International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(6): 87-99

www.veterinarypaper.com
Received: 05-06-2023
Accepted: 04-07-2023

Sneha A Patel
Department of Veterinary Pathology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Priti D Vihol
Department of Veterinary Pathology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Jignesh M Patel
Department of Veterinary Pathology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Rasesh D Varia
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Hitesh C Parmar
Department of Veterinary Clinical Complex College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Jatin H Patel
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Jitendra K Raval
Livestock Research Station Kamdhenu University, Navsari, Gujarat, India

Parth D Baraiya
Department of Veterinary Pathology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Pranav R Panzade
Department of Veterinary Pathology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Corresponding Author:
Sneha A Patel
Department of Veterinary Pathology, College of Veterinary Science and AH, Kamdhenu University, Navsari, Gujarat, India

Protective effects of hesperidine against organ toxicity and oxidative stress induced by piroxicam in wistar rats

Sneha A Patel, Priti D Vihol, Jignesh M Patel, Rasesh D Varia, Hitesh C Parmar, Jatin H Patel, Jitendra K Raval, Parth D Baraiya and Pranav R Panzade

DOI: https://doi.org/10.22271/veterinary.2023.v8.i6b.817

Abstract
The present study was carried out to evaluate protective effects of flavonoid Hesperidine against pathological effects and oxidative stress induced by Piroxicam. Forty Wistar rats were divided into five groups namely vehicle control, low dose toxicity control, high dose toxicity control, protective group for low dose toxicity and protective group for high dose toxicity. At the end of an experiment, gross and microscopic examination of liver, kidney, intestine and stomach was done and oxidative stress was also measured. In piroxicam high dose group, gross examination of liver, kidney, stomach and intestine released inflammation, gastric erosions and ulcers, intestinal necrosis and erosions as well as degenerative/necrotic and/or inflammatory changes Where's the SOD values decreased and LPO was increased significantly. However, pathological changes in organs as well as oxidative stress were considerably found reduced which were produced by piroxicam high dose. According to the present findings, hesperidine protected the organs like liver, kidney and intestine as well as stomach it also showed promising antioxidant activity.

Keywords: Liver, kidney, intestine and stomach

Introduction
Piroxicam, the first member of the oxicam or enolic acid derivatives, a nonspecific COX (cyclooxygenase) inhibitor which is commonly prescribed for the musculoskeletal disorders, ankylosing spondylitis, dysmenorrhea, acute gout and rheumatoid arthritis in humans (Jackson and Morrow, 2001) [1]. Piroxicam has long been used as an antineoplastic agent to treat transitional cell carcinoma, and it is now also being used as an adjunct therapy in other chemotherapy regimens to treat squamous cell carcinoma and mammary carcinoma (Robertson, 2007) [2]. It has also gained attention as an effective therapy for tumors like colorectal and invasive bladder cancers (Nadendla, 2003) [3]. However, it is reported that prolong use of this drug in humans and animals causes severe gastrointestinal toxicity, ulcerogenic gastropathy, renal hemostatic abnormalities, foetotoxicity, hepatotoxicity, nephrotoxicity, etc. (Brune and Lindner, 1992 [4]; Murray and Brater, 1993 [5]; Ebaid *et al.*, 2007 [6]; Aithal, 2011 [7]). Earlier studies indicated that piroxiacm induced organ damage is possibly due to oxidative stress built up by free hydroxyl radical (•OH) generation and gastro-protective PGE_2 synthesis inhibition *in vivo* in the course of its metabolism or action (Abdeen *et al.*, 2019) [21].
Hesperidin (HES, 5,7,3′ -trihydroxy-4′ -methoxyflavanone-7- rhamnoglucoside) is one of the major dietary flavanones which are abundantly found in many citrus fruits such as orange (Citrus sinensis), grapefruit (Citrus paradise), tangerine (*Citrus reticulata*), lime (*Citrus* × *aurantiifolia*) and lemon (*Citrus* × *limon*), (Kawser *et al.*, 2016) [8]. Hesperidin is one of the boon compound from the nature as it has many important beneficial pharmacological and biological properties including antioxidative, anti-carcinogenic, anti-inflammatory, lipid-lowering activities, anti-allergic, anti-bacterial, anti-viral,

neuroprotective and vascular protective (Cetin *et al.*, 2016) [9]. It also exerts reasonable protective effects against several drug induced toxicities (Thenmozhi *et al.*, 2015; Cetin *et al.*, 2016) [10, 9].

Association of high amount of •OH generation on oral administration of piroxicam has triggered the search for a nutritional component which can be effective in •OH scavenging. Therefore, the remedy is sought to be located in the inclusion of antioxidants like hesperidin in regular diet. In this study, we have in consequent phases determined the alterations in the oxidative stress parameters and pathology induced by piroxicam at two different doses. However, the protective role of hesperidin against piroxicam induced toxicityhas not been investigated. Hence we proposed to investigate whether oral administration of hesperidin provides protection against piroxicam induced injury in various organs and also whether it reduced oxidative stress.

Materials and Methods

Experimental animals

The present study was performed with a total of 40 male Wistar rats of weighing 200-350 gms were procured from Jai Research Foundation, Vapi, Gujarat. After randomization, rats were acclimatized in the animal house for 10 days prior to the experiment. During the experiment, the rats were maintained and housed in polypropylene cages (four rats in each) in the departmental animal house under room temperature 22±3 (℃) with 12 hours light and dark circle and provided with standard pellets and water *ad libitum*. The experiments were approved by Institutional Animal Ethical Committee (IAEC) under the project no. 108-VCN-VPP-2022.

Experimental design

Rats were randomly assigned into 5 groups comprising 8 rats each. Group I; rats that received 1% Carboxy methyl cellulose (CMC) served as the vehicle control. Group II; rats were injected with piroxicam at the dose rate of 10 mg/kg b. wt. Group III; rats were given piroxicam at dose rate of 25 mg/kg b. wt. Group IV; rats in this group were received both piroxicam (10 mg/kg b. wt.) and hesperidin (160 mg/kg b. wt.). Group V; rats of this group were received both piroxicam (25 mg/kg b. wt.) and hesperidin (160 mg/kg b. wt.). All compounds were administered once orally daily for 28 consecutive days. The drug, piroxicam was procured from the Merck & Co., Inc, and Hesperidin from the Sigma-Aldrich company. The piroxicam and hesperidin suspensions were made in 1% Carboxy methyl cellulose (CMC) and administered through oral gavage at the volume of 1ml/100gm b. wt. The formulation was prepared freshly just before dosing each day.

Sample collection

Oxidative stress parameters

Blood samples were taken from retro-orbital plexus using microcapillaries on 29th day of experiment. Blood was collected in sterile vial containing K_3 EDTA for lipid peroxidation (LPO) estimation; and plain vacutainers for serum collection (Plain vacutainers were initially kept at room temperature for 30 minutes and later centrifuged at 1000 rpm for 10 minutes at 4 ℃ to extract serum) used for superoxide dismutase (SOD) estimation.

Necropsy: At the end of the experiment, rats of all the experimental groups were subjected to necropsy. Prior to sacrifice, the animals were fasted overnight. The rats were sacrificed by overdosing of carbon dioxide/carbon dioxide plus chloroform anesthesia. The standard necropsy procedure was followed and gross if any was noted and tissue samples *viz*., liver, kidneys, stomach and intestine were collected from each animal for histopathology in 10% neutral buffered formalin.

Statistical Analysis

Oxidative stress parameters were subjected to statistical analysis using SPSS 20.0 statistical software (SPSS, Inc., 2009). One-way analysis of variance (ANOVA) followed by Duncan's test was performed to determine intergroup differences. The criterion for statistical significance was $p<0.05$.

Results and Discussion

The results of the oxidative stress parameters studied are presented in the table 1.

Table 1: Details of LPO and SOD levels in the experimental groups

Parameters	Group I (N=8)	Group II (N=8)	Group III (N=8)	Group IV (N=8)	Group V (N=8)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
LPO (nM/ml)	7.21^{a}±0.17	7.90^{ab}±0.68	9.17^{b}±0.45	7.58^{a}±0.45	7.91^{ab}±0.50
SOD (ng/ml)	4.28^{a}±0.55	3.47^{a}±0.45	2.46^{a}±0.62	4.07^{a}±0.54	3.90^{a}±2.30

Note: Means bearing different superscripts between groups differ significantly ($p<0.05$).
N = Number of animals

In group III, piroxicam at dose rate of 25 mg/kg b. wt./day showed significant ($p<0.05$) lipid peroxidation damage while only slight increase in LPO was noted at 10 mg/kg b. wt. in group II as compared to control group. The mean value of group II revealed non significantly increase in LPO in comparision to rats of group I. Groups treated with hesperidin along with low and high dose of piroxicam (group IV and V) showed decrease in the mean value of LPO concentration as compared to groups II and III, respectively and this proved antioxidant effect of hesperidin. The non significant decrease in SOD level was noted in animals treated with low and high dose of piroxicam. Whereas mean SOD values of groups IV and V showed increased in comparision with group II and III. Present results supports the findings of earlier workers who also reported good antioxidant properties of HES agaist various toxicities *viz*. NMU, cisplatin etc (Urkude *et al.*, 2021; Sorathiya, 2021) [14, 19].

It is widely recognised that when the levels of ROS (Reactive oxygen species) surpass the endogenous antioxidant capacity, the cellular antioxidant state gets changed, resulting in an oxidative distress condition. Lipid peroxidation of the cell and mitochondrial membranes, ATP depletion, protein oxidation and unfolding, DNA oxidation and ultimately cell death are symptoms of oxidative stress. The major ROS produced during oxidative stress are hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet}$) and hydroxyl radicals ($OH^{\bullet}$), (Klaunig *et al.*, 2018) [11]. Among them, hydroxyl radicals are said to be the most prevalent and harmful species that directly degrades

the lipid content of the cell membrane, producing lipid peroxidation. Lipid peroxidation is indicated by a rise in the amount of lipid peroxide in the body and decrease in SOD and other anti-oxidant enzymes' levels. Hesperidin works as hydrogen-donor and free-radical scavenger as well as it acts as a potential chain breaking antioxidant and it also chelates transition of metal ions hence can inhibit free radical formation and the propagation of free-radical reactions (Mohamed *et al.*, 2015) [13]. It is reported that HES acts by scavenging free radicals and by maintaining intracellular superoxide dismutase (SOD) and glutathione levels, thereby prevents lipid peroxidation and tissue damage (Urkude *et al.*, 2021) [14].

In present study, no any noticeable gross pathological lesions were observed in animals of groups I, II and IV. In group II and III mild congestion of kidneys and mild degenerative changes in the liver were noted. Gross examination of liver in group III animals revealed paleness and swelling with rounded edges with an accentuated lobular pattern while stomach examination revealed focal erosions as well as ulcers in glandular part of stomach and in these animals mild congestion of kidneys were also noted. However, no gross changes were noted in intestine of group III animals. In comparison to group III animals, animals of group V revealed minimal pathological changes in various organs *viz.*, stomach, kidney and liver.

No gross lesion of pathological significance was observed in any organ during necropsy in groups I, II, IV and V. However, in group III degenerative changes in liver, erosions and ulcers in stomach, as well as congestion in kidney were noted. Our observations were in accordance with earlier worker Vihol (2010) [20] who also noted paleness and slight enlargement of liver; enlargements of kidneys; hemorrhagic ulcers and necrosis in small intestine after administration of piroxicam at dose rate of 15 mg/kg b. wt./day to rats, however he did not find any ulcers on stomach. It was also reported that piroxicam at dose rate of 1 and 7 mg/kg b. wt. did not induce any noticeble gross lesions (Vihol, 2010) [20].

The histopathological changes of various organs like liver, kidney, stomach and intestine are discussed in details.

Liver

In group I, liver section revealed normal architecture. In group II and V, mild congestion, inflammation and degenerative changes were noted. The histopathological lesions found in liver of group III rats revealed marked engorgement of central veins (Fig. 1) and hepatic veins with sinusoidal dilations, marked hepatic hydropic degeneration (Fig. 2), focal obliteration of sinusoidal space, mild focal necrosis (Fig. 3), disorganization of hepatocytes with disruption of hepatic cords. Moderate inflammatory cells infiltration in portal trade (Fig. 4) as well as moderate fatty changes (Fig. 5) were also noted focally. In some animals, few hepatocytic nuclei were enlarged, hyperchomic and binucleated. At places, apoptosis was also noted in hepatocytes. In the Group V, these changes were less pronounced with mild central vein and sinusoidal congestion (Fig. 6) as well as minimal to mild degeneration, restoration of hepatic cord organization and minimal to mild infiltration of inflammatory cells (Fig. 7). Due to enhanced generations of ROS the mitochondria, endoplasmic reticulum and DNA might be damaged, resulting in altered protein synthesis and gene expression which caused necrosis of liver and disturbance in liver function. The mechanism of piroxicam hepatotoxicity relates both to impairment of adenosine triphosphate synthesis by mitochondria and production of active metabolites, particularly 5-hydroxy piroxicam, which causes direct cytotoxicity. Induction of mitochondrial membrane permeability transition has also been shown to be important in NSAID-induced liver injury, resulting in the generation of ROS, mitochondrial swelling and oxidation of nicotinamide adenine dinucleotide phosphate and protein thiols (Sahu, 2016) [15]. Hesperidin increases concentration of antioxidant enzymes which in turn decreases the attack of free radicals on bio-molecules including DNA and membrane lipids and by this way HES reduces the deleterious effects on the liver (Kalpana *et al.*, 2011) [16].

Kidney

In group I, kidney revealed normal architecture. Microscopic examination of kidney collected from group II animals revealed mild tubular dilation, mild glomerular and peritubular congestion (Fig. 8), focal minimal necrosis and minimal cast formation in renal tubules and minimal degenerative changes. On other hand, in group IV no any noticiable lesions were noted showing complete protective effect of hesperidin in low toxicity group of piroxicam. In higher dose piroxicam treated group of rats (Group III) marked congestion (Fig. 9), moderate tubular necrosis (Fig. 10), mild cast formation in tubules (Fig. 11), focal inflammatory cells infiltration, minimal to mild glomerular and peritubular capillary dilation and congestion (Fig. 12), tubular dilation in Medullla (Fig. 13), focal area of glomerular necrosis and atrophy as well as decreased urinary spaces (Fig. 14) and mild degenerative changes (Fig 15) were noted. In group V, mild intensity pathological cahnges like tubular dilation, inflammatory cells infiltration, degenerative changes, congestion, were discernible. Piroxicam caused renal injury in the experimental animals by commonly affecting PCTs and it was suggested that this drug causes severe organ toxicities through metabolic activation of highly reactive free radicals including superoxidase and oxygen reactive species. Increase LPO in renal cell membrane, causes disintegrated brush border, which leads to tubular impairment. Piroxicam inhibits renal endogenous prostaglandin production, decreases renal afferent vasodilation and raises afferent resistance in kidney and hence together all of these causes the glomerular capillary pressure to fall below normal values and decline in glomerular filtration rate (Bancroft and Gamble, 2008). It was reported that hesperidin suppresses the generation of ROS, lipid peroxidation, pro-inflammatory cytokine and opined that hesperidin excellently worked as nephro-protective phytochemical in piroxicam-induced renal injury (Sahu *et al.*, 2013) [18].

Stomuch

In group I, stomach section reveled normal histoarchitecture (Fig. 24). In group II mild epithelial erosions, mild ulceration, minimal to mild inflammatory cells infiltration in the muscularis layer of glandular stomach and focal hemorrhages were present. In group III moderate epithelial erosions (Fig. 25), congestion, marked inflammatory cells infiltration and focal ulcers (Fig. 26) were discernible. Microsections of stomach in group IV revealed normal histology of stomach (Fig. 27). In group V which was treated with higher dose of piroxicam along with hesperidin mild infiltration of inflammatory cells was discernible (Fig. 28). Piroxicam is well known to induce gastric ulceration by interfering with the synthesis of prostaglandins via inhibition of COX1, which converts unsaturated fatty acids (which are released by cell

injury) such as arachidonic acid to prostaglandins. In the stomach, prostaglandin synthesis induces protective mechanism as a result of enhanced mucosal blood flow and stimulation of mucus and bicarbonate secretion (Bandyopadhyay *et al.*, 2004) [22]. NSAID treatment causes elevation of inducible nitric oxide synthase (iNOS). It was reported that nitric oxide plays an important role in protecting the gastric mucosa and is necessary for optimal mucosal functions. High level of iNOS is activated by inflammatory cytokines which leads to disturbance of microcirculation and gastric mucosal damage. The excessive production of iNOS induces inflammation and leads to gastric injuries through the generation of reactive oxygen species. ROS production causes damage to gastric mucosa through disruption of the gastric cytoskeleton protein, α-actinin (Abdeen *et al.* 2019) [21]. NSAIDs may also increase the synthesis of TNF-α and leukotrienes. TNF-α and IL-6 are important mediators in NSAID-induced gastropathy and its production is normally inhibited by an increase in PGE2 levels (Mousa and Fahmy, 2023) [23].

Hesperidin suppresses the generation of ROS, lipid peroxidation, oxidative stress, pro-inflammatory cytokine reduces DNA damage in stomach tissue (Sahu *et al.*, 2013) [18]. The obtained results showed that hesperidin protects the stomach mucosa.

Intestine

In group I, normal architecture of intestinal villi was observed (Fig. 29). In lower dose piroxicam group (Group II) loss of surface epithelium, focal necrosis at tip of villi, fusion of villi and inflammatory cells infiltration in submucosa was noted. In group III, microscopic examination of intestine revealed focally shortening as well as disappearance of villi (Fig. 30), focal loss of epithelium, degeneration and atrophy in intestinal glands. At place in submucosa, moderate mononuclear cells infiltration was also noted. Minimal fusion of villi (Fig. 31) and moderate to marked necrosis at the tip (Fig. 32) and widening of spaces between villi (Fig. 33) were also noted in group III. However, in group V mild inflammatory cells infiltration, focal mild necrosis at tips with apperantly normal villi were dicsernible. In group IV, minimal inflammatory cells infiltrations were noted. Piroxicam is a nonselective inhibitor of cyclooxygenase enzyme. It inhibits constitutive COX-1 isoform and the inducible COX-2 isoform. In the GIT, COX-1 is the predominant isoform (found in gastric fundus, pylorus, duodenum, jejunum, ileum, cecum and colon) and is expressed normally in rodents, canine, humans and primates while the COX- 2 isoform is nearly absent in these species, except for low levels in the large intestine. The anti-inflammatory benefits of piroxicam are primarily derived from COX- 2 inhibition, while inhibition of COX-1 often elicits gastrointestinal toxicity (Koki *et al.*, 2002) [24]. In present study, in group co-administered with hesperidin and high dose of piroxicam comparatively normal intestinal villi were discernible in comparison with high dose group of piroxicam.

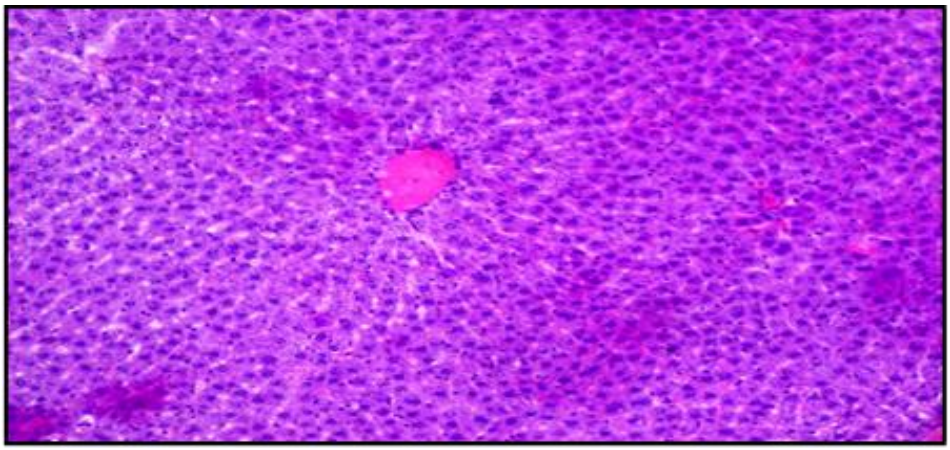

Fig 1: Liver (group III) micro section revealing marked congestion of central vein. H&E, 200X.

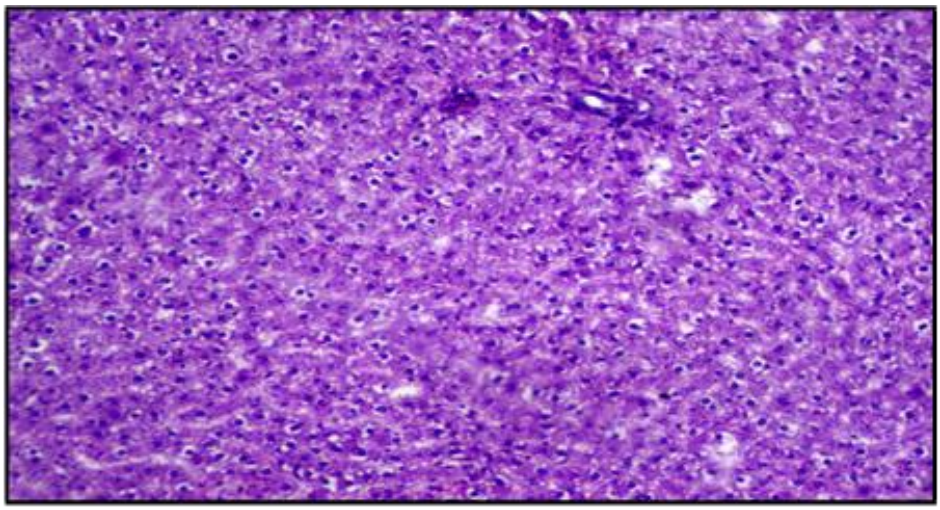

Fig 2: Liver (group III) micro section revealing marked hydropic degeneration of hepatocytes. H&E, 200X.

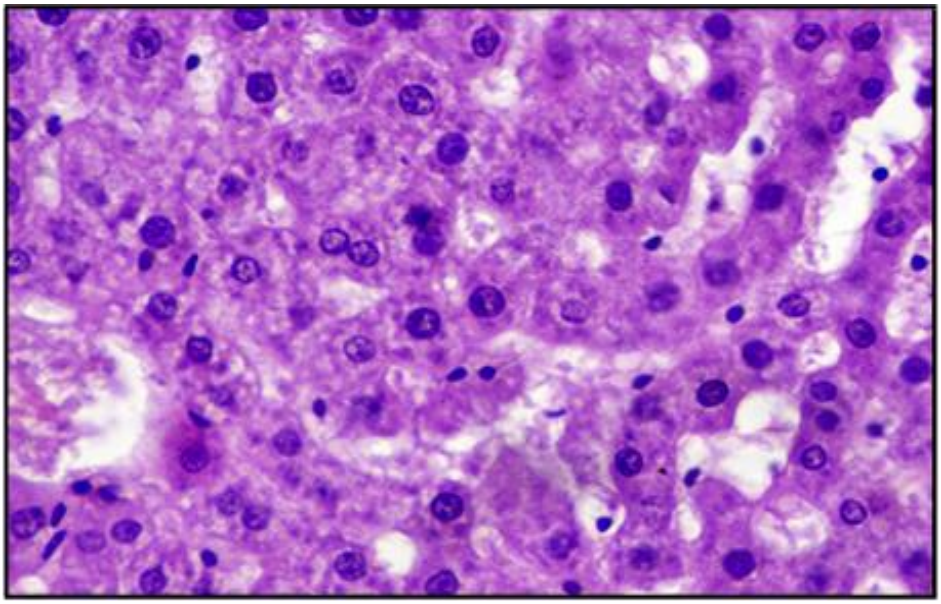

Fig 3: Liver (group III) microsection revealing focal necrosis and obliterated sinusoids. H&E, 800X.

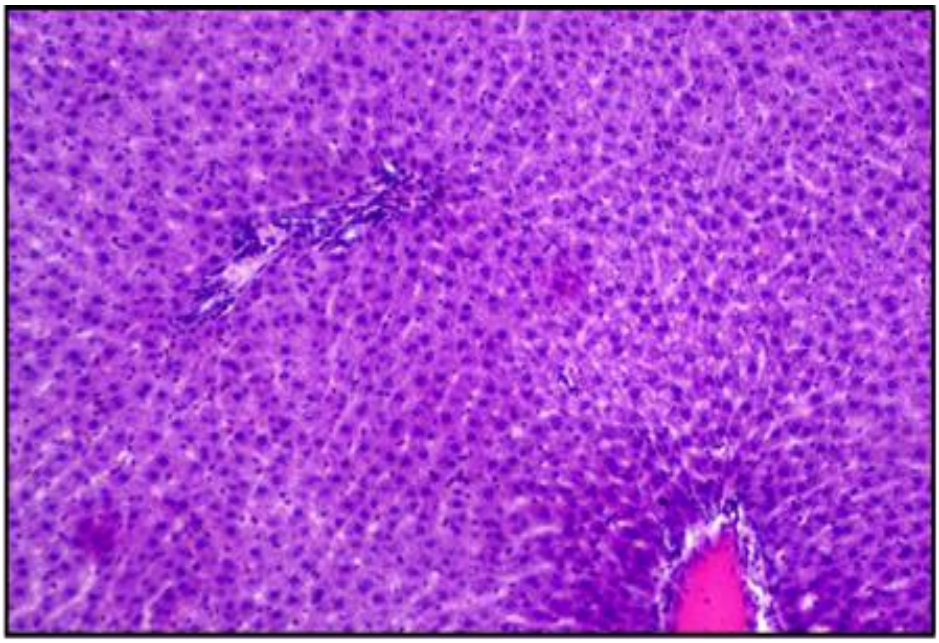

Fig 4: Liver (group III) microsection revealing moderate infiltration of inflammatory cells in portal triad. H&E, 200X.

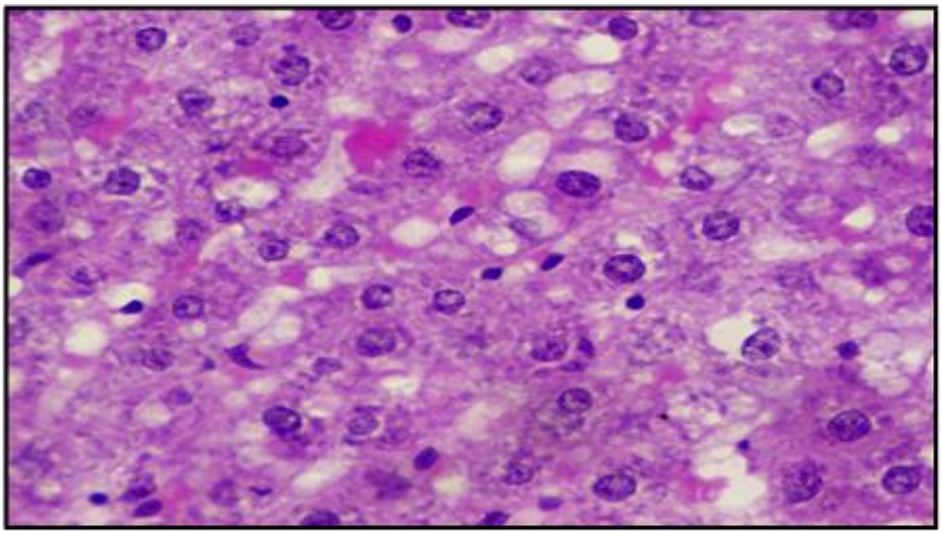

Fig 5: Liver (group III) microsection revealing fatty changes. H&E, 800X.

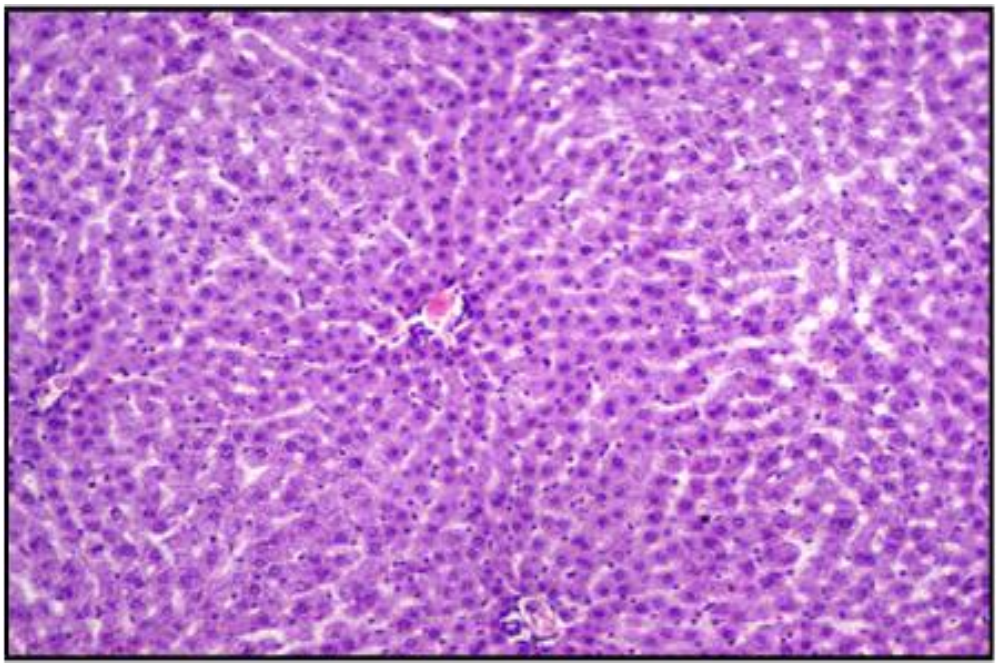

Fig 6: Liver (group V) microsection revealing mild congestion of central vein, sinusoids and hydropic degeneration. H&E, 200X.

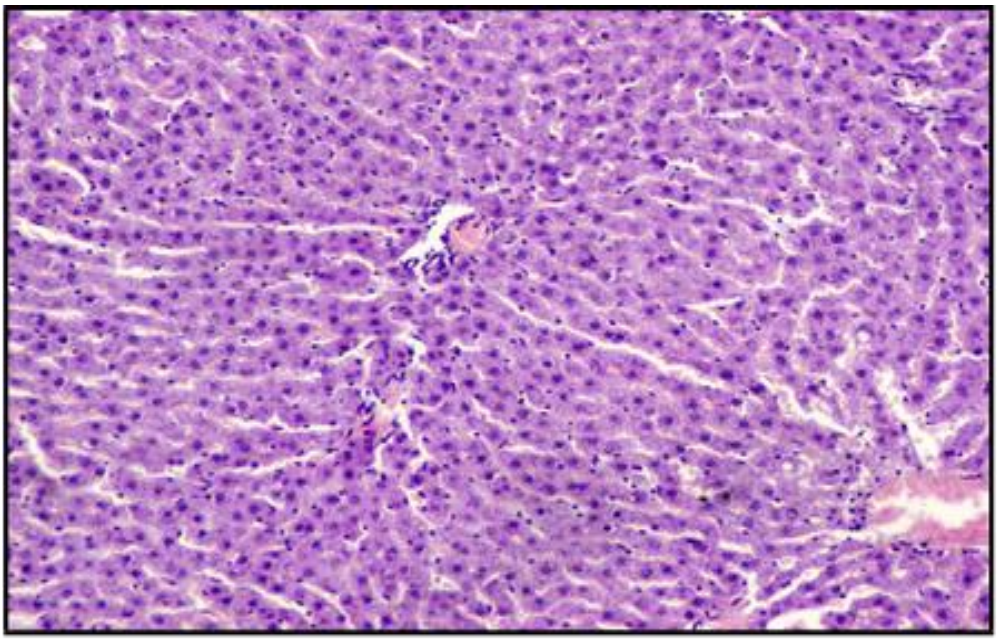

Fig 7: Liver (group V) microsection revealing mild infiltration of inflammatory cells and apparently normal hepatic cords. H&E, 200X.

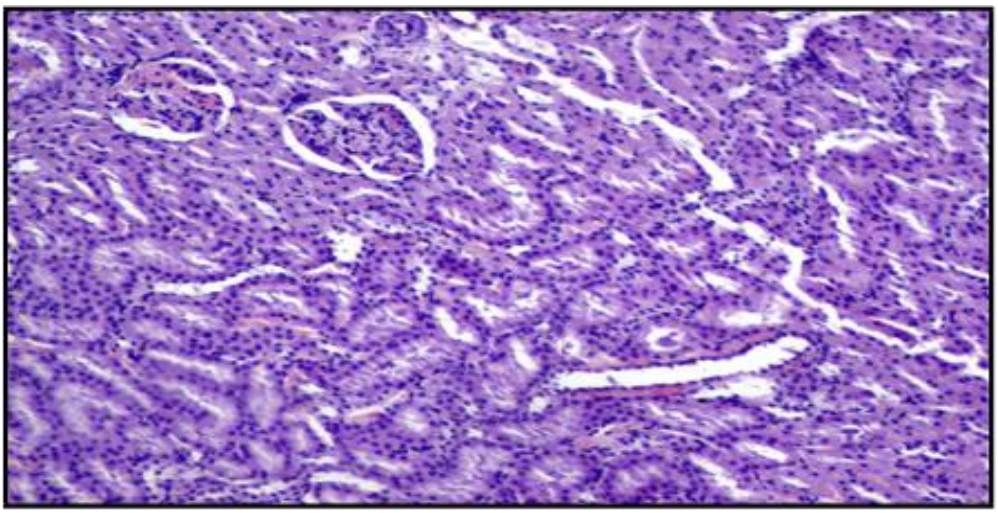

Fig 8: Kidney (group II) microscopic section revealing mild glomerular and peritubular capillary congestion. H&E, 200X.

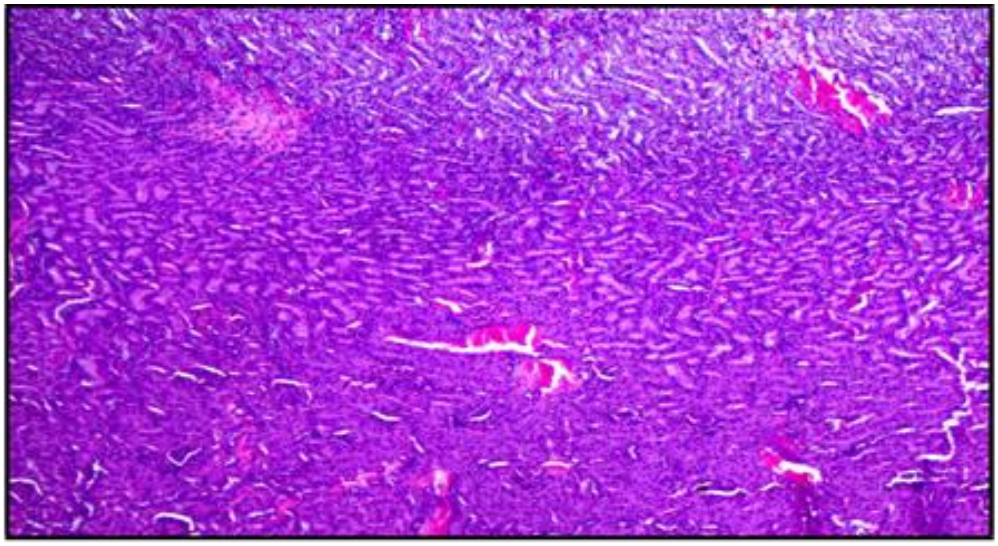

Fig 9: Kidney (group III) microsection revealing marked congestion. H&E, 80X.

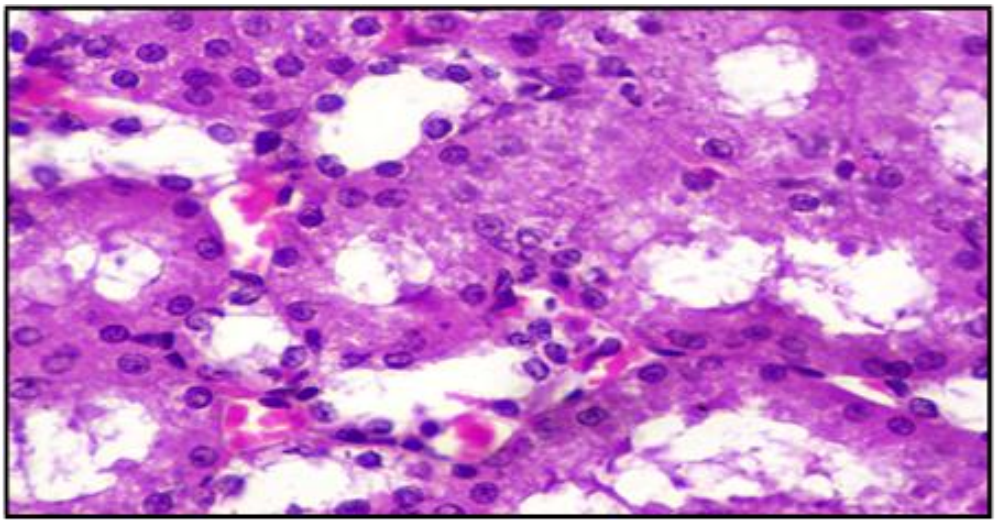

Fig 10: Kidney (group III) microsection revealing focal areas of necrosis and degeneration in tubules. H&E, 800X.

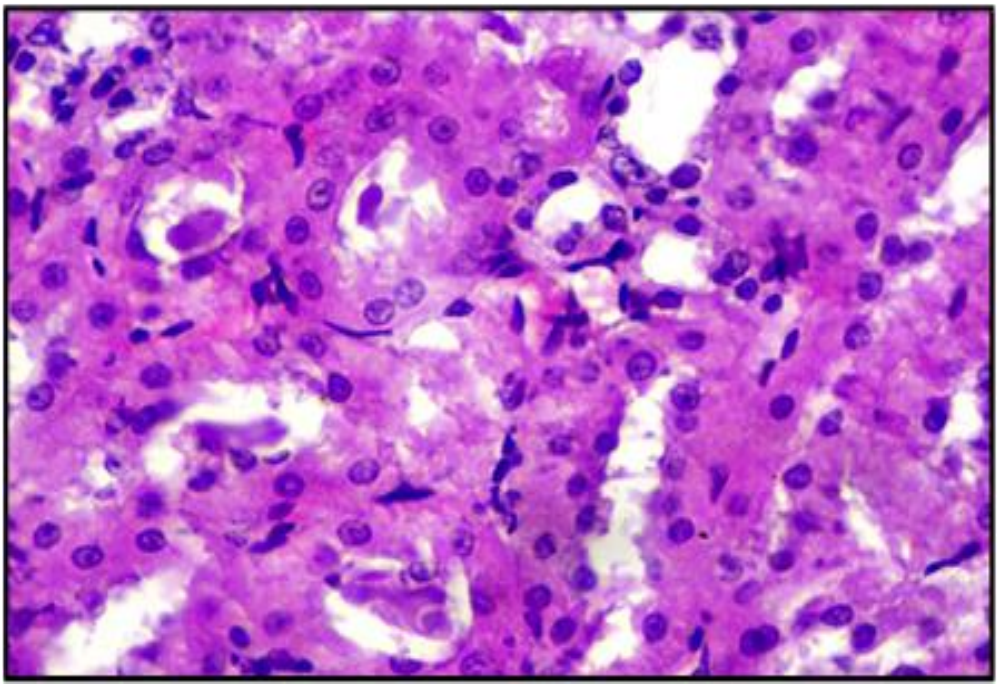

Fig 11: Kidney (group III) microsection revealing tubular cast formation. H&E, 800X.

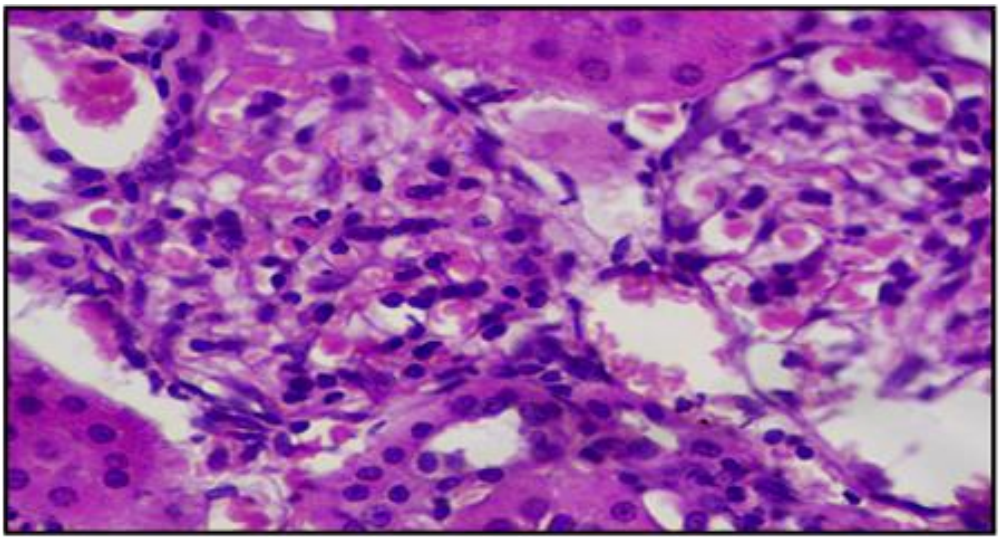

Fig 12: Kidney (group III) microsection revealing dilation and congestion of glomerular capillaries. H&E, 800X.

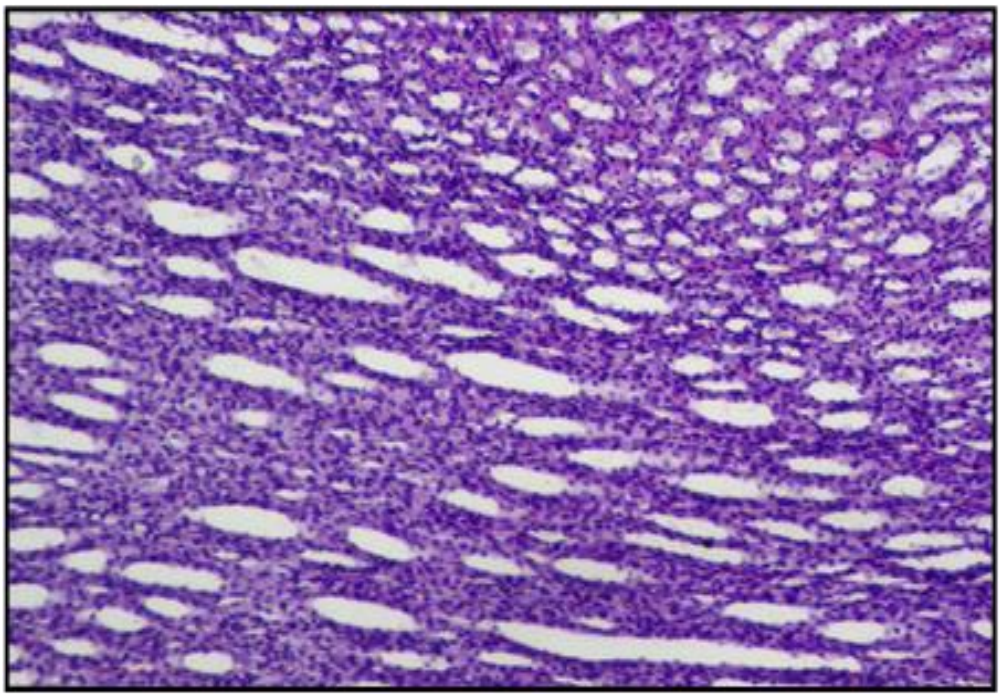

Fig 13: Kidney (group III) microsection revealing medullary tubular dilation. H&E, 200X.

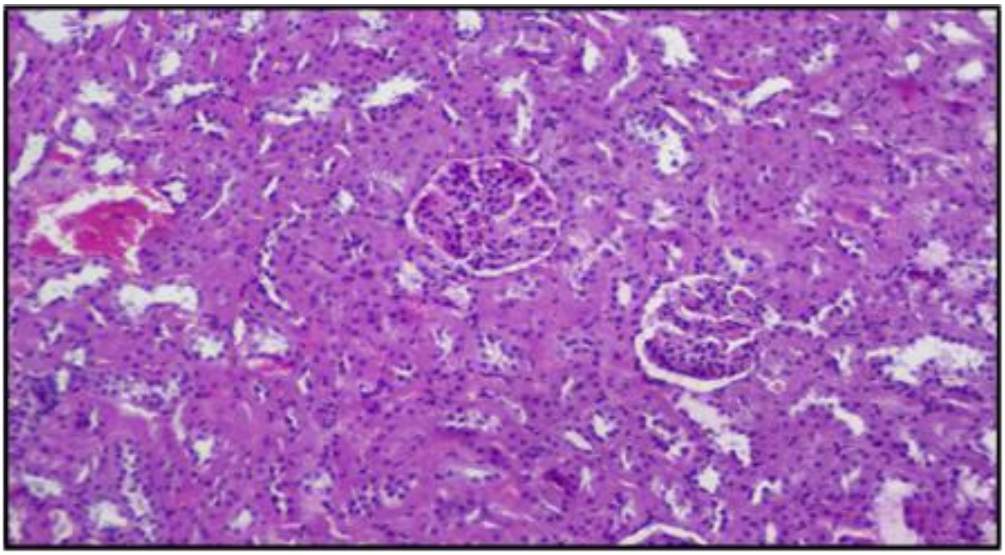

Fig 14: Kidney (group III) microsection revealing decreased urinary space in glomeruli. H&E, 200X.

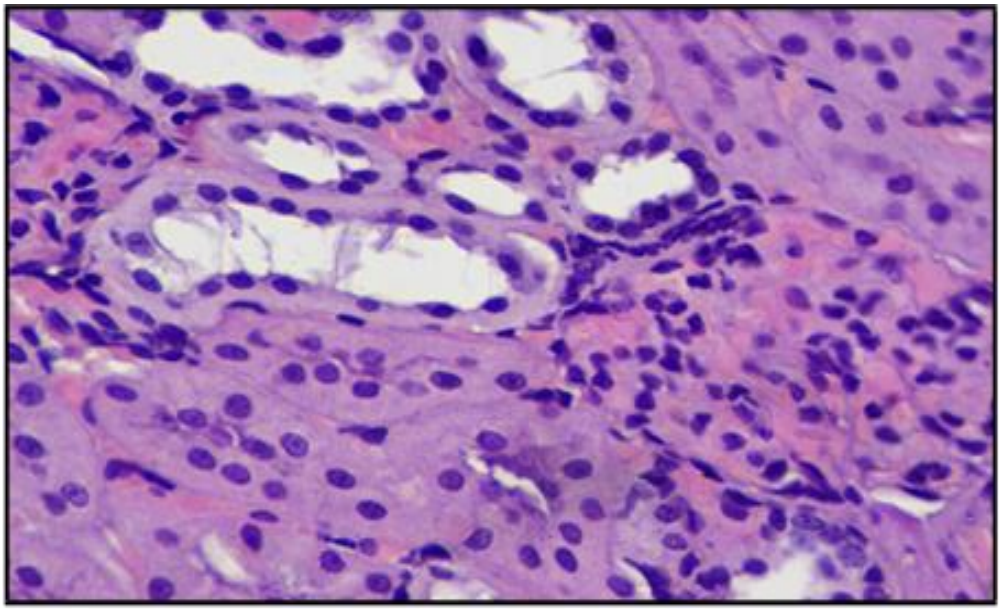

Fig 15: Kidney (group III) microscopic section revealing degenerative tubular epithelial cells

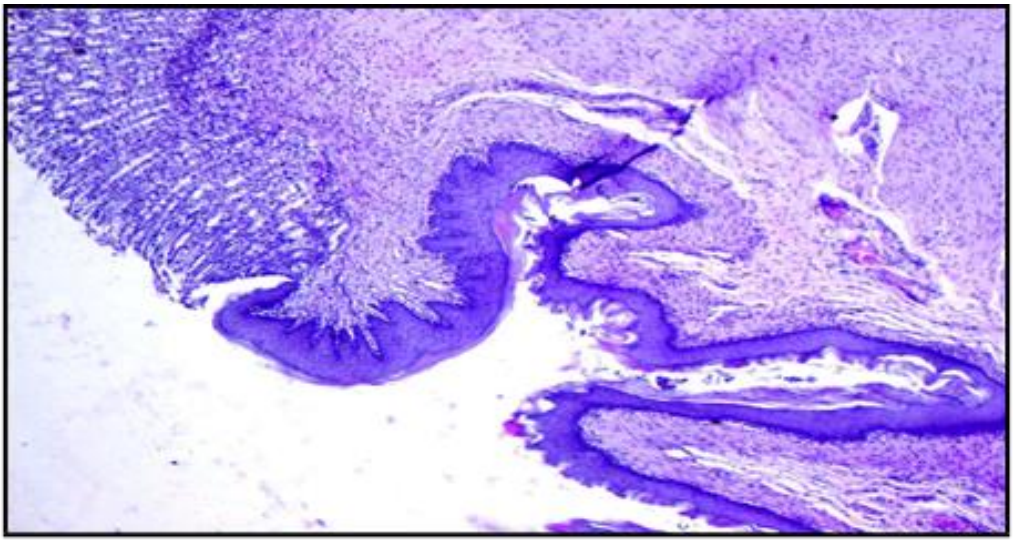

Fig 24: Stomach (group I) microsection revealing normal histology of stomach. H&E, 80X.

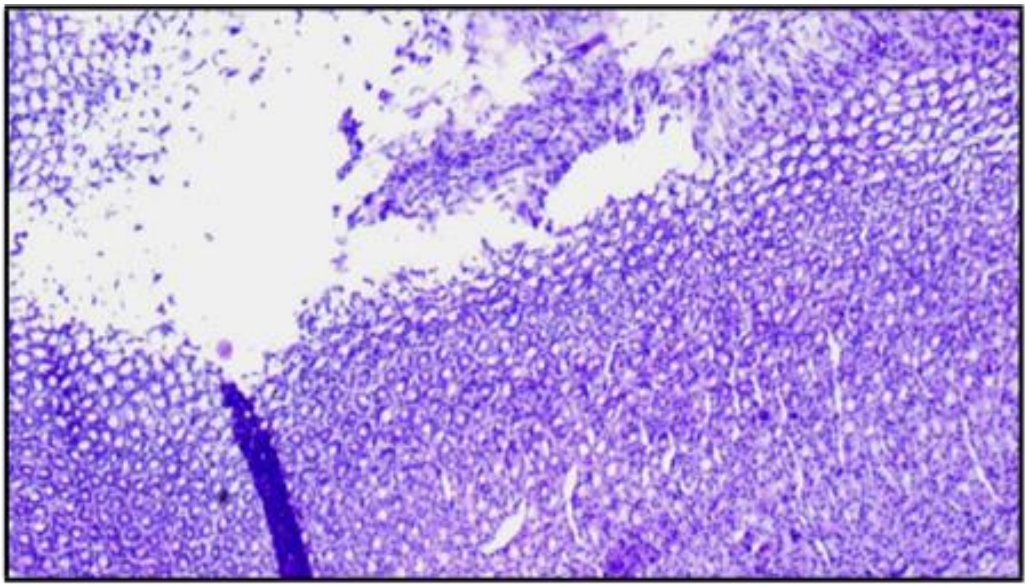

Fig 25: Stomach (group III) microsection revealing focal erosion in gastric mucosa. H&E, 200X.

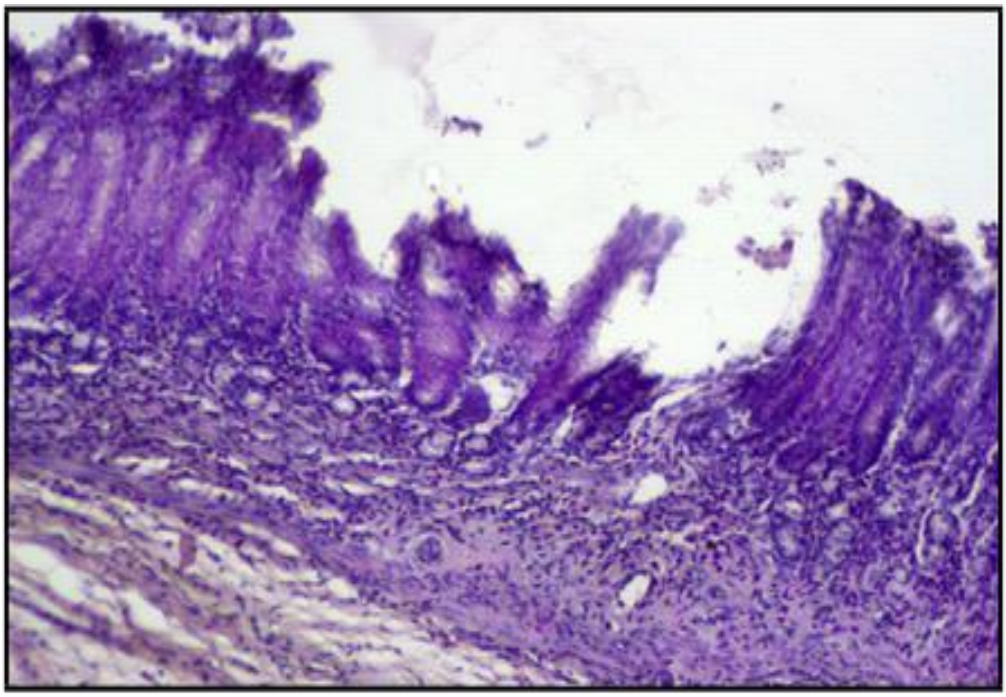

Fig 26: Stomach (Group III) microsection revealing ulcer and underlying mononuclear cell infiltration in gastric mucosa. H&E, 200X.

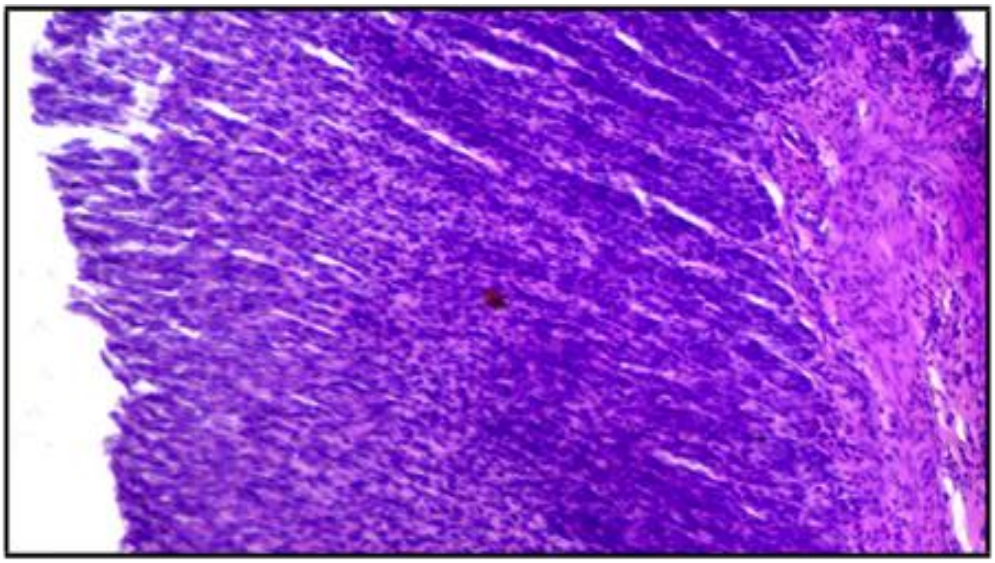

Fig 27: Stomach (group IV) microsection revealing restoration of gastric mucosa. H&E, 200X.

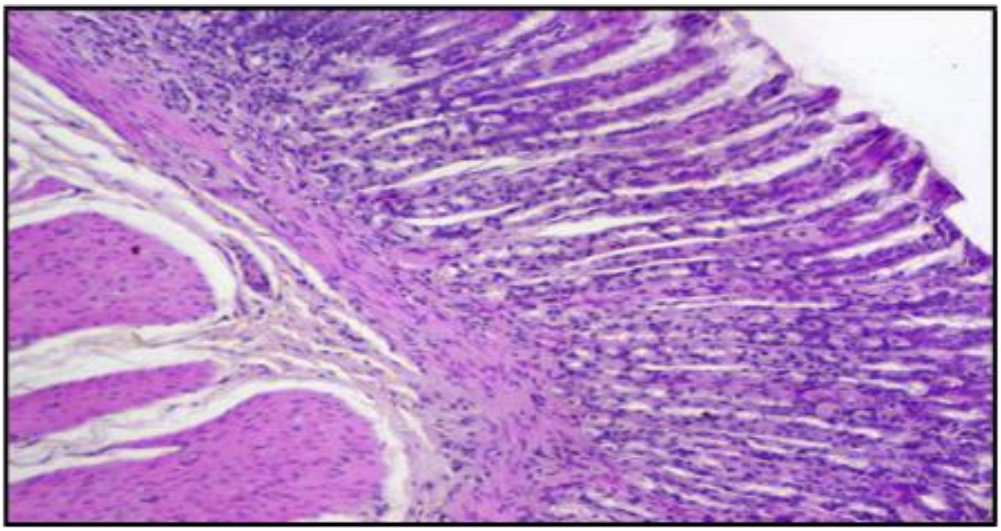

Fig 28: Stomach (group V) microsection revealing inflammatory cells infiltration. H&E, 200X.

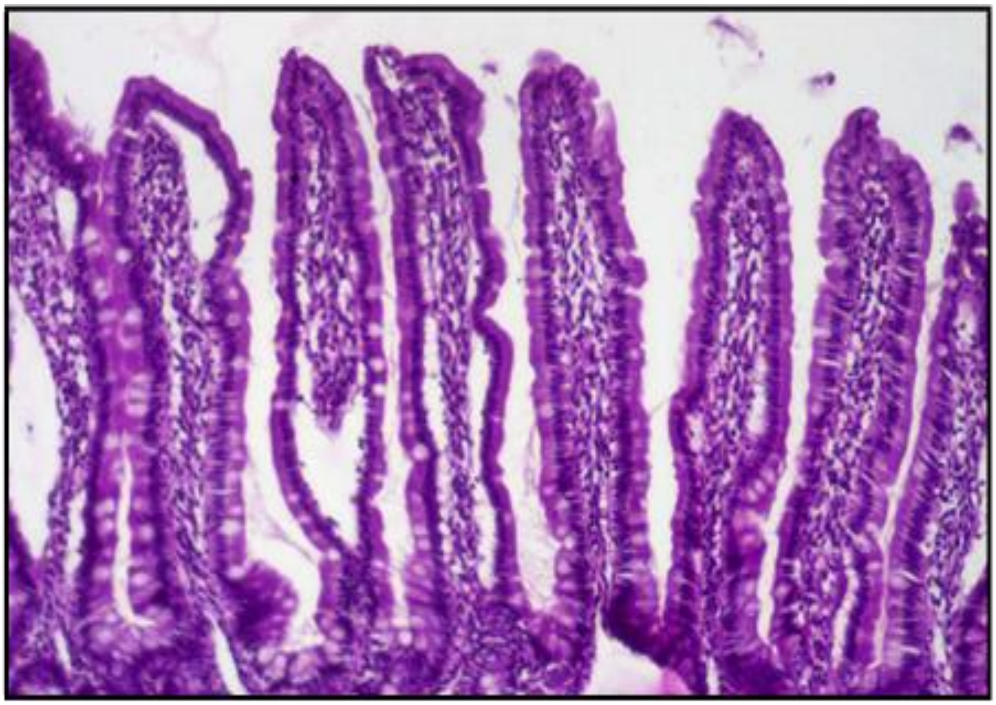

Fig 29: Intestine (group I) microsection revealing normal intestinal villi. H&E, 200X.

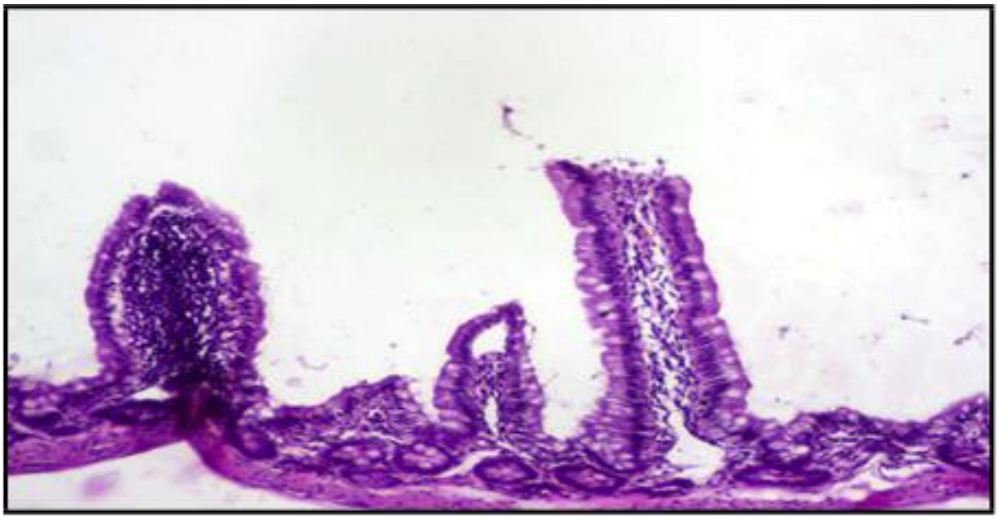

Fig 30: Intestine (group III) microsection revealing shortening of intestinal villi. H&E, 200X.

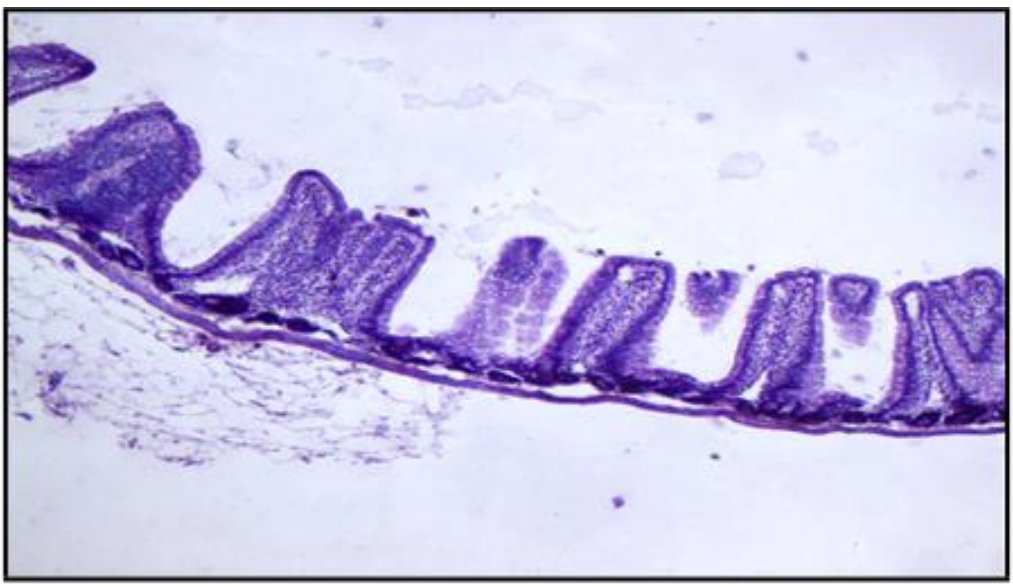

Fig 31: Intestine (group III) microsection revealing fusion of villi. H&E, 200X.

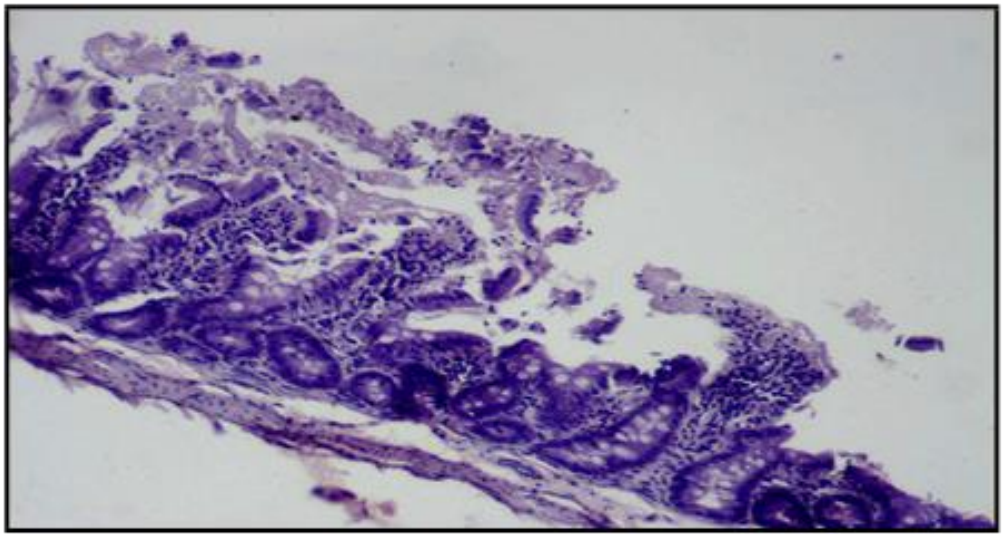

Fig 32: Intestine (group III) microscopic section revealing necrosis of tip of intestinal villi. H&E, 200X.

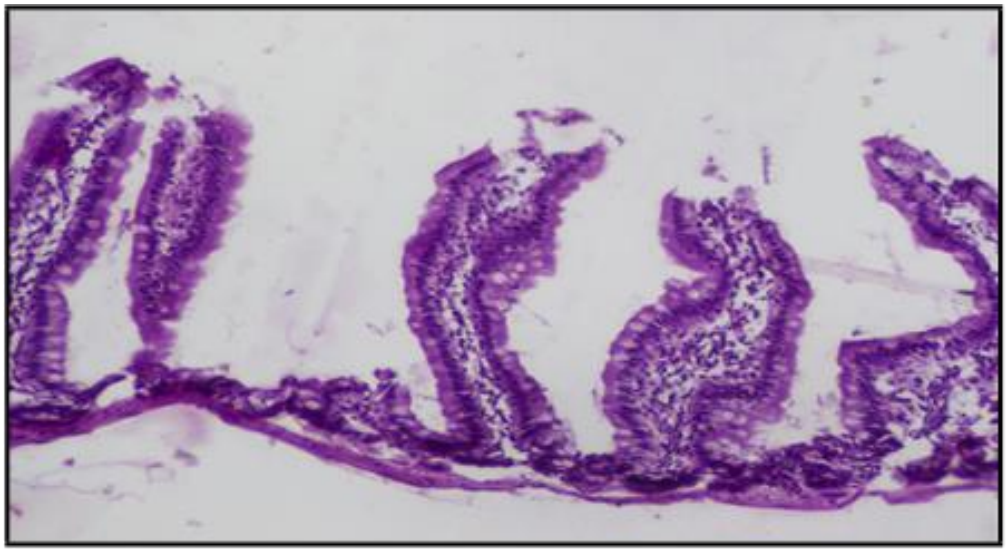

Fig 33: Intestine (group III) microsection revealing widening and loss of villi. H&E, 200X

Conclusions

The distinguishable pathomorphological lesions discernible in animals administered with piroxicam at different doses revealed inflammation, gastric erosions and ulcers, intestinal necrosis and erosions as well as degenerative/necrotic and/or inflammatory changes in liver kidneys, stomach and intestine in dose dependent manner. Additionally, piroxicam at high dose also induced odxidative stress which was reduced by co-administration of HES at 160 mg/kg b.wt. Hesperidine also was found to be hepato-protective, nephro-protective and GIT-protective effects against organ toxicities caused by piroxicam at both the doses as evidenced by present results. So, antioxidant property of HES might be responsible for these protective effects.

Acknowledgement

We are highly thankful to Principal, College of Veterinary Science & Animal Husbandry, Kamdhenu University, Navsari, Gujarat for providing the necessary facility.

References

1. Jackson L, Morrow JD. Analgesic, antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In Grosser T, Smyth E, FitzGerald GA, (Eds.) Goodman and Gilman's the pharmacological basis of therapeutics. (12th Ed.). New York: McGraw-Hill; c2001. p. 687-732.
2. Robertson E. Piroxicam. In Enna SJ, Bylund DB, (Ed.) Xpharm: The comprehensive Pharmacology Reference. Elsevier; c2007. p. 1-6.
3. Nadendla RR. Chemistry of non-steroidal anti–inflammatory drugs–a review. The Antiseptic. 2003;100(8):292-296.
4. Brune K, Lindner J. Increased liver toxicity of diclofenac by paracetamol: results and possible mechanisms. In Rainsford KD, Velo GP, (Eds.) Side-Effects of Anti-Inflammatory Drugs 3 (5th Ed.). Springer; c1992. p. 198-203.
5. Murray MD, Brater DC. Renal toxicity of the nonsteroidal anti-inflammatory drugs. Annual Review of Pharmacology and Toxicology. 1993;33:435-465.
6. Ebaid H, Dkhil MA, Danfour MA, Tohamy A, Gabry MS. Piroxicam-induced hepatic and renal histopathological changes in mice. Libyan Journal of Medicine. 2007;2(2):82-89.
7. Aithal GP. Hepatotoxicity related to antirheumatic drugs. Nature Reviews Rheumatology. 2011;7(3):139-150.
8. Kawser HM, Abdal DA, Han J, Yin Y, Kim K, Saha SK, *et al.* Molecular mechanisms of the anti-obesity and

antidiabetic properties of flavonoids. International Journal of Molecular Sciences. 2016;17(4):569.
9. Cetin A, Ciftci O, Otlu A. Protective effect of hesperidin on oxidative and histological liver damage following carbon tetrachloride administration in Wistar rats. Archives of Medical Science. 2016;12(3):486-493.
10. Thenmozhi AJ, Raja TRW, Janakiraman U, Manivasagam T. Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats. Neurochemical Research. 2015;40(4):767-776.
11. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicologic Pathology. 2010;38(1):96-109.
12. Bhalchandra W, Alqadhi YA. Administration of honey and royal jelly ameliorate cisplatin induced changes in liver and kidney function in rat. Biomedical and Pharmacology Journal. 2018;11(4):2191-2199.
13. Mohamed WR, Arafa ESA, Shehata BA, El Sherbiny GA, Elgendy ANA. Beneficial effects of hesperidin against cisplatin-induced nephrotoxicity and oxidative stress in rats. British Journal of Pharmacology and Toxicology. 2015;6(3):56-63.
14. Urkude AP, Vihol PD, Patel JM, Patel JH, Varia RD, Saurathiya VL, *et al.* Chemo preventive Efficacy of Hesperidin against N-methyl N-nitrosourea Induced Mammary Tumors in SD Rats. International Journal of Current Microbiology and Applied Sciences. 2021;10(06):344-355.
15. Sahu CR. Mechanisms involved in toxicity of liver caused by piroxicam in mice and protective effects of leaf extract of Hibiscus rosa-sinensis L. Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders, 9, CMAMD-S29463; c2016.
16. Kalpana KB, Devipriya N, Srinivasan M, Vishwanathan P, Thayalan K, Menon VP. Evaluating the radio protective effect of hesperidin in the liver of swiss albino mice. European Journal of Pharmacology. 2011;658(2-3):206-212.
17. Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier Health Sciences; c2008.
18. Sahu BD, Kuncha M, Sindhura GJ, Sistla R. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. Phytomedicine. 2013;20(5):453-460.
19. Sorathiya VL. Evaluation of ameliorative effects of Hesperidin in Cisplatin treated rats. M.V.Sc. Thesis. College of Veterinary Science & Animal Husbandry, Navsari Agricultural University, Navsari; c2021.
20. Vihol KA. Sub-acute toxicity study of piroxicam following repeated oral administration in Wistar rats, M.V.Sc. Thesis, Anand Agricultural University, Anand, India; c2010.
21. Abdeen A, Aboubakr M, Elgazzar D, Abdo M, Abdelkader A, Ibrahim S, *et al.* Rosuvastatin attenuates piroxicam-mediated gastric ulceration and hepato-renal toxicity in rats. Biomedicine & Pharmacotherapy. 2019;110:895-905.
22. Bandyopadhyay D, Ghosh G, Bandyopadhyay A, Reiter RJ. Melatonin protects against piroxicam-induced gastric ulceration. Journal of Pineal Research. 2004;36(3):195-203.
23. Mousa HR, Fahmy N. Gastro protective and Healing Effect of Naringin and Quercetin in Experimentally Induced Gastric Ulcer by Diclofenac Sodium in Adult Male Albino Rat: A Histological and Immuno-histochemical Study. The Egyptian Journal of Hospital Medicine. 2023;90(1):213-225.
24. Koki A, Khan NK, Woerner BM, Dannenberg AJ, Olson L, Seibert K, *et al*. Cyclooxygenase-2 in human pathological disease. Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury. 2002;5:177-184.