Protective effect of *Nigella sativa* against paracetamol induced hepatic and renal damages

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INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs. They are prescribed commonly as an analgesic, antipyretic, anti-inflammatory, and antiplatelet agent. They have significant adverse effect on liver and kidney besides damaging stomach. Their effect on liver and kidney are of serious concern. Hence, we have decided to study the preventive effect of *Nigella sativa* against paracetamol induced hepatic and renal damages.

ABSTRACT

**Background:** Non-steroidal anti-inflammatory drugs are very commonly used as an analgesic, antipyretic, anti-inflammatory, and antiplatelet agent. They have significant adverse effect on liver and kidney besides damaging stomach. Their effect on liver and kidney are of serious concern. Hence, we have decided to study the preventive effect of *Nigella sativa* against paracetamol induced hepatic and renal damages.

**Methods:** Ethanolic and aqueous extracts of *N. sativa* were prepared with the help of Soxhlet’s apparatus. Totally, 36 wistar albino rats (150-200 g) of either sex were divided into six groups of six each. Group I was administered with distilled water, Group II-VI were treated with paracetamol 750 mg/kg i.p. Group III-VI were test groups also treated with *N. sativa* aqueous extract (200 and 400 mg/kg p.o) and ethanolic extract (200 and 400 mg/kg p.o), respectively. The treatment was given daily for 7 days and on 8th all the rats were sacrificed and the blood was analyzed for hepatic and renal function tests and tissue was preserved for histopathological examination.

**Results:** Paracetamol administration caused a marked hepatic and renal damage, which is evidenced by the increase in liver and renal function test parameters in the negative control group. *N.sativa* extracts prevented this damage. The protective was seen maximum in ethanolic extract followed by the aqueous extract in dose-dependent manner.

**Conclusion:** Ethanolic extract showed significant protection against paracetamol-induced and renal damage.

**Keywords:** *Nigella sativa*, Paracetamol, Hepatoprotection, Nephroprotection
due to the inhibition of renal prostaglandin (PG) synthesis. The risk of ARF is fourfold in NSAID users as compared to general population. Current users of NSAIDs had a relative risk for ARF of 3.2 (95% CI: 1.8-5.8), and the risk declined after treatment was discontinued. Increased risk was present with both short- and long-term therapy and was slightly greater among users of high doses.

Acetaminophen (Paracetamol; N-acetyl-p-aminophenol) is an active metabolite of phenacetin. It has analgesic and antipyretic effects but has only an weak anti-inflammatory effect. It is a weak non-selective cyclooxygenase inhibitor which has an excellent oral bioavailability. It is metabolized by hepatic microsomal enzymes and excreted in urine as glucuronide, sulfated, cysteine, hydroxylated conjugates, which are inactive. A small portion undergoes CYP-450 mediated N-hydroxylation to form N-acetyl-p-benzoquinoneimine (NAPQI), which is highly reactive intermediate. Acetaminophen is well-tolerated at therapeutic doses but at overdose and chronic use may cause hepatic and renal damage.

*Nigella sativa* commonly known as “Kalonji/Black Cumin” belongs to family ranunculaceae is an annual herb. Seeds are small dicotyledous, trigonus, angular, regulose-trabecular, and black externally with white inside. The odor of seeds is aromatic with a bitter taste. It is reported to have various pharmacological activities such as antidiabetic, analgesic and anti-inflammatory, antioxidant, antitumor, wound healing.

Till date no any drug is available to prevent the hepatic and renal damage caused by NSAIDs. Newer drugs, which have minimal adverse effects with maximum benefit, are continuously searched worldwide to prevent the hepatic and renal damage induced by NSAIDs. Hence, in this regard study was planned to evaluate the protective effect of *N. sativa* against paracetamol induced hepatic and renal damage.

**METHODS**

**Institutional Animal Ethical Committee (IAEC) approval**

The study protocol was approved by the IAEC, Jawaharlal Nehru Medical College, Aligarh Muslim University (A.M.U); Aligarh, Uttar Pradesh on 13.04.2012 (Registration no. 401/CPCSEA dated 08.05.2012). All animal experiments were carried out as per the rules and regulations of IAEC and CPCSEA under the “Guidelines for Care and Use of Animals in Scientific Research” (INSA 1992 and 2000).

**Plant material and extraction**

The seeds of *N. sativa* were purchased from pharmacy store of Ajmal Khan Tibbiya College, A.M.U; Aligarh, Uttar Pradesh and authenticated by Dr. (Mrs) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum (RHMD) of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. The sample specimen was deposited at RHMD, NISCAIR; New Delhi bearing ref. no. NISCAIR/RHMD/Consult/2013/2316/96. Seeds were powdered with the help of a grinder. For aqueous extract, 100 g of seed powder was extracted separately with 300 ml of distilled water for 72 hrs by using Soxhlet’s apparatus. The extract so obtained was filtered. Then the filtrate was collected in Petri dish and evaporated till dryness in an incubator. Similarly, for ethanolic extract finely powdered, 100 g of powdered seeds extracted separately in 300 ml of absolute ethanol for 72 hrs using Soxhlet’s apparatus. The extract so obtained was collected in Petri dish and evaporated till dryness at 35-40°C in an incubator. The yields of aqueous and ethanolic extracts were found to be 12.62% and 48.50%, respectively.

**Chemicals and drugs**

All chemicals and drugs used were of analytical grade.

Chemicals: Formaldehyde (Central Drug House Pvt. Ltd.).

Drugs: Injection Paracetamol (Tablets India Ltd.).

**Kits**

Bilirubin and Blood urea kits from M/S Excel Diagnostics Pvt. Ltd., India. Aspartate transaminase (AST), alanine transaminase (ALT), and serum creatinine kits from Span Diagnostics Ltd., India.

**Animal**

Totally, 36 wistar albino rats (150-200 g) of either sex procured from Central Animal House, Jawaharlal Nehru Medical College; A.M.U; Aligarh, Uttar Pradesh and were housed under standard condition (temperature 27±2°C, humidity 30-70% and 12 hrs light/dark cycles), and fed with standard pellet diet and water *ad libitum*. They were acclimatized to the laboratory condition for 1-week prior to experimental study.

**Experimental design**

All the rats were randomly divided into six groups each containing six. Hepatic and renal damage was induced by paracetamol 750 mg/kg i.p daily for 7 days. Test doses were selected on the basis of previous toxicity studies on *N. sativa*. A sub-acute toxicity study in Malaysia found 1 g/kg/day dose of *N. sativa* safe in rats for 28 days. Similarly, in Iran another acute and sub-acute toxicity study revealed dose up to 6 g/kg/day for 14 days safe in mice. The nephroprotective and hepatoprotective effects of *N. sativa* extracts were documented in a range between 50 mg/kg and 500 mg/kg; however, the toxicity inducing agents were different.
Thus, based on the aforementioned evidence we have chosen the two test doses (200 and 400 mg/kg) for evaluation of hepatoprotective and nephroprotective effect of *N. sativa*.

Different groups were treated as below:
- Group I (Normal control): distilled water 0.5 ml daily p.o for 7 days.
- Group II (Negative control): distilled water 0.5 ml daily p.o + paracetamol 750 mg/kg i.p daily for 7 days.
- Group III (N. sativa aqueous extract [NSAE] 200): NSAE (200 mg/kg/day p.o) + paracetamol (750 mg/kg/day i.p) × 7 days
- Group IV (NSAE 400): NSAE (400 mg/kg/day p.o) + paracetamol (750 mg/kg/day i.p) × 7 days
- Group V (N. sativa ethanolic extract [NSEE] 200): NSEE (200 mg/kg/day p.o) + paracetamol (750 mg/kg/day i.p) × 7 days
- Group VI (NSEE 400): NSEE (400 mg/kg/day p.o) + paracetamol (750 mg/kg/day i.p) × 7 days

On 8th day, all the rats were sacrificed under sodium pentobarbitone (50 mg/kg i.p) anesthesia, and dissection was done.

**Blood collection and biochemical analysis**

After sacrifice blood had been collected in vacutainer and centrifuge under 5000 rpm for 10 mins then serum was separated and analyzed for:
- Total serum bilirubin - Modified Jendrassik and Grof’s method
- Indirect bilirubin - Modified Jendrassik and Grof’s method
- Serum AST - 2,4 dinitrophenyl hydrazine (DNPH)/ Reitman and Frenkel method
- Serum ALT - DNPH/Reitman and Frenkel method
- Serum creatinine - Alkaline Picrate method
- Blood urea - Berthelot method

Finally, the tissue was preserved in 10% Formalin for histological examination.

**Histological examination**

The tissue was processed and 7 μm thick paraffin sections were cut, slides were stained with hematoxyline and eosin and the histological changes were observed under light microscope (Olympus BX40, Japan), and relevant findings were recorded on ×400 magnification.

**Statistical analysis**

All the values were presented as a mean±standard error of mean (SEM). The groups were compared by one-way analysis of variance followed by post-hoc “Dunnett’s multiple comparison test” to analyze statistical significance. p<0.05 was considered to be significant.

**RESULTS**

After 7 days of treatments, all the groups were sacrificed on 8th day. Blood was collected and analyzed for different parameters of hepatic and renal functions while the tissues (liver and kidney) were taken out for histological analysis.

**Biochemical analysis**

On hepatic function evaluation (serum total bilirubin, indirect bilirubin, AST, and ALT) it was found that administration of paracetamol causes massive damage to liver in Group II rats, evidenced by highly significant (p<0.001) increase in serum bilirubin (total and indirect), and transaminases levels (AST and ALT) as compared to Group I (normal control rats). The *N. sativa* (extracts and oil) treated rats try to prevent this damage. Among the test groups, it was found that Group VI showed highest preventive effect against the rise of above parameters as compared to Group II (p<0.001). This was followed by Group V as compared to Group II (p<0.01). Although, the aqueous extract treated groups also showed some preventing effect on the hepatic damage but only the Group IV showed a significant decrement as compared to Group II (p<0.05) (Table 1).

On kidney functions, evaluation the parameters used were serum creatinine and blood urea levels whose rise is the indication for renal damage. It was found that paracetamol causes a significant increase in these parameters in Group II rats as compared to Group I (p<0.001). The test drug (*N. sativa* extracts) tried to prevent the rise in above renal parameters. Among the test drug group animals, maximum protection was seen in Group VI, which was significant as compared to Group II animals (p<0.001). This was followed by Group V as compared to Group II animals (p<0.01). The aqueous extract treated group animals also showed preventive effect but among them only Group IV showed a significant protection as compared to Group II (p<0.05) (Table 2).

**Histological analysis**

The rat liver tissue of Group I animals on staining with hematoxylin and eosin show normal micro architecture in the form of intact hepatic laminae, hepatocytes having well-defined border with centrally placed nuclei and sinusoids (Figure 1a). While the Group II, animals show marked distorted hepatic architecture. Though it did not show apparent hepatocellular necrosis but certainly there was enough stretching of hepatocytes and hepatic laminae, congestion and dilatation of sinusoids to that extent causing compression on hepatocytes. It was also associated with an increase in number of kupffer cells as compared to the Group I animals (Figure 1b). Administration of *N. sativa* extracts showed a dose-dependent improvement in the distorted architecture of the liver tissue. Among the test...
groups, NSEE treated groups, (Groups VI and V) showed marked improvement in the hepatic architecture and they were approaching toward the normal (Figure 1c and e). While NSAE treated, Group IV showed relatively less improvement in the architecture (Figure 1d).

The hematoxylin and eosin stained normal rat tissue (i.e., Group I) showed organized renal corpuscles with their renal glomeruli and tubules. The renal tubules also revealed intact epithelial cells (Figure 2a). The paracetamol only treated stained renal tissue (i.e., Group II) showed disorganization of renal microarchitecture in terms of glomerular and tubular congestion, shrunken renal corpuscles, dilated tubules and their cloudy swelling, and even loss of tubular epithelium (Figure 2b). Among the test groups, Group VI showed marked improvement in the distorted renal microarchitecture. There is relatively less glomerular and tubular congestion. The integrity of tubular epithelia cells is maintained almost similar to the normal group (Figure 2c). However, the administration of NSEE at a dose of 200 mg/kg shows intermediate improvement while the aqueous extract at 400 mg/kg showing micro-architecture near to the Group II (Figure 2c and d).

### DISCUSSION

NSAIDs are one of the most commonly used drugs worldwide. It is estimated that more than 20 million people take NSAIDs daily. These drugs are used for a wide variety of conditions, and many are available as over-the-counter drugs without prescription. The essential medicine lists of 100 countries published on the WHO website included

| Groups | Group name | Total bilirubin (mg/dl) | Indirect bilirubin (mg/dl) | AST (IU/L) | ALT (IU/L) |
|--------|------------|-------------------------|---------------------------|------------|------------|
| I      | Normal control | 0.809±0.039 | 0.149±0.022 | 80.46±2.11 | 40.23±4.17 |
| II     | Negative control | 2.629±0.126*** | 2.346±0.139*** | 328.69±10.96*** | 195.11±8.34*** |
| III    | NSAE 200 | 2.458±0.175 | 2.082±0.164 | 296.98±8.23 | 173.66±6.49 |
| IV     | NSAE 400 | 2.183±0.162* | 1.781±0.129* | 254.12±9.49* | 166.52±8.67* |
| V      | NSEE 200 | 2.029±0.094** | 1.721±0.086** | 231.02±30.92** | 138.06±15.78** |
| VI     | NSEE 400 | 1.143±0.069*** | 0.833±0.047*** | 132.68±10.48*** | 79.78±13.36*** |

NSAE (200 and 400): Nigella sativa aqueous extract (200 and 400 mg/kg), NSEE (200 and 400): Nigella sativa ethanolic extract (200 and 400 mg/kg). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control; *p<0.05, **p<0.01, ***p<0.001 when compared with negative control

| Groups | Group name | Serum creatinine (mg/dl) | Blood urea (mg/dl) |
|--------|------------|-------------------------|-------------------|
| I      | Normal control | 0.413±0.021 | 37.391±1.381 |
| II     | Negative control | 1.527±0.150*** | 78.033±4.501*** |
| III    | NSAE 200 | 1.443±0.071 | 72.775±3.509 |
| IV     | NSAE 400 | 1.104±0.134* | 62.980±1.579* |
| V      | NSEE 200 | 0.918±0.157** | 61.135±6.581** |
| VI     | NSEE 400 | 0.500±0.026*** | 41.791±2.425*** |

NSAE (200 and 400): Nigella sativa aqueous extract (200 and 400 mg/kg), NSEE (200 and 400): Nigella sativa ethanolic extract (200 and 400 mg/kg). *p<0.05, **p<0.01, ***p<0.001 when compared with normal control; *p<0.05, **p<0.01, ***p<0.001 when compared with negative control

Table 1: Effect of *Nigella sativa* on hepatic damage induced by paracetamol.

Table 2: Effect of *Nigella sativa* on renal damage induced by paracetamol.

Figure 1: Photomicrographs of rat liver stained with H and E on 400×, (a) Normal control group showing normal histological architecture with well-defined hepatocytes (h), (b) negative control group showing marked distorted hepatic architecture with congested (cv), ill-defined hepatocytes, increased Kupffer cells (arrow marked) with hemorrhagic areas (hg), (C.) *Nigella sativa* ethanolic extract (NSEE) (200 mg/kg) group is showing hepatocytes (h) with increased spaces, (d) *N. sativa* aqueous extract (400 mg/kg) group is showing hepatocytes (h) with increased spaces, (d) N. sativa aqueous extract (400 mg/kg) group is showing relatively distorted hepatic architecture, ill-defined hepatocytes, and some areas of hemorrhage (hg) also, (e) NSEE (400 mg/kg) group showing well maintained the hepatic architecture, which is comparable to the normal.
approximate six agents of NSAIDs and most commonly recommended were aspirin, ibuprofen, diclofenac, indomethacin, and naproxen in 88, 90, 74, 56, and 27 countries, respectively. One study in U.K estimated that 5-7% of hospital admissions are related to adverse effects of drugs. Approximately 30% of these hospitalizations were due to gastrointestinal, nervous system, hepatic, renal or allergic effects of aspirin or non-aspirin NSAIDs. In a study, it was found that the risk of clinically apparent liver damage was in the range of 1/10000-1/10000 in NSAIDs treated patients. This damage may be potentially serious as it can lead to acute liver failure requiring liver transplantation or leading to death. Nearly, all NSAIDs have been reported to cause an asymptomatic elevation in aminotransferases that is often not clinically relevant and are returns to normal after cessation of treatment. However, this elevation in aminotransferases predispose to hepatic damage with other hepatotoxic agents. The overall incidence of ARF in patients with paracetamol poisoning is &lt;2% and ARF occur in 10-40% of patients with severe hepatic necrosis. Besides these, synthetic drugs have limitations in terms of their abusive and incorrect use, chronic use which results in adverse drug reactions and toxicities. Hence, the adverse drug reactions and organ damages due to NSAIDs give rise to an increase in morbidity, which results in poor quality of life. Besides this, there is also an increase in financial burden to the community and the health system. The use of herbal medicines with therapeutics properties has been around since the dawn of human civilization. Sheng-Nongs Herbal Book, one of the earliest sources of folk knowledge on the use of herbs in China, dated back to 3000 B.C. included knowledge of 365 plants, animals, and minerals useful as medication. Besides that a large number of possible new drug targets have already outgrown the number of existing compounds that could potentially serve as drug candidates and the field of chemistry has limitation when it comes to synthesizing new drug structures.

In this study, we have evaluated the hepatoprotective and nephroprotective effects of N. sativa with the different doses (aqueous [200 and 400 mg/kg] and ethanolic [200 and 400 mg/kg]) of extracts against paracetamol (750 mg/kg i.p) induced hepatopo- and nephro-toxicities, a 7 days study.

In our study, paracetamol at a dose of 750 mg/kg i.p for 7 days was used to induce hepatotoxicity. In the negative control group which was treated only with paracetamol showed almost three times increase in total bilirubin (2.629 mg/dl), 1.5 times in indirect bilirubin (2.346 mg/dl), four times in AST (328.69 IU/L), and five times in ALT (195.11 IU/L) levels to that of normal control group (0.809 mg/dl, 0.149 mg/dl, 80.46 IU/L and 40.23 IU/L, respectively) and the rise was statistically significant (p<0.001). All the above parameters signified liver damage. Increase in serum transaminases (AST and ALT) levels highly signifies hepatocellular injury and is clinically significant when the levels are ≥3 times than the reference values. ALT is more specific as compared to AST for hepatic injury. The study also revealed that paracetamol-only treated group showed this type of elevation in transaminases levels, which signified hepatocytes damage, which was also evidenced by histological changes (Figure 1b). The damage is probably due to the formation of a highly reactive free radical NAPQI which later conjugates with glutathione (GSH) resulting in detoxification. Beside NAPQI other free radicals such as superoxide (O--.) and hydroxyl (OH-) free radicals generated by futile cyclic P-450. Study also revealed that N. sativa extracts (ethanolic and aqueous) administration for 7 days along with paracetamol showed protective effect against paracetamol induced hepatic damage in a dose-dependent manner. Among the test groups, NSEE co-administration at a dose of 400 mg/kg p.o showed maximum protection as evidenced by the improvement in liver function test parameters by preventing the rise in bilirubin (total and indirect) and transaminases levels. Figure 2: Photomicrographs of rat kidney stained with H and E on 400×, (a) Normal control group showing normal histological architecture with normal renal tubules (t) with well-defined and intact epithelial cells, (b) Negative control group showing disorganization of renal microarchitecture in terms of shrunken renal corpuscles dilated renal tubules and loss of tubular epithelial cells and presence of hyaline cast (arrow mark), (c) Nigella sativa aqueous extract (400 mg/kg) showing somehow improvement in the renal architecture, interstitial bleeding, and cloudy swelling of tubular epithelial cells. There is also presence of distorted glomerulus (g), (d) N. sativa ethanolic extract (NSEE) (200 mg/kg) showing relatively better arranged micro architecture with defined tubules (t) as compared to c, (e) NSEE (400 mg/kg) showing improvement in terms of well-organized microarchitecture, well-defined glomerulus (g), and only a few areas having hyaline casts.
indirect) and transaminases levels (<2 fold rise) as compared to normal control group and histological findings (Figure 1e). This signifies that the levels were reaching toward the normal on co-administration of N. sativa extracts with paracetamol. The hepatoprotection may be due to presence of thymoquinone and thymol in N. sativa as previously reported.\textsuperscript{35-37} The probable mechanism of hepatoprotection can be explained on the basis of direct and indirect effects of thymoquinone and thymol. These active constituents of N. sativa not only act themselves as anti-oxidants but also enhances the activity of antioxidants like GSH, catalase (CAT), superoxide dismutase (SOD) etc., thus augmenting body’s antioxidant mechanism against oxidative stress.\textsuperscript{38,39} Further research is needed to enlighten their precise mechanistic actions.

In this study, paracetamol at a dose of 750 mg/kg i.p for 7 days was used to induce nephrotoxicity. The renal function was evaluated by using serum creatinine and blood urea levels. The test drug (N. sativa) was administered along with paracetamol for the same period of time. Paracetamol resulted in significant increase in serum creatinine and blood urea levels as compared to normal control group (p<0.001) (Table 2). The histological findings also showed a major architectural change as compared to normal control (Figure 2a). These results were similar to earlier reported findings.\textsuperscript{33,34} Role of PGs in maintaining the renal homeostasis is well-documented. Moreover, α-aminophenol and p-phenoxyl free radical may potentially damage the kidney. Thus, nephrotoxicity following paracetamol administration can either be due to inhibition of PG synthesis e.g. PGE\textsubscript{2}, and PG\textsubscript{1} or formation of deacetylated products of paracetamol i.e., α-aminophenol and p-phenoxyl free radical or both.\textsuperscript{34,41,42} In the study, we found that the test drug i.e., among N. sativa extracts (ethanolic and aqueous) showed a protective effect against paracetamol-induced damages in the kidney. Although, all the test groups showed nephroprotective effect in a dose-dependent manner but the maximum protection was shown by NSEE (400 mg/kg p.o) as evidenced by significant decrease in serum creatinine and blood urea levels as compared to negative control group (p<0.001). Further, this test drug dose produced almost comparable values to the normal control group. This nephroprotection was followed by NSEE at a dose of 200 mg/kg p.o and was significant as compared to negative control group (p<0.01). Among the aqueous extract groups only 400 mg/kg p.o dose showed a significant nephroprotection as compared to negative control group (p<0.05) (Table 1). Histological findings were also following the same order of improvement as compared to paracetamol only treated group (Figure 2c and d). The probable mechanism of nephroprotection achieved by N. sativa occurs at two levels. First, it enhances prostaglandin synthesis, which results inadequate renal perfusion. Second, it detoxifies the free radicals action by either acting itself as antioxidant or indirectly by significantly improving serum enzymatic activities of SOD, GSH peroxidase, and CAT thus further augmenting the antioxidant mechanism.\textsuperscript{32,33}

Further preclinical and clinical studies are required to confirm and elucidate the protective effect of N. sativa against NSAIDs induced damages.

**CONCLUSION**

Hence, in view of the above findings it may be concluded that N. sativa ethanolic and aqueous seed extracts has significant protective effects against paracetamol induced hepatic and renal damages.

**ACKNOWLEDGMENTS**

The authors acknowledge the help of Dr. Umme Aiman, Dr. Mohammed Fayazuddin, Dr. Md Azmat Rana, and Dr. Abdul Haseeb M.

**Funding:** No funding sources

**Conflict of interest:** None declared

**Ethical approval:** The study was approved by the Institutional Animal Ethics Committee

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Cite this article as: Hasan MN, Khan RA, Nasiruddin M, Khan AA. Protective effect of Nigella sativa against paracetamol induced hepatic and renal damages. Int J Basic Clin Pharmacol 2015;4:503-9.