World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN ADULT MALE WISTAR RATS FOLLOWING ACUTE ADMINISTRATION OF IBUPROFEN

Okwakpam F.N.[1*], Omeodu S.I.[2], Nkomadu V.D.[1] and Ajie P.[3]

[1]Department of Biochemistry, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.
[2]Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Nigeria.
[3]Department of Anatomy, Faculty of Basic Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

***Corresponding Author: Okwakpam F.N.**
Department of Biochemistry, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Article Received on 29/01/2023 **Article Revised on 17/02/2023** **Article Accepted on 09/03/2023**

ABSTRACT

Ibuprofen is a non-steroidal anti-inflammatory drug commonly used worldwide for the treatment of inflammation and pains in man and animals. Despite these benefits, there are concerns that acute administration of this drug may have adverse effect on kidney and liver. More so there are conflicting roles of ibuprofen in pathologies of diseases associated with tissue damage. This study investigated the effect of acute administration of ibuprofen on some biochemical parameter in male albino rats and histological changes in liver and kidney induced by ibuprofen were also investigated. Twenty albino rats were divided into four groups of five animals per group. Group 1 served as control and was given feed and water only. Group 2, 3 and 4 rats were treated with 200mg/kg, 400mg/kg and 500mg/kg ibuprofen orally once a day for 7 days respectively. The result showed that there was a significant ($p<0.05$) difference in serum creatinine (mg/dl) and serum urea (mg/dl) for Group 2, Group 3 and Group 4 at $p\leq0.05$ when compared with group 1 the control rats. More so, there was no significant ($p<0.05$) difference in total protein (g/L) and total bilirubin (g/L) in all the Groups. There was also a significant ($p<0.05$) difference in albumin (g/L) in Group 4 only. There was significant ($p<0.05$) difference in serum Na^+ (mg/dl), K^+ (mg/dl) and Cl^- (mg/dl) for Group 2, Group 3 and Group 4 at $p\leq0.05$. Furthermore, Histopathological alterations were found in liver and kidney tissues. Acute Ibuprofen show potentially harmful effect to the liver, kidney and heart of the albino rats.

KEYWORDS: Ibuprofen, Kidney, Liver, Heart and Histopathological alterations.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively used as analgesics and anti-inflamatory drugs (Okwakpam *et al*., 2020). They exert their anti-inflammatory, anti-pyretic and analgesic effect via the suppression of prostaglandins synthesis, by inhibiting the enzyme cyclooxygenase which has two isoform, COX-1 and COX-2 (Horl, 2010). They inhibit both COX-1 and COX-2, the rate limiting enzymes for the production of prostaglandin and thromboxane (Harris, 2006). COX-1 functions mainly in the control of renal haemodynamics and glomerular filtration rate, while COX-2 functions primarily affect salt and water excretion (Weir, 2002). Inhibition of cyclooxygenase pathway plays an important role in the pain-relieving mechanism as well as also in conversion of arachidonic acid into thromboxanes and prostaglandin.

Among the NSAIDs, Ibubrofen (2-(p-isobutylphenyl) propionic acid is a widely circulated drug used by both human and animals for fast and effective relief of headache, backache, menstrual pain, toothache, fever, inflammation from muscle strain and sprains (Diamond, 2000). The major mechanism of action of ibuprofen is the non-selective, reversible inhibition of the "COX enzymes COX-1 and COX-2" (Prusakiewicz *et al*., 2009). Ibuprofen is totally metabolized; the main route of elimination is oxidative metabolism by cytochrome p2C9 enzymes into inactive metabolites. Urinary excretion of the two major metabolites, "carboxy-ibuprofen" and "2-hydroxy-ibuprofen" represents 25% of the administered dose (Rudy *et al*., 1992).

Besides been a common prescription drug by care professionals, it is also available over the counter. This implies that a large number of people are exposed to the drug. Despite its beneficial effect, with increasing general consumption, more attention is being directed

toward their risk profile for gastrointestinal, renal, and cardiovascular side effects (Okwakpam *et al*., 2020). Non selective NSAIDs continue to be nephrotoxic much like the conventional NSAIDs. Several studies conducted around the world indicate that NSAIDs may be associated with adverse effects on the liver and kidneys (Leach *et al*., 1999; Abatan *et al*., 2006; Refael *et al*., 2006; Basavray *et al*., 2012; Bolat and Selcuk, 2013; El-Maddawy and El-Ashmawy, 2013). The spectrum of nephrotoxicity includes acute tubular necrosis, acute tubulointerstitial nephritis, glomerulonephritis, renal papillary necrosis, chronic renal failure, salt and water retention, hypertension, hyperkalaemia and hypereninaemic hypoaldosteronism. Despite the fact that nephrotoxicity attributed to NSAIDS has been reviewed in the past, NSAID induced hepatoxicity still remains a debatable issue. Thus, there are increasing concerns among medical practitioners that treatment of pains and inflammation with Ibuprofen might cause hepatocellular and renal injuries in humans.

There are reports that this risk may be associated with duration of use and/or dose administered (Meunier *et al*., 2018) Thus, the need to investigate the effect of short term administration of ibuprofen by measuring biochemical and histological alterations of the liver and kidney.

MATERIALS AND METHODS

Reagents and Drug Materials

Ibuprofen tablets manufactured by Larborate Pharmaceutical, India and purchased at TSK global Pharmacy, Uniport, Choba. Randox urea, creatinine, bilirubin assay kits, manufactured by Randox laboratories limited, United Kingdom. SPECTRUM albumin and total protein assay kits, manufactured by Egyptian Company for Biotechnology, Egypt. All other reagents were commercially available analytical-grade chemicals.

Experimental Animals

Twenty male Wistar rats weighing between 150-200g was used. The rats were purchased from the University of Part Harcourt and housed within the premises of the animal house of Department of Biochemistry, Faculty of Science, Rivers State University. The rats were left to acclimatize for seven days, the animals were housed under standard conditions of animal husbandry (30 $\pm$ 2^0C, 60-70% relative humidity). The rats were fed on rat diet (flour 55.6%, meat 35%, edible oil 7.5%, sodium chloride 1.2%, Vitamins and minerals 0.7%) and water provided *ad libitum.*

Experimental Design

The experimental design and protocol for this study was in accordance with the standard guide for the care and use of laboratory animals (National Research Council, 2011). The Wistar rats were divided into 4 groups of 5 animals per group (n=5). The control groups were administered the vehicle (distilled water) while groups 2-4 were treated with 200, 400 ad 500 mg/kg body weight of Ibuprofen The drug was administered orally for 7days. A 7-day period was chosen because it has been reported that even short term administration of NSAIDs may induce biochemical changes (Okwakpam *et al*., 2020).

Sample Collection

At the end of the 7th day, the animals were left to fast overnight and the next day they were sacrificed by cervical dislocation and blood and tissue samples were collected. The blood was centrifuged at 4000rpm for 10minutes and serum were separated from the blood cells. The sera collected was used to determine the following biochemical parameters; TP (total protein), AB (albumin), T.B (total bilirubin), Creatinine, Urea, Serum Electrolytes (Sodium (Na), Potassium (K), Chlorine (Cl). The liver and kidney tissues were fixed separately in 10% formal-alkaline solution to preserve the cell content. Thereafter, the tissues were sectioned and stained routinely with haematoxylin and eosin for microscopy.

BIOCHEMICAL ANALYSIS

Determination of Blood Urea level

Urea was estimated using the Berthelot Method. The principle is based on the Berthelot reaction. The enzyme urease splits urea into ammonia and carbon dioxide. The ammonia then reacts with salicylate in the presence of hypochlorite and nitroprusside to form 2, 2 –dicarboxyl indophenols.

Determination of Blood Creatinine level

Blood creatinine was measured using Jaffe's method. Creatinine reacts with picric acid in an alkaline solution to form a reddish coloured complex. The reaction is commonly known as the Jaffe reaction and the red colored product as the Janovski complex.

Determination of serum Albumin

Serum Albumin was estimated using the BromoCresol green method (Monica, 2010). Bromocresol green is an indicator which is yellow between pH 3.5- 4.2. When it binds to albumin, its colour changes from yellow to blue-green. The intensity of the blue-green color is directly proportional to the concentration of albumin in the sample. The absorbance of the color produced is measured by a spectrophotometer at 632nm wavelength.

Determination of serum electrolyte

Serum electrolyte (Soduim (Na), Potassium (K), and Chlorine (Cl) was estimated using ISE method (Ion Selective Electrode) technology. The instrument measures the electrode potentials and the data is then processed by the microprocessor to obtain the concentration of a given ion. The measure method is called "standard comparison".

Determination of Bilirubin level

Principle

Bilirubin and the diazo reagent form an azobilirubin complex, which can be measured calorimetrically. The colour of the azobilirubin varies with pH. It is based on the following principle: conjugated (direct) bilirubin is water soluble and therefore will react with diazo reagent in a water solution, and unconjugated bilirubin is not water soluble, therefore, alcohol is necessary to put the unconjugated bilirubin in solution so that it can react in the diazo reaction.

Statistical Analysis

Statistical package for social science (SPSS), version 23.0 was used for statistical analysis. Results were expressed as mean ± standard error of mean (SEM), (n=5) and statistically analyzed by a one-way analysis of variance (ANOVA) followed by a Turkey's multiple comparison test as a post-test. Analysis at $p \leq 0.05$ was considered to indicate statistical significance.

RESULTS

Table 1 shows the effect of Ibuprofen on selected renal biomarkers. The result obtained shows that there was a significant increase in the concentration of creatinine, urea, sodium and chloride in the serum of group 2, 3 and 4 animals that were treated with different doses of ibuprofen when compared to control in a dose dependent manner. Table 2 shows the effect of Ibuprofen on selected Liver biomarkers. The result obtained shows that there was no significant change in the serum concentration of albumin in group 2 (35.67±1.20) and group 3 (36.67±2.03) when compared to control (40.00±1.00) but there was a significant decrease in Group 4.There was also no variation in the serum levels of total bilirubin and total protein level in group 2, 3 and 4 animals that were treated with different doses of ibuprofen when compared to control.

Table 1: Effect of Ibuprofen on serum renal biomarkers.

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Na^+ (mg/dl)	K^+ (mg/dl)	Cl^-(mg/dl)
Group 1 (Control)	54.33 ± 3.48^a	3.27 ± 0.23^a	138.67 ± 0.67^a	3.53 ± 0.27^a	98.33 ± 0.33^a
Group 2 (200mg/kg Ibuprofen)	85.00 ± 3.4^b	4.43 ± 0.19^c	134.67 ± 0.88^b	4.77 ± 0.89^b	79.67 ± 2.03^b
Group 3 (400mg/kg Ibuprofen)	$101.67 \pm 2.60^{b,c}$	5.87 ± 0.18^b	131.33 ± 0.81^c	5.30 ± 0.21^c	76.00 ± 2.30^b
Group 4 (500mg/kg Ibuprofen)	115.00 ± 5.00^c	4.87 ± 0.09^b	128.67 ± 0.33^c	6.03 ± 0.26^c	73.67 ± 2.19^b

Values are expressed as Mean ± Standard error of mean (SEM), n=5. Values with the same superscript within a column are not significantly different at ($p<0.05$).

Table 2: **Effect of Ibuprofen on Liver biomarkers.**

Groups	Albumin (g/L)	Total bilirubin level (g/L)	Total protein level (g/L)
Group 1 (Control)	40.10 ± 1.10^a	7.00 ± 0.59^a	64.00 ± 0.58^a
Group 2 (200mg/kg Ibuprofen)	35.67 ± 1.20^a	9.33 ± 0.88^a	58.33 ± 10.20^a
Group 3 (400mg/kg Ibuprofen)	36.67 ± 2.03^a	9.00 ± 0.58^a	65.33 ± 0.88^a
Group 4 (500mg/kg Ibuprofen)	28.33 ± 0.88^b	8.00 ± 0.58^a	62.67 ± 1.45^a

Values are expressed as Mean ± Standard error of mean (SEM), n=5. Values with the same superscript within a column are not significantly different at ($p<0.05$).

Histological Examination

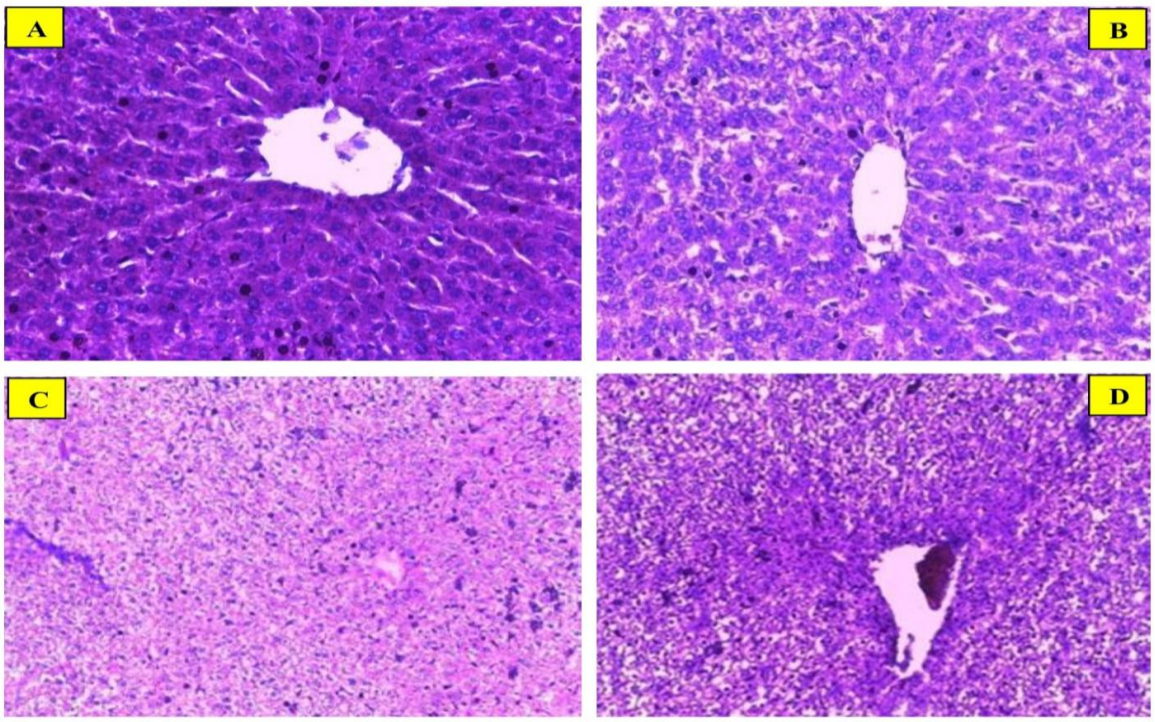

Plate 1: Representative photomicrographs from the liver showing the effect of Ibuprofen in rats. (A) Control: No visible lesions seen (B) 200mg/kg Ibuprofen: There is very mild fatty change (arrows). (C) 400mg/kg Ibuprofen: There is a moderate fatty change in the liver (arrows). (D) 500mg/kg Ibuprofen: There is a severe fatty change in the liver (arrows). H & E; magnification X400.

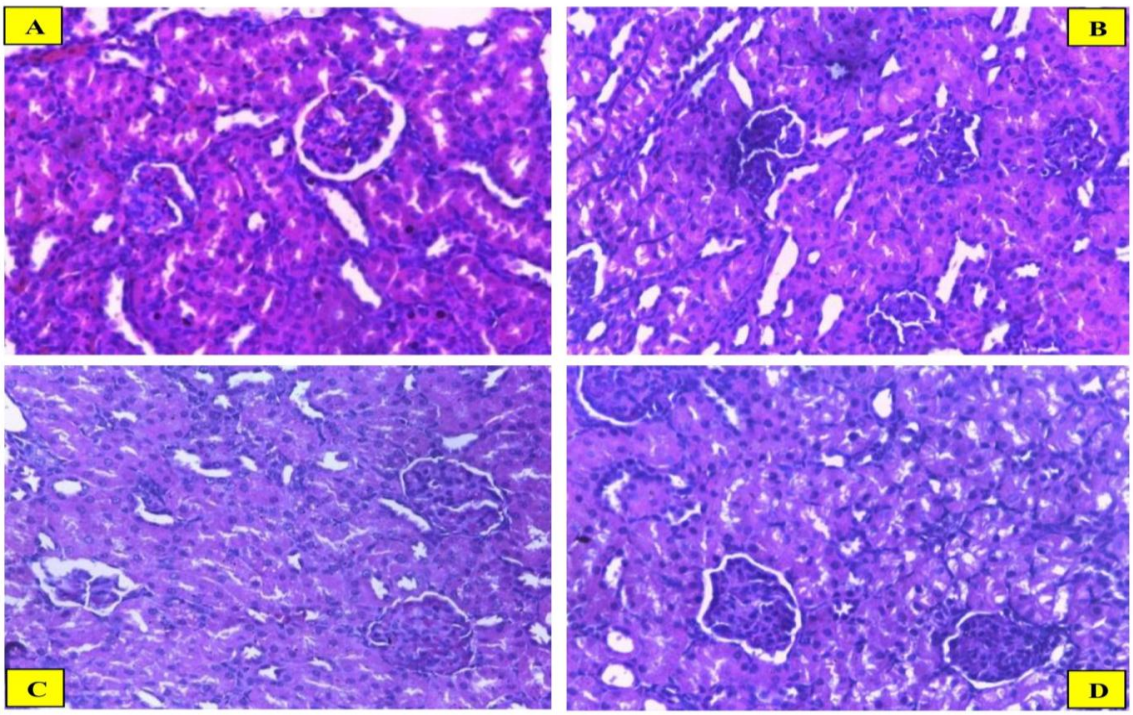

Plate 2: Representative photomicrographs from the kidney showing the effect of Ibuprofen in rats. (A) Control: No visible lesion seen (B) 200mg/kg Ibuprofen: No visible lesion seen (arrows). (C) 400mg/kg Ibuprofen: There is a loss of nuclei, desquamation of cell; lipid accumulation in places, mesangial proliferation in the glomerulus (arrows). (D) 500mg/kg Ibuprofen: There is a loss of nuclei, desquamation of cell; lipid accumulation in places. Focal collapsed glomerulus, mesangial proliferation in the glomerulus (arrows). H & E; magnification, X400.

DISCUSSION

The present study was performed to investigate the effect of ibuprofen on some biochemical parameters of Wistar rats. There was a significant increase in the concentration of creatinine in the serum in all test groups when compared to control. This could be due to nephrotoxic effect of the drug leading to reduced renal function. The test for creatinine is sensitive and a primary biomarker of the kidney damage (Vasudevan and Sreekumari, 2007). Thus, elevated serum levels of creatinine may indicate kidney injury with resultant reduced glomerular filtration. Also, there was increase in urea concentration in all test groups when compared to control. Urea represents the principal waste product of protein catabolism. Ibuprofen probably causes a decrease in glomerular filtration rate, resulting in decreased excretion of urea, which may produce an increase in the concentration of the blood urea. It was earlier reported that ibuprofen inhibits cyclooxygenase, thereby suppressing the production of prostaglandins which play an important role in maintaining glomerular filtration rate of the kidneys (Horl, 2010; Dhanvijay *et al*., 2013) thus, ibuprofen at given doses may alter renal functions through its effect on renal prostaglandins leading to reduced GFR and accumulation of both creatinine and urea in blood.

There was significant difference in serum electrolyte level in all the test groups when compared to control. Failure in the regulatory functions of the kidney leads to abnormally high or low levels of these ions in the blood, due to reduced GFR and rate of secretion of these ions. The inhibition of renal prostaglandins synthesis by NSAIDs causes various electrolyte and acid -base disturbance including sodium retention, hyponatremia, hyperkalemia, hypochloridemia and decreased renal function (Kim and Joo, 2007). Hyperkalemia is seen in decreased urinary output, increased hemolysis and tissue necrosis, since the normal level of potassium ions is kept at a very narrow margin, even a small increase is life threatening. Thus, the observed difference in serum electrolyte level compared to control may be as a result of reduced renal excretion or severe tissue injury, due to the doses of ibuprofen administered. These findings are in agreement with Juhlin *et al*. (2004) who demonstrated that acute administration of diclofenac induces significant decrease in GFR, urine flow, excretion of sodium and potassium in patients with congestive heart failure.

Serum levels of these biomarkers' albumin, total bilirubin level and total protein level are markedly used as indicators of severe liver damage. There was no significant decrease in the serum concentration of albumin in group 2 and group 3 (36.67±2.03) when compared to control but there was a significant decrease in Group 4 (28.33±0.88) .The significant decrease in serum level of albumin in the animals treated with the highest dose of ibuprofen in this study may be indicative of liver impairment in the synthesizing function of the liver (Vasudevan and Sreekumari, 2007). Decrease in serum albumin may also be due to hemorrhage in the gastrointestinal tract as a result of toxic effect of the drug (Chen *et al*., 2017). These findings are in agreement with El-Maddawy and El-Ashmawy (2013), who observed significant decrease in serum albumin levels in rats treated with diclofenac sodium at a dose of 13.5mg/kg for 14 days and compatible with an earlier scientific report (Basavraj *et al*., 2012). There was no significant difference in the serum concentration of total protein and total bilirubin in the test groups when compared with control. However, from the data, it could be observed that there was an increase in the total bilirubin concentration in all the groups when compared with control. Bilirubin is a product of heme within the reticuloendothelial system; its elevation in the blood stream can be adduced to over production, increased hemolysis, decreased conjugation or impaired bilirubin transport (Mishima *et al*., 2019). Bilirubin is an index that is used to assess the normal functioning of the liver instead of the extent of hepatocellular injury. This may be indicative of mild liver impairment.

Histological findings in liver and kidney tissue go side by side with the biochemical alterations. Histopathological investigation of liver revealed no damage in the control animals but changes were observed in the tissues of the animals that received varying doses of ibuprofen respectively. Also, histopathological investigation of kidney revealed no damage in control and group 2 animals but changes were observed in groups 3 and 4 that received higher doses of ibuprofen. These findings suggest that a high dose of ibuprofen may cause cell damage and acute hepatitis, also it may adversely affect the kidney function and may play a role in the induction of membranous nephropathy (Gokcimen *et al*., 2001). These findings are in agreement with the work of El-Maddawy and El-Ashmawy (2013) whose result showed that administration of diclofenac induced adverse effect on hematological, biochemical, oxidative parameters as well as histology of liver and kidney. The result also agrees with the reports of Gabriel and Renato (2020) who stated that NSAIDS have the ability to cause injury in virtually any renal compartment; its main effects are described in most renal side effects are observed on all subclasses; however, disagrees with the report that the incidence of nephrotic syndrome could be more related to the use of the non-selective type, especially when for more than 15 days and up to 2 years after exposure to the drug.

CONCLUSION

These results have shown that short term administration of ibuprofen has potentially harmful effect on the liver and kidneys of Wistar rats especially at high.

REFERENCES

1. Okwakpam, F.N., Abarikwu, S., and Monanu, M.O. (2020). Evaluation of Stress Enzymes Activities and

Lipid Peroxidation in Heart Homogenates of Male Wistar.Rats Following the Administration of Diclofenac. *Asian Journal of Research in Biochemistry*, 6(3): 10-16.
2. Horl, W.H. (2010) Non-steriodal Anti-inflamatory Drugs and the kidney, *Pharmaceuticals*, 3: 2291-2321
3. Harris, R.C. (2006). Cox-2 and the kidney. *Journal of cardiovascular pharmacology*, 47: 37-42.
4. Weir M.R. (2002). Renal effect of non-selective Non-steriodal Anti-inflamatory Drugs and coxibs. *Cleveland Clinical Journal medical*, 69(1): 53-58.
5. Diamond, S. (2000). Ibuprofen versus aspirin and placebo in the treatment of muscle contraction headache. *Headache*, 23: 206-210.
6. Prusakiewicz, J.J., Duggan, K.C., Rouzer, C.A., Marnett, L.J. (2009). Differential sensitivity and mechanism of inhibition of COX-2 oxygenation of arachidonic acid and 2-arachidonoylglycerol by ibuprofen and mefenamic acid. *Biochemistry*, 48:7353-7355.
7. Rudy, A.C., Knight, P.M., Brater, D.C., and Hall, S. (1992). Stereoselective metabolism of R-, S- and racemic ibuprofen. *Journal of Pharmacology and Experimental Therapeutics*, 259(3): 1133-1139.
8. Leach, W. M., Doyle W.F., Mark R.B., and Ellen W. E. (1999). Renal changes associated with Naproxen sodium administration in cynomogus. *Toxicologic pathology*, 27(3): 295-306.
9. Abatan , M.O., Lateef I., and Taiwo V.O. (2006). Toxic effect of Non-steriodial Anti-Inflammatory Agents in rats, *African Journal of Biomedical Research*, 9: 219 -223.
10. Refael, S.M.D., Emilia, L.M.D., Adrain, I.M.D., and Dan C.M.D (2006). Renal effects of low dose of Aspirin in Elderly patients. *Israel Medical Association Journal*, 8: 679-682.
11. Basavray, S.T., Dhaval T.F., Kantibhai S.P., Jivani B.M., Ketan B.T., Bholanath P.J., and Vishal V.U. (2012). Haemato-biochemical alterations induced by Diclofenac Sodium toxicity in Swiss Wistar mice, *Veterinary World*, 5(7): 417-419
12. Bolat, D. and Selcuk, M.L. (2013). Stereological and biochemical evaluation of diclofenac-induced acute nephrotoxicity in rats. *Revised Medical Vertenary*, 164(6): 290-294
13. El-Maddawy, Z.K., and El-Ashmawy I.M. (2013). Hepato-Renal and hematological effects of diclofenac sodium in rats, *Global Journal of Pharmacology*, 7(2): 123-132.
14. Meunier, L. and Dominique, L (2018). Recent Advances in Hepatotoxicity of Non-Steroidal Anti-Inflammatory Drugs. *Annals of Hepatology*, 17(2): 187-191.
15. National Research Council (2011). Guide for the care and use of laboratory animals, 8th edition. The National Academies Press, Washington
16. Monica, C. (2010). District Laboratory Practice in tropical countries, 2nd edition update, Cambridge University press, Singapore, pp: 355-357.
17. Vasudevan D.M. and Sreekumari S. (2007). Textbook of biochemistry for medical student (5th edition), *Jaypee Brothers Medical Publishers Limited*, New Delhi, pp: 266-280.
18. Dhanvijay, P., Misra, A. K., & Varma, S. K. (2013). Diclofenac induced acute renal failure in a decompensated elderly patient. *Journal of pharmacology & pharmacotherapeutics*, *4*(2): 155–157.
19. Kim, S and Joo, K.W. (2007). Electrolyte and Acid-base disturbances associated with Non-steriodal Anti-inflammatory drugs. *Electrolytes and Blood Pressure*, 5: 116-125.
20. Juhlin, T., Bjorkman, S., Gunnersson, B., Fyge, A., Roth, B., and Hogland, P. (2004). Acute administration of diclofenac but possibly not long-term low dose aspirin, causes detrimental renal effects in heart failure patients treated with ACE-inhibitors. *European Journal of Heart Failure*, 6(7): 909-916.
21. Chen, R.C., Cai, Y.-J., Wu, J.M., Wang, X.D., Song, M., Wang, Y.Q., Zheng, M.H., Chen, Y.P., Lin, Z., and Shi, K.Q (2017). Usefulness of albumin-bilirubin grade for evaluation of long-term prognosis for hepatitis B-related cirrhosis. *Journal of Viral Hepatitis*, 24: 238-245.
22. Mishima, M., Koda, M., Tsui, W.M.S. (2019). Granulomatous liver diseases. In: Hashimoto, E., Kwo, P., Suriawinata, A., Tsui, W., Iwai, M. (Eds.), Diagnosis of Liver Disease. Springer, Singapore.
23. Gokciman, A., Acrogen M., and Karoaz E. (2000). Structural and biochemical changes in liver and renal tissues induced by an acute high dose of diclofenac Sodium in rats, *Biomedical Research*, 11: 293-302.
24. Gabriel, T.M and Renato, D.F. (2020). Review Article: Drug-induced nephrotoxicity. *Revista da Associacao Medica Brasileira*, 66(1): doi.10.1590/1806-9282.