ORIGINAL ARTICLE

Histological Effects of *Nigella Sativa* on Aspirin-Induced Nephrotoxicity in Albino Rats

Sania Asif[1], Sitwat Mudassir[2] and Rabia Sajjad Toor[3]

ABSTRACT

Objective: To determine the histological effects of *Nigella sativa* on kidneys against aspirin-induced nephrotoxicity in albino rats.

Study Design: An experimental study.

Place and Duration of Study: Anatomy Department, University of Health Sciences, Lahore, from January 2014 to December 2015.

Methodology: Thirty-two rats were equally divided into four groups A, B, C and D of eight animals each. Group A (control) was provided with single oral dose of 10 mg/100 gm body weight of 1% methyl cellulose. Group B (experimental group) and C (self recovery group) were provided with single oral dose of 1000 mg/kg aspirin. Group D was provided with 250 mg/kg ethanolic extract of *Nigella sativa* followed by 1000 mg/kg aspirin by oral gavage, after that only extract of *Nigella sativa* was given for seven days. Animals of groups A, C and D were sacrificed on the 8th day of experiment and that of group B on the second day of experiment. Kidneys were excised and fixed in 10% formalin solution. Hematoxylin, eosin and periodic acid Schiff's reagents were used to stain the kidney tissues. Histological slides were prepared to study proximal convoluted tubules.

Results: Histological parameters were normal in control group A. Significant impairment was present in group B. There was no self-recovery in group C. Significant improvement was present in all the parameters of group D.

Conclusion: *Nigella sativa* has protective effect on aspirin-induced nephrotoxicity in rats.

Key Words: *Aspirin, Kidneys, Nigella sativa, Proximal convoluted tubules, Methyl cellulose, Rat model.*

INTRODUCTION

Kidneys play a vital role in the excretion of medicines and are susceptible to different forms of damaging insults.[1] Acetyl salicylic acid is a well-known anti-pyretic, anti-platelet and analgesic agent.[2] It affects kidneys by disturbing the vasomotor functions of glomerular blood vessels and salt preservation.[3] Its anti-inflammatory and analgesic role is different from that of steroids and opiates, respectively.[4] Salicylate medications had been used during 19th century; but they went into disrepute due to their side effects, especially gastric irritation.[5]

Aspirin inhibits cyclooxygenase (COX) enzyme system.[6] Cyclooxygenase-1 is liable for the homeostatic functions while Cyclooxygenase-2 is responsible for the inflammatory processes. By inhibiting the action of COX enzymes, aspirin reduces the production of prostaglandins; which have protective role on gastric lining against hydrochloric acid.[5] Hepatic and renal problems are produced due to its excessive usage.[4]

[1] *Department of Anatomy, King Edward Medical University, Lahore, Pakistan.*

[2] *Department of Anatomy, University College of Medicine and Dentistry (UCMD), Lahore, Pakistan.*

[3] *Department of Anatomy, Sahara Medical College, Mureedkey Road, Narowal, Pakistan.*

Correspondence: Dr. Sania Asif, Department of Anatomy, King Edward Medical University, Lahore, Pakistan.
E-mail: sania_1asif@yahoo.com

Received: December 07, 2017; Accepted: June 22, 2018.

Absorption of aspirin from the stomach and small intestine is *via* diffusion across the intestinal wall in the gastrointestinal mucosa and plasma. It is rapidly hydrolyzed to salicylic acid by the action of estrases within about 1 to 2 hours of its oral or rectal administration. Maximum plasma levels are obtained. It is dispersed throughout the body, having maximum concentrations in the kidney, lungs and heart.[7] Average half-life in the plasma is about 15-20 minutes.[8]

Nigella sativa (NS) is a member of Ranunculaceae family. The flowers of NS plant have a dark blue color.[9] It is commonly known by name of *Kalvanji* and *black cumin*.[8] It has been used as a flavour and food preservative as well as for the treatment of respiratory and gastrointestinal problems.[10] Fixed and essential oils can be extracted from its seeds, which in turn have anti-inflammatory, nephroprotective and antihypertensive effects.[11]

In 1997, flavonoids thymoquinone were isolated from the NS seeds, which were found to have anti-inflammatory and anti-oxidant effects.[12] It is being used for the treatment of cough, asthma, abdominal pain, and diarrhea in traditional medicines.[13]

NS is known to have wide spectrum of activities which includes anti-diabetic, anti-hypertensive, analgesic, anticancer, anti-inflammatory, anti-oxidant, and diuretic.[14] Protective effects of NS against ischemia reperfusion injury of kidney have been proven to be due to its ability to reduce serum urea, creatinine levels, and total

oxidative stress on kidneys.[15] This study was conducted to evaluate the histological effects of NS in proximal convoluted tubules against aspirin-induced nephrotoxicity in albino rats.

METHODOLOGY

Thirty-two female albino rats weighing 175-225 grams obtained from inbred colony of University of Health Sciences were used. The experiment was conducted in the Anatomy Department, University of Health Sciences, Lahore, from January 2014 to December 2015. The Ethical Review Committee considered the ethical aspects of the project and was satisfied with the undertaking of the investigators.

Randomise control study was conducted. Rats were kept in cages, under controlled room temperature (23 ±2ºC), humidity (50 ±5%), and light and dark cycle of 12 hours each. The animals were fed on standard rat diet and water *ad libitum*. The experiment was started after a period of 2-3 days of acclimatisation; all procedures were carried out in standard laboratory conditions. Simple random sampling was done to divide animals into four groups, A, B, C and D of eight rats each. The experiment was of eight days.

The control group A was given 10 mg/100 g body weight of 1% methyl cellulose orally as a single dose and sacrificed on the 8th day of experiment. Group B was given 1000 mg/kg aspirin suspended in 10 mg/100 g body weight of 1% methyl cellulose orally as a single dose and sacrificed on the second day of experiment. Recovery group C was given 1000 mg/kg aspirin suspended in 10 mg/100 g body weight of 1% methyl cellulose orally as a single dose and sacrificed on the 8th day of experiment. Group D was given 250 mg/kg NS extract (NSE) and 1 hour later 1000 mg/kg of single dose of aspirin orally, then NSE was given till the 7th day and sacrificed on the 8th day of experiment.

After completely anesthetising the animal, it was placed on the dissection board with paws fixed by using thumb pins. Ten percent buffered formalin was prepared and put in labelled containers before starting the dissection. A midline incision was made from manubrium sterni to pubic symphysis, incising skin, muscles and bisecting sternum. Additional transverse incision was made midway between xiphoid process and pubic symphysis, incising skin and muscle. Four flaps were reflected and clipped to dissection board with thumb pins, exposing the heart and abdominal viscera. Kidneys were then identified, removed and then washed with normal saline and cut with knife into 3 to 5 millimeter cube sized pieces, which were then placed for fixation in 10% buffered formalin solution for 48 hours.

Both transverse and longitudinal sections of kidney tissue pieces were placed in single plastic tissue cassette, labelled properly and processed in an automatic tissue processor (Histotech III-USA) for processing. The tissue cassettes were then shifted to embedding station (Model: SM C1-044-SO). Parrafin blocks were prepared by placing the tissue pieces in a metallic trough, having molten paraffin. Then the labelled plastic tissue cassette, with lid removed, was placed on the metallic trough and paraffin was poured to the brim and shifted immediately to cold plate and allowed to solidify and then stored in refrigerator.

Hematoxylin and Eosin (H&E) staining was used to study tissue at the histological level. For the glycogen accumulation and basement membrane integrity, periodic acid Schiff's (PAS) staining technique was used.

Slides were randomly selected from each animal and were studied under a light microscope (Leica, DM 1000) at X400 magnification. Tubular parameters for proximal convoluted tubules (PCT) were determined by using three slides from each animal. Tubules were selected from five randomly selected non-overlapping fields from cortical part of each section for studying disrupted brush border, epithelial necrosis, intraluminal protein casts and broken basement membrane.

The data was entered and analysed using SPSS 20.0. Frequencies and percentages were given for qualitative variables like basement membrane, brush border, epithelial necrosis, and intraluminal protein casts. Pearson Chi-square / Fisher exact test was applied to find association between groups and histological parameters. P-value of <0.05 was considered as statistically significant.

RESULTS

The rats were observed daily for assessment of health and growth, and they were found to be healthy during the entire period of experiment.

In PCT disrupted brush border, epithelial necrosis, intraluminal protein casts, and basement membrane

Table I: Comparison of percentage of histological parameters among different groups.

Parameters	Group A n=8 (%)		Group B n=8 (%)		Group C n=8 (%)		Group D n=8 (%)		Fisher exact test	p-value
	Present	Absent	Present	Absent	Present	Absent	Present	Absent		
Disrupted brush border	0.00 (0%)	8.00 (100%)	8.00 (100%)	0.00 (0%)	6.00 (75.00%)	2.00 (25.00%)	1.00 (12.50%)	7.00 (87.50%)	23.28	<0.001
Epithelial necrosis	1.00 (12.50%)	7.00 (87.50%)	8.00 (100%)	0.00 (0%)	7.00 (87.50%)	1.00 (12.50%)	2.00 (25%)	6.00 (75%)	18.80	<0.001
Intraluminal protein casts	1.00 (12.50%)	7.00 (87.50%)	7.00 (87.50%)	1.00 (12.50%)	7.00 (87.50%)	1.00 (12.50%)	0.00 (0%)	7.00 (87.50%)	21.63	<0.001
Broken basement membrane	0.00 (0%)	8.00 (100%)	7.00 (87.50%)	1.00 (12.50%)	7.00 (87.50%)	1.00 (12.50%)	1.00 (12.50%)	7.00 (87.50%)	21.63	<0.001

**p<0.05is considered statistically significant.*

integrity were calculated in terms of percentages with significant results ($p \leq 0.001$, Table I). Haematoxylene, eosin and periodic acid Schiff's staining showed statistically significant impairment in these parameters of groups B and C ($p \leq 0.001$). These parameters were normal in group A. Group D showed significant improvement in these parameters ($p \leq 0.001$). The histological appearances are shown in Figures 1a-h.

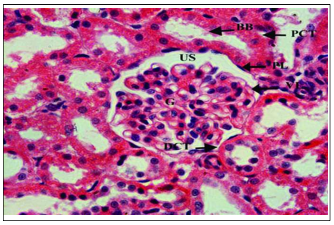

Figure 1-a: (Group A): Photomicrograph showing cortical part of the kidney of control group A. Proximal convoluted tubules (PCT) are with intact brush border (BB). Normal distal convoluted tubules (DCT), parietal layer (PL), visceral layer (VL),urinary space (US) and glomerulus (G) are also shown. H & E stain X400.

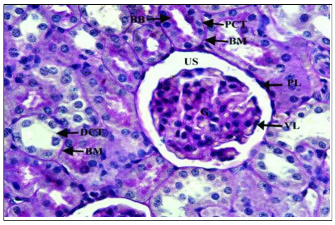

Figure 1-b: (Group A): Photomicrograph showing cortical part of the kidney of control group A. PCT are with intact brush border (BB) and basement membrane (BM). PAS stain X400.

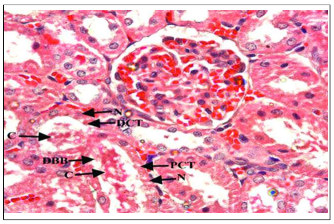

Figure 1-c: (Group B): PCT are with disrupted brush border (DBB), epithelial necrosis (N) and intraluminal protein casts (C). H & E stain X400.

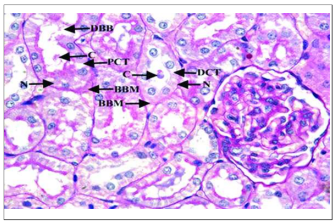

Figure 1-d: (Group B): PCT are with disrupted brush border (DBB), broken basement membrane (BBM) and intraluminal protein cast (C). PAS stain X400.

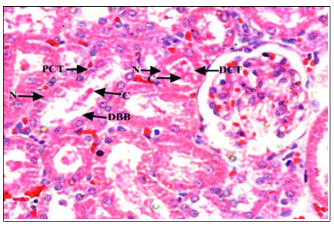

Figure 1-e: (Group C): PCT are with disrupted brush border (DBB), epithelial necrosis (N) and intraluminal protein casts (C). H & E stain X400.

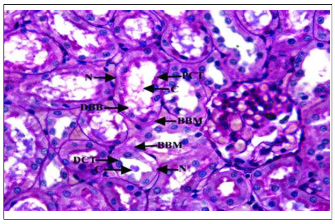

Figure 1-f: (Group C):PCT are with disrupted brush border (DBB), epithelial necrosis (N) intraluminal protein casts (C) and broken basement membrane (BBM). PAS stain X400.

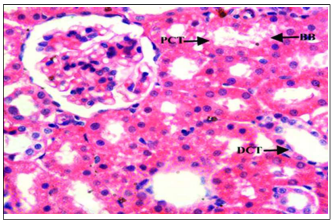

Figure 1-g: (Group D): PCT are with intact brush border (BB). H & E stain. X400.

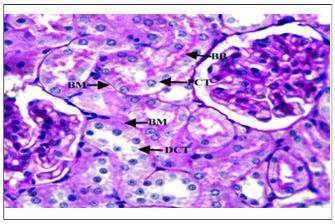

Figure 1-h: (Group D): PCT are with intact brush border (BB) and basement membrane (BM). PAS stain. X400.

DISCUSSION

The role of *Nigella sativa* (NS) as a medicinal herb is well documented. Alemi *et al.* described that the anti-inflammatory and antioxidant role of NS was due to its thymoquinone content.[16] In the present study, its protective role on aspirin-induced nephrotoxicity was studied by evaluating histological parameters of proximal convoluted tubule (PCT) like disrupted brush border, epithelial necrosis, intraluminal protein casts, and broken basement membrane.

In PCT of groups B and C, significantly impaired histological changes in brush border, epithelial necrosis, intraluminal protein casts and basement membrane were present (Figures 1c-f); these were normal in group A (Figure a and b, Table I). These changes were similar to those reported by Owen and Heywood.[17] Aspirin is known to be responsible for the leakage of fluid from the damaged tubules, thus producing inflammatory reaction.[18] Aspirin decreases the synthesis of prostaglandins which are required for the protection of gastric lining from the harmful effects of acid.[5] That is why the disrupted brush border, intraluminal protein casts, broken basement membrane and epithelial necrosis were seen in aspirin treated groups B and C.

In group D, statistically significant improvement was present in all these parameters showing protective role of NS (Figures 1-g, 1-h, Table I). No abnormal histological changes were observed in a study when NS was given to rats for a period of five weeks.[19] The ethanolic extract of NS has free radical scavenging effects.[20] Essential fatty acids also act against free radicals, as NS contains almost 50% essential fatty acids. Thus it has free radical scavenging activity.[21] This proved protective effects of NS on aspirin-induced abnormal histological findings on the cortical part of kidney.

When all the four groups were compared, it was found that PCT of group A had an intact basement membrane and brush border. In experimental group B, broken basement, intraluminal protein casts, and epithelial necrosis were observed. In the recovery group C, there was no self-recovery. In the protective group D, significant improvement in all the histological parameters of PCT was present. As aspirin was responsible for the inflammatory effects and NS has anti-inflammatory and anti-oxidant effects.,[16] this proved the protective effects of NS on aspirin-induced nephrotoxicity in rats.

The main limitation of this study was micrometry due to limited time. Next study will focus on glomerular diameter and urinary space in similar circumstances.

CONCLUSION

Aspirin produced marked impairment in histological parameters of groups B and C. There was no statistically significant self-recovery in group C. There was statistically

significant improvement in all the parameters of group D. Thus, it was concluded that NS has protective effect on aspirin-induced nephrotoxicity in rats.

REFERENCES

1. Perazella MA. Renal vulnerability to drug toxicity. *Clin J Am Soc Nephrol* 2009; **4**:1275-83.
2. Al Janabi AS, A Izohyri AM. Al-Rubayai FK. Pharmacological effects of low-dose of aspirin on corpus luteum functions in mature cycling female mice. *Middle East Fertil Soc J* 2005; **10**:150-62.
3. Nwanjo HU, Okolie NJC, Oze G, Okafor MC, Nwosu D, Anyaehie CAB, *et al.* Changes in some biochemical parameters of kidney functions in rats co-administered with chloroquine and aspirin. *Res J Med Sci* 2007; **1**:106-9.
4. Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res* 2003; **110**:255-8.
5. Fuster V, Sweeny JM. A historical and contemporary therapeutic overview. *Am Heart Assoc* 2011; **123**:768-78.
6. Katzung BG. Non-steroidal anti-inflammatory drugs. Basic and clinical pharmacology. 10th ed, San Francisco: McGraw Hill; 2006.
7. Marica LB. Use of aspirin in children with cardiac disease. *Pediatr Pharmacother* 2007; **13**:2.
8. Patron C, Rodgriguez LAG, Landolfi R, Baigent C. Low dose aspirin for the prevention of atherothrombosis. *New Eng J Med* 2005; **353**:2373-83.
9. Jain N, Shrivastava R, Raghuwanshi AK, Shrivastava VK. Aspirin-induced changes in serum ACP, ALP, GOT, GPT, bilirubin and creatinine in correlation with histopathological changes in liver and kidney of female albino rat. *International J Appl Pharmaceut* 2012; **4**:9-11.
10. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seeds. *Int Immunopharmacol* 2005; **5**: 1749-70.
11. Ahmad A, Husain 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**:337-52.
12. Merfort I, Wray V, Barakat HH, Hussein SAM, Nawwar MAM, Willuhn G. Flavonol triglycosides from seeds of *Nigella sativa*. *Phytochemistry* 1997; **2**:359-63.
13. Alhaj NA, Shamsudin MN, Alipiah NM, Zamri HF, Bustamam A, Ibrahim S. *et al.* Characterisation of *Nigella sativa* L. essential oil- loaded solid lipid nanoparticles. *Am J Pharmacol Toxicol* 2010; **5**:52-7.
14. Ahmad A, Husain 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**:337-52.
15. Yildiz F, Coban S, Terzi A, Savas M, Bitiren M, Celik H, *et al.* Protective effects of *Nigella sativa* against ischemia reperfusion injury of kidney. *Ren Fail* 2010; **32**:126-31.
16. Alemi M, Sabouni F, Sanjarian F, Haghbeen K, Ansari S. Anti-inflammatory effect of seeds and callus of *Nigella sativa* L. Extracts on mix glial cells with regard to their thymoquinone content. *Am Assoc Pharmaceut Sci* 2013; **14**:160-7.
17. Owen RA, Heywood R. Age-related susceptibility to aspirin-induced nephrotoxicity in female rats. *Toxicol Lett* 1983; **18**: 167-70.
18. Robbins and Cotran's. Pathologic basis of diseases. 8th ed, Philadelphia: Saunders; 2009.
19. Dollah MA, Parhizkar S, Izwan M. Effect of *Nigella sativa* on the kidney function in rats. *Avicenna J Phytomed* 2013; **3**: 152-8.
20. Majeed N, Tahir M. Effect of *Nigella sativa* extract on renal functions in amphotericin b induced nephrotoxicity in mice. *Biomedica.* 2014; **30**:1.
21. Mahmood T, Faizy FA, Sharma P, Jawad K. Free radical scavenging and cyto-protective activity of ethanolic extract of *Nigella sativa* Seeds. *Int Arch BioMediClin Res* 2016; **2**:51-4.