International Journal of Basic & Clinical Pharmacology
Okoroama CE et al. Int J Basic Clin Pharmacol. 2023 Sep;12(5):631-639
http://www.ijbcp.com
pISSN 2319-2003 | eISSN 2279-0780

DOI: https://dx.doi.org/10.18203/2319-2003.ijbcp20232557

Original Research Article

Evaluation of the protective role of antioxidants: α-tocopherol, vitamin C, and quercetin, against ibuprofen-induced renal damage in male Wistar rats

Casmir E. Okoroama[1]*, Prince C. Unekwe[1], Linda C. Okoroama[2], Samuel U. Okparaoka[3], Godwin C. Akuodor[1]

[1]Department of Pharmacology and Therapeutics, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria
[2]Department of Public Health, Imo State University, Owerri, Imo State, Nigeria
[3]Department of Histopathology, Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria

Received: 31 May 2023
Revised: 06 July 2023
Accepted: 07 July 2023

***Correspondence:**
Dr. Casmir E. Okoroama,
Email: emekaokoroama@yahoo.co.uk

ABSTRACT

Background: Ibuprofen, commonly used in management of pains and inflammatory conditions, has been associated with renal damage. The protective role of antioxidants against Ibuprofen-induced renal damage in wistar rats were investigated in this study.
Methods: The study was designed in two parts; first, to induce kidney damage and secondly, to determine protective role of antioxidants against ibuprofen-induced kidney injury. In the first phase, two groups of animals were used; one group treated with 120mg/kg ibuprofen daily for 14 days, while the other served as control and had distilled water. Serum malondialdehyde and kidney parameters were estimated after treatment and kidneys harvested for histology. In the protection study, animals were divided into five groups, with groups 1-4 having three sub-groups treated with 120mg/kg Ibuprofen and graded doses of vitamin E, vitamin C and quercetin respectively, while group five served as control. After 14days, antioxidant enzymes and kidney parameters were estimated, and the kidneys harvested for histology.
Results: Showed significant ($p<0.05$) increase in malondialdehyde, urea, creatinine, and uric-acid levels after ibuprofen administration. Conversely, there was significant ($p<0.05$) reduction in kidney parameters after co-administration of antioxidants with ibuprofen, with significant increase in antioxidant enzymes. Estimated antioxidant's percentage protection showed vitamin E gave highest protection on the kidneys against ibuprofen-induced damage amongst others. Histology revealed atrophied glomeruli with widened capsular space, desquamated tubular epithelial cells and infiltrating lymphocytes after ibuprofen administration, but showed normal histo-architecture after co-administration with antioxidants.
Conclusion: Antioxidants such as α-tocopherol, vitamin C, quercetin, protected against ibuprofen-induced renal damage in Wistar rats.

Keywords: Ibuprofen, Antioxidants, α-tocopherol, Vitamin C, Urea, Creatinine

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are commonly used agents in our society. They are over-the-counter drugs, used primarily in controlling different types of pains, such as acute pains, osteoarthritis, dysmenorrhea, and other chronic pain management as well as antipyretics and anti-inflammatory agents.[1] Concerns of

the adverse effects of ibuprofen and other NSAIDs have been raised, which includes: dyspepsia, stomach ulcers, upper gastrointestinal bleeding, allergic reactions, liver, and kidney damage.[2,3] Irrespective of these adverse effects, their frequent use remain inevitable, especially in chronic pain management. Although, some measures have been suggested to mitigate some of these adverse effects; for instance giving NSAIDs with proton pump inhibitors has minimized the incidence of upper gastrointestinal effects however, effects such as kidney damage remains a challenge.[4]

Prolonged and indiscriminate use of ibuprofen and other NSAIDs is almost always unavoidable because those taking them need to keep a pain-free state. Therefore, such individuals are at risk of kidney derangement and other adverse effects. It has been suggested that one of the mechanisms by which organ derangement can manifest during use of non-steriodal anti-inflammatory drugs, is through the generation of oxidative stress at the intracellular levels.[5] This intracellular oxidative stress is thought to precede organ derangements, and plays a significant role in early pathogenesis of ibuprofen-induced organ damages.[5,6] Oxidative stress has generally been linked to different organ dysfunctions especially when the homeostatic balance is not maintained.[7]

Studies have shown that antioxidants play vital role in preventing organ dysfunction arising from oxidative stress.[8,9] Since oxidative stress is one of the earliest pathophysiological processes underlying ibuprofen-induced kidney damage, maintaining a homeostatic balance between antioxidants and free radicals can avert such derangement.[7,10] It is therefore possible to proffer a preventive approach by co-administering ibuprofen with antioxidants, so as to protect the kidneys. Preventive measures are easy, affordable and less demanding, when compared to treating kidney diseases. In this study, antioxidants such as vitamin E, vitamin C, and quercetin, which are commonly available and quantifiable in their supplements, will be co-administered with ibuprofen to determine their protective effects against ibuprofen-induced kidney damage in Wistar rats.

The findings from the research may provide an opportunity to mitigate the incidence of kidney injury resulting from use of ibuprofen and other NSAIDs, and possibly other nephrotoxic drugs, considering the current rise in kidney diseases in our society.[10,11] By extension, natural foods rich in antioxidants may provide equivalent protection as their supplements; hence, such diets may become routine nutritional requirements for those taking ibuprofen regularly.

Objective

The necessity to prevent diseases at the earliest possible time is the rationale for this study.

METHODS

Study type, place and duration

This is a protection study conducted at department of pharmacology and therapeutics, faculty of basic clinical sciences and department of pharmacology and toxicology, faculty of pharmaceutical sciences, Nnamdi Azikiwe University, Anambra State, Nigeria from February 2022 to June 2022

Procedure

Animal selection: Seventy (70) adult male Wistar rats of 8-10 weeks old and weighing about 150-180 gram were used for this study. The animals were kept in cages, five animals per cage, for seven days to acclimatize to the laboratory conditions. Test drugs: The drugs used in the study included ibuprofen, and antioxidant supplements such as vitamin E, vitamin C, and quercetin. Experimental design: Study was designed in two parts, first to induce kidney damage from ibuprofen administration, and secondly to determine if co-administration of ibuprofen with antioxidants will prevent kidney damage in the Wistar rats.

Induction of kidney damage: The method described by Gomaa was adopted to induce kidney injuries in the animals.[12] This was achieved by administering high dose of ibuprofen, 120mg/kg body weight daily. The dose is about five times less than the LD_{50} of ibuprofen which is 636mg/kg in rats.[12,13] The principle is that NSAIDs with short half-lives, such as ibuprofen, may produce renal decompensation in a matter of days as reported by Bindu, Mazumder and Bandyopadhyay.[14] The animals were divided into two groups of five animals each and one group treated with 120 mg/kg of ibuprofen daily in three divided doses for fourteen (14) days, while the other group served as control and received 2ml/kg of distilled water per oral. The serum malondealdehyde (a marker of oxidative-stress) and biochemical parameters of the kidney were estimated; the kidneys were harvested and the histological examination carried out. Elevated kidney parameter when compared to the control is an indication of organ damage.[15]

Protection study with antioxidants against ibuprofen-induced renal damage

The animals were randomly divided into five experimental groups of fifteen animals each; which included: Treatment group I, II, III, IV, and V. The drugs (antioxidants and ibuprofen) were dissolved in sterile water and administered per oral for fourteen days. Varying doses of the different antioxidants were administered simultaneously with similar dose of ibuprofen used in induction of kidney damage (120 mg/kg).

Treatment group I had three sub-groups of five animals in each set; group 1a, 1b and 1c respectively. Each sub-group was given 120mg/kg of Ibuprofen daily in three divided

doses, together with graded doses of vitamin E as follows: 1a-50 IU per kg daily, 1b-100 IU /kg daily, 1c-500 IU /kg daily. Treatment group II had three sub-groups of five animals in each (2a, 2b, and 2c). Each sub-group received 120mg/kg of Ibuprofen in three divided doses, with graded doses of vitamin C, as follows: 2a-100 mg/kg daily, 2b-200 mg/kg daily, 2c-500 mg/kg daily. Treatment group III was divided into three sub-groups of five animals in each (3a, 3b, and 3c). They were given 120mg/kg of Ibuprofen in three divided doses, together with graded doses of quercetin, as follows: 3a-125mg/kg daily, 3b-250 mg/kg daily, and 3c-500 mg/kg daily. Treatment group IV had three sub-groups of five animals in each (4a, 4b, and 4c). Each set was given 120 mg/kg of Ibuprofen in three divided doses, with vitamin E, vitamin C, and quercetin in graded doses, as follows: 4a-50 IU vitamin E + 100 mg vitamin C + 125 mg quercetin; 4b-100 IU vitamin E + 200 mg vitamin C + 250 mg quercetin; and 4c-500 IU vitamin E-500 mg vitamin C + 500 mg quercetin. Group V served as control and received distilled water (2 ml/kg). Some antioxidant enzymes and biochemical parameters of the kidney estimated after treatment. From the biochemical parameters obtained, the percentage protection of individual antioxidant on the kidneys was calculated as follows;

Antioxidant's percentage protection (APP)

APP=Value of the parameter in the induction of organ damage – the value in the protection study X 100 / Value of the parameter in the induction of organ damage.

Again, the kidneys were harvested, and histological examination carried out.

Collection of blood samples: Blood samples were collected after the treatment period through ocular puncture into a plane bottle. The blood samples collected were allowed to clot and then centrifuged at 1500revolution per minute, for 15minutes in other to obtain a clear serum. The serum samples collected were used to determine the kidney parameters and malondialdehyde after the induction of organ damage; while the kidney parameters and some antioxidant enzymes were estimated after the protection study. Determination of markers for oxidative stress: The serum level of malondialdehyde, a marker of oxidative stress, was estimated using the following analytical method.

Malondialdehyde (MDA) estimation

The spectrophotometric method described by Lefevre et al was used to assay the plasma malondialdehyde level.[16] Principle: Malondialdehyde is the main product of lipid peroxidation, and an indicator of oxidative stress. The method is based on the reaction of malondialdehyde with thiobarbituric acid (TBA), which leads to the formation of MDA-TBA2 adduct called thiobarbituric acid reactive substances (TBARS). TBARS yields a red-pink color whose intensity is a measure of MDA level.

Determination of antioxidant enzymes

The activity of some antioxidant enzymes were measured by the following analytical methods;

Analysis of Supraoxide dismutase (SOD) activity

The spectrophotometric method was used to assay for the activity of SOD in the serum as described by Paoletti, Aldinucci, Mocali and Caparrini.[17] Principle: This is based on the inhibition of a superoxide-driven NADH oxidation. The assay consists of reaction sequence which involves EDTA, Mn (II), mercaptoethanol, and molecular oxygen. The decrease of the rate of NADH oxidation is a function of enzyme concentration.

Total Glutathione perioxidase (GSSH) activity

The total glutathione perioxidase activity was determined according to the enzymatic method by Tipple and Roggers.[18] Principle: Reduced glutathione (GSH) is oxidized by 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) resulting in the formation of GSSG and 5-thio-2-nitrobenzoic acid (TNB). GSSG is then reduced to GSH by glutathione reductase using reducing equivalent provided by NADPH. The rate of TNB formation is proportional to the sum of GSH and GSSG present in the sample and is determined by measuring the formation of TNB at 412 nm.

Analysis of kidney parameters

The serum levels of urea, creatinine, and uric acid were determined by the following analytical methods;

Analysis of serum urea: The Berthelot enzymatic method was used for estimation of serum urea.[19] Principle: Urea is enzymatically converted to ammonia and carbon dioxide by urease, as shown in the equation: Urea + H_2O $\rightarrow$Urease$\rightarrow$ $2NH_3 + CO_2$. The free ammonia in an alkaline pH is then converted to dicarboxy endo-phenol which is a blue coloured compound, whose intensity is directly proportional to the amount of urea in the serum.

Analysis of serum creatinine

The Jaff reaction which is a cholorimetric method was used to determine the level of creatinine in the serum.[20] Principle: The assay is based on the reaction of creatinine with sodium picrate. Creatinine reacts with alkaline picrate forming a red complex. The intensity of the colour change is directly proportional to the creatinine concentration in the sample.

Analysis of serum uric acid

The enzymatic method proposed by Kayamori, Katayama, Matsuyama and Urata was used to assay the uric acid in serum.[21] Principle: An enzyme coupled reaction involving the uricase-catalase-FADH with the new tetrazolium salt

produces a water-soluble formazan dye in the presence of uric acid. The formazan dye produced is proportional to the uric acid present.

Histology preparations/analysis of kidney specimen

The method described by Titford was used in the histological analysis.[22] The harvested tissues were fixed with 10% formalin solution, and immersed in a series of ethanol solutions of increasing concentrations. Then, the tissues were immersed in three different xylene immersions, infiltrated with molten paraffin wax in the three different solutions, and allowed to cool. The tissues were secured on the microtome and cut into sections. The sections were attached to a glass slide, smeared, and allowed to dry. These were then be stained with hematoxylin and counter stained with eosin dye. The slides were mounted in glycerin jelly and observed at varying magnifications of a light microscope.

Statistical analysis

Results were expressed as the mean ± standard deviation (SD) with N= 5 (where N is number of animals per group), in descriptive statistics. Data was analyzed using Statistical Package for the Social Sciences (SPSS IBM version 23.0) and Microsoft excel 2019 edition. One-way analysis of variance (ANOVA) was used to compare the differences between groups followed by Fischer's Least Significant Difference Post Hoc test. Confidence interval was set at 95%, and values of $p<0.05$ were considered statistically significant.

RESULTS

The levels of kidney parameters and malondialdehyde after administration of ibuprofen alone

The results of the kidney parameters (urea, creatinine, and uric acid) and malondialdehyde obtained after administration of 120mg ibuprofen for 14 days were illustrated in multiple bar charts (Figures 1,2). There was significant increase ($p<0.05$) in the serum levels of urea ($p=0.002$), creatinine ($p=0.002$) and uric acid ($p=0.014$), respectively after administration of 120mg/kg ibuprofen when compared to the control (Figure 1). The result also showed a significant increase ($p=0.004$) in serum level of malondialdehyde after ibuprofen administration (Figure 2).

The levels of kidney parameters after co-administration of ibuprofen with antioxidants

The results of the kidney parameters obtained after protection study with doses of antioxidants were illustrated in multiple bar charts (Figures 3-5). There was a significant decline ($p<0.05$) in the serum levels of urea, creatinine, and uric acid after co-administration of 120 mg/kg ibuprofen with varying doses of vitamin E, vitamin C, and quercetin, as well as their combination, when compared to the animals given 120 mg/kg ibuprofen alone in the induction of organ damage phase (Figure 3-5). The decline in the values of kidney parameters was noticed to be independent on the dose of antioxidant administered. Combining different antioxidants showed no significant superior effect on the kidney parameters apart from uric acid level which was further reduced in combination of antioxidants (Figure 5).

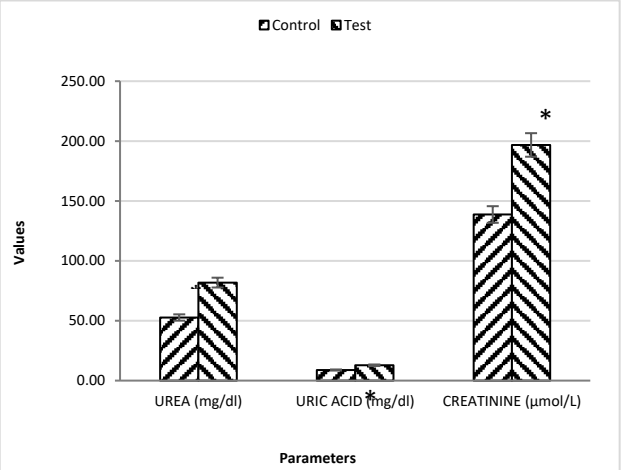

Figure 1: The serum levels of kidney parameters of the control and test group after induction of organ damage with 120mg/kg of ibuprofen. the black arrows represent the standard deviation of the mean; bars marked with asterisk (*) differ significantly from control group (*p<0.05).

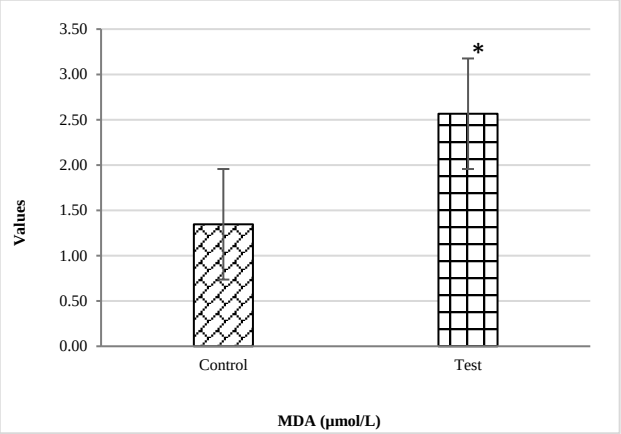

Figure 2: The serum levels of malondialdehyde of the control and test group after induction of organ damage with 120mg/kg of ibuprofen. The black arrows represent the standard deviation of the mean; bars marked with asterisk (*) differ significantly from control group (p<0.05).

The average percentage protection of the antioxidants on the kidney against ibuprofen-induced damage

From the biochemical parameters above, the average percentage protection of the antioxidants on the kidneys

against ibuprofen-induced damage was calculated, and presented in (Table 1). This was calculated by obtaining the difference between the value of the parameter during the induction of organ damage and that obtained during the protection study, then divided with the value during the induction of organ damage and converted to percentage as below:

APP = Value of the parameter in the induction of organ damage – the value in the protection study X 100 / Value of the parameter in the induction of organ damage.

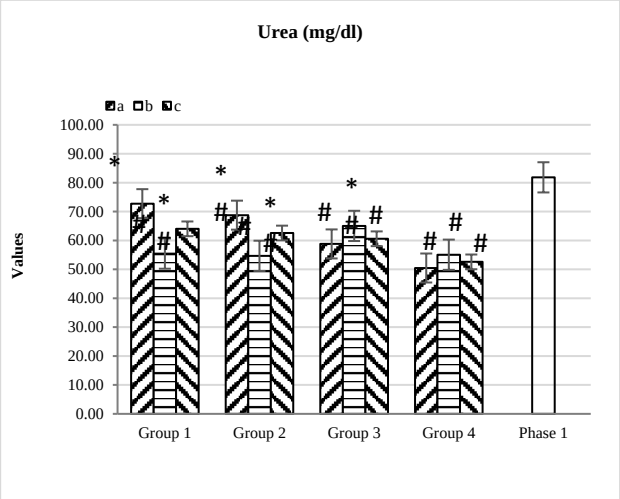

Figure 3: The serum urea levels after co-administration of 120mg/kg ibuprofen with doses of antioxidants and their combination. Each value represents mean±SD. Values marked with asterisk (*) differ significantly from control group (p<0.05) while those marked with (#) differ significantly from the phase 1 test group (p<0.05).

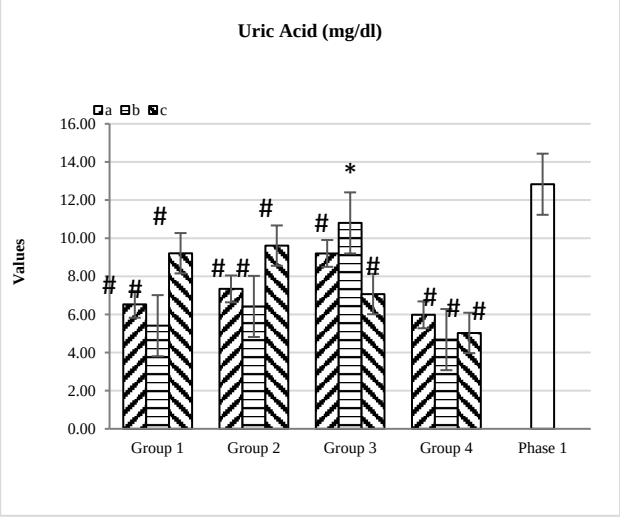

Figure 4: The serum uric acid levels after co-administration of 120mg/kg ibuprofen with doses of antioxidants and their combination.

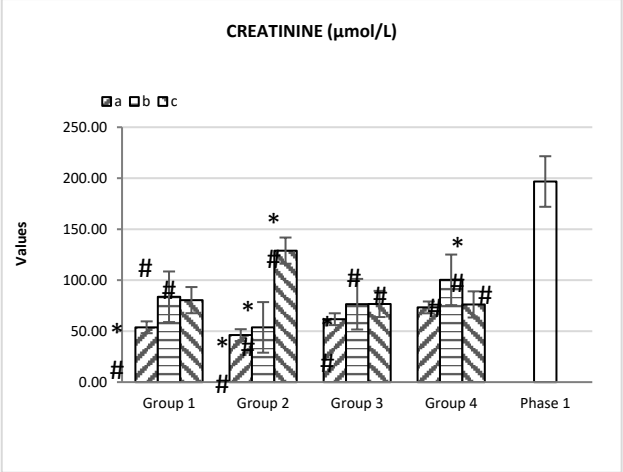

Figure 5: The Serum creatinine levels after co-administration of 120mg/kg ibuprofen with doses of antioxidants and their combination.

It quantifies the degree of kidney protection derived from each antioxidant, as well as that derived when the antioxidants are combined. The result showed that all the antioxidants offered some degree of protection to the kidneys against-ibuprofen induced damage, though with some variations; while their combination gave the highest average percentage protection to the kidneys against ibuprofen-induced damage (Table 1).

The levels of antioxidant enzymes after co-administration of ibuprofen with antioxidants:

The result of some antioxidant enzymes (including total Glutathione peroxidase and Suproxide dismutase) were obtained after protection study with the antioxidants and illustrated in multiple bar-charts (Figures 6, 7). There was significant increase *(*$p<0.05$*)* in the serum levels of total Glutathione peroxidase and Suproxide dismutase, after co-administration of ibuprofen and antioxidants, when compared to the control. However, there was no significant effect of increasing doses of the antioxidants on the antioxidant enzymes.

Histology of the kidney tissues after administration of ibuprofen

The kidney histology of the control showed normal renal histo-architecture renal cortex with normal shaped glomeruli surrounded by capsular space (Figure 8). The proximal and distal convoluted tubules were present, and collecting ducts with blood vessels present in between. The kidney tissues of the animals given ibuprofen showed kidney cortex containing atrophied glomeruli, with widened capsular space, the proximal and distal convoluted tubules containing desquamated epithelial cells from the luminal surface. Also, heavy infiltrating lymphocytes around the glomeruli and tubules were other observable histopathological changes as well as presence of massive aggregation of inflammatory cells (Figure 9).

Table 1: The Average percentage protection of the antioxidants on the kidney against ibuprofen-induced damage.

Antioxidants	Average percentage protection on the kidney (%)			Average
	Urea	Uric acid	Creatinine	
Vitamin E	21.67	45.15	63.31	43.38
Vitamin C	24.2	39.31	61.47	41.66
Quercetin	24.85	29.69	63.84	39.46
Vitamin E+Vitamin C+Quercetin	35.54	59.27	57.88	50.9

Histology of the kidney tissues after co-administration of ibuprofen with antioxidants

Slide preparations of the kidney tissues after co-administration of ibuprofen with doses of different antioxidants showed normal renal histo-architecture both in the treatment groups and the control, similar to that in Figure 8. The renal cortex contained normal shaped glomeruli, normal proximal and distal convoluted tubules around the glomeruli. Similar normal histological findings were observed in the kidney tissues of the different treatment categories, irrespective of the dose, the type, and single administration or combination of the antioxidants.

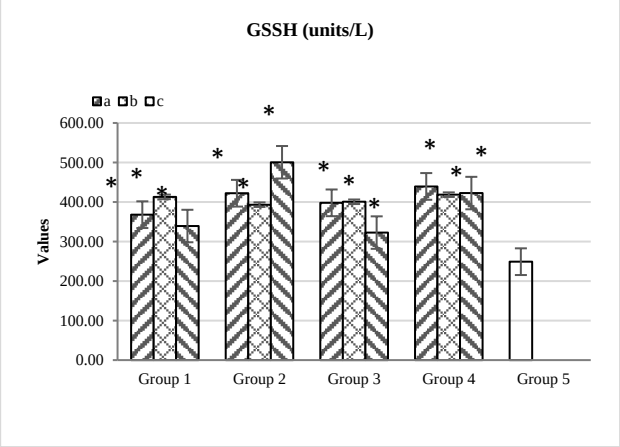

Figure 6: The serum levels of total glutathione peroxidase after co-administration of ibuprofen with doses of antioxidants. Each value represents mean±SD, Values marked with asterisk (*) differ significantly from control group (p<0.05).

DISCUSSION

The hike in oxidative stress and reduced endothelial NO synthesis activity have been shown to contribute in the pathogenesis of ibuprofen–induced kidney.[23] In this study, the significant increase in the level of malondialdehyde, a marker of oxidative stress, is in consonance with findings from other studies, which reported that ibuprofen causes increased generation of reactive oxygen species that has been shown to contribute to the pathogenesis of ibuprofen–induced kidney.[5,23-25] Studies have also shown that ibuprofen and most other NSAIDs cause mitochondrial alteration especially in the proximal tubules of the nephron, which are early events in drug induced tubular necrosis.[24-26]

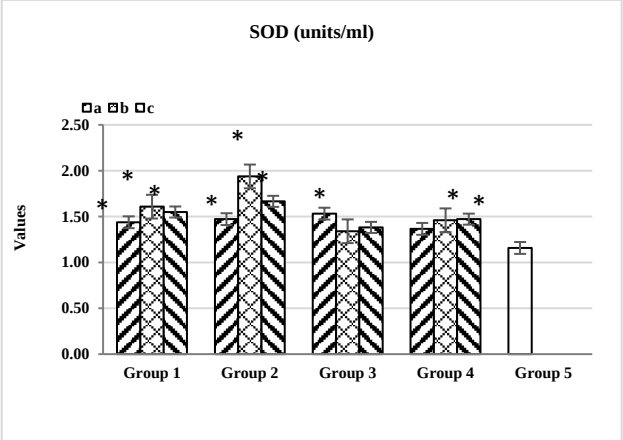

Figure 7: The serum levels of suproxide dismutase (SOD) after co-administration of ibuprofen with doses of antioxidants.

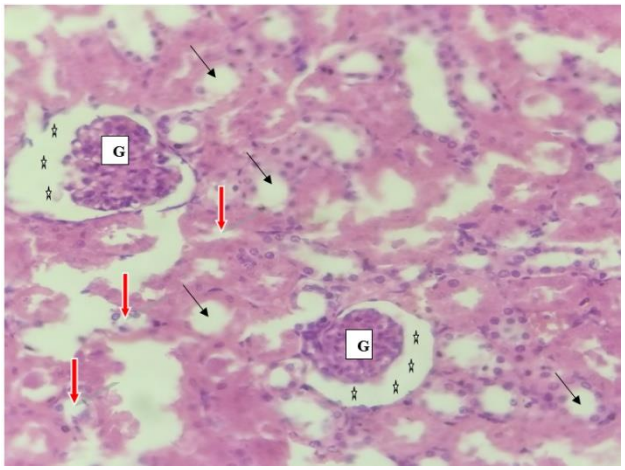

Figure 8: Kidney histology of Control; demonstrating the normal glomerulus (G) surrounded by capsular space (star) and great number of proximal convoluted tubules (red arrow) and distal convoluted tubules (black arrow) (Hematoxylin and Eosin stain 10X).

The significant increase in serum urea, uric acid, and creatinne after administration of ibuprofen for 14 days, indicated that ibuprofen may induce kidney injury even at short term administration, as reported by Lucas et al and

Su et al.[25,26] The histo-pathological studies correlate with the alterations in the biochemical parameters after ibuprofen administration. The kidney histology findings in this study is similar to the findings by Nagappan et al who in their study reported that NSAIDs, such as indomethacin and ibuprofen, cause abnormal structure of the glomerulus, tubular necrosis, and presence of interstitial inflammation, which lead to reduced renal perfusion with subsequent renal damage.[27]

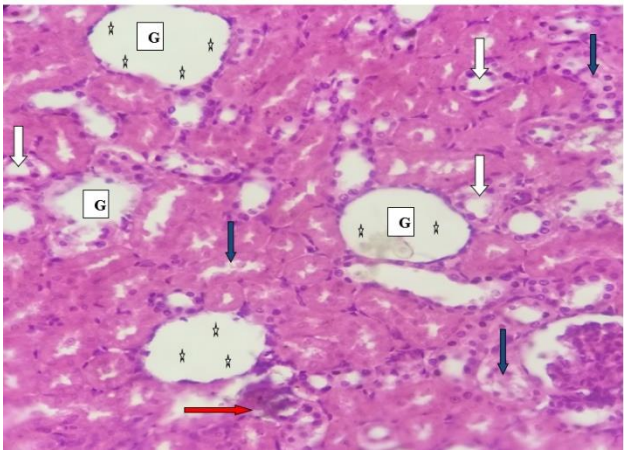

Figure 9: Kidney histology of animals given ibuprofen alone; with renal cortex demonstrating atrophied glomerulus (G), wide capsular space (star) desquamated cells in the lumen of the proximal convoluted tubules (black arror) and distal convoluted tubules (white arrow), and aggregation of inflammatory cells (red arrow) (H & E, 10X).

Fortunately, recent advances in nutritional science based on food supplements, medicinal herbs, and antioxidants has significantly been employed in controlling and modulating acute and chronic diseases in human beings. [8, 28] In this study, the results obtained when ibuprofen was co-administered with antioxidants showed significant increase ($p<0.05$) in the serum levels of antioxidant enzymes. This is similar with the findings from nutritional sciences that dietary antioxidants boost the levels of antioxidants enzymes, which counters the elevated oxidative stress induced by ibuprofen administration, and by extension protects against organ injury.[28-30] Therefore, antioxidants can forestall the initial pathophysiological processes.[30] The results showed that increasing the doses of antioxidants or combining different antioxidants provided little or no superior effect on the levels of antioxidant enzymes, when compared to single administration of antioxidants. Furthermore, there is significant reduction in the levels of kidney parameters after co-administration of ibuprofen with antioxidants, when compared to those given ibuprofen alone. This suggests that antioxidants offer protection to the kidneys against ibuprofen-induced damage, as reported previously.[31,32] Increasing doses of antioxidants showed no superior effect on the kidney parameters, meanwhile, combining different antioxidants showed significant reduction on the kidney parameters, especially the uric acid level when compared to single antioxidant therapy. This suggests that any antioxidant may be able to offer protective effect on the kidneys against ibuprofen-induced injury, whether given as single regimen or combination, as reported in some previous studies.[32,33] However, the estimated average percentage protection showed some differences among the antioxidants in their potency to protect the kidneys against ibuprofen-induced injury. Quecertin had 39.46% average protection, vitamin E and C had 43.38% and 41.66% respectively, while combination of antioxidants provided highest average protection (50.90%) in the kidneys, which supports the finding that antioxidants may potentiate the activity of one another.[34] The normal renal histology after co-administration of ibuprofen with antioxidants in this study, supports the findings from the biochemical parameters, and suggests a protective effect of antioxidants as posited by, and Borran et al and Dennis and Witting.[32,35]

CONCLUSION

From the results of this study, it can be concluded that exogenous antioxidants such as α-tocopherol, vitamin C, and quercetin protected against ibuprofen-induced renal damage in Wistar rats. Meanwhile, the average percentage protection of the individual antioxidants varied, with vitamin E providing the highest percentage protection on the kidneys when compared to other antioxidants, while combination of these antioxidants gave a higher percentage protection on the kidneys than individual antioxidants. Therefore, one effective antioxidant can obviate kidney injuries that may occur from taking ibuprofen; however, combining antioxidants provided far more protection to the kidneys than single antioxidants.

ACKNOWLEDGEMENTS

The authors are grateful Mr. Ike Chibueze of the Department of Pharmacology/Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, for his technical assistance.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Machado GC, Maher CG, Ferreira PH, Day RO, Pinheiro MB, Ferreira ML. Non-Steroidal inflammatory drugs for spinal pain: a systemic review and meta-analysis. Ann Rheumat Dis. 2017;76 (7): 1269-78.
2. Solomon DH, Husni ME, Libby PA. The risk of major nsaid toxicity with celecoxib, ibuprofen, or naproxen: a secondary analysis of the precision trial. Am J Med. 2017;130(12):1415-22.

3. Varrassa G, Pergolizzi JV, Dowling P, Paladini S, Hernaz D, Paladini A. Ibuprofen Safety at the Golden Anniversary: Are all NSAIDs the Same? A Narrative Review. Adv Ther. 2020;37:61-82.
4. Yeomans ND, Graham DY, Husni ME. Randomised clinical trial: gastrointestinal events in arthritis patients treated with celecoxib, ibuprofen or naproxen in the precision trial. Alim Pharmacol Therap. 2018; 47(11):1453-63.
5. Mahmood KS, Ahmed J, Jawad A. Non-steroidal anti-inflammatory drugs (NSAIDs), free Radicals and reactive oxygen species (ROS): A review of literature. Med J Basrah Uni. 2019;27(1):46-53.
6. Ahmad MH, Fatima M, Hossain M, Mondal AC. Evaluation of naproxen-induced oxidative stress, hepatotoxicity and *in-vivo* genotoxicity in male Wistar rats. J Pharm Anal. 2018;8(6):400-6.
7. Akbar A, Jelodar G, Nazifi S, Afsar T, Nasiri K. Oxidative stress as the underlying biomechanism of detrimental outcomes of ionizing and non-ionizing radiation on human health: antioxidant protective strategies. J Res Med Sci. 2019;21(4):e85655.
8. Galli F, Azzi A, Birringer M, Cook-Mills JM, Eggersdorfer M, Frank J. Vitamin E: Emerging aspects and new directions. J Free Rad Biol Med. 2017;102:16-36.
9. Liu D, Shi J, Ibarra AC, Kakuda Y. The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and carotene mixtures on the DPPH free radical. J Food Sci Technol. 2018;41(7):1344-9.
10. Shao Q, Yin X, Lui H, Zhao B, Huang J, Li Z. Kidney injury following ibuprofen and acetaminophen: a real-world analysis of post-marketing surveillance data. Front Pharmacol. 2021;12:750108.
11. Nagy J, Kovács T. A brief review on the rising incidence of chronic kidney diseases and non-alcoholic fatty liver disease. Physiol Int J. 2019; 106(4):305-10.
12. Gomaa S. Adverse effects induced by diclofenac, ibuprofen, and paracetamol toxicity on immunological and biochemical parameters in Swiss albino mice. J Basic Applied Zool. 2018;79(5):72-92.
13. Bushra R, Aslam N. An overview of clinical pharmacology of Ibuprofen. Oman Medical J. 2010; 25(3):155-61.
14. Bindu S, Mazumder S, Bandyopadhyay U. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. J Biochem Pharmacol. 2020;180:114-7.
15. Zoubek ME, Lucena MI, Andrade RJ, Stephens C. Systematic review: ibuprofen-induced liver injury. Aliment Pharmacol Therap. 2020;51:603-11.
16. Lefevre G, Bonneau C, Rahma S, Chanu B, Brault D, Couder R. Determination of plasma pro tein-bound malondialdehyde by derivative spectro-photometry. Eur J Clin Chem Clin Biochem. 1996;34(8):631-6.
17. Paoletti F, Aldinucci D, Mocali A, Caparrini A. A sensitive spectrophotometric method for the determination of superoxide dismutase activity in tissue extracts. Analyt Biochem.1986;154(2):536-41.
18. Tipple TE, Rogers LK. Methods for the determination of plasma or tissue glutathione levels. Methods Mol Biol. 2012;889:315-24.
19. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. J Clin Pathol. 1960;13(2): 156-9.
20. Joris RD, Marijn MS. Creatinine determination according to Jaffe what does it stand for? Nephrol Dial Transplant Plus. 2011;4(2):83-6.
21. Kayamori Y, Katayama Y, Matsuyama T, Urata T. Enzymatic method for assaying uric acid in serum with a new tetrazolium salt produces water-soluble formazan dye. Clin Biochem. 1997;30(8):595-9.
22. Titford M. Progress in the development of microscopical techniques for diagnostic pathology. J Histotechnol. 2009;32:9-19.
23. Gyurászová M, Gurecká R, Bábíčková J, Tóthová L. Oxidative stress in the pathophysiology of kidney disease: implications for monitoring and identification of biomarkers. Oxid Med Cell Long. 2020;20:547.
24. Duni A, Liakopoulos V, Roumeliotis S, Peschos D, Peschos S, Dounousi E. Oxidative stress in the pathogenesis and evolution of chronic kidney disease: untangling Ariadne's thread. Int J Mol Sci. 2019; 20(15):3711.
25. Lucas GNC, Leitão ACC, Alencar RL, Xavier RMF, Daher EDF, da Silva GB. Pathophysiological aspects of nephropathy caused by non-steroidal anti-inflammatory drugs. Brasilian J Nephrol. 2019;41(1): 124-30.
26. Su L, Li Y, Xu R, Luo F, Gao Q, Chen R. Association of Ibuprofen Prescription with Acute kidney Injury Among Hospitalized Children in China. J Am Med Assoc. 2021;4(3):e210775.
27. Nagappen AS, Varghese J, Pranesh GT, Jeyaseelan V, Jacob M. Indomethacin and Ibuprofen inhibit activation of endothelial nitric oxide synthase in the rat kidney; Possible role of this effect in the pathogenesis of NSAIDs-induced renal damage. J Chem Biol Interact. 2014;221:77-87.
28. Zehiroglu C, Sarikaya SBO. The importance of antioxidants and place in today's scientific and technological studies. J Food Sci Technol. 2019; 56(11):4757-74.
29. Wang Y, Quan F, Cao Q, Lin Y, Yue C, Cui X, et al. Quercetin alleviates acute kidney injury by inhibiting ferroptosis. J Adv Res. 2021;28:231-43.
30. Hong YA, Lim JH, Kim MY, Kim Y, Park HS, Kim HW, et al. Extracellular superoxide dismutase attenuates renal oxidative stress through the activation of adenosine monophosphate-activated protein kinase in diabetic nephropathy. Antioxidant Redox Sig. 2018;28:1543-61.
31. Marriott BP, Birt DF, Stallings VA, Yates AA. Vitamin C present knowledge in nutrition. 11th ed. London: Academic Press (Elsevier); 2020:155-70.
32. Dennis JM, Witting PK. Protective Role for Antioxidants in Acute Kidney Disease. Nutrients. 2017;9(7):718.

33. Xu D, Hu MJ, Wang YQ, Hu M, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019;24(6):1123.
34. Traber MG, Stevens JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. Free Rad Biol Med. 2011;51(5):1000-13.
35. Borran M, Dashti-Khavidaki S, Alamdari A, Naderi N. Vitamin C and kidney transplantation: Nutritional status, potential efficacy, safety, and interactions. Clin Nutr. 2020;41:1-9.

Cite this article as: Okoroama CE, Unekwe PC, Okoroama LC, Okparaoka SU, Akuodor GC. Evaluation of the protective role of antioxidants: α-tocopherol, vitamin C, and quercetin, against ibuprofen-induced renal damage in male Wistar rats. Int J Basic Clin Pharmacol 2023;12:631-9.