hepatocytes and vasculature, distinct hepatic cords and central vain

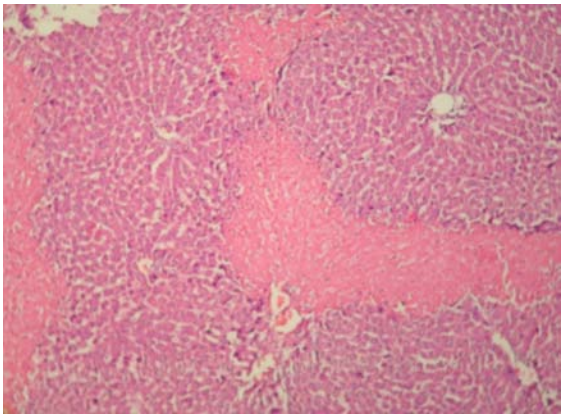

Fig. 2: Photomicrograph from paracetamol treated rats showing extensive necrosis, fraying of cell margins and portal triditis with mononuclear lymphoplasmocytic inflammatory infiltration

Histopathologic changes: In control group animals, normal liver parenchyma and normal architecture of hepatocytes and vasculature, distinct hepatic cords and central vain was seen (Fig. 1). However, animals treated with PCM (3 g kg^{-1} p.o.) showed extensive necrosis, fraying of cell margins, portal triditis with mononuclear lymphoplasmocytic inflammatory infiltration (Fig. 2, 3). This effect was significantly decreased in animals pretreated with NSE in both doses (Fig. 4).

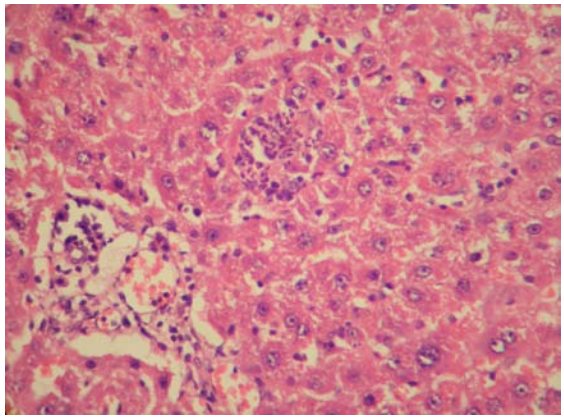

Fig. 3: Photomicrograph from paracetamol treated rats showing Strip focal necrosis, feathery degeneration (in the mid zone) involving several hepatocyte, necrosis of several hepatocyte extended in band like fashion in the mid zonal area and occasional apoptotic bodies

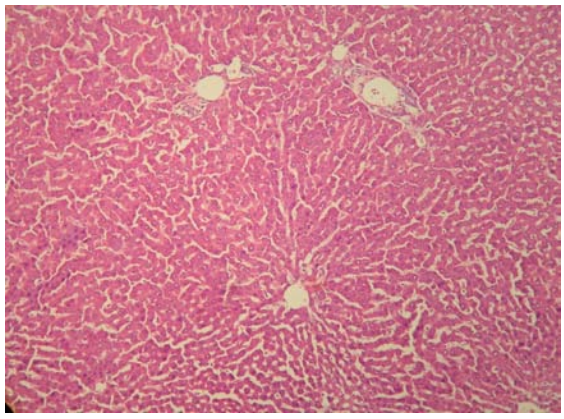

Fig. 4: Liver section from rat orally treated NSE 500 mg kg^{-1}, showing protective effects of plant extract

NSE pretreatment caused resumption of normal liver architecture with decrease in lymphocytic infiltration.

These results show that *N. sativa* protects against paracetamol induced hepatotoxicity possibly by preventing reduction of glutathione, levels and lymphocytic infiltration and may lead to further research in prevention of drug induced hepatotoxicity.

DISCUSSION

Our results suggest that oral administration of NSE might protect the liver against PCM induced acute toxicity. PCM induced hepatotoxicity manifested biochemically by significant elevation of serum levels of liver enzymes such as ALP, SGOT and SGPT coupled with decrease in GSH level. Marked destruction of hepatic structure further evidenced the liver cell damage. Pretreatment with NSE (250, 500 mg kg^{-1} p.o.) significantly ameliorated the elevated level of these enzymes which were comparable to control group.

PCM is readily detoxified by hepatic phase 2 drug metabolizing systems in the liver by glucuronidation and sulfation (Henderson *et al*., 2000), with a small portion undergoing a cytochrome P-450-mediated conversion to a highly reactive electrophilic arylating intermediate N-acetyl-p-benzoquinoneimine (NAPQI) (Dahlin *et al*., 1984). NAPQI is detoxified principally by conjugation with reduced glutathione (GSH) spontaneously or via., glutathione transferase (GST)-mediated reactions to the 3-glutathione-S-yl-PCM conjugate (Henderson *et al*., 2000).

If there is an overdose of PCM, the high levels of NAPQI produced eventually exhaust GSH, UDP-glucuronic acid and inorganic sulfate, inhibit GSH synthesis (Hazelton *et al*., 1986; Lauterburg and Mitchell, 1982) and decrease cytosolic GST activity (Yonamine *et al*., 1996). NAPQI also causes hepatocellular damage and centrilobular hepatic necrosis (James *et al*., 2003). Since, GSH plays a key role in the detoxification of reactive toxic metabolites of PCM, liver necrosis begins when GSH stores are markedly depleted (Mitchell *et al*., 1973). As expected PCM treatment caused remarkable depletion of liver GSH levels in our study. These results agree with other reports pertaining to PCM-induced GSH depletion.

Nigella sativa has antioxidant properties and can elevate reduced glutathione level in oxidative stress (Abdel-Sater, 2009). Present data shows that *Nigella sativa* improves GSH level which is in conformity to results of previous studies (Abdel-Sater, 2009; Neveen and Iman, 2010). In order to find weather NSE alone is increasing GSH levels or pretreatment with NSE blocking the decrease in GSH levels, we administered NSE alone in one group. No significant difference between this group and control group shows that pretreatment with NSE blocks the depletion of GSH by Paracetamol toxicity. Another mechanism of PCM induced hepatotoxicity is the formation of reactive oxygen species such as superoxide anion, hydrogen peroxide, hydroxyl radical, reactive nitrogen species such as nitric oxide and peroxynitrite and peroxidation reaction products (James *et al*., 2003; Reid *et al*., 2005; Bessems and Vermeulen, 2011). Burits and Bucar (2000) tested the essential oil of *Nigella sativa* L., for antioxidant activity and showed that thymoquinone, active constituent of *Nigella sativa* and the components carvacrol, t-anethole and 4-terpineol demonstrated high free radical scavenging property.

Several studies have reported hepatoprotective activity of *Nigella sativa* and its active constituent Thymoquinone against CCL_4 induced liver damage (Oyagbemi and Odetola, 2010). In the present study we induced liver cell damage by PCM, a common over the counter drug. Further research is required before *Nigella sativa* can be used in humans.

CONCLUSION

Further research is warranted to explore individual effects of its active components and effect of *N. sativa* in hepatic damage caused by other drugs where oxidative damage or lymphocytic infiltration is involved.

A limitation of the study is that the only two doses of extract have been used as for establishing dose-response relationship three or more doses are required. Furthermore in the present study NSE was supplemented only before PCM administration and another study is required to find out if the plant will be curative when given after PCM-induced hepatotoxicity.

Nigella sativa is effective in protecting against PCM induced hepatotoxicity possibly via protecting depletion of GSH and cellular injury by Paracetamol.

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