ORIGINAL ARTICLE

MITIGATIVE EFFECTS OF NIGELLA SATIVA ON THE HISTOLOGY OF KIDNEYS AGAINST ASPIRIN-INDUCED TOXICITY

Sania Asif[1], Rabia Sajjad Toor[2], Sitwat Amna[3], Areeba Haider[1], Sarah Khan[1]

[1]Department of Anatomy, King Edward Medical University, Lahore, [2]Department of Anatomy, Sahara Medical College, Narowal, [3]Department of Anatomy, Allama Iqbal Medical College, Lahore-Pakistan

Background: Aspirin is one of the popular, economical, easily accessible and commonly used drugs all over the world. Injudicious use of this over-the-counter available drug is a common cause of nephrotoxicity. The aim of the present study is to assess the protective effects of Nigella Sativa (NS) on the histology of kidneys against aspirin-induced toxicity. It was an experimental study that included comparative analysis of control and experimental groups, conducted in the department of Anatomy, University of Health Sciences, Lahore, from October 2016 to December 2016. **Methods:** The study included thirty-two female albino rats which were equally distributed into 4 groups. Group A was run as control and given single oral dose of 1% methyl-cellulose (10mg/100gm body weight of rat). Group B and **C** were treated with a single oral dose of aspirin (1000mg/kg body weight) dissolved in 1% methyl-cellulose (10mg/100gm body weight). Group C animals were left untreated for 7 days. Group D was pre-treated on day 1 with oral dose of Nigella Sativa (NS) extract (250mg/kg body weight) followed one hour later by a single oral dose of aspirin (1000mg/kg body weight), subsequently NS extract was administered till day 7. Rats of group B were euthanized and dissected on 2nd day of experiment while those of groups A, C and D on 8th day. Kidneys were dissected out, weighed and fixed in 10% formalin. 5µm thick sections were yielded after tissue processing and stained with haematoxylin, eosin (H&E staining) and periodic acid Schiff's reagent (PAS staining). Histological parameters of distal convoluted tubules (DCT) were observed. **Results:** All histological parameters were normal in group A. Group B showed marked increase in epithelial necrosis, intraluminal protein casts and broken basement membranes of distal convoluted tubules. Group C showed no self-recovery. Statistically significant improvement was observed in the histology of distal convoluted tubules with treatment of Nigella Sativa extract in aspirin-ingested rats in group D. **Conclusion:** Nigella Sativa extract has shown protective effects on kidneys against aspirin-induced damage as shown by improvement in the histological parameters of distal convoluted tubules.

Keywords: Kidneys; Nigella Sativa; Aspirin; Distal convoluted tubules; Methyl-cellulose

Citation: Asif S, Toor RS, Amna S, Haider A, Khan S. Mitigative Effects of Nigella Sativa on the Histology of Kidneys against Aspirin-Induced Toxicity. J Ayub Med Coll Abbottabad 2021;33(1):39–43.

INTRODUCTION

Acetyl salicylic acid, also known as Aspirin, is one of the oldest and most widely used drugs in medicine.[1] Aspirin is a type of non-steroidal anti-inflammatory drugs (NSAIDs) used for pain, fever and inflammation at high doses up to 1g.[2] The anti-inflammatory and antithrombotic effects of salicylic acid have COX (cyclooxygenase enzymes 1 & 2)-dependent mechanisms leading to irreversible inhibition of arachidonic acid and prostaglandin synthesis pathways.[3] Owing to its anti-thrombotic effects, it is now frequently recommended in low doses and life-long therapies for patients with cardiovascular diseases (CVD) or those at high risk of developing the diseases.[4] Data also shows its use in cancer prevention.[5] A study shows that in some cases aspirin is over-used by a number of patients who are recommended its use for primary and secondary prevention of cardiovascular events.[6] Injudicious use of aspirin is a common cause of hepatic and renal toxicity as well as damage to gastrointestinal mucosa leading to ulcers and bleeding.[7] Mechanisms that produce drug-induced nephrotoxicity include changed hemodynamic, tubulonephritis and inflammation; persons with risk factors such as old age are more susceptible to drug induced renal failure.[8] Other than these mechanisms, aspirin-induced renal and hepatic damage has also been related to increased oxidative stress; addition of anti-oxidants have shown improvement and reversal of these deteriorative effects.[9]

Nigella Sativa, also known as 'black seed', is an annual herb belonging to the family Ranunculaceaeis. It has been a part of the aboriginal medicaments used over centuries worldwide.[10] It has also been widely used as a spice for seasoning and food additive especially in Mediterranean and Middle East areas.[11] NS seeds and oil have a wide spectrum of beneficial effects including anticancer, analgesic,

anti-inflammatory, hepato-protective, reno-protective and antioxidant properties mostly attributed to the presence of a bioactive component thymoquinone, other than the small amounts of other beneficial components like flavonoids, saponins etc.[12,13] Nigella sativa extract has shown promising result in protecting renal tissue under conditions of high oxidative stress and also is as potent in significantly reversing the state as the known antioxidants like ascorbic acid.[14] As increasing oxidative stress is one of the major tissue injury mechanism associated with aspirin, this study has been conducted to assess the effect of Nigella Sativa extract on the histology of kidneys in aspirin-induced toxicity.

MATERIAL AND METHODS

The study included thirty-two female Wistar albino rats of age 6–8 weeks and weight 175–225 gm., taken from the inbred colony of University of Health Sciences, Lahore (UHS). The study was conducted in Anatomy department, UHS after approval from Ethical Review Committee. Rats were randomly divided into four groups A, B, C and D of eight rats each by using balloting method (randomized control study). The rats were kept in four separate cages for each group and permitted to adapt for a week before the start of experiment. Free access of food and water was provided at room temperature ($24^{o}\pm5^{o}$), humidity (45%±5%) and a light and dark cycle of 12 hours.

Aspirin was acquired in powder form from Sigma Aldrich USA and suspension in 1% methylcellulose was prepared as stock solution.

Nigella sativa seed, obtained from a local store, were washed, dried, crushed and soaked in ethanol for 4 days. The seeds were filtered and alcohol was evaporated from the filtrate using a rotary evaporator. NSE was stored in refrigerator till use.

All rats of the four groups were treated at 9 am every day. Group A was used as control. The rats were administered a single oral dose of 1% methylcellulose (10mg/ 100gm body weight) and sacrificed on the 8^{th} day of experiment. Group B (Aspirin only) was given a single oral dose of Aspirin (1gm/ kg body weight) as a suspension in 1% methylcellulose (10mg/ 100gm body weight) and sacrificed on the 2^{nd} day. Group C (recovery) was given a single oral dose of Aspirin (1gm/kg) and sacrificed on the 8^{th} day. Group D (Aspirin + NSE) was pre-treated with oral dose of NSE (250mg/ kg body weight) on the first day followed after one hour by administration of oral dose of Aspirin (1gm/ kg body weight). Same dose of NSE was administered orally till the 7^{th} day and rats were sacrificed on the 8^{th} day of experiment. The rats were euthanized and pinned to the dissection board. Abdomen was opened via a midline vertical incision and then a transverse incision to expose the kidneys. Kidneys were dissected out, weighed, cut into pieces (3–5 mm^3) and placed in 10% buffered formalin-filled containers. After tissue processing, 4–5µm thick sections were yielded and stained with haematoxylin & eosin (H&E staining) and periodic acid Schiff reagent (PAS staining). Three randomly selected slides were used from each rat and studied under light microscope (Leica, DM 1000) at the X400 magnification. Five non-overlapping fields from each section were observed for epithelial necrosis and intraluminal protein casts in distal convoluted tubules (DCT) in H&E stained slides. Basement membranes of DCT were observed in three randomly selected PAS stained slides of each rat in five non-overlapping fields. The data was analysed in SPSS 20.0. Fisher exact was used to analyse the epithelial necrosis, intraluminal protein casts and basement membranes of distal convoluted tubules. Percentages and frequencies were given for these qualitative parameters.

RESULTS

In the present study, rats of all four groups showed normal appetite, behaviour and remained healthy till the completion of experiment. Epithelial necrosis was calculated in terms of percentages and difference was statistically significant (p-value <0.001). Tissues of groups A, B, C and D showed 12.5%, 100%, 87.5% and 25% epithelial necrosis respectively. Absence of it in groups A, B, C and D was calculated to be 87.5%, 0%, 12.5% and 75% respectively (Table 1). Group C was comparable with group A in which 7 animals had necrosis. Microscopically it was present in all the 8 animals of group B (Figure-3). Percentages of intraluminal protein casts in DCT of renal tissue of groups A, B, C and D were calculated to be 12.5%, 87.5%, 75% and 12.5% in respectively and results showed that the difference was statistically significant (p-value<0.001). Absent intraluminal casts were calculated to be 87.5%, 12.5%, 25% and 87.5% in groups A, B, C and D respectively (Table-2). Groups A and D were comparable with group B showing no casts in 7 animals of these two groups. Histologically, in group B 7 animals showed intraluminal protein casts while in group D only 1 animal showed casts (Figure 3 & 4). Observations showing breach in BM were recorded and percentages were calculated. Results showed that there was significant difference (p-value<0.001) among groups. In groups A, B, C and D presence of broken basement membrane was calculated to be 0%, 87.5%, 75% and 0 respectively. Its absence in groups A, B, C and D was 100%, 12.5%, 25% and 100% respectively (Table-3). Group D had significant improvement and was comparable with group A in having all the 8 animals with intact and normal looking basement membrane. Microscopic picture of these two groups had normal intact basement membrane (Figure 2 & 8).

Table-1: Comparison of percentage of epithelial necrosis in DCT among different groups.

Epithelial necrosis	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	Total
Present	1.00 (12.5)	8.00 (100)	7.00 (87.5)	2.00 (25)	18.00 (56.2)
Absent	7.00 (87.5)	0.00 (0)	1.00 (12.5)	6.00 (75)	14.00 (43.8)
Total	8.00 (100)	8.00 (100)	8.00 (100)	8.00 (100)	32.00 (100)
Fisher exact test = 18.80			***p*-value**<0.001*		**n=8**

*$p \leq 0.05$was considered statistically significant.

Table-2: Comparison of percentage of Intraluminal protein casts in DCT among different groups

Intraluminal protein casts	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	Total
Present	1.00 (12.5)	7.00 (87.5)	6.00 (75)	1.00 (12.5)	15.00 (46.9)
Absent	7.00 (87.5)	1.00 (12.5)	2.00 (25)	7.00 (87.5)	17.00 (53.1)
Total	8.00 (100)	8.00 (100)	8.00 (100)	8.00 (100)	32.00 (100)
Fisher exact test =14.97.		**n=8.**		***p*-value** <0.001*	

*$p \leq 0.05$was considered statistically significant.

Table-3: Comparison of percentage of broken basement membrane among different groups.

Broken basement membrane	Group A n (%)	Group B n (%)	Group C n (%)	Group D n (%)	Total
Present	0.00 (0)	7.00 (87.5)	6.00 (75)	0.00 (0)	13.00 (40.6)
Absent	8.00 (100)	1.00 (12.5)	2.00 (25)	8.00 (100)	19.00 (59.4)
Total	8.00 (100)	8.00 (100)	8.00 (100)	8.00 (100)	32.00 (100)
Fisher exact test= 22.40.		**n=8**	***p*-value**	<0.001*	

*$p \leq 0.05$was considered statistically significant.

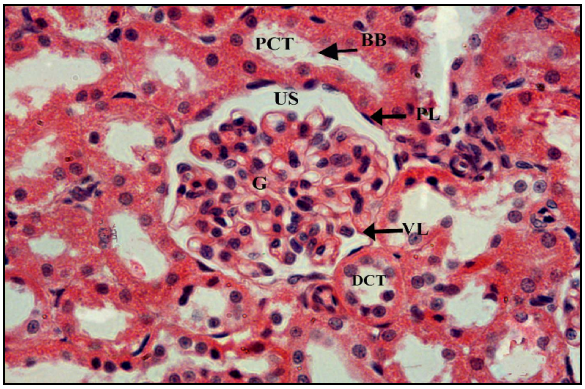

Figure-1: Photomicrograph showing cortical part of the kidney of control group A. Normal distal convoluted tubules (DCT), parietal layer (PL), visceral layer (VL), urinary space (US) and glomerulus (G) are also shown. H & E stain. X400.

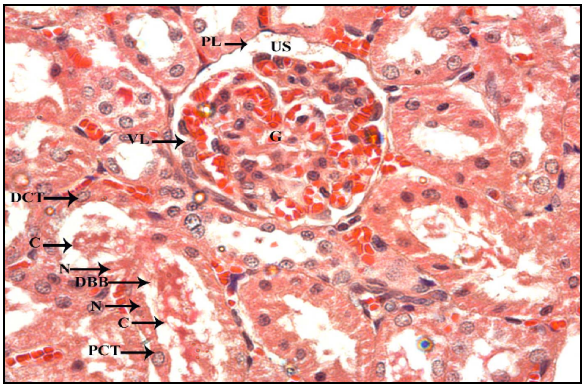

Figure-3: Photomicrograph showing cortical part of the kidney of experimental group B. Distal convoluted tubules (DCT) are with epithelial necrosis (N) and intraluminal protein casts (C). Parietal layer (PL), visceral layer (VL) and urinary space (US) are normal. There is haemorrhage in the glomerulus (G). H & E stain. X400.

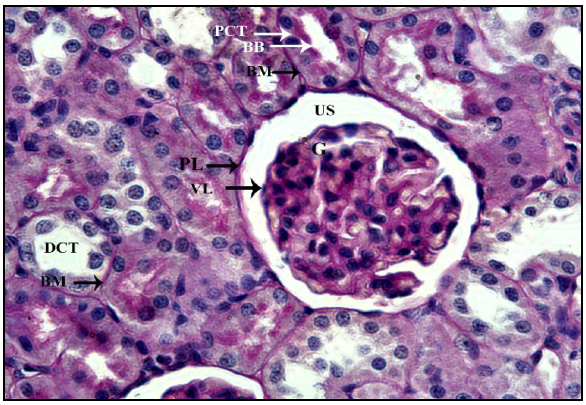

Figure-2: Photomicrograph showing cortical part of the kidney of control group A. Distal convoluted tubules (DCT) are also with intact basement membrane (BM). Normally looking parietal layer (PL), visceral layer (VL) urinary space (US) and glomerulus (G) are also shown. PAS stain. X400.

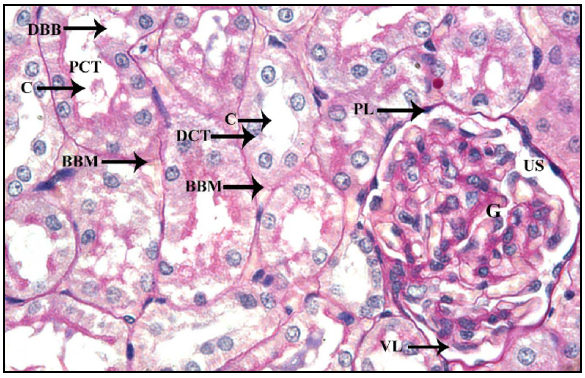

Figure-4: Photomicrograph showing cortical part of the kidney of experimental group B. Distal convoluted tubules (DCT) are also with broken basement membrane (BBM) and intraluminal protein cast (C). Normally looking parietal layer (PL), visceral layer (VL), urinary space (US) and glomerulus (G) are also shown. PAS stain. X400.

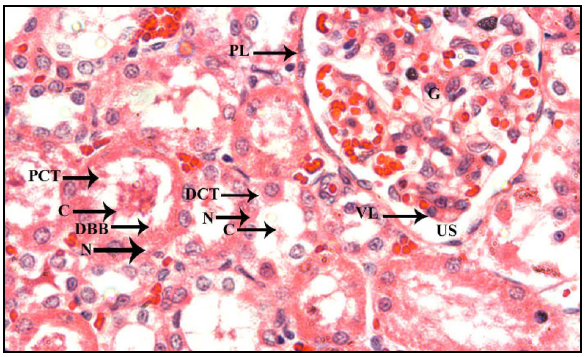

Figure-5: Photomicrograph showing cortical part of the kidney of experimental group C. Distal convoluted tubules (DCT) are with epithelial necrosis (N) and intraluminal protein casts (C). Disrupted parietal layer (PL) and visceral layer (VL) are shown. Urinary space (US) is normal. Glomerulus (G) has haemorrhage. H & E stain. X400.

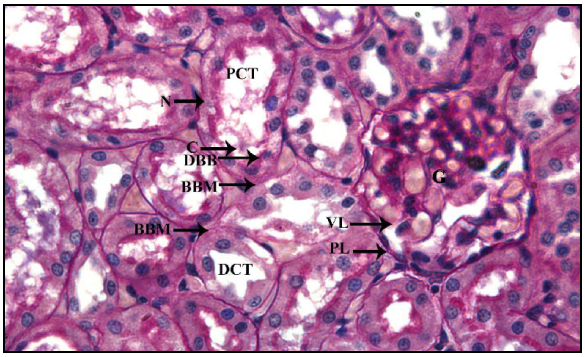

Figure-6: Photomicrograph showing cortical part of the kidney of experimental group C. Distal convoluted tubules (DCT) are with epithelial necrosis (N) intraluminal protein casts (C) and broken basement membrane (BBM). Parietal layer (PL), visceral layer (VL) and glomerulus (G) are also shown. PAS stain. X400.

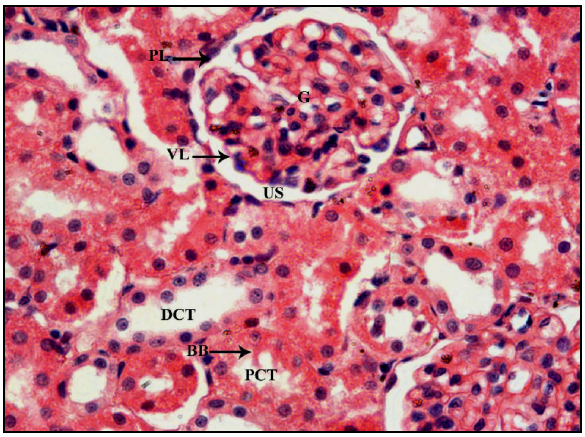

Figure-7: Photomicrograph showing cortical part of the kidney of protective group D. Normally looking distal convoluted tubules (DCT), parietal layer (PL), visceral layer (VL), urinary space (US) and glomerulus (G) are also shown. H & E stain. X400.

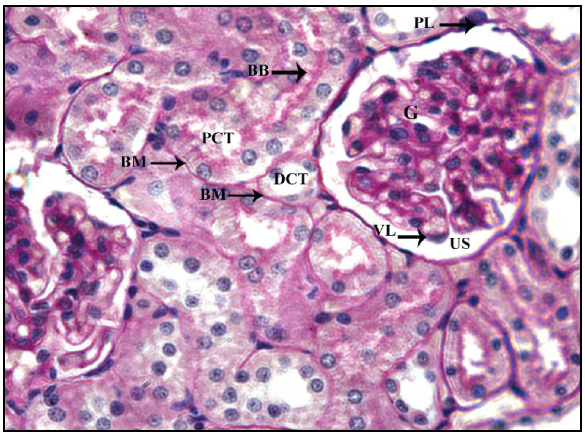

Figure-8: Photomicrograph showing cortical part of the kidney of protective group D. Distal convoluted tubules (DCT) are also with intact basement membrane (BM). Normally looking parietal layer (PL), visceral layer (VL), urinary space (US) and glomerulus (G) are also shown. PAS stain. X400.

DISCUSSION

Aspirin is an over-the-counter use non-steroidal anti-inflammatory drug which is more extensively used when compared to other drugs.[15] It is one of the key drugs used in primary as well as secondary prevention of Stroke and Myocardial Infarction.[16] Its use have been associated with gastrointestinal bleeding and haemorrhagic shock.[17] Aspirin and other NSAIDs have also been linked with nephrotoxicity.[18] Increased oxidative stress is an important mechanism by which Aspirin causes deteriorative effects on renal tissue,[9] therefore, adding a potent antioxidant like NS may prove beneficial.

In the present study, DCT of group A showed no epithelial necrosis, intraluminal protein casts and broken basement membrane. The work of Boon *et al*. on methylcellulose showed similar findings.[19] In group B and C, statistically significant increase in epithelial necrosis, intraluminal protein casts and broken basement membranes was recorded. In a study, Jain et al. used 100mg/kg aspirin for 15 days and found degenerated and atrophic tubules in cortical part of kidneys of female albino rats.[20] Inhibition of cyclooxygenase and decrease in prostaglandin levels in renal tissue causes vasoconstriction and decreased blood flow in kidneys, which is probably the main mechanism of the damage to the tissue.[21]

In group D, statistically significant improvement was present in all the parameters of DCT showing protective role of NS. NS (100 and 200 mg/kg body weight) showed protective effects when given as pre-treatment for 5 days and co-treatment on 6th day to rats against cisplatin-induced nephrotoxicity.[22] Study of Havakhah and co-workers showed that alcoholic extract of NS (150 and 300 mg/kg of body weight) given to rats

in preventive and treatment groups not only decreased oxidative stress in renal tissue as determined by the markers but also protected the tissue morphology and DNA structure.[23] The results of this study demonstrated the protective effect of the NS extract against Aspirin-induced nephrotoxicity.

CONCLUSION

The results in this study indicate that Nigella sativa have a protective role in kidneys. Its administration along with a known nephrotoxic drug aspirin improved the histological parameters of DCT in kidneys. Further studies in long term treatments and on genetic levels can unlock more mechanisms behind its beneficial effects. Clinical trials may further assess the effect of adding NS as an adjuvant therapy to patients on aspirin treatment.

AUTHORS' CONTRIBUTION

SA: Conceptualization of study design and methodology. SA & RST: Data collection, analysis and results. RST: contribution in literature review. SM: Contribution in discussion and review of references (citation). AH: Revision of draft according to JAMC criteria. SK: Proof reading and approval for submission.

REFERENCES

1. Fuster V, Sweemy JM. Apirin, a historical and contemporary therapeutic overview. Circulation 2011; 123(7):768–78. https://doi.org/10.1161/CIRCULATIONAHA.110.963843
2. Zhou Y, Boudreau DM, Freedman AN. Trends in the use of aspirin and non-steroidal anti-inflammatory drugs in general U.S. population. Pharmacol Drug saf 2013;23(1):43–50.
3. Milland MA, Hernandez-vila EA. What do the guidelines really say about aspirin? Tex Heart Inst J 2018;45(4):228–30.
4. Ittaman SV, VanWormer JJ, Rezkalla SH. The role of aspirin in the prevention of cardiovascular disease. Clin Med Res 2014;12(3-4):147–54.
5. Chubak J, Whitlock EP, Williams SB, Kamineni A, Burda BV, Buist DS, *et al*. Aspirin for the prevention of cancer incidence and mortality: systemic evidence reviews for the U.S. Preventive Services Task Force. Ann Intern Med 2016;164(12):814–25.
6. Vanwormer JJ, Greenlee RT, McBride PE, Peppard PE, Malecki KC, Che J, *et al*. Aspirin for primary prevention of CVD: are the right people using it? J Fam Pract 2012;61(9):525–33.
7. Bozimowski G. A review of non-steroidal anti-inflammatory drugs. AANA J 2015;83(6):425–33.
8. Shahbazi F, Khavidaki SD, Khalili H, Pezeshki ML. Potential renoprotective effects of silymarin against nephrotoxic drugs: a review of literature. J Pharm Pharm Sci 2012;15(1):112–23.
9. Bouzema H, Dhibi S, Samout N, Rjeibin I, Talamin H, Elfeki A, *et al*. The protective effect of citrus limon essential oil on hepatotoxicity and nephrotoxicity induced by aspirin. J Biomed Pharmacother 2016;83:1327–34.
10. 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(5):337–52.
11. Amin B, Hosseinzadeh H. Black cumin (nigella sativa) and its active constituent, thymoquinone: an overview on the analgesic and anti-inflammatory effects. Planta Med 2016;82(1-2):8–16.
12. Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H. Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone. J Pharmacopuncture 2017;20(3):179–93.
13. Heiss AG, Stika HP, DeZorzi N, Jursa M. Nigella in the mirror of time a brief attempt to draw a genus' ethnohistorical portrait. Offa 2012;69(70):147–69.
14. Beheshti F, Norouzi F, Abareshi A, Khazaei M, Alikhani V, Moussavi S, *et al*. Nigella Sativa prevented liver and renal tissue damage in lipopolysaccharide-treated rats. Saudi J Kidney Dis Transpl 2018;29(2):554–66.
15. O'Brien CW, Juraschek SP, Wee CC. Prevalence of aspirin use for primary prevention of cardiovascular disease in the united states: results from the 2017 national health interview survey. Ann Int Med 2019;171(8):596–98. Doi: 10.7326/M19-0953.
16. Gaziano JM, Brotons C, Coppolecchia R, Cricelli C, Darius H, Gorelick PB, *et al*. Use of aspirin to reduce the risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomized double-blind, placebo-controlled trial. Lancet 2018;392(10152):1036–46.
17. Chubak J, Whitlock EP, Williams SB, Kamineni A, Burda BU, Buist DS, *et al*. Aspirin for the prevention of cancer incidence and mortality: systemic evidence reviews of the U.S. Preventive Services Task Force. Ann Intern Med 2016;164(12):814–25.
18. Khalil MAM, Khalil MSUD, Khamis SSA, Alam S, Daiwajna RG, Rajput AS, *et al*. Pros and cons of aspirin prophylaxis for prevention of cardiovascular events in kidney transplant and review of evidence. Adv Prev Med 2019;2019:e6139253.
19. Boon AC, Lam AK, Gopalan V, Benzie IF, Briskey D, Coombes JS, *et al*. Endogenous elevated bilirubin modulates kidney function & protection from circulating oxidative stress in a rat model of adenine induced kidney failure. Sci Rep 2015;5:15482.
20. Jain NE, Shrivastava RE, 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. Int J Appl Pharm 2012;4(3):9–11.
21. Okada S, Morimoto T, Ogawa H, Mio S, Soejima H, Nakayama M, *et al*. Is long term low dose aspirin therapy associated with renal dysfunction in patients with type 2 diabetes? JPAP2 cohort study. J PLoS One 2016;11(1):e0147635.
22. Hosseinian S, Rad KA, Hadizadeh MAR, Roshan NM, Havakhah S, Shafiee S. The protective effect of Nigella sativa against cisplatin-induced nephrotoxicity in rats. Avicenna J Phytomed 2016;6(1):44–54.
23. Havakhah S, Sadeghnia HR, Hajzadeh MR, Mohammadian N, Safiee S, Hosseinzadeh H, *et al*. Effect of nigella sativa on ischemia-reperfusion induced rat kidney damage. Iran J Basic Med Sci 2014;17(12):986–92.

Submitted: January 2, 2020	*Revised: March 26, 2020*	*Accepted: May 1, 2020*

Address for Correspondence:

Rabia Sajjad Toor, Department of Anatomy, Sahara Medical College, Narowal-Pakistan
Email: rabiaehsan22@gmail.com