Ameliorative Effect of *Nigella sativa* Oil against Paracetamol Induced Hepatic and Renal Damages in Rats

Mohammed Nazer Hasan[1*], Rahat Ali Khan[2], Mohammed Nasiruddin[2] and Aijaz Ahmed Khan[3]

[1]*Department of Pharmacology, Maulana Azad Medical College, New Delhi, India.*
[2]*Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh, India.*
[3]*Department of Anatomy, Jawaharlal Nehru Medical College, Aligarh, India.*

Authors' contributions

This work was carried out in collaboration between all authors. Authors MNH and RAK collaboratively created the idea of the study. Author MNH managed the literature search, designed the study, wrote the protocol, execute the study, analyzed the data and drafted the manuscript. Author AAK has significant contribution in histological analysis. Author MN has contribution in data analysis and proof reading of the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: To evaluate the protective effect of *Nigella sativa* oil against Non-steroidal anti-inflammatory drug induced hepatic and renal damages in rats.
Methodology: Study was conducted on 24 Wistar albino rats (150-200 g) of either sex. They were divided into four groups each containing six rats. Group I was treated with distilled water only, Group II treated with Paracetamol (750 mg/kg) only and Group III to IV were test groups treated with Paracetamol (750 mg/kg) and *Nigella sativa* oil (1 & 2 ml/kg respectively) for 7 days. On 8th day all rats were sacrificed and blood were collected for liver and renal function tests and tissue

---

Corresponding author: E-mail: hasan.nazar@gmail.com;

samples for histo-pathological examination.

Results: Paracetamol caused marked hepatic and renal damages which were prevented by *Nigella sativa* oil in dose dependent manner.

Conclusion: *Nigella sativa* oil showed significant hepato- and reno-protection against Paracetamol induced damages.

1. INTRODUCTION

Paracetamol was discovered in 1889 [1]. First used clinically by Von Mering in 1893 and commercially available in US & UK by 1950 & 1956 respectively [2]. It is an active metabolite of phenacetin, which has good analgesic and antipyretic properties. Paracetamol is very well tolerated drug, commonly used as an over the counter for fever and different conditions of pain.

Although, Paracetamol is a safe at therapeutic doses but toxicity due to overdose, poisoning or chronic use are very common in clinical practice. The most common organ damaged by Paracetamol is liver. Clinically, in 1966 hepatotoxicity was first reported in two individuals who were died after two days following overdose [3]. In UK it is one of the most common agents of intentional self harm. From 2000 to 2008 there were 90-155 deaths from Paracetamol poisoning every year in UK [4]. In US from 1998 to 2003 out of total reported accidental poisoning 48% were Paracetamol related cases (131 of 274) [5]. A population based surveillance study in US estimated that around 1600 cases of Acute Liver Failure (ALF) annually and the most common etiology of this was Paracetamol [6]. Beside hepatotoxicity Paracetamol also damages the renal system. Acute renal failure occurs in less than 2% of all acetaminophen poisonings and 10% of severely poisoned patients, which manifest as acute tubular necrosis [7]. The incidence of acute renal failure in severe hepatic necrosis caused by Paracetamol is about 10-40% [8]. In a retrospective study it was found that out of 45 adolescents (12-18 years) acetaminophen-related nephrotoxicity occurred in 4 (8.9%) with severe overdoses [9].

Nigella sativa Linn is an annual herbaceous plant belonging to the Ranunculaceae family growing in countries bordering the Mediterranean Sea, Pakistan, India and Iran [10]. Commonly known as "Black cumin". It has long been used in folk medicine in the Arabian Gulf region, Far East Asia, and Europe. Black Seed is also mentioned as the curative "black cumin" in the Holy Bible

and is described as Melanthion by Hippocrates and Dioscorides and as Gith by Pliny [11]. Traditionally it was recommended for a wide range of ailments, including fever, cough, bronchitis, asthma, chronic headache, migraine, dizziness, chest congestion, dysmenorrhea, obesity, diabetes, paralysis, hemiplagia, back pain, infection, inflammation, rheumatism, hypertension, and gastrointestinal problems such as dyspepsia, flatulence, dysentery, and diarrhea. It has been used as a stimulant, diuretic, emmenagogue, lactagogue, anthelmintic, and carminative in Arabian Gulf region [12]. Of all the plant parts it is only the seeds which attracted most of the researchers. Seeds are the source of oil. *Nigella sativa* oil (NSO) composed of fixed oil and volatile oil. The major portion of NSO is fixed oil while around 0.4 to 0.5% is volatile oil obtained by steam distillation of crushed *Nigella sativa* seeds & extraction by diethyl ether. However, soxhlet extraction of seeds with petroleum ether gives around 35% oil which on steam distillation yield 1.5% of volatile oil [13]. It has many medicinal properties including gastroprotective, antidiabetic, antibacterial, hepatoprotective, anti-tumor, immune-modulatory effects [13-15]. Based on the above folklore & traditional uses as well as medicinal properties we have taken *Nigella sativa* oil for our study.

Therefore, the aim of this work was to evaluate the role of *Nigella sativa* oil against Paracetamol induced hepatotoxicity and nephrotoxicity in rats.

2. MATERIALS AND METHODS

Before starting the study, protocol was approved by the Institutional Animal Ethical Committee (IAEC). 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).

2.1 Chemicals and Drugs

All chemicals and drugs used were of analytical grade.

Nigella sativa oil (NSO): Procured from local market of Aligarh, which was manufactured by Mohammedia products, Aamir nagar, Shah Sahab Mohalla, Karimnagar-505001, A.P, India. As per manufacturer's information, it was prepared by steam distillation. Packing size was 200 ml, Batch no. 020 and Date of manufacture was Feb., 2011.

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

Drugs: Injection Paracetamol (Tablets India Ltd.)

2.2 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.

2.3 Animals

Study was conducted on 24 adult healthy Wistar albino rats (150-200 g) of either sex and was 6-8 weeks of age. They were procured from Central Animal House, Jawaharlal Nehru Medical College; A.M.U; Aligarh, Uttar Pradesh and were housed under standard condition (temperature $27\pm2°C$, 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.

2.4 Experimental Design

All the rats were randomly divided into four groups each containing six rats. Hepatic and renal damage was induced by Paracetamol 750 mg/kg i.p daily for 7 days [16]. Test dose was selected on the basis of previous toxicity studies and the doses documented in on the other beneficial effects of *Nigella sativa* oil. In a 12-week chronic study it was found that oral administration of *Nigella sativa* oil at a dose 2 ml/kg/day is safe which was evidenced by very high level of LD_{50} (28.8 ml/kg) [17]. It was also found in many animal studies that *Nigella sativa* oil showing hepatic- and reno-protective potential in a wide range of doses (0.2 ml/kg to 2 ml/kg) however, the toxicity inducing agents were different [18-21]. Thus, based on the aforementioned evidence we have chosen the two test doses (1 and 2 ml/kg) for evaluation of

hepatoprotective and nephroprotective effect of *Nigella sativa* oil (NSO).

Different groups were treated as below:

- Group I (Normal control): Distilled water 0.5 ml/day p.o for 7 days.
- Group II (Negative control): [Distilled water 0.5 ml/day p.o + paracetamol 750 mg/kg/day I.P] × 7 days.
- Group III (NSO-I): [*Nigella sativa* Oil (1 ml/kg/day p.o) + Paracetamol (750 mg/kg/day I.P)] × 7 days
- Group IV (NSO-II): [*Nigella sativa* Oil (2 ml/kg/day p.o) + Paracetamol (750 mg/kg/day I.P)] × 7days

The two different formulations were administered at two different times 6 hrs apart.

On 8th day, all the rats were sacrificed under Sodium pentobarbitone (50 mg/kg I.P) and dissection was done.

2.5 Blood Collection and Biochemical Analysis

After sacrifice blood had been collected in vacutainer and centrifuge under 5000 rpm for 10 minutes then serum was separated and analyzed for:

- Total serum Bilirubin (TSB)- Modified Jendrassik and Grof's method [22-23].
- Indirect bilirubin (IB)- Modified Jendrassik and Grof's method [22-23].
- Serum AST - 2,4 dinitrophenyl hydrazine (DNPH)/Reitman and Frenkel method [24]
- Serum ALT - DNPH/Reitman and Frenkel method [24].
- Serum creatinine - Alkaline Picrate method [25].
- Blood urea - Berthelot method [26].

Finally, the tissues were preserved in 10% Formalin for histological examination.

2.6 Histological Examination

The tissues were 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.

2.7 Statistical Analysis

The results were presented as Mean ± Standard Error of Mean (SEM). The groups were compared by one way analysis of variance (ANOVA) followed by post hoc "Dunnett's Multiple comparison test" to analyze statistical significance. P<0.05 was considered to be significant.

3. RESULTS AND DISCUSSION

3.1 Results

After 7 days of treatment all rats were sacrificed on 8th day. Blood was collected for biochemical analysis while the tissues were taken out for histological examination.

3.1.1 Biochemical analysis

On liver functions evaluation, we evaluate Total Serum Bilirubin (TSB), Indirect Bilirubin (IB), AST and ALT levels (Table 1). It was found that administration of paracetamol causes massive damage to liver in Group II rats which was 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). *Nigella sativa* oil (NSO) tried to prevent this

damage to a significant level. Among the test groups it was found that *Nigella sativa* oil (2 ml/kg) administered group rats [i.e. Group IV] showed highest protection followed by the rats of Group III which were administered *Nigella sativa* oil (1 ml/kg). The deranged liver function parameters (TSB, IB, AST and ALT) significantly prevented in Group IV rats as compared to Group II (p<0.001). In Group III rats, *Nigella sativa* Oil (1 ml/kg) also tried to prevent in derangement of liver function parameters as compared to Group II (p<0.01), but this was less as compared Group IV.

On kidney functions evaluation we evaluate Serum creatinine (SC) and Blood urea (BU) levels [Table 2]. Among all the groups it was found that in Group II rats there was a massive damage in kidney evidenced by almost 2-3 fold rise in serum creatinine and blood urea as compared to Group I rats (p<0.001). This damage was prevented by co-administration of *Nigella sativa* Oil seen in test groups (Group III & IV). Among the test groups it was found that maximum protection was seen in Group IV (NSO-II) followed by Group III (NSO-I). In Group IV rats *Nigella sativa* oil prevented significantly the rise in SC and BU levels as compared to Group II rats (p<0.001). Group III rats also showed a significant improvement in the kidney function parameters as compared to Group II rats (p<0.01).

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

Group no.	Group name	Total serum bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	AST (IU/L)	ALT (IU/L)
I.	Normal control	0.81 ± 0.04	0.15 ± 0.02	80.46 ± 2.11	40.23 ± 4.17
II.	Negative control	2.63 ± 0.13[c]	2.35 ± 0.14[c]	328.69 ± 10.96[c]	195.11 ± 8.34[c]
III.	NSO I	1.98 ± 0.12[y]	1.68 ± 0.14[y]	224.45 ± 30.76[y]	129.88 ± 17.10[y]
IV.	NSO II	1.25 ± 0.04[z]	0.94 ± 0.03[z]	154.80 ± 14.72[z]	84.96 ± 12.71[z]

[NSO I- Nigella sativa oil 1ml/kg and NSO II- Nigella sativa oil 2 ml/kg. [a] (p<0.05), [b] (p<0.01) and [c] (p<0.005) when compared with Normal control; [x] (p<0.05), [y] (p<0.01) and [z] (p<0.005) when compared with Negative control. Comparisons were made within the column]

Table 2. Effect of *Nigella sativa* oil on renal damage induced by Paracetamol

Group no.	Group name	Serum creatinine (mg/dl)	Blood urea (mg/dl)
I.	Normal control	0.41 ± 0.02	37.39 ± 1.38
II.	Negative control	1.53 ± 0.15[c]	78.03 ± 4.50[c]
III.	NSO I	0.86 ± 0.14[y]	58.20 ± 4.12[y]
IV.	NSO II	0.52 ± 0.04[z]	44.37 ± 2.28[z]

[NSO I- Nigella sativa oil 1 ml/kg and NSO II- Nigella sativa oil 2 ml/kg. [a] (p<0.05), [b] (p<0.01) and [c] (p<0.005) when compared with Normal control; [x] (p<0.05), [y] (p<0.01) and [z] (p<0.005) when compared with Negative control. Comparisons were made within the column]

3.1.2 Histological analysis

After staining with haematoxylin and eosin of Group I rat liver showed normal micro-architecture with intact hepatic laminae, well defined hepatocytes and sinusoids [Fig. 1A]. Group II rat liver showed marked distorted architecture. There were marked stretching of hepatocytes with inconspicuous nucleus and distorted hepatic lamina. Within sinusoids there were marked congestion and dilatation leads to compression of hepatocytes. Besides these, there were increase in number of kupffer cells and areas of hemorrhage [Fig. 1B]. *Nigella sativa* oil co-administration tried to prevent these damages caused by paracetamol as evidenced by improved hepatic architecture of test groups (Group III and IV). Among the test groups there was dose dependent protection [Figs. 1C and 1D]. The Group III rat liver showed improved hepatic architecture while Group IV rat liver showed even more improved architecture as compared to Group II liver. The Group IV rat liver relatively maintained the conspicuous architecture with well defined hepatocytes, which was comparable to Group I rat liver [Fig. 1D].

The haematoxylin and eosin stained Group I rat kidney tissue showed normal organized architecture. Renal corpuscles, glomeruli and tubules were in organized array. The renal tubules showed intact and conspicuous epithelial cells [Fig. 2A]. The Paracetamol-only treated rats (Group II) showed massive disorganized micro-architecture. The renal corpuscles were shrunken; glomerulus and tubules were congested with cloudy swelling and breach in the tubular epithelial lining, focal necrosis of tubular lining and marked interstitial hemorrhage [Fig. 2B]. The test groups (Group III & IV) rat kidney tissue showed improved micro-architecture. Among them, Group IV rat kidney showed maximum improvement in micro-architecture. There was relatively less glomerular and tubular congestion, and the integrity of tubular epithelial cells maintained which can be comparable to the normal rat kidney tissue [Fig. 2D]. Group III rat kidney also showed improvement in the tissue architecture but it was relatively less as compared to Group IV [Fig. 2C]. The histological analysis also showed a dose-dependent improvement in the micro-architecture when the rats were co-administered with *Nigella sativa* oil.

3.2 Discussion

Paracetamol is used commonly as an analgesic and antipyretic agent. It is one of the most frequently used drugs in self prescribing situations and as an over the counter [27]. Many of the studies showed that the prescribing pattern of paracetamol is high as analgesic and antipyretic agent [28-29]. In a study in UAE it was found that paracetamol was included in 35.5% of all prescription, out of which 58.5% were children under 12 years of age [30]. It is also one of the common off label drug used in children. Approximately 20% of paracetamol prescriptions issued to children by their General Practioners are off label as primary care and most common reason is either too small or too high dose [31]. Because of ease of availability and off label use persons are prone to its toxicity and poisoning. A study in UK & France revealed that there were strong correlation between paracetamol sell and non-fatal overdose poisoning and suicide [32]. Paracetamol is a known toxin to both the liver and extra-hepatic tissues. It causes fulminant hepatic necrosis and nephrotoxicity in both humans and experimental animals when used in high doses. In human it produce acute centrilobular hepatic necrosis, plasma aminotransferase (AST or ALT) activity increase dramatically with prolongation of the prothrombin time ratio and rise in bilirubin level owing to jaundice [33]. Among the extra-hepatic tissues renal tissues affected commonly. Renal insufficiency occurs in approximately 1-2% of patients with acetaminophen overdose [8,34,35] Acute renal failure occurs in less than 2% of all paracetamol poisoning and 10% of severely poisoned patients [7]. At present, drug- or chemical-induced liver and kidney injuries have become a major clinical problem. The precise mechanisms underlying drug- or chemical-induced hepato-toxicity and nephro-toxicity are gradually elucidated. However, there is still lack of effective therapeutic strategies or specific medicines for such diseases. Hence, in this regard we planned the study to evaluate the potential protective effect of *Nigella sativa* oil against paracetamol induced hepato- and nephro-toxicity.

In the present study, rat model of paracetamol-induced liver and kidney damage was adopted to investigate the possible protective effects of *Nigella sativa* oil on the liver and kidney after 7 days of intervention.

Results revealed that rats which were treated with Paracetamol-only (Negative control) indicated deterioration of the hepatic as evidenced by marked elevation of AST, ALT, Bilirubin (total and indirect) levels. There was almost three times increase in total Bilirubin, four times in AST and five times in ALT levels to that of normal control group. 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 [36]. Serum ALT is often used as monitoring parameter for hepatic damage. Histological data also showed the massive

derangements in hepatic micro-architecture as compared to normal group rats. All these signify that paracetamol caused massive damages to the liver [16,37,38]. This damage is mainly due to excessive production of N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is highly reactive electrophilic intermediate responsible for hepatotoxicity. At normal doses only a trace amount of the NAPQI is formed. And in the presence of hepatic reduced glutathione (GSH) NAPQI can either be reduced back to Paracetamol or covalently linked to GSH to form a 3-glutathione-S-yl-paracetamol conjugate (PAR-SG). This results in detoxification of
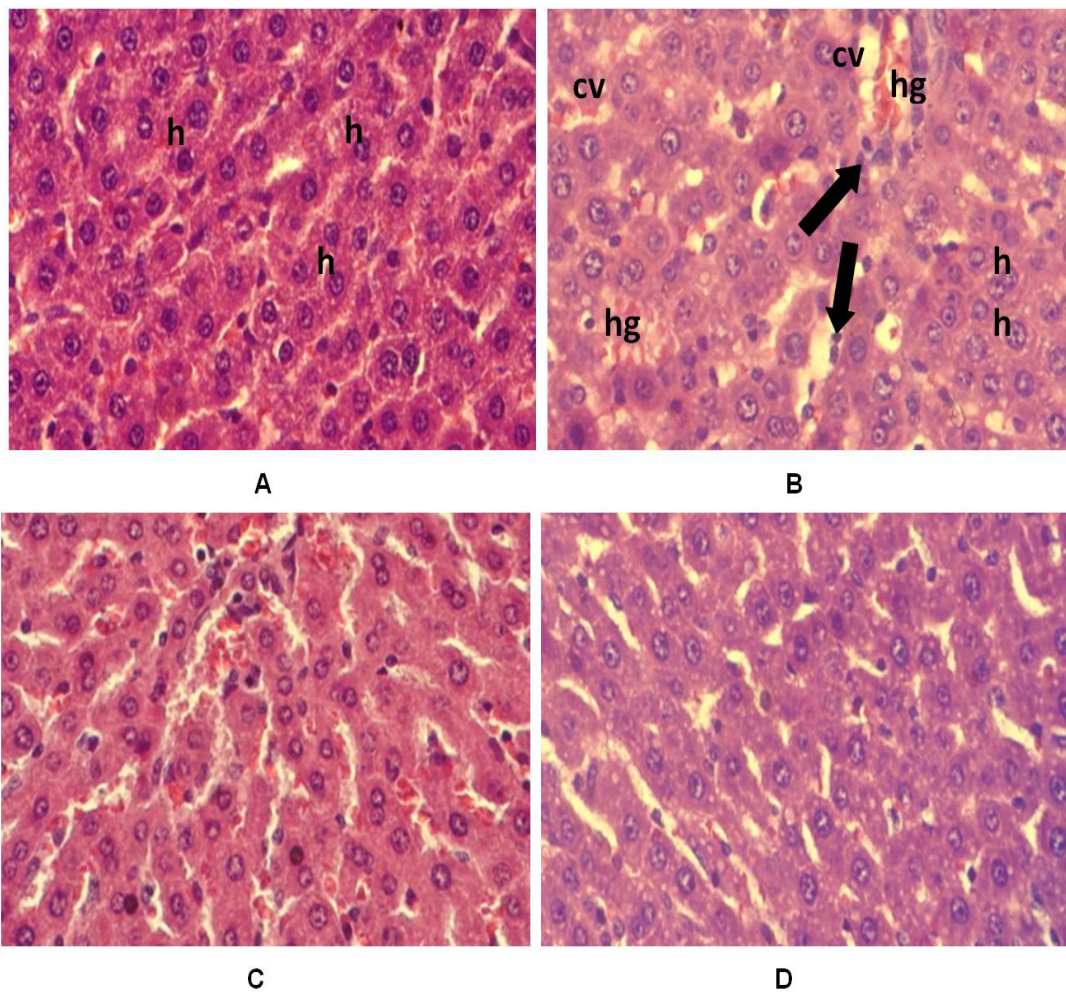
Fig. 1. Microscopic photograph of rat liver stained with H&E on 400X
A. Normal control group showing normal histological architecture with well defined hepatocytes (h). B. Negative control group showing marked distorted hepatic architecture with congested central vein (cv), ill defined hepatocytes with increased spaces, increased kupffer cells (arrow marked) with hemorrhagic areas (hg). C. NSO-I (Nigella sativa oil 1 ml/kg) group showing hepatocytes (h) with relatively less spaces & fewer hemorrhagic areas. D. NSO-II (Nigella sativa oil 2 ml/kg) group showing relatively maintained hepatic architecture with decreased spaces and defined hepatocytes
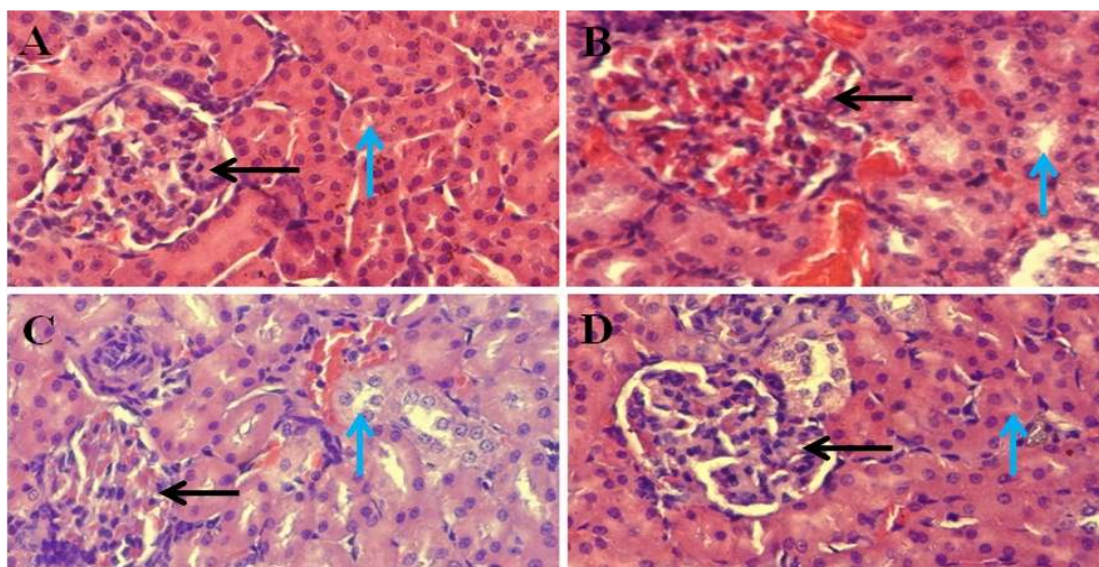
Fig. 2. Microscopic photograph of rat kidney stained with H&E on 400X

Normal control group [A] and negative control [B], NSO-1 and NSO-2 groups [C & D] respectively. In all images, the black horizontal arrow (←) indicates renal corpuscles and blue vertical arrow (↑) indicates renal tubules.
A. *Normal control group- the renal corpuscles and renal tubules reveal normal histo-architecture with well-defined and intact epithelial cells.* ***B.*** *Negative control group shows disorganization of renal micro-architecture in the form of edema & congestion of renal corpuscle, focal necrosis of tubular lining and marked interstitial hemorrhage.*
C. *NSO-I (Nigella sativa oil 1 ml/kg) group reveals improved condition in terms of only occasional site of interstitial hemorrhage and tubular necrosis and casts.* ***D.*** *NSO-II (Nigella sativa oil 2 ml/kg) group shows histo-architecture very close to group A except that damage to some tubular epithelium can be noticed*

NAPQI. However, an overdose of Paracetamol leads to massive production of NAPQI results in exhaustion of hepatic GSH which results in depletion of hepatic GSH and can no longer compensate for a massive production of NAPQI [39]. Our results demonstrate that co-administration of *Nigella sativa* oil for 7 days ameliorates the hepatic injuries, as evidenced by biochemical and histological analysis. In the test group rats (NSO-I & -II) we found that there were dose-dependent protective effect which was evidenced by significant improvement in deranged hepatic biochemical parameters and histological architecture as compared to Paracetamol-only (Negative control) rats. And this improvement was approaching towards normal values. These results are in accordance with previous studies also [40-41]. The hepatoprotective activity of *Nigella sativa* oil may be due to its active constituents. The probable mechanism for protection may be defined at different levels. The active constituents may have the direct role to convert the NAPQI to some another non-toxic metabolite or indirectly augmenting the hepatic anti-oxidant system. Further work to be needed to explore the detailed mechanism for hepatoprotection by *Nigella sativa* oil.

Beside, the above we also found that renal function parameters were also markedly hampered in Paracetamol-only treated rats (Negative control) as evidenced by 4 times rise in serum creatinine and 2 times rise in blood urea as compared to normal control group rats. The histological assessment of renal tissues of negative control group rats also revealed marked distortion in the architecture of renal tissue as compared to normal group rats. These findings were coincided with other investigator's results [42]. In the previous study reports it was found that the damage due to excessive production of NAPQI and another metabolite p-aminophenol (PAP). PAP after oxidation converted to p-aminophenoxy free radical and subsequently 1,4-benzoquinoneimine, which can covalently bind to renal tissue macromolecules. In normal doses of Paracetamol these metabolites are conjugated with the help of glutathione and detoxification occurs, but excessive production of PAP & NAPQI leads to exhaustion of glutathione [39,43]. In the test group rats (NSO-I &-II) we found that there were dose-dependent decrease in serum creatinine and blood urea levels as compared to Paracetamol-only (Negative control) rats, which quite significant. And the deranged

values were approaching towards the normal levels. The histological examination of renal tissues also showed improved architecture as compared to paracetamol only treated rats. These findings supported the previous studies [44-45]. The nephroprotection is also may be due to *Nigella sativa* oil's active constituents, which may directly or indirectly take part in detoxification mechanism. Further studies needed to elucidate the mechanism of renoprotection.

4. CONCLUSION

The findings of the current study illustrated that *Nigella sativa* oil protected the rat liver and kidney against paracetamol induced damages when co-administered with paracetamol. Hence, *Nigella sativa* oil can be considered as a potential protective agent against paracetamol induced toxicities. Further studies required to find out more with this regard and the mechanistic aspect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was taken from Institutional Animal Ethical Committee (IAEC) Jawaharlal Nehru Medical College, A.M.U; Aligarh (U.P), before starting the study [Registration no. 401/CPCSEA dated 08.05.2012].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Prescott LF. Paracetamol: Past, present, and future. Am J Ther. 2000;7(2):143-147.
2. Davidson DG, Estham WN. Acute liver necrosis following overdose of paracetamol. Br Med J. 1966;2:497-499.
3. Hawton K, et al. Impact of different pack sizes of paracetamol in the United Kingdom and Ireland on intentional overdoses: A comparative study. BMC Public Health. 2011;11:460.
4. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Lee WM, et al. Acute Liver Failure Study Group (ALFSG). Acetaminophen-induced acute liver failure: Results of a United States multicenter, prospective study. Hepatology. 2005; 42(6):1364-72.
5. Bower WA, Johns M, Margolis HS, Williams IT, Bell B. Population-based surveillance for acute liver failure. Am J Gastroenterol. 2007;102:2459-2463.
6. 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.
7. McJunkin B, Barwick KW, Little WC, Winfield JB. Fatal massive hepatic necrosis following acetaminophen overdose. JAMA. 1976;236(16):1874-1875.
8. Boutis K, Shannon M. Nephrotoxicity after acute severe acetaminophen poisoning in adolescents. J Toxicol Clin Toxicol. 2001; 39(5):441-445.
9. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res. 2003;17:299–305.
10. El-Tahir H. Kamal El-Din, Bakeet DM. The Black seed *Nigella sativa* linnaeus - A mine for multi cures: A plea for urgent clinical evaluation of its volatile oil. J T U Med Sc. 2006;1(1):1-19.
11. Tariq M. *Nigella sativa* seeds: Folklore medicine in modern day medicine. Saudi J Gastroenterol. 2008;14(3):105–106.
12. Nadkarni AK. Indian materia medica. 3rd ed. Mumbai: Popular Prakashan Pvt. Ltd; 1976;301–340.
13. Khan MA. Chemical composition and medicinal properties of *Nigella sativa* Linn. Inflammopharmacology. 1999;7(1):15-35.
14. Ahmad A, Hussain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed. 2013;3(5):337-352.
15. Musa D, Dilsiz, N, Gumushan H, Ulakoglu G, Bitiren M. Antitumor activity of an ethanol extract of *Nigella sativa* seeds. Biologia, Bratislava. 2004;59:735-740.
16. 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):261-270.
17. Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch K, Hassar M. Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomedicine. 2002;9:69–74.

18. Yaman I, Balikci E. Protective effects of *Nigella sativa* against gentamicin-induced nephrotoxicity in rats. Experimental and Toxicologic Pathology. 2010;62:183–190.

19. 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): 6684-6688.

20. Al-Khafaji NM. Protective effect of crude oil of *Nigella sativa* on liver in male albino mice treated with low toxic dose of paracetamol. Medical Journal of Babylon. 2014;10:4.

21. Ebru U, Bayrak O, Efkan U, Kaya A, Bayrak R, Burak U, Turgut FH, Bavbek N, Kanbay M, Akcay A. *Nigella sativa* oil for prevention of chronic cyclosporine nephrotoxicity: An experimental model. Am J Nephrol. 2008;28(3):517-522.

22. Jendrassik L, Grof P. Vereinfachte photometrische methode zur bestimmung des blutbilirubins. Biochem Z. 1938;297: 81.

23. Mori L. Modified jendrassik – Grof method for bilirubins adapted to the abbott bichromatic analyzer. Clin Chem. 1978; 24(10):1841-1845.

24. 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.

25. Lustgarten JA, Wenk RE. Simple, rapid, kinetic method for serum creatinine measurement. Clin Chem. 1972;18(11): 1419-1422.

26. 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):46-49.

27. Samarawickrama AAHS, Suraweera RK, Sivayoganathan C, Sakeena MHF. A study on paracetamol consumption by undergraduate students in the Faculty of Allied Health Sciences, University of Peradeniya. International Journal of Scientific and Research Publications. 2014;4(6).

28. James LP, Mayeux PR, Hinson J. Acetaminophen-induced hepatotoxicity. Drug Metabolism and Disposition. 2003; 31:1499–1506.

29. Mohammed TCH, Beegum IM, Perumal P. Prescribing pattern of analgesics in a tertiary care hospital. Int. J. Pharm Tech Res. 2011;3(3):1521-1529.

30. Dawson KP, McIlvenny S, Harron DWG. Paracetamol prescribing- an epidemic? Family Practice. 1996;13:179-181.

31. Kazouini A, Mohammed BS, Simpson CR, Helms PJ, McLay JS. Paracetamol prescribing in primary care: Too little and too much? Br J Clin Pharmacol. 2011; 72(3):500-504.

32. Gunnell G, Hawton K, Murray V, Garnier R, Bismuth C, Fagg J, Simkin S. Use of paracetamol for suicide and non-fatal poisoning in the UK and France: Are restrictions on availability justified? J Epidemiol Community Health. 1997;51: 175-179.

33. Prescott LF. Paracetamol overdosage pharmacological considerations and clinical management. Drugs. 1993;25(3): 290-314.

34. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: Pathophysiology, clinical manifestations and management. J Med Toxicol. 2008;4(1):2-6.

35. Zhao YL, Zhou GD, Yang HB, Wang JB, Shang LM, Li RS. Rhein protects against acetaminophen-induced hepatic and renal toxicity. Food and Chemical Toxicology. 2011;49:1705-1710.

36. Pratt DS, Kaplan MM. Evaluation of liver functions. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, et al. (eds.) Harrison's Principles of Internal medicine, 18th edition. New York: McGraw Hill. 2012;2527-31.

37. Corcoran GB, Racz WJ, Smith CV, Mitchell JR. Effects of n-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. J Pharmacol Exp Ther. 1985;232(3):864–872.

38. Gardner CR, Laskin JD, Dambach DM, Sacco M, Durham SK, Bruno MK, et al. Reduced hepatotoxicity of acetaminophen in mice lacking inducible nitric oxide synthase: Potential role of tumor necrosis factor-alpha and interleukin-10. Toxicology and Applied Pharmacology. 2002;184:27–36.

39. Bessems GJM, Vermeulen NPE. Paracetamol (Acetaminophen) induced toxicity: Molecular and biochemical mechanisms, analogues and protective approaches. Critical Reviews in Toxicology. 2001;31(1):55–138.

40. Abuelghasim AI, Omer EA, Elmahdi B. The effectiveness of *Nigella sativa* against liver damages in rats. Res J Med Plant. 2008; 2(1):43-47.

41. Mollazadeh H, Hosseinzadeh H. The protective effect of *Nigella sativa* against liver injury: A review. Iran J Basic Med Sci. 2014;17(12):958-966.

42. Hamid ZA, Budin SB, Jie NW, Hamid A, Husain K, Mohamed J. Nephroprotective effects of *Zingiber zerumbet* smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats. J Zhejiang Univ Sci B. 2012;13(3): 176–185.

43. Dio K, Ishida K. Diabetes and hypertriglyceridemia modify the mode of acetaminophen induced hepatotoxicity and nephrotoxicity in rats and mice. J Toxicol. Sci. 2009;34(1):1-11.

44. Abdelaziz I, Kandeel M. The protective effects of *Nigella sativa* oil and *Allium sativum* extract on amikacin induced nephrotoxicity. Int J Pharmcol. 2011;7(6): 697-703.

45. Hadjzadeh MAR, Keshavarzi Z, Yazdi SAT, Shirazi MG, Rajaei Z, Rad AK. Effect of alcoholic extract of *Nigella sativa* on cisplatin-induced toxicity in rats. Iranian Journal of Kidney Diseases. 2012;6(2):99-104.