Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.03.044

Histological and Biochemical Changes of Rats kidneys post Indomethac in Administration

Ban Ismael Sedeeq[1*]
[1]Tikrit University / College of Dentistry
***Corresponding author:** Ban Ismael Sedeeq, Tikrit University / College of Dentistry, Email: banasnan@tu.edu.iq

Submitted: 18 November 2022; Accepted: 10 December 2022; Published: 12 January 2023

ABSTRACT

Non-steroidal Anti-inflammatory Drugs (NSAIDs) are one of the most widely used drugs in medicine and their uses has dramatically increased in recent years. They are a class of drugs that reduce pain and lowering the body temperature. The metabolism and excretion of indomethacin was conducted in kidney, so as a result, biochemical parameter and kidney tissues may altered. The present study was designed to evaluate the biochemical, and histological changes induced by indomethacin on the rat's kidney , Thirty adult male albino Sprague-Dawley rats were used in this experimental study. Rats were divided into three equal groups [Ten in each]. All rats were kept in a quite non stressful environment, provided with food ad libitum and free access to water. Normal saline (1 ml) was given intramuscularly for the control group (I). Experimental groups (II, and III) were injected with indomethacin intramuscularly equivalent to 5 and 10 mg/kg body weight/day respectively for two weeks. Results revealed that indomethacin administration significantly increased the levels of blood urea, uric acid and creatinine when compared with control rats. This study confirmed the risk of increased oxidative stress, and nephrotoxicity due to indomethacin administration. Although indomethacin is reported to be effective in pain management, its toxicity should be kept in mind

Keywords: *Histological, Administration, Biochemical, Post*

.

INTRODUCTION

Kidney has a vital role in renalclearance, maintenance of blood pressure, elimination of toxic products and formation of prostaglandins.Certain medications are known to causerenalinjuryon its frequent usage and high dosage(1).

Non-steroidal Anti-inflammatory Drugs (NSAIDs) are one of the most widely used drugs in medicine and their uses has dramatically increased in recent years. They are a class of drugs that reduce pain and decrease the inflammation (2).

Indomethacin is 1-(p-chlorobenzoyl)-5-methoxy-2-methyllindole3-acetic acid ,an important member of the NSAIDs family. is a methylated indole acetic acid derivative and commonly used in clinical therapy as anti-inflammatory, analgesic and anti-pyretic

drugs. Its extensive utility is arisen from the rapid absorption, rapid and efficient crossing blood brain barrier (3,4).

The present study was conducted to evaluate the effects of the indomethacin on kidney tissues and biochemical parameter of albino rats.

MATERIALS AND METHODS

Thirty experimental animals albino Sprague-Dawley male rats weighing 200-225 g were used. These rats kept for two weeks at the laboratory animal house center of the Faculty of Veterinary Medicine, University of Tikrit. All rats were kept in a quite non stressful environment and were maintained under standard housing conditions: temperature (27 ±1 °C), humidity (55- 60%), light/dark cycle (12:12h) and had free access to standard rodent pellet diet ad libitum and free access to water.

Normal saline (1 ml) was given intramuscularly as placebo for the control group (I). Experimental groups (II, and III) were injected with indomethacin intramuscularly equivalent to 5 and 10 mg/kg body weight/day respectively for two weeks.

After two weeks, blood samples were collected from rats hearts, then all animals were sacrificed. Blood urea, uricacidandcreatinine were estimated using a commercial diagnostic kit. Estimation of serum cystatin C was measured by immunoturbidimetric method.

Statistical analysis

The data were expressed as the mean ± S.E.M. and were analyzed by means of one-way analysis of variance (ANOVA). Statistical evaluation of data was done following Student's t-test. A difference was considered significant at $P < 0.01$.

Kidney specimens (1 cm thick) of different groups were immediately fixed in 10% neutral buffered formalin, dehydrated with graded series of ethyl alcohol and embedded in paraffin of 5 µm thickness. The sections were stained with hematoxylin and eosin(5).

RESULTS

Biochemical parameters

TABLE 1: Effect of indomethacin Administration on Biochemical Profile of Male Rats

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Cystatin C (mg/dl)
Control	0.32±0.08	37.19±1.7[a]	8.20±0.68a	0.83 ± 0.26
Indomethacin(5mg/kg)	0.67±0.08[a]	43.3±12.7	15.0± 9.0	1.12 ± 0.06
Indomethacin(10mg/kg)	0.79±0.13[a]	52±15.13*	23.7± 0.7	1.56 ± 0.34

Histopathological examination of the kidney

Kidney of the control group (I)

The cortex of the kidney contained the glomeruli, which was appeared normal in shape as a tuft of capillaries inside Bowman's capsule surrounded by capsular space. The proximal convoluted tubules were present with distal convoluted tubules around the renal glomeruli (Fig.1). The renal medulla contained the renal tubules and collecting ducts with henle's loops and blood vessels were present in between (Fig.2).

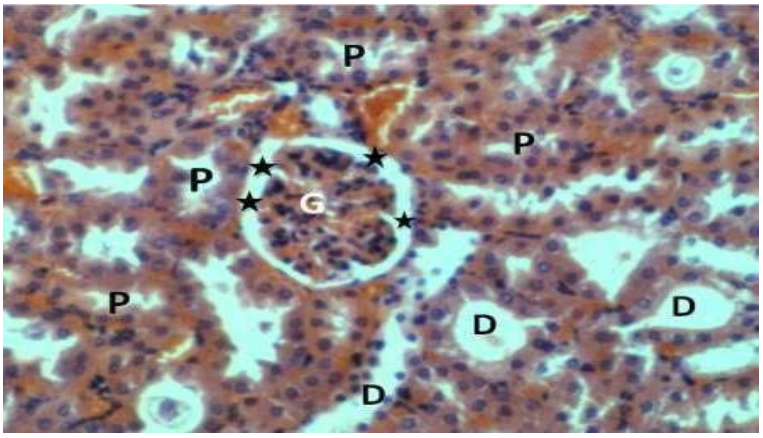

FIG 1: Renal cortex in control group G1(I), demonstrating the shape of glomerulus (G), surrounded by capsular space (stars) and a great number of proximal convoluted tubules (P) and distal convoluted tubules (D). (H &E X20).

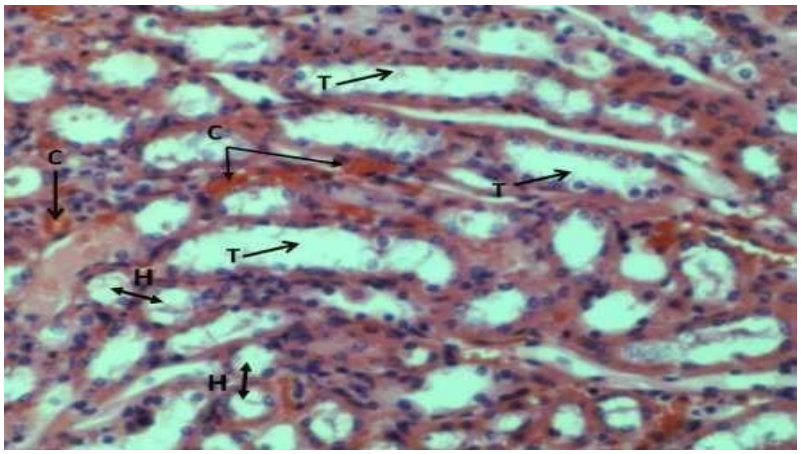

FIG 2: Renal medulla in control group G1, shows, renal tubules (T), Henle's loops (H), Blood capillaries (C).(H &E ×20).

Experimental group (II)/ This group receive Indomethacin 5mg/kg.

Kidney cortex contains atrophied glomeruli, so the capsular space was wide. Most of the proximal and distal convoluted tubules were containing desquamated epithelial cell from its luminal surface. Other interesting histopathological changes was the heavy infiltrating of lymphocytes around the glomeruli, proximal and distal convoluted tubules(Fig. 3 & 4).

The renal medulla was characterized by the presence of massive aggregation of lymphocytic and other WBC inflammatory cells in the pelvis of the kidney and around the renal tubules, which also appeared containing desquamated cells in its lumens (Fig 4 , 5 & 6).

J Popul Ther Clin Pharmacol Vol 30(3):e426–e432; 12 January 2023.

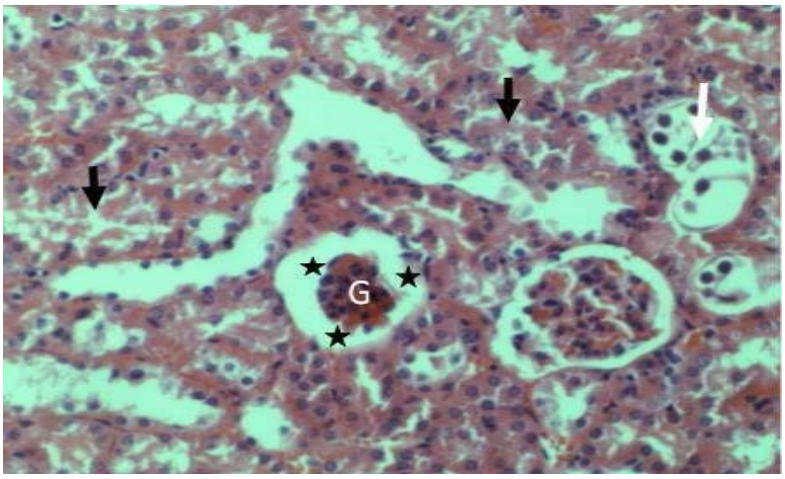

FIG 3: G2 (II) Renal Cortex shows, Atrophied glomeruli (G). Wide capsular space (stars). Desquamated cells in the lumen of proximal (black arrows) and distal tubules (white arrow) . (H &E ×20).

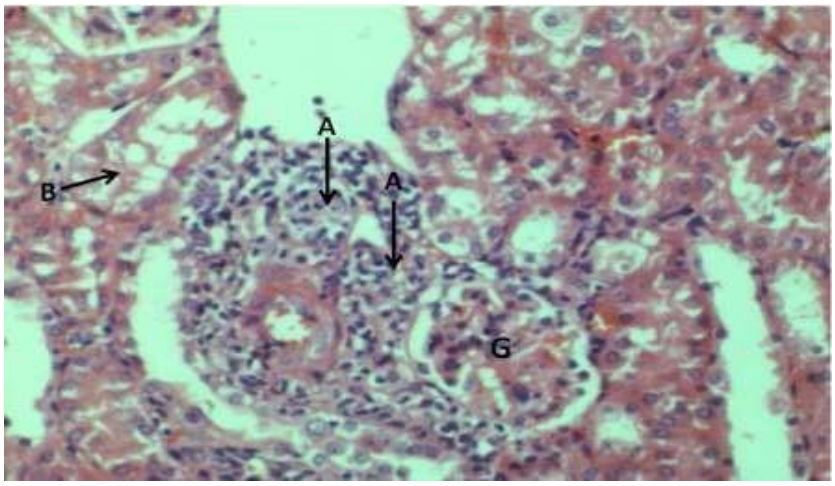

FIG 4: Renal cortex of experimental (II), Lymphocytic cells aggregation (A), desquamated cells in the lumen of proximal convoluted tubules (B). (H &E ×20).

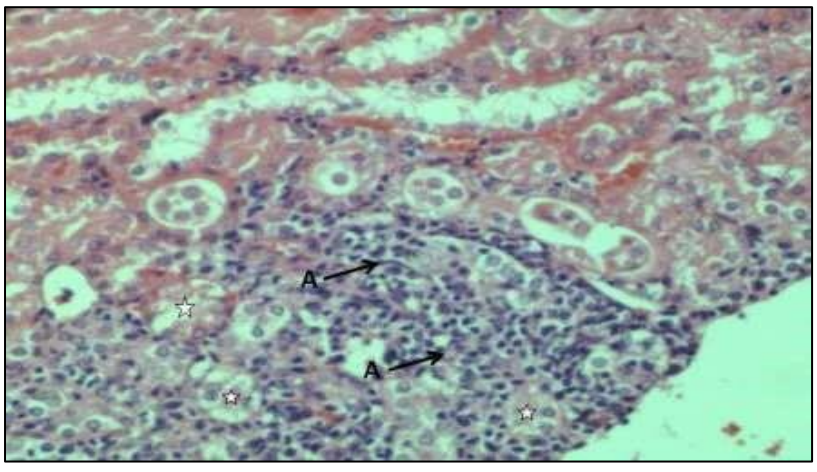

FIG 5: Renal medulla of experimental group (II), showing massive aggregation of lymphocytic and other WBC in the pelvis (A), and degeneration in renal tubules (stars). (H & E ×20).

J Popul Ther Clin Pharmacol Vol 30(3):e426–e432; 12 January 2023.

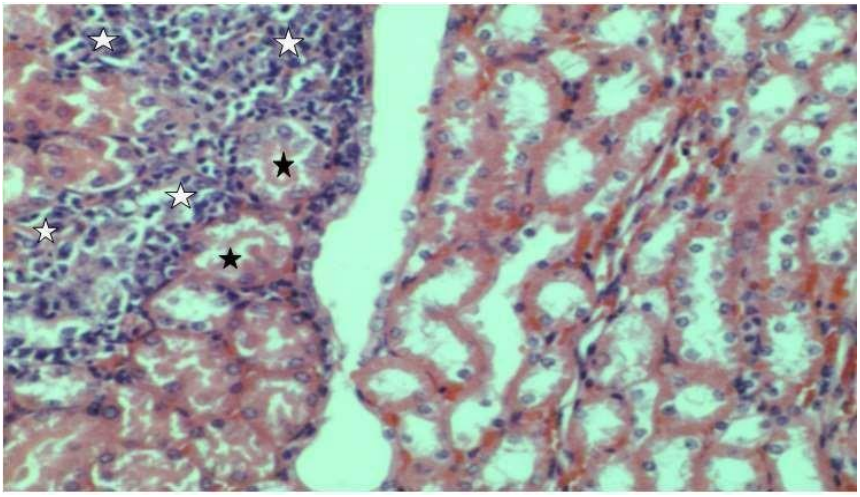

FIG 6: Renal medulla experimental group (II), showing lymphocytes and other inflammatory cells in between renal tubules (white stars) with degeneratin medullary tubules (black stars) . (H & E ×20).

Experimental group (III)/This group receive Indomethacin 10 mg/kg bwt

The renal cortex shows atrophied glomeruli. The blood vessles of cortex aadjecent to the glomeruli and convoluted tubules were severly congested, renal medulla showed thickening of Henles loop wall. (Fig 7 & 8).

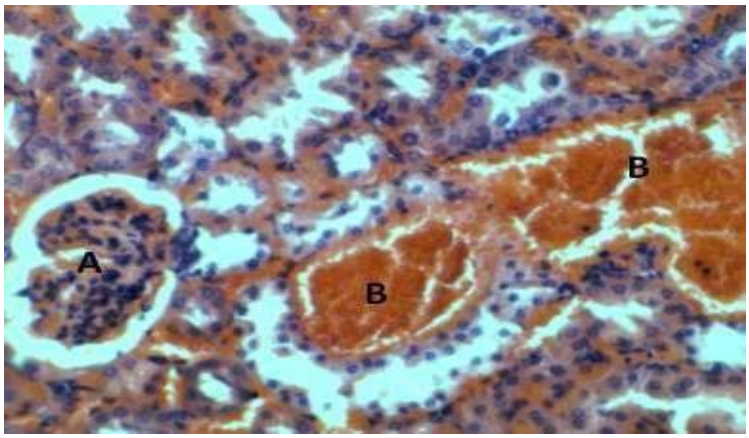

FIG 7: renal cortex shows, atophiedglumerulas (A). severe congestion of B.V (B). (H&E ×20)

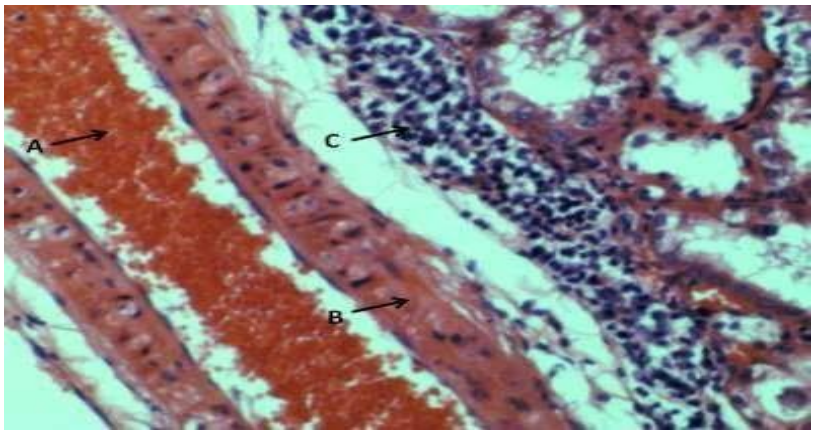

FIG 8: Renal medulla in group (III) shows, congestion of blood vessels (A)Thickening of blood vessles wall (B). Sheath of lymphocytes infilterationadjecent to the blood vessles (C)(H &E ×20).

DISCUSSION

The kidneys are involved in the secretion of several, toxins and therefore they are liable to liberate high quantities of free radicals which contribute to high oxidative stress that is involved in Causing kidney damage.In recent years, the science of nutrition has advanced significantly based on food supplements and medicinal herbs employed in controlling and modulating chronic diseases in human beings(6).

The indomethacin elevated oxidative stress and reduced endothelial nitric oxide syntheses activity have been demonstrated to contribute to the pathogenesis of this drug- induced kidney injuries (7).

Blood tests for Blood urea nitrogen (BUN) and creatinine are the simplest way to monitor kidney function. These substances are normal metabolic waste products that are excreted by the kidneys. Urea is a byproduct of protein breakdown (8).

Creatinine and urea levels are used as markers of kidney function, but the test for creatinine is more sensitive than urea (8).Creatinine is the breakdown product of creatinine phosphate is released from skeletal muscle at a steady rate. It is filtered by the glomerulus, and a small amount is also secreted into the glomerular filtrate by the proximal tubule (9-11) .

As the excretory function of kidney is impaired, urea and Cr excretion is hampered leading to its increased levels in blood (12), so that our results showed that statistical significant increase of urea and creatinine is as a result of administration of indomethacin which may indicate kidney injury, with resultant reduced glomerular filtration. The results of this study disagree with the findings of KIM et al (13) which found that indomethacin did not significantly alter the plasma creatinine clearance. BOLAT et al (14) indicate that NSAIDs induced nephrotoxicity may be due to the inhibitory effect of these drugs on prostaglandin synthesis, thus causing kidney ischemia.

Certainly, cystatin-c jointly with blood urea and serum creatinine is used for the assessment of renal function and GFR (15). It is a single chain, nonglycosylated basic, low-molecular-weight protein of 12 KDa synthesized by all nucleated cells, it is filtered solely by the glomerulus, is not handled by the renal tubules, and is generated at a constant rate by all cells in the body. The utility of cystatin C as a renal marker suggested that cystatin C is more sensitive and superior to creatinine for estimating GFR (16). The study shows significant increase in serum cystatin C levels in treated group compared to controls, due to renal impairment.

Uric acid is the final product of purine metabolism in humans. It is formed in the liver, can be degraded by the hepatic enzyme uricase and converted to allantoin, and excreted in urine through the kidney. Uric acid is a byproduct of the continual process in the body, where old cells are broken down and new one are formed(17).

The increased uric acid level observed in this study, following indomethacin administration might be due to hyperuricemia and gout because kidneys plays an important role in elimination of uric acid and creatinine.

Due to the defense mechanisms against free radical-created oxidative damage causes an increase in the concentration of uric acid (electron donors) in order to reduce free radicals. This effect may aggravate the condition of renal damage resulted from uric acid (18). The mechanism of kidney destruction because of the oxidative stress involves the secretion of cytokines, mainly tumor necrosis factor TNF-α, interleukin IL-1, and IFN-c(19).

Our histopathological findings in kidneys tissues go side by side with the obtained biochemical alterations. Histopathological examinations showed severity of the lesions increased with increased the dose of drug. The renal lesions in the same rats shows atrophied glomeruli of renal cortex, with partial segmenation. The blood vessles of cortex a round the glomeruli and convoluted tubules were severly congested with blood, renal medulla showed heavy infiltration of lymphocytes and other WBCs. The results of present study agree with Al –mayaliet al (20), their study revealed abnormal structure of glomerulus with tubular necrosis and presence of interstitial inflammetion. And also agree with Nagappanet el (7) results, they were concluding that indomethacin reduced renal perfusion, and this was leading to glomerular and tubular injury with subsequent renal damage.

J Popul Ther Clin Pharmacol Vol 30(3):e426–e432; 12 January 2023.

REFERENCES

1. Simon J.P., Evan P. S. Diclofenac-induced renal toxicity in female Wistar albino rats is protected by the pre-treatment of aqueous leaves extract of Madhucalongifolia through suppression of inflammation, oxidative stress and cytokine formation. Biomed Pharmacoth. 2018;98(1): 45-51.
2. Alabi Q.K., Akomolafe R.O., Adefisayo M.A., OlukiranO.S. ,Nafiu A.O. , Fasanya M.K. , Oladele A.A., Kolaviron attenuates diclofenac-induced nephrotoxicity in male Wistar rats. Applied Physiology, Nutrition, and Metabolism. 2018;43(9):956968.
3. EntedharRifaatSarhat, SihamAjmeeWadee, Ban Ismael Sedeeq, ThuraiaRifaatSarhat. Biochemical and Histological Evaluation of Indomethacin-induced Hepatotoxicity in Rats.
4. Lucas S: the pharmacology of indomethacin. Headache. 2016;56(2):436-64.
5. Buthayna A. A., Entedhar R. S. ,Siham A. W. Study of The Effect of Castor Seeds(RicinusCommunislinn.)on Ovary Functions and Characters of Female Rabbits.. Assiut Vet. Med. J. 2017; 63(152):62-65.
6. Buthyna .A .Abdullah, Siham .A. Wadi2, Entedhar R. Sarhat. Histological Study Effects of Paracetamol on Livers and Kidneys of Adult Mice .Journal Tikrit Univ. for Agri. Sci.,2017; 17;Special : 6th Scientific Conference for Agricultural Researches, 28-29:97-103.
7. Nagappan AS, Varghese J, Pranesh GT, Jeyaseelan V, Jacob M. Indomethacin inhibits activation of endothelial nitric oxide synthase in the rat kidney: possible role of this effect in the pathogenesis of indomethacin-induced renal damage. ChemBiol Interact. 2014;221:77-87.
8. Dabla PK. Renal function in diabetic nephropathy. World J Diabetes. 2010;1(2):48-56.
9. Entedhar R. Sarhat. Practical Medical Biochemistry.1st rev. ed. Tikrit. Tikrit University Press.2019;425p.
10. Orinya A.O., Adenkola A.Y. and OgbeR.J.. Hematological and biochemical studies
11. on the effect of diclofenac sodium on WistarRattusnorvegicus. Int. J. Biol. Chem. Sci. 2016;10(5): 2231-2242.
12. BamanikarSA ,Bamanikar AA, Arora A., Study of Serum urea and Creatinine in Diabetic and nondiabetic patients in in a tertiary teaching hospital. JMR 2016; 2(1): 12-15.
13. EntedharRifaatSarhat, and Nawal Abdullah Murtadha. Biochemical Changes in Chronic Renal Failure Pre and Post Hemodialysis. Journal of Environmental Science and Engineering A.2016; 5(4):190-195
14. Kim S.W., Kim J.W., Choi K.C., Ma S.K., OH Y., Jung J.Y., JIN Kim J, and Lee J., Indomethacin Enhances Shuttling of Aquaporin-2 Despite Decreased Abundance in Rat Kidney. J Am SocNephrol.2004; 15: 2998-3005.
15. Bolat D. and Slcuk M.L., Stereological and biochemical evaluation of diclofenac – induced acute nephrotoxicity in rats, Revue Med Vet., 2013;164(6), 290-294.
16. Al-Kuraishy HM, Al-Gareeb AI, Hussien NR. Synergistic effect of berberine and pentoxifylline in attenuation of acute kidney injury. Int J CritIllnInjSci2019;9:6974.
17. Alaje AK, Adedeji TA, Adedoyin AR, Idogun SE. Creatinine and cystatin C-based evaluation of renal function among obese subjects in Benin City, Nigeria. Saudi J Kidney Dis Transpl. 2019;30:648-54.
18. XiaoniShao , Wenjie Lu, FabaoGao , Dandan Li , Jing Hu, Yan Li , ZepingZuo , HuiJie , Yinglan Zhao, and Xiaobo Cen. Uric Acid Induces Cognitive Dysfunction through Hippocampal Inflammation in Rodents and Humans. The Journal of Neuroscience. 2016 ;36(43):10990-11005.
19. Sivaraj R, Umarani S. Diclofenac-induced biochemical changes in nephrotoxicity among male Albino rats. Int J Basic ClinPharmacol2018;7:640-3.
20. EntedharRifaatSarhat, Siham A. Wadi, Ban I Sedeeq. Thuraia R. Sarhat, Nawar.A. Jasim. Study of histopathological and biochemical effect of PunicagranatumL.extract on streptozotocin - induced diabetes in rabbits. Iraqi Journal of Veterinary Sciences, 2019; 33(1):189-194.
21. Al-mayali Z.K., JaffatH.S.and Mohammed J.A., effect of the indomethacin drug on kidney histology in male Albino Rats, Int J pharm Quality Assurance, 2019, 10(3).
22. Mahdi, E. M., & Mustafa, M. A. (2022). Effect of different concentrations of extract of Urticadioica and Cladosporiumcladosporiodes on Triboliumcastaneum or: Coleoptera after 24-48 hours of exposure in Samarra City/Iraq. HIV Nursing, 22(2), 3207-3210.
23. Mustafa, M. A., Kadham, S. M., Abbass, N. K., Karupusamy, S., Jasim, H. Y., Alreda, B. A., ... & Ahmed, M. T. (2023). A novel fuzzy M-transform technique for sustainable ground water level prediction. Applied Geomatics, 1-7.
24. Kadham, S. M., Mustafa, M. A., Abbass, N. K., &Karupusamy, S. (2022). IoT and artificial intelligence–based fuzzy-integral N-transform for sustainable groundwater management. Applied Geomatics, 1-8.

J Popul Ther Clin Pharmacol Vol 30(3):e426–e432; 12 January 2023.