Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.18203/2319-2003.ijbcp20150029

Research Article

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

Mohammed N. Hasan[1]*, Rahat A. Khan[2], Mohammed Nasiruddin[2], Aijaz A. Khan[3]

[1]Department of Pharmacology, National Institute of Medical Sciences & Research, Jaipur, Rajasthan, India, [2]Department of Pharmacology, [3]Department of Anatomy, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Received: 14 March 2015
Revised: 03 April 2015
Accepted: 24 April 2015

***Correspondence to:**
Dr. Mohammed Nazer Hasan, Email: hasan.nazar@gmail.com

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

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs. They are prescribed commonly as an analgesic, antipyretic, and anti-inflammatory. In historical prospective, it was the Hippocrates who explored the ability of extract from willow bark and leaves to treat fever and inflammation around 400 B.C. In 17th century, the active ingredient of willow bark salicin was identified in Europe.[1] Drug-induced liver injury (DILI) is leading cause of acute liver failure. Antibiotics and NSAIDs are the most common drugs causing liver injury.[2] In a study, it was found that DILI is most commonly caused due to antibiotics followed by NSAIDs.[3] The incidence of liver disease induced by NSAIDs reported in clinical studies is fairly ranging from 0.29/100,000 (95% confidence interval [CI]: 0.17-051) to 9/100,000 (95% CI: 6-15). However, compared with these results, a higher risk of liver-related hospitalizations was reported (3-23 per 100,000 patients). NSAIDs exhibit a broad spectrum of liver damage ranging from asymptomatic, transient increase in liver enzymes to fulminant hepatic failure.[4]

NSAIDs also have significant effect on kidney functions which may be in the form of reduction in renal blood flow and glomerular filtration rate, acute tubular necrosis, allergic interstitial nephritis, renal papillary necrosis, salt and water retention, and impairment of blood pressure control and even acute renal failure (ARF) also.[5] The most common toxic effect is hemodynamically mediated renal insufficiency

due to the inhibition of renal prostaglandin (PG) synthesis.[6] The risk of ARF is fourfold in NSAID users as compared to general population.[7] 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.[8]

Acetaminophen (Paracetamol; N-acetyl-*p*-aminophenol) is an active metabolite of phenacetin. It has analgesic and antipyretic effects but has an only 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.[9]

Nigella sativa commonly known as "Kalonji/Black Cumin" belongs to family ranunculacea is an annual herb. Seeds are small dicotyledonous, trigonus, angular, regulose-trabecular, and black externally with white inside. The odor of seeds is aromatic with a bitter taste.[10] 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.[11] 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.[12] Similarly, in Iran another acute and sub-acute toxicity study revealed dose up to 6 g/kg/day for 14 days safe in mice.[13] 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.[14-17]

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[18,19]
- Indirect bilirubin - Modified Jendrassik and Grof's method[18,19]
- Serum AST - 2,4 dinitrophenyl hydrazine (DNPH)/ Reitman and Frenkel method[20]
- Serum ALT - DNPH/Reitman and Frenkel method[20]
- Serum creatinine - Alkaline Picrate method[21]
- Blood urea - Berthelot method[22]

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

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

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

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

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

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 2e). 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).

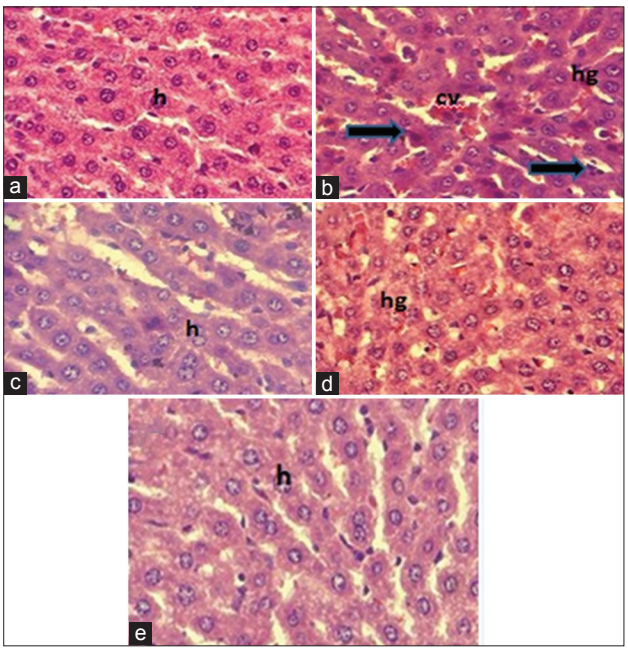

Figure 1: Photomicrographs of rat liver stained with H and E on 400×, (a) Normal control group showing normal histological architecture with well-defined hepatocytes (h), (b) negative control group showing marked distorted hepatic architecture with congested (cv), ill-defined hepatocytes, increased Kupffer 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 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.

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.[23] The essential medicine lists of 100 countries published on the WHO website included

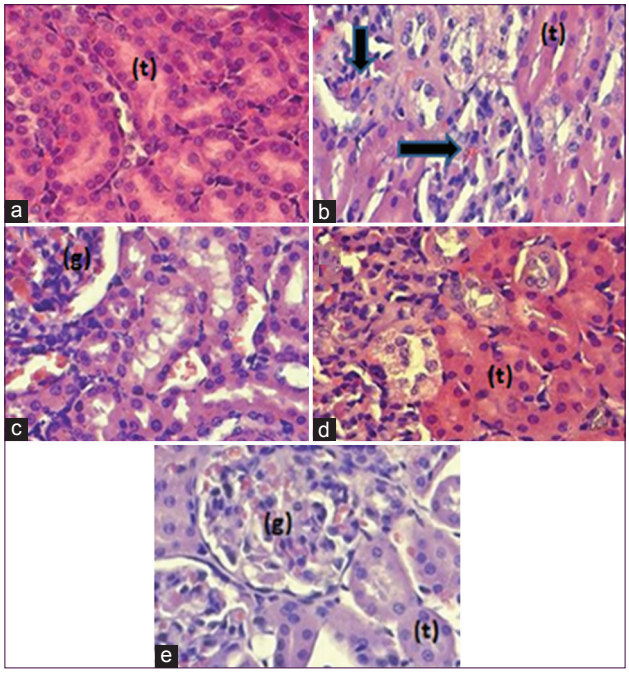

Figure 2: Photomicrographs of rat kidney stained with H and E on 400×, (a) Normal control group showing normal histological architecture with normal renal tubules (t) with well-defined and intact epithelial cells, (b) Negative control group showing disorganization of renal microarchitecture in terms 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.

approximately six agents of NSAIDs and most commonly recommended were aspirin, ibuprofen, diclofenac, indomethacin, and naproxen in 88, 90, 74, 56, and 27 countries, respectively.[24] 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.[25] In a study, it was found that the risk of clinically apparent liver damage was in the range of 1/100000-1/10000 in NSAIDs treated patients.[26] 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.[27] The overall incidence of ARF in patients with paracetamol poisoning is <2%[28] and ARF occur in 10-40% of patients with severe hepatic necrosis.[29] Besides these, synthetic drugs have limitations in terms of their abusive and incorrect use, chronic use which results in adverse drug reactions and toxicities.[30] 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.[31] 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.[32]

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 hepato- and nephro-toxicities, a 7 days study.[11]

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.[33] 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.[34] 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 (<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.[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.[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[11,40] Role of PGs in maintaining the renal homeostasis is well-documented. Moreover, p-aminophenol and p-phenoxy free radical may potentially damage the kidney. Thus, nephrotoxicity following paracetamol administration can either be due to inhibition of PGsynthesis e.g. PGE_2 and PGI_2 or formation of deacetylated products of paracetamol i.e., p-aminophenol and p-phenoxy free radical or both.[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.[43,44]

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 with 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

REFERENCES

1. Rao P, Knaus EE. Evolution of nonsteroidal anti-inflammatory drugs (NSAIDs): cyclooxygenase (COX) inhibition and beyond. J Pharm Pharm Sci. 2008;11:81s-110.
2. Björnsson E. Review article: drug-induced liver injury in clinical practice. Aliment Pharmacol Ther. 2010;32:3-13.
3. Andrade RJ, Lucena MI, Fernandez MC, Pelaez G, Pachkoria K, Garcia-Ruiz E, et al. On behalf of Spanish group for study of drug-induced liver. Gastroenterology. 2005;129:512-52.
4. Bessone F. Non-steroidal anti-inflammatory drugs: what is the actual risk of liver damage? World J Gastroenterol. 2010;16:5651-61.
5. Linton AL. Adverse effects of NSAIDs on renal function. Can Med Assoc J. 1984;131(3):189-91.
6. Bush TM, Shlotzhauer TL, Imai K. Nonsteroidal anti-inflammatory drugs. Proposed guidelines for monitoring toxicity. West J Med. 1991;155(1):39-42.
7. Pérez Gutthann S, García Rodríguez LA, Raiford DS, Duque Oliart A, Ris Romeu J. Nonsteroidal anti-inflammatory drugs and the risk of hospitalization for acute renal failure. Arch Intern Med. 1996;156:2433-9.
8. Huerta C, Castellsague J, Varas-Lorenzo C, García Rodríguez LA. Nonsteroidal anti-inflammatory drugs and risk of ARF in the general population. Am J Kidney Dis. 2005;45(3):531-9.
9. Grosser T, Smyth E, FitzGerald GA. Anti-inflammatory, antipyretic, and analgesic agents; Pharmacotherapy of Gout: introduction. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 12th Edition. New York: McGraw Hill Medical Publication; 2011: 959-1004.
10. Rajsekhar S, Kuldeep B. Pharmacognosy and pharmacology of *Nigella sativa* - A review. Int Res J Pharm. 2011;2(11):36-9.
11. Abdel-Zaher AO, Abdel-Hady RH, Mahmoud MM, Farrag MM. The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage. Toxicology. 2008;243(3):261-70.

12. Dollah MA, Parhizkar S, Latiff LA, Bin Hassan MH. Toxicity effect of *Nigella sativa* on the liver function of rats. Adv Pharm Bull. 2013;3(1):97-102.
13. Vahdati-Mashhadian N, Rakhshandeh H, Omidi A. An investigation on LD50 and subacute hepatic toxicity of *Nigella sativa* seed extracts in mice. Pharmazie. 2005;60(7):544-7.
14. El-Daly ES. Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on Cisplatin induced toxicity in rats. J Islam Acad Sci. 1996;9(4):105-18.
15. Hadjzadeh MA, Keshavarzi Z, Tabatabaee Yazdi SA, Ghasem Shirazi M, Rajaei Z, Khajavi Rad A. Effect of alcoholic extract of *Nigella sativa* on cisplatin-induced toxicity in rat. Iran J Kidney Dis. 2012;6(2):99-104.
16. Al-Ghamdi MS. Protective effect of *Nigella sativa* seeds against carbon tetrachloride-induced liver damage. Am J Chin Med. 2003;31(5):721-8.
17. Abuelgasim AI, Omer EA, Elmahdi B. The effectiveness of *Nigella sativa* against liver damage in rats. Res J Med Plant. 2008;2(1):43-7.
18. Jendrassik L, Grof P. Vereinfachte photometrische methode zur bestimmung des blutbilirubins. Biochem Z. 1938;297:81.
19. Mori L. Modified jendrassik – Grof method for bilirubins adapted to the abbott bichromatic analyzer. Clin Chem. 1978;24(10):1841-5.
20. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28(1):56-63.
21. Lustgarten JA, Wenk RE. Simple, rapid, kinetic method for serum creatinine measurement. Clin Chem. 1972;18(11):1419-22.
22. Ngo TT, Phan AP, Yam CF, Lenhoff HM. Interference in determination of ammonia with the hypochlorite-alkaline phenol method of bertholet. Anal Chem. 1982;54(1):46-9.
23. Majithia R, Johnson DA, Ryan MJ, Wootton FT, Willis J, Hornbuckle K & Brooks W. Underreported use of non-steroidal anti-inflammatory drugs in an outpatient gastroenterology practice: a prospective office-based survey. Gastroenterology Insights. 2013; 5(1), e3: 09-11.
24. McGettigan P, Henry D. Use of non-steroidal anti-inflammatory drugs that elevate cardiovascular risk: an examination of sales and essential medicines lists in low-, middle-, and high-income countries. PLoS Med. 2013;10(2):e1001388.
25. Pirmohamed M, James S, Meakin S, Green C, Scott AK, Walley TJ, et al. Adverse drug reactions as cause of admission to hospital: prospective analysis of 18 820 patients. BMJ. 2004;329(7456):15-9.
26. Rostom A, Goldkind L, Laine L. Nonsteroidal anti-inflammatory drugs and hepatic toxicity: a systematic review of randomized controlled trials in arthritis patients. Clin Gastroenterol Hepatol. 2005;3(5):489-98.
27. Bjorkman DJ. Current status of nonsteroidal anti-inflammatory drug (NSAID) use in the United States: risk factors and frequency of complications. Am J Med. 1999;107(6A):3S-8.
28. Blakely P, McDonald BR. Acute renal failure due to acetaminophen ingestion: a case report and review of the literature. J Am Soc Nephrol. 1995;6(1):48-53.
29. McJunkin B, Barwick KW, Little WC, Winfield JB. Fatal massive hepatic necrosis following acetaminophen overdose. JAMA. 1976;236(16):1874-5.
30. Rates SM. Plants as source of drugs. Toxicon. 2001;39(5):603-13.
31. Sheng-Ji P. Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. Pharm Biol. 2001;39 Suppl 1:74-9.
32. Mohamed I, Shuid A, Borhanuddin B, Fozi N. The application of phytomedicine in modern drug development. The Internet Journal of Herbal and Plant Medicine. 2012; 1(2). https://ispub.com/IJHPM/1/2/14278.
33. Pratt DS, Kaplan MM. Evaluation of liver functions. Harrison's Principle of Internal Medicine. 18th Edition. Vol. 2. Ch. 302. 2012: 2527-31.
34. Bessems JG, Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol. 2001;31(1):55-138.
35. Alsaif MA. Effect of thymoquinone on ethanol induced hepatotoxicity in wistar rats. J Med Sci. 2007;7(7):1164-70.
36. Lebda FM, Ahmed MA, Abd-El-Samad AA, Shawky MK. Protective effect of Thymoquinone against D-Galactosamine-Induced Liver injury in rats. Aust J Basic Appl Sci. 2011;5(2):49-58.
37. Janbaz KH, Saeed SA, Gilani AH. Hepatoprotective effect of thymol on chemical induced hepatotoxicity in rodents. Pak J Biol Sci. 2003;6(5):448-51.
38. 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(42):6684-8.
39. Yildiz F, Coban S, Terzi A, Ates M, Aksoy N, Cakir H, et al. *Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver. World J Gastroenterol. 2008;14(33):5204-9.
40. Salah-Eldin O, Abd-El-Azim SA, Eldeib KM, Barakat MM. Is lysosomal enzymes changes important in the pathogenesis of Liver and Kidney injury induced by short and long term administration of some NSAID' drugs in rats? Life Sci J. 2012;9(4):1102-13.
41. Horl WH. Nonsteroidal anti-inflammatory drugs and the kidney. Pharmaceuticals. 2010;3:2291-321.
42. Fischer V, West PR, Nelson SD, Harvison PJ, Mason RP. Formation of 4-aminophenoxyl free radical from the acetaminophen metabolite N-acetyl-p-benzoquinone imine. J Biol Chem. 1985;260(21):11446-50.
43. Bayrak O, Bavbek N, Karatas OF, Bayrak R, Catal F, Cimentepe E, et al. *Nigella sativa* protects against ischaemia/reperfusion injury in rat kidneys. Nephrol Dial Transplant. 2008;23(7):2206-12.
44. Salama RH, Abd-El-Hameed NA, Abd-El-Ghaffar SK, Mohammed ZT, Ghandour NM. Nephroprotective effect of *Nigella sativa* and *Matricaria chamomilla* in Cisplatin induced renal injury. Int J Clin Med. 2011;2:185-95.

doi: 10.18203/2319-2003.ijbcp20150029
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.