

(RESEARCH ARTICLE)



The protective role of α -tocopherol, vitamin C and quercetin against ibuprofen-induced liver damage in male Wistar rats

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GSC Biological and Pharmaceutical Sciences, 2023, 24(02), 170–184

Publication history: Received on 25 June 2023; revised on 07 August 2023; accepted on 10 August 2023

Article DOI: <https://doi.org/10.30574/gscbps.2023.24.2.0308>

Abstract

Aim: Ibuprofen which is one of the frequently used analgesics has been associated with some adverse effects, including liver injury. The protective role of α -tocopherol, vitamin C and quercetin against ibuprofen-induced liver damage in male Wistar rats were investigated in this study.

Method: The study design included; induction of liver damage using ibuprofen, and secondly, determination of the protective role of α -tocopherol, vitamin C and quercetin against ibuprofen-induced liver injury. In the first phase, two groups of animals were used; one group treated with 120 mg/kg ibuprofen daily for 14 days, while the other served as control and given distilled water. After treatment, the serum malondialdehyde, liver enzymes and total bilirubin were estimated, and the liver harvested for histology. In the protection study, there were four treatment groups, each having three sub-groups, treated with 120 mg/kg Ibuprofen and graded doses of vitamin E, vitamin C and quercetin respectively, while the fifth group given distilled-water served as control. After 14 days, antioxidant enzymes and liver parameters were estimated, while the liver harvested for histology.

Results: Showed increase in aspartate transaminase and Alkaline phosphatase activities, and significant ($p < 0.05$) increase in malondialdehyde levels, alanine transaminase and total bilirubin, after ibuprofen administration. Conversely, there was significant ($p < 0.05$) reduction in liver parameters after co-administration of antioxidants with ibuprofen, and significant ($p < 0.05$) increase in the activity of total glutathione and catalase enzymes. The estimated antioxidant's percentage protection showed the antioxidants offered variable degree of protection on the liver against ibuprofen-induced damage. Histology revealed cellular and portal infiltration of inflammatory cells, macrophage aggregation, and some parenchymal necrosis, 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 liver damage in Wistar rats.

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Keywords: Ibuprofen; α -tocopherol; Vitamin C; Quercetin; Alanine transaminase; Aspartate transaminase; Alkaline phosphatase

1. Introduction

Ibuprofen is a propionic acid class of the non-steroidal anti-inflammatory drugs (NSAIDs) commonly used in controlling different types of pains, both acute and chronic pain management [1]. It is also used as antipyretics and anti-inflammatory agents. NSAIDs are generally over the counter drugs, which are readily available and frequently misused by individuals suffering from pains. Concerns of the adverse effects and complications of ibuprofen and other NSAIDs have been raised, which includes: dyspepsia, stomach ulcers, upper gastrointestinal bleeding, allergic reactions, liver, and kidney damage [2, 3, 4]. In as much as these adverse effects are apparent, their frequent use remains inevitable in pain management. Although, several control measures have been suggested to mitigate some of the adverse effects; for instance giving NSAIDs with proton pump inhibitors has ameliorated the upper gastrointestinal effects [5], however, other effects such as liver damage remains a challenge.

Prolonged and indiscriminate use of ibuprofen and other NSAIDs is almost always unavoidable because they are readily available, cheap, and those taking them need to keep a pain-free state. Therefore, such individuals are at the risk of some organ derangements (such as liver injury) and other adverse effects [2]. It has been suggested that one of the mechanisms by which organ derangement can manifest during use of ibuprofen, is through the formation of metabolites that leads to increased generation of oxidative stress at the intracellular level [6, 7]. This hike in intracellular oxidative stress precedes organ derangements and plays a significant role in the early pathogenesis of ibuprofen-induced liver damage [6]. Oxidative stress has generally been linked to a lot of organ dysfunctions especially when the homeostatic balance is not maintained [8].

Studies have shown that antioxidants play vital role in preventing organ dysfunction arising from oxidative stress, through sustaining homeostatic balance with free radicals [9, 10]. This suggests that it is possible to proffer a preventive approach by co-administering ibuprofen and antioxidants, in order to avert organ injury resulting from use of this medication. Preventive measures are easy, affordable and less demanding, when compared to treating liver diseases. In this study, the antioxidants – 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 male Wistar rats.

The findings from the research will generally provide an opportunity to mitigate the incidence of liver damages resulting from use of ibuprofen and other NSAIDs. 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. The necessity to prevent diseases at the earliest possible time is the rationale for this study.

2. Material and methods

2.1. Animals

Seventy-five (75) adult male wistar rats of 8 – 10 weeks old and weighing about 180 – 200 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.

2.2. Drugs

The drugs used in the study included ibuprofen, and antioxidant supplements such as vitamin E, vitamin C, and quercetin.

2.3. Experimental design

The study was designed in two parts; first, induction of liver damage from ibuprofen administration, and secondly determination of the protective effect of antioxidants on the liver against ibuprofen-induced damage in the Wistar rats, when ibuprofen is co-administered with antioxidants.

2.4. Induction of liver damage

The method described by Gomaa [11] was adopted to induce liver injuries in the Wistar rats. This was achieved by administering 120 mg/kg body weight of ibuprofen (high dose) daily for duration of 14 days. This dose is about five times less than the LD₅₀ of ibuprofen which is 636 mg/kg in rats [11, 12]. The principle is that NSAIDs with short half-

lives, such as ibuprofen, may produce renal and liver injury in a matter of days as reported by Bindu, Mazumder and Bandyopadhyay [13]. Ten (10) animals were divided into two groups of five animals in each. Group one was given 120 mg/kg of ibuprofen daily in three divided doses for fourteen days, while group two served as control and received 2 ml/kg of distilled water per oral. The serum malonaldehyde (a marker of oxidative-stress) and biochemical parameters of the liver function were measured, while the liver was harvested and the histological examination carried out.

Elevated liver enzyme when compared to the control is an indication of organ damage [14].

2.5. Protection study with antioxidants against ibuprofen-induced liver damage

The animals were randomly divided into four experimental groups of fifteen animals in each and a control group of five (5) animals, categorized as; Group I, II, III, IV, and V (the control). Groups I to IV had three (3) sub-groups each and were given 120 mg/kg of Ibuprofen with graded doses of vitamin E, vitamin C and quercetin. The drugs (ibuprofen and antioxidants) were dissolved in sterile water and administered orally for fourteen days. Varying doses of the different antioxidants were administered simultaneously with similar dose of ibuprofen (120 mg/kg) used in induction of liver damage as shown below;

- **Treatment group I** was divided into three sub-groups of five animals in each; group 1a, 1b and 1c respectively. Each sub-group was given 120 mg/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 120 mg/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 120 mg/kg of Ibuprofen in three divided doses, together with graded doses of quercetin, as follows: **3a** – 125 mg/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/kg vitamin E + 100 mg/kg vitamin C + 125 mg/kg quercetin; **4b** – 100 iu/kg vitamin E + 200 mg/kg vitamin C + 250 mg/kg quercetin; and **4c** – 500 iu/kg vitamin E + 500 mg/kg vitamin C + 500 mg/kg quercetin.
- **Group V** served as control and received distilled water (2 ml/kg).

The biochemical parameters of the liver and some antioxidant enzymes were estimated after treatment. From the biochemical parameters obtained, the percentage protection of individual antioxidant on the liver was calculated as follows;

2.6. Antioxidant's percentage protection =

$$\frac{\text{Value of the parameter in the induction of organ damage} - \text{the value in the protection study} \times 100}{\text{Value of the parameter in the induction of organ damage}}$$

Again, the liver was harvested, and histological examination carried out.

2.7. Collection of blood samples

Blood samples were collected through ocular puncture into a plane bottle after each treatment period, respectively. The blood collected were allowed to clot and then centrifuged at 1500 revolution per minute, for 15 minutes to obtain a clear serum. The sera obtained were used to determine the liver parameters and malondialdehyde after the induction of organ damage; while the liver parameters and antioxidant enzymes were estimated after the protection study.

2.8. Determination of markers for oxidative stress

The serum level of malondialdehyde, a marker of oxidative stress, was estimated using the following analytical method;

2.8.1. Malondialdehyde (MDA) estimation

The spectrophotometric method described by Lefevre et al. [15] was used to assay the plasma malondialdehyde level.

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.

2.9. Determination of antioxidant enzymes

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

2.9.1. Analysis of catalase activity

The spectrophotometric method described Hadwan and Abed [16] was used in the measurement of catalase activity.

Principle: This is based on the fact that catalase enzyme enhances the conversion of hydrogen peroxide (H_2O_2) to water and oxygen:



Undecomposed H_2O_2 reacts with ammonium molybdate to produce a yellow color, and the intensity of the color can be related to the activity of the catalase enzyme

2.9.2. Total Glutathione peroxidase (GSSH) activity

The total glutathione peroxidase activity was determined according to the enzymatic method by Tipple and Roggers [17].

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.

2.10. Analysis of liver enzymes

The activities of liver enzymes and total bilirubin concentration were determined by the following analytical methods;

2.10.1. Determination of Aspartate transaminase (AST) activity

Principle: AST is an enzyme of the transferase class that catalyses the reversible transfer of an amino group from aspartate to α - ketoglutarate to form glutamate and oxaloacetate. It is also called Serum glutamic oxaloacetate transaminase (SGOT). The serum aspartate aminotransferase (AST) was estimated by measuring the amount of oxaloacetate produced by forming 2,4-dinitrophenylhydrazine, according to the colorimetric method [18].

2.10.2. Determination of Alanine transaminase (ALT) activity

Principle: ALT is an enzyme of the transferase class that catalyses the reversible transfer of an amino group from alanine to α - ketoglutarate to form glutamate and pyruvate. It is also called Serum glutamic pyruvic transaminase (SGPT). The serum alanine aminotransferase (ALT) was estimated by measuring the amount of pyruvate produced by forming 2,4-dinitrophenylhydrazine, according to the colorimetric method [18].

2.10.3. Determination of Alkaline phosphatase (ALP) activity

Principle: ALP is an enzyme of the hydrolase class that catalyses the cleavage of orthophosphate (an intracellular anion otherwise called inorganic phosphate, P_i) from orthophosphoric monoesters under alkaline condition. The Colorimetric Assay technique was used in the analysis of the serum ALP, and it measures the amount of orthophosphate produced, according to the method described by Stratford, Castro and Daffinrud [19].

2.10.4. Total bilirubin estimation:

The Malloy and Evelyn method using diazo reagent was used in the colorimetric estimation of serum total bilirubin [20].

Principle: This method for bilirubin estimation is based on Van Den Bergh reaction. In this reaction, bilirubin reacts with diazotized sulphanic acid to produce azobilirubin which is purple in color. Intensity of color is directly proportional to the amount of bilirubin in the serum.

2.11. Histology preparations/analysis of liver specimen

The histology preparation and analysis was carried out according to the method described by Titford [21]. 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.

2.12. Statistical analysis

Results were expressed as the mean \pm standard deviation (S.D) 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.

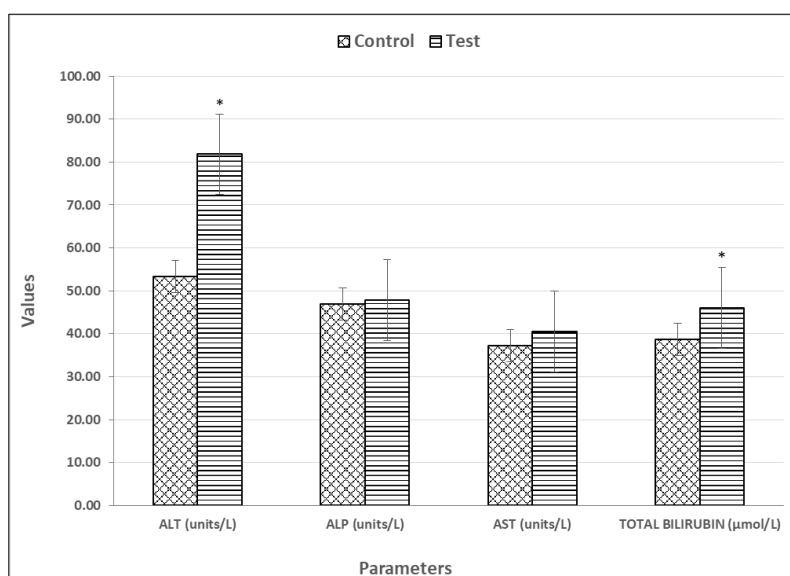
3. Results

3.1. The levels of liver parameters and malondialdehyde after induction of organ damage with ibuprofen:

The results of the liver enzymes (Alanine transaminase - ALT, Aspartate transaminase - AST, Alkaline phosphatase - ALP, and total bilirubin), and malondialdehyde – MDA, obtained after administration of 120 mg/kg of ibuprofen for 14 days were illustrated in multiple bar charts (Figures 1 and 2, respectively).

There was significant increase ($P < 0.05$) in the serum ALT level ($p = 0.008$) and the total bilirubin ($p = 0.046$). Meanwhile, the increase in ALP and AST, were not significant ($p > 0.05$) after administration of 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).



Note: The black arrows represent the standard deviation of the mean; bars marked with asterisk (*) differ significantly from control group ($*p < 0.05$); while the legends below represent

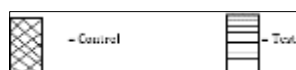
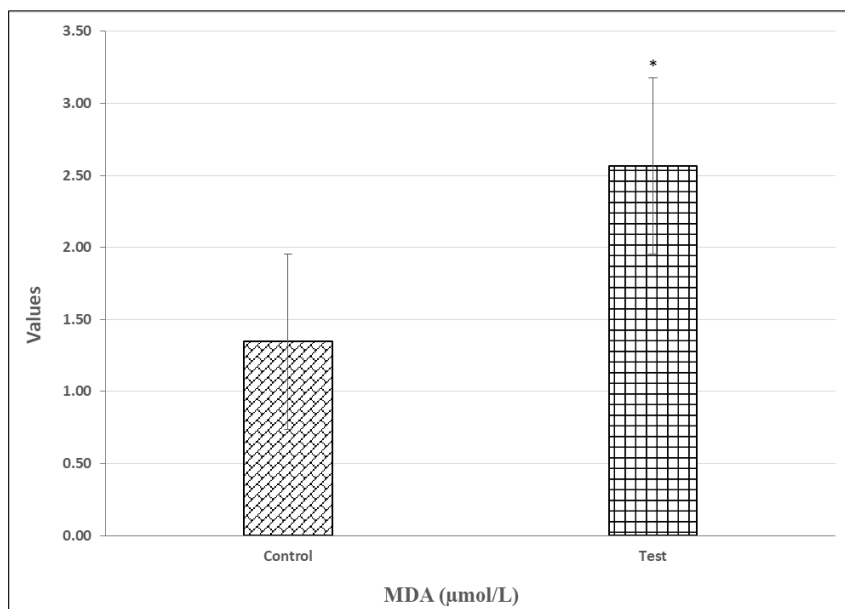


Figure 1 The Serum Levels of Liver Enzymes and Total Bilirubin of the Control and Test Group after Induction of Organ Damage with 120 mg/kg of Ibuprofen

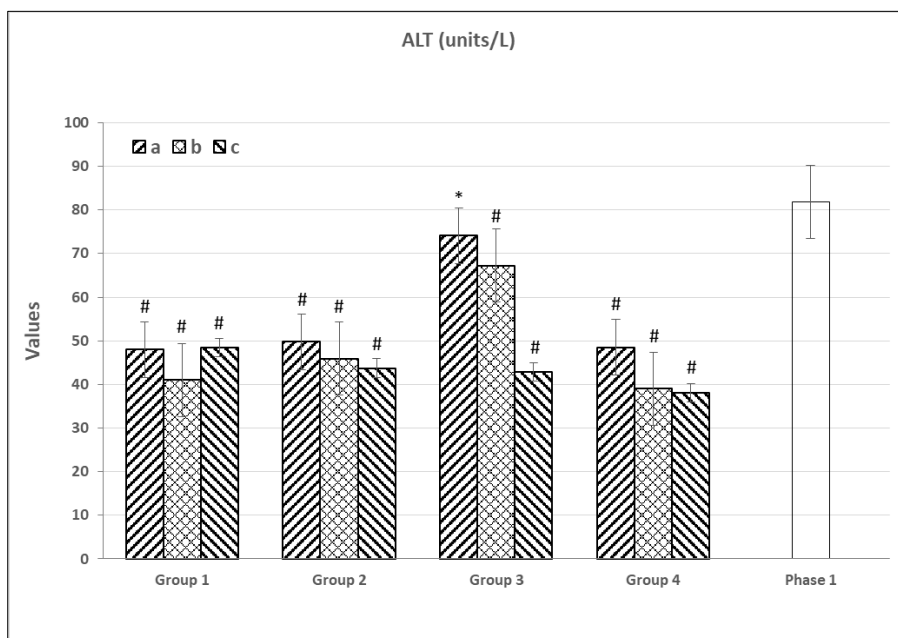


Note: The black arrows represent the standard deviation of the mean; bars marked with asterisk (*) differ significantly from control group ($*p < 0.05$)

Figure 2 The Serum Levels of Malondialdehyde of the Control and Test Group after Induction of Organ Damage with 120 mg/kg of Ibuprofen

3.2. The levels of liver enzymes and total bilirubin after co-administration of ibuprofen with antioxidants:

The results of the liver enzymes and total bilirubin after protection study with graded doses of different antioxidants were illustrated in multiple bar charts (Figures 3, 4, 5 and 6).



Note: 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 ($#p < 0.05$) The legends below represent;

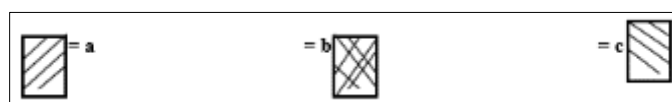
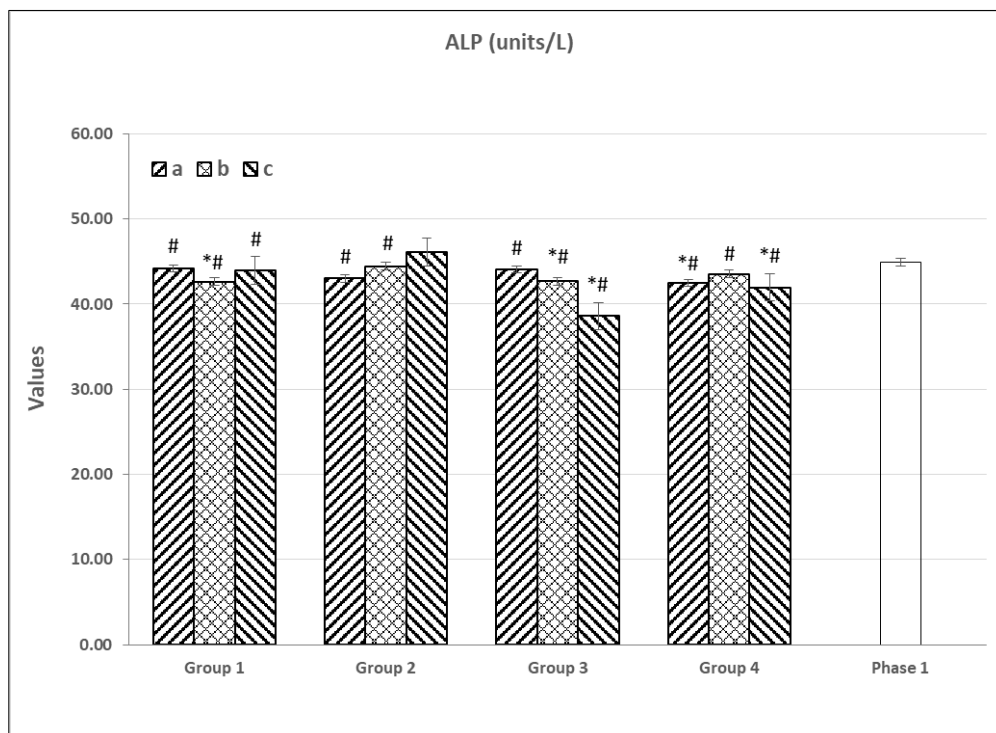


Figure 3 The Serum Alanine transaminase Levels after Co-Administration of 120 mg/kg of Ibuprofen with Doses of Antioxidants and their Combinations

There was a significant decline ($p < 0.05$) in the levels of the ALT, AST, ALP, and total bilirubin 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 during the induction of organ damage (Figure 3, 4, 5 and 6). However, the decrease is more pronounced in the serum ALT and AST (Figure 3 and 4). The decline in the values of kidney parameters was noticed to be independent on the dose of antioxidant administered, and not affected by combining the antioxidants.



Note: Each value represents mean \pm 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 (# $p < 0.05$) The legends below represent;

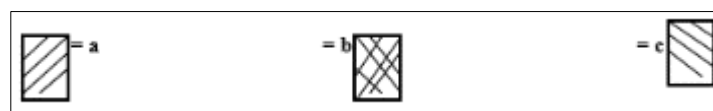


Figure 4 The Serum Levels Alkaline phosphatase after Co-Administration of 120 mg/kg of Ibuprofen with Doses of Antioxidants and their Combinations

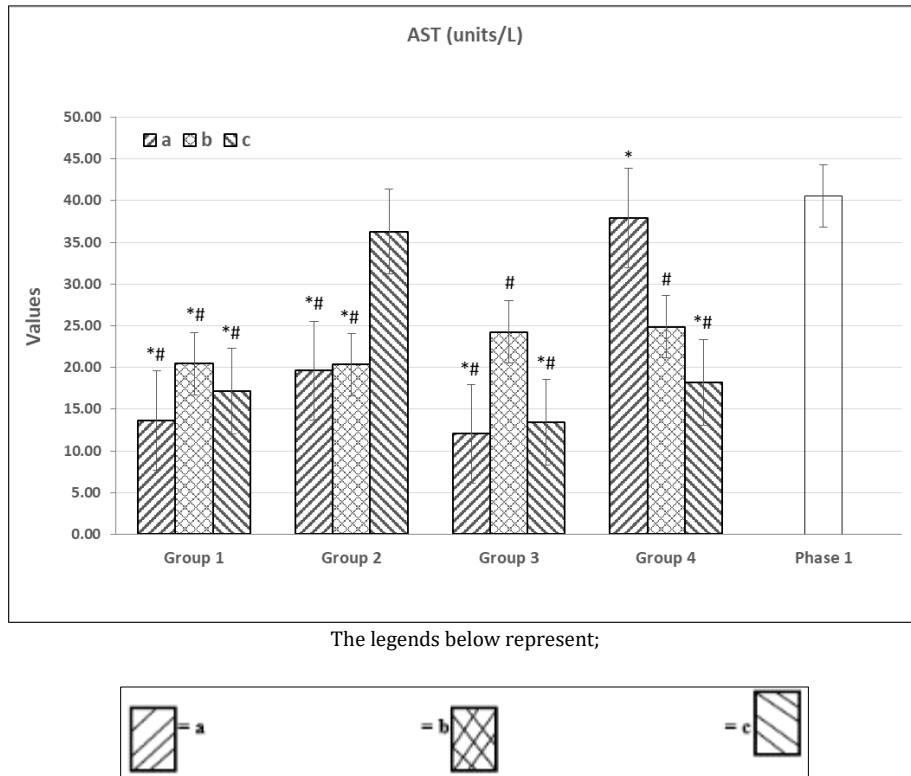


Figure 5 The Serum Levels Aspartate transaminase after Co-Administration of 120 mg/kg of Ibuprofen with Doses of Antioxidants and their Combinations

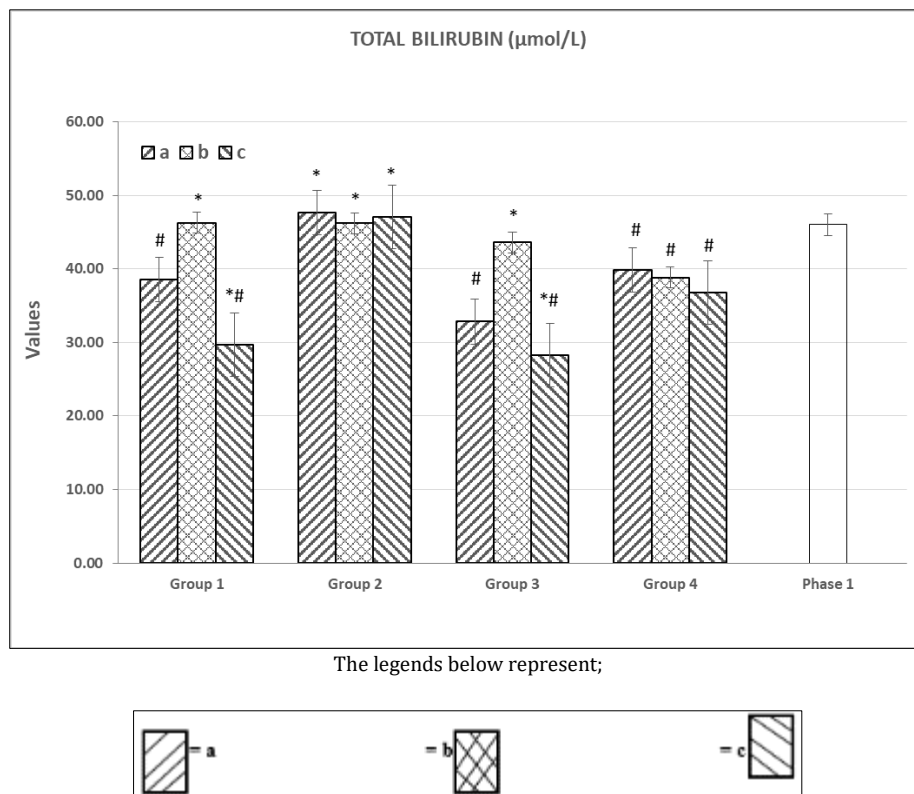


Figure 6 The Serum Levels Total Bilirubin after Co-Administration of 120 mg/kg of Ibuprofen with Doses of Antioxidants and their Combinations

3.3. The average percentage protection of the antioxidants on the liver against ibuprofen-induced damage

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

Antioxidant's percentage protection =

$$\frac{\text{Value of the parameter in the induction of organ damage} - \text{the value in the protection study} \times 100}{\text{Value of the parameter in the induction of organ damage}}$$

This quantifies the extent at which the antioxidant protects the liver against ibuprofen-induced injury. The result showed that all the antioxidants showed some degree of protection on the liver with vitamin E giving the highest average protection, while vitamin C had the least (Table 1).

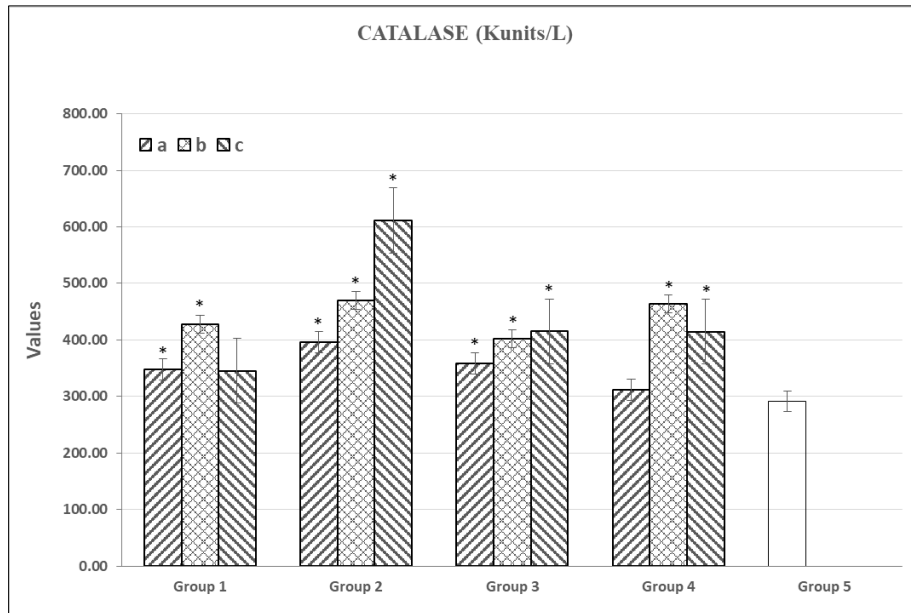
able 1 The Average Percentage Protection of the Antioxidants (Vitamin E, Vitamin C, and Quercetin) on the Liver against Ibuprofen-Induced Damage after 14days

Antioxidants	Average percentage protection on the liver (in %)				Average
	ALT	ALP	AST	TB	
Vitamin E	43.94	8.98	76.04	17	36.49
Vitamin C	43.13	21.09	37.3	-2.13	24.85
Quercetin	24.93	12.7	59.13	24.06	30.21
Vitamin E + Vitamin C + Quecetin	48.73	12.1	33.41	16.31	27.64

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

The result of some antioxidant enzymes (including the catalase and total Glutathione peroxidase activities) were obtained after co-administration of ibuprofen with the antioxidants in the protection study, and illustrated in multiple bar-charts (Figures 7, and 8).

There was significant increase ($p < 0.05$) in the serum levels of Catalase enzyme ($p = 0.035$) and total Glutathione peroxidase ($p = 0.025$) 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, and superior effect single dosing of antioxidant over their combined administration.



Each value represents mean±SD, Values marked with asterisk (*) differ significantly from control group (*p < 0.05) The legends below represent;

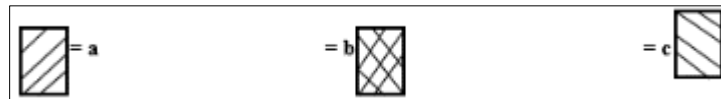
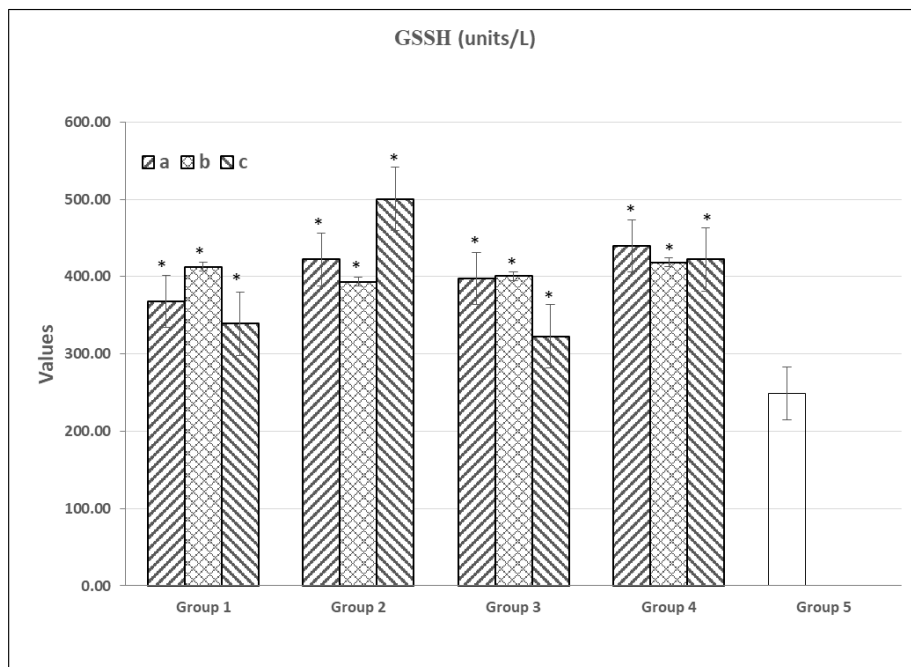


Figure 7 The Serum Levels of Catalase Enzyme after Co-Administration of 120 mg/kg of Ibuprofen with Doses of Antioxidants



Each value represents mean±SD, Values marked with asterisk (*) differ significantly from control group (*p < 0.05), The legends below represent;

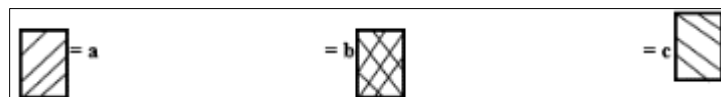


Figure 8 The Serum Levels of Total Glutathione peroxidase after Co-Administration of 120 mg/kg Ibuprofen with Doses of Antioxidants

3.5. Histology of the liver tissues after administration of ibuprofen

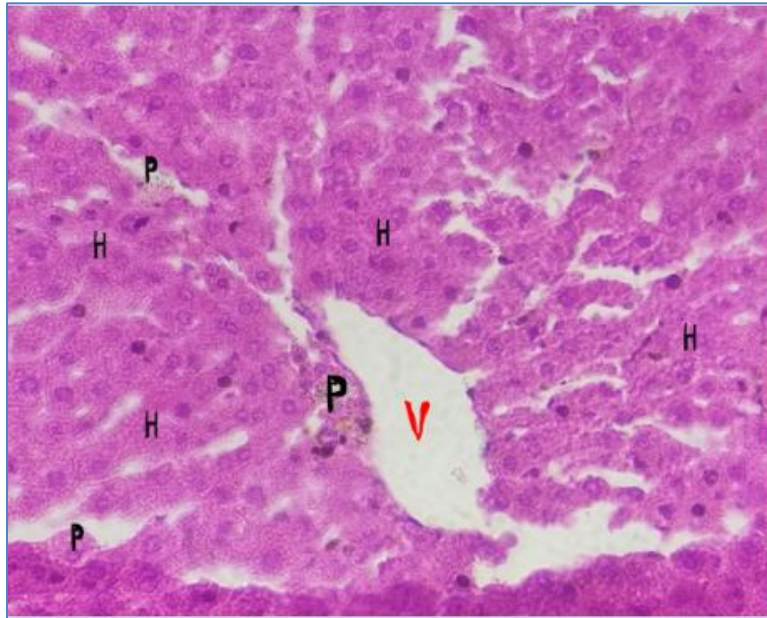


Figure 9 Liver histology of Control; demonstrating normal hepatic architecture of the laboratory animal, with central vein (V) surrounded by normal hepatocytes (H) arranged in chords and radiating towards the portal area (P) composed of the hepatic vein, hepatic artery, and bile duct. [Hematoxylin and Eosin stain X 10]

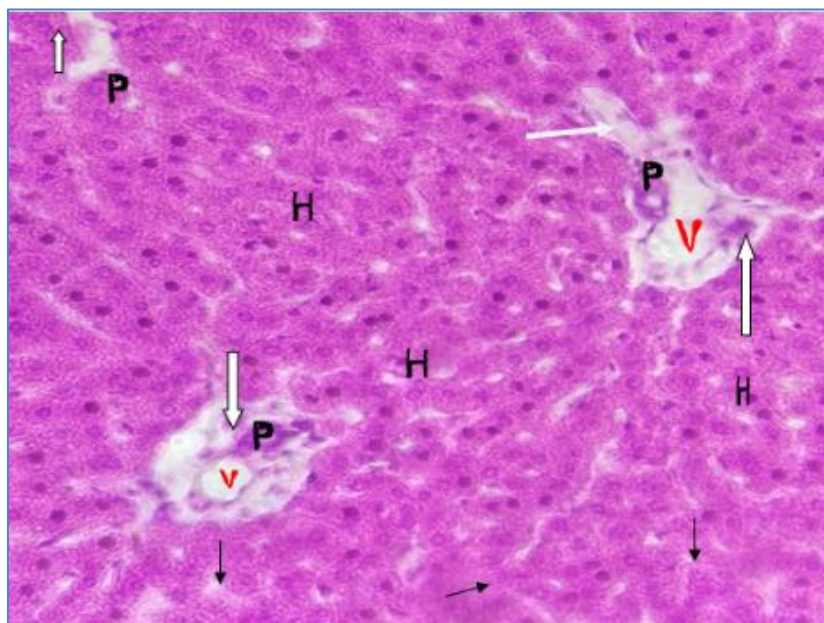


Figure 10 The liver histology of the animals given ibuprofen alone; showing periportal infiltration of inflammatory cells (white arrow), parenchymal necrosis (black arrow), the portal area (P), and the central vein (V). [Hematoxylin and Eosin stain X 10].

The histology of the control showed the normal hepatic histo-architecture; hepatic lobules with central vein surrounded by normal hepatocytes arranged in chords, radiating towards the portal area composed of the hepatic vein, hepatic artery, and bile duct (Figure 9).

The liver tissues of the animals given ibuprofen showed infiltration of inflammatory cells into the portal area, as well as centrilobar infiltrations, aggregation of macrophages, and some range of parenchymal necrosis and hepatocellular damage (Figure 10).

3.6. Histology of the liver tissues after co-administration of ibuprofen with antioxidants

Slide preparations of the liver tissues after co-administration of ibuprofen with doses of different antioxidants showed normal hepatic histo-architecture both in the treatment groups and the control, similar to that in Figure 9. There were normal hepatic lobules with central vein, surrounded by normal hepatocytes arranged in chords and radiating towards the periphery of the lobules, portal triad with normal outline. Similar normal histological findings were observed in the liver tissues of the different treatment categories, irrespective of the dose and type of antioxidant administered.

4. Discussion

Ibuprofen which is a class of propionic acid family of non-steroidal anti-inflammatory drugs has been found to be associated with liver injury [3, 22]. This drug-induced damage is said to be linked with the formation of metabolites that leads to increase generation of reactive oxygen species (ROS) in the body [6]. The hike in oxidative stress has been shown to contribute in the pathogenesis of ibuprofen-induced liver injury [2]. In this study, the significant increase in the level of malondialdehyde, a marker of oxidative stress, is in consonance with findings from other studies [6, 23], which reported that ibuprofen causes increased generation of reactive oxygen species that has been shown to contribute to the pathogenesis of ibuprofen-induced liver damage [2, 14]. Studies have also shown that ibuprofen and most other NSAIDs cause impairment with futile consumption of NADP may lead to acute hepatitis and irreversible cell changes in high doses of NSAIDs [2, 14, 24].

There was a significant increase in the serum levels of alanine transaminase, total bilirubin, and aspartate aminotransaminase after administration of ibuprofen for 14 days, indicated that ibuprofen may induce liver injury even at short term administration, as reported by Sriutha *et al.* [22]. The histological study correlated with the alterations in the biochemical parameters after ibuprofen administration. The liver histological findings in this study is similar to the findings by Schmeltzer *et al.*, [25] who in their study reported that ibuprofen and other NSAIDs causes mitochondrial injury, hepatocellular necrosis, and intrahepatic cholestasis. Also, Moorthy *et al.* [26] reported that administration of high dose of ibuprofen in mice resulted in distorted hepatocellular architecture due to hepatocytes degeneration, infiltration of the portal areas and central veins, and formation of wide area in the sinusoidal gap. However, several investigations have attempted to clarify the mechanism of NSAID-induced hepatotoxicity, and it has been reported to be associated with idiosyncratic reactions to an extent [24].

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 [9, 10, 27, 28]. In this study, the results obtained when ibuprofen was co-administered with antioxidants showed a 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 antioxidant enzymes by up-regulating gene responses linked to generation of antioxidant enzymes and protecting glutathione by maintaining the level of reduced glutathione [10, 27, 18], which counters the elevated oxidative stress induced by ibuprofen administration, and by extension protects against organ injury [29]. However, the results showed that increasing the doses of the various antioxidants or combining different antioxidants provided little or no superior effect on the levels of antioxidant enzymes, when compared to single administration of antioxidants.

There was a significant reduction ($p < 0.05$) in the activities of the serum ALT, AST, ALP, and total bilirubin after co-administration of ibuprofen with antioxidants, when compared to those given ibuprofen alone. This could suggest that antioxidants offer protective effect against ibuprofen-induced liver damage, as stated by Adak [30] and Panchal *et al.* [31] that vitamin E and other antioxidants significantly lowered the circulating aminotransferase levels in nonalcoholic hepatitis, suggesting improvement in liver function. This may be related to the free radical scavenging characteristics of antioxidants and their inhibition of lipid peroxidation [27, 30]. Although, the decline in the activity of liver enzymes was independent on the dose of antioxidant; however, it selectively varied with each antioxidant. The decrease is more pronounced in ALT and AST than other liver parameters, when compared to those given ibuprofen alone. ALT and AST are known to be a specific liver enzyme [32], which suggest that the antioxidants exerts protective effect on the intrinsic activities of the liver, as supported by previous studies [30, 33]. Vitamin E and C had 36.49% and 24.85% average protection on the liver, respectively; quercetin had 30.21%, while the combination of antioxidants had 27.64% average protection on the liver against ibuprofen-induced damage in wistar rats. The normal liver 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 reported in some studies [30, 12, 34], which could be as a result of the ROS scavenging properties of the antioxidants, enhanced antioxidant enzymes activity and anti-inflammatory functions of quercetin, vitamin E and C [30].

5. 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 liver damage in wistar rats. Meanwhile, the average percentage protection of the individual antioxidants varied, with vitamin E providing the highest percentage protection on the liver, when compared to other antioxidants. Therefore, one effective antioxidant can obviate liver injuries that may occur from taking ibuprofen; however the combination had no superior protective effect when compared to that of single antioxidants on the liver.

Compliance with ethical standards

Acknowledgments

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

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Statement of ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Also, all procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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