Protective Effects Of *Vitis vinifera* And *Carica papaya* Seed Extracts On Diclofenac-Alcohol Combination Induced Kidney Damage In Albino Rats

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ABSTRACT

Diclofenac is a non-steroidal anti-inflammatory drug commonly used in the treatment of pain and inflammatory conditions in the tropics. Its easy accessibility without prescription has made it a commonly abused medication. The abuse has recently become worrisome as young adults in Nigeria have been observed to use diclofenac and alcohol for non-medical purposes concomitantly. This study was undertaken to study the nephrotoxic effect associated with the combined use of alcohol and diclofenac using an animal model and the protective effect of *Vitis vinifera* (Grape) seed and *Carica papaya* (pawpaw) seed. Adult albino rats were divided randomly into groups of six rats each, and each group was treated for 90 days with 5% or 45% alcohol with 2mg/kg diclofenac or 10mg/kg diclofenac, and some rats were treated with grape seed and/or pawpaw seed concomitantly. Diclofenac and 45% alcohol combination induced nephrotoxicity in rats and oxidative stress characterized by elevated serum urea and creatinine, increased malondialdehyde and decrease in reduced glutathione, catalase and superoxide dismutase in kidney homogenate, as well as moderate interstitial congestion and hemorrhage in rat kidney histology section stained with Haematoxylin and Eosin, which was significantly ameliorated by the grape seed and pawpaw seed. There was a 1.32% increase in relative kidney weight in comparison with control rats. The severity of the nephrotoxicity observed with the diclofenac-alcohol combination makes it a very unsafe practice. There is a need to explore the nephroprotective benefits of *Vitis vinifera* seed and *Carica papaya* seed for maximum benefit to man.

KEYWORDS: Diclofenac, Alcohol, Toxicity, Grape Seed, Pawpaw Seed.

ABBREVIATIONS

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs, COX: Cyclooxygenase, GSP: Grape Seed Proanthocyanidins, GSE: Grape Seed, PSE: Pawpaw Seed, MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide Dismutase, H&E: Hematoxylin and Eosin

1. INTRODUCTION

Diclofenac is an NSAID that is among the most commonly prescribed painkillers in the world, as it is easily accessible over the counter; it also possesses anti-inflammatory and antipyretic properties [1]. NSAIDs are associated with 12,000 hospital admissions per year in order to treat side effects, and they reportedly contribute to 2600 deaths in the United Kingdom per annum [2]. The molecular target of NSAIDs is the blockade of the COX enzymes in the arachidonic acid cascade. Inhibition of COX-1 accounts for most of the side effects, while COX-2 inhibition produces therapeutic effects [3]. Current literature describes variability in the genetic expression of these COX isoforms with functional and sometimes clinically relevant results [4].

Studies have shown that Nigerian youths in urban, semi-urban and rural areas, with different socio-economic backgrounds, have indulged in the use and abuse of drugs and substances [5]. When this fact is viewed against the background that youths are often larger in number as compared to other age groups in any society, much cause for alarm and concern is raised. The harmful consumption of alcohol is responsible for 7.1% and 2.2% of the global burden of disease for males and females, respectively. It is the leading risk factor for premature deaths and disability among people between the ages of 15 to 49 years, accounting for Ten percent (10%) of all mortalities in this age group [6]. In recent times, opioid and alcohol concomitant use has become more prevalent in Nigeria [7], and government restrictions on opioids have made diclofenac, which is accessible without prescription, a ready alternative.

GSEs are by-products of grapes (*Vitis vinifera*) left from the industrial production of grape wine and juice. The GSEs are rich and potent sources of flavonoids and proanthocyanidins, which are mainly composed of dimers, trimers, and oligomers of monomeric catechins or polymers of polyhydroxy falvan- 3-ol units, such as (+)-catechin, (−)-epicatechin [8]. GSP are potent antioxidants and are able to serve as free radical scavengers. The flavonoids have several health-promoting benefits, including the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals. Their antioxidant activity is 20 times more potent than vitamin C and 50 times more potent than vitamin E [8]. In a study by Valli-Kanagarla *et al*., co-administration of GSE extract (75 mg/kg) and Marjoram volatile oil (0.16 ml/kg) prevented oxidative damages and resulted in a reduction of the hazardous effects of ethanol toxicity on male fertility liver, and brain tissues. Also, pretreatment with resveratrol (10 mmol) prevented ethanol-induced disruption of embryonic development in blastocysts and ESC-B5 embryonic stem cells [9].

Carica papaya is a popular fruit which belongs to the family *Caricaeceae.* It is a plump and round fruit that comes in various sizes. It is believed to have originated from the tropics of the Americas but is now commonly cultivated in several tropical countries, including Nigeria. The fruit, which is the known edible part, is seen in and out of seasons. Various parts of the *papaya* plant are commonly used worldwide in the treatment and management of different diseases of humans and animals. Latex is incorporated in Asian folk medicine as an antiseptic in dressing wounds, in treating dyspepsia and as an abortifacient. In the treatment of piles, some venereal diseases and yaws, infusion of the papaya root is commonly utilized in Africa. Anti-parasitic activities have been reported for the pulverized seeds against parasites such as *Dirofilaria immitis* and *Entamoeba histolytica* [10]. Studies have shown that *Carica papaya* seed has significant nephroprotective benefits. In a study by Naggayi *et al*. [11] on the protective effects of aqueous extract of *Carica papaya* seeds in paracetamol-induced nephrotoxicity in male Wistar rats, it was revealed that *Carica papaya* contains nephroprotective phytochemicals and may be useful in preventing kidney damage induced by paracetamol. From the study, the nephroprotective property of the extract was confirmed by significant improvement of the kidney architecture by reversing the nephrotoxic effects of paracetamol, such as glomerular congestion, interstitium with inflammatory cells, tubular necrosis, peritubular necrosis and presence of intra-luminal casts suggesting massive total necrosis. Literature has shown medicinal plants with nephroprotective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids they contain [12].

2. METHOD(S)

Adult male albino rats weighing between 200 grams to 240 grams were obtained from the animal house of the Faculty of Basic Medical Sciences, College of Medical Sciences, Ambrose Alli University, Ekpoma, Nigeria. The rats were left to acclimatize for three weeks (21 days). The rats were de-wormed and kept in wire mesh cages elevated from the ground, and the animal beds were changed weekly with fresh sawdust. During the acclimatization period, the rats were fed with grower's marsh and water ad libitum. The house was swept daily and disinfected on weekends to prevent the rats from being infected in accordance with the standard guide for the care and use of laboratory animals. The animals were kept under suitable laboratory conditions throughout the period of investigation.

Fresh unripe *Carica papaya* (pawpaw) and *Vitis vinifera* (Grapes) fruits were purchased from the local fresh fruits market in Ekpoma, Edo State and identified using the PlantNet App version 3.17.4 by plantnet.org. The pawpaw and grapes were sliced open and the Seeds removed and air dried. After drying, the seeds were pulverised using an electric blender and the fine powdered PSE and GSE were measured using an electric weighing balance. Each small measure was packed in a drug envelope and exposed to ultraviolet light overnight to avoid contamination, from which aliquots were reconstituted with distilled water to obtain liquid and semi-solid forms for proper administration.

Fresh 5% w/v beer grade grain alcohol and 45% w/v alcohol was obtained from the Dublos supermarket in Ekpoma South-South Nigeria, and recently manufactured K+-Diclofenac was obtained from the pharmacy. The K+-Diclofenac was dissolved in the alcohol, and the concentrations were adjusted appropriately.

2.1 EXPERIMENTAL DESIGN

Rats were divided randomly into groups of six (6) rats each. Six rats served as control. Each group was treated for 90 days as follows: Control Group received distilled water orally, a group received a combination of Grape and PSE orally, rats in another group received 5% w/v alcohol (4g/kg) and K+-Diclofenac (2 mg/kg) orally, another group was administered 45% w/v alcohol (4g/kg) and K+-Diclofenac (2 mg/kg) orally, another given GSE (150 mg/kg) concomitantly with 2 mg/kg K+-Diclofenac and 4g/kg of 45% w/v alcohol. A group of albino rats was given PSE (150 mg/kg) concomitantly with 2 mg/kg K+-Diclofenac and 4g/kg of 45% w/v alcohol; another group of albino rats was given a combination of GSE and PSE (150mg/kg) concomitantly with 2 mg/kg Diclofenac and 4g/kg of 45% w/v alcohol intraperitoneally trice weekly (Monday, Wednesday and Friday). A group of rats (Recovery group) received K+-Diclofenac (10 mg/kg) and 4g/kg of 45%

w/v alcohol intraperitoneally for 45 days, after which it was discontinued. Consequently, the GSE and PSE combination was given to the rats for the next 45 days.

The LD50 of the various substances (five doses) were tested each for Diclofenac and alcohol combination, GSE and PSE. The LD50 was calculated using linear interpolation between the response of the dose below the LD50 and the dose above the LD50. The Oral LD50 (Male, albino rats between 200 to 240 grams) for Diclofenac combined with 45% w/v alcohol (4g/kg) was 26mg/kg, the Oral LD50 for GSE was >4000mg/kg and 1800mg/kg for unripe PSE. All doses used for this study were less than the LD50.

Animals were weighed, and blood samples were obtained from albino rats under light anesthesia by diethyl ether from the retro-orbital vein of each rat, according to the method of Cocchetto and Bjornsson [13]. Blood was collected into plain sample bottles and allowed to coagulate and then centrifuged at 3000 rpm for 5 minutes. The obtained serum was used to estimate the activities of Sodium, Potassium, Bicarbonate, Chloride, Urea and Creatinine. Animals were sacrificed following standard ethical protocols for animal handling. Kidney weights were measured after the animals were dissected. The weight of the kidney was subtracted from each corresponding rat to obtain the kidney-free estimate of the body weight of each rat. The left lobe of each kidney was dissected and placed in 10% formalin in saline; the other weighed part of each kidney was homogenized, using a homogenizer to prepare 20% w/v homogenate. The homogenate was centrifuged at 4000 rpm for 5 minutes to remove cell debris. The aliquot was used for the assessment of reduced glutathione, Catalase, SOD and MDA.

2.2 ETHICAL CONSIDERATION

Ethical approval was obtained from Ambrose Alli University Ekpoma, Health Research Ethics Committee (NHREC/12/06/2013) with assigned number 014/19. Also, we observed the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education issued by the New York Academy of Sciences Adhoc Committee on Animal Research.

2.3 DETERMINATION OF LABORATORY PARAMETERS

Reduced GSH content in liver homogenate was determined according to the colorimetric method of Ellman [14], and absorbance was read using a Labtech UV-Vis Spectrophotometer UV 9100-Series. The data was expressed as µmol/ml.

Lipid peroxidation is usually assayed by the measurement of MDA generated during the peroxidation of lipids according to the colorimetric method of Uchiyama and Mihara [15]. Absorbance was measured at 532 nm against a reagent blank using a Labtech UV-Vis Spectrophotometer UV 9100-Series, which was also used for the determination of Catalase and SOD levels.

Determination of serum Potassium, Sodium, Chloride and Bicarbonate was based on the ion-selective electrode methods. The determination of creatinine was based on the Jaffe-Slot modified alkaline picrate colorimetric method used for Creatinine level estimation. After deproteinisation, creatinine in an alkaline solution forms a yellow-red complex with picric acid. The determination of urea was based on the Urease-Berthelot colorimetric method. Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically by Berthelot's reaction [16].

Histology was done based on the method described by Bancroft and Gamble [17]. The kidneys from all groups were removed and immediately fixed in 10% neutral buffered formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. 4-5µm thick sections were prepared and stained with H&E for photomicroscopic observation.

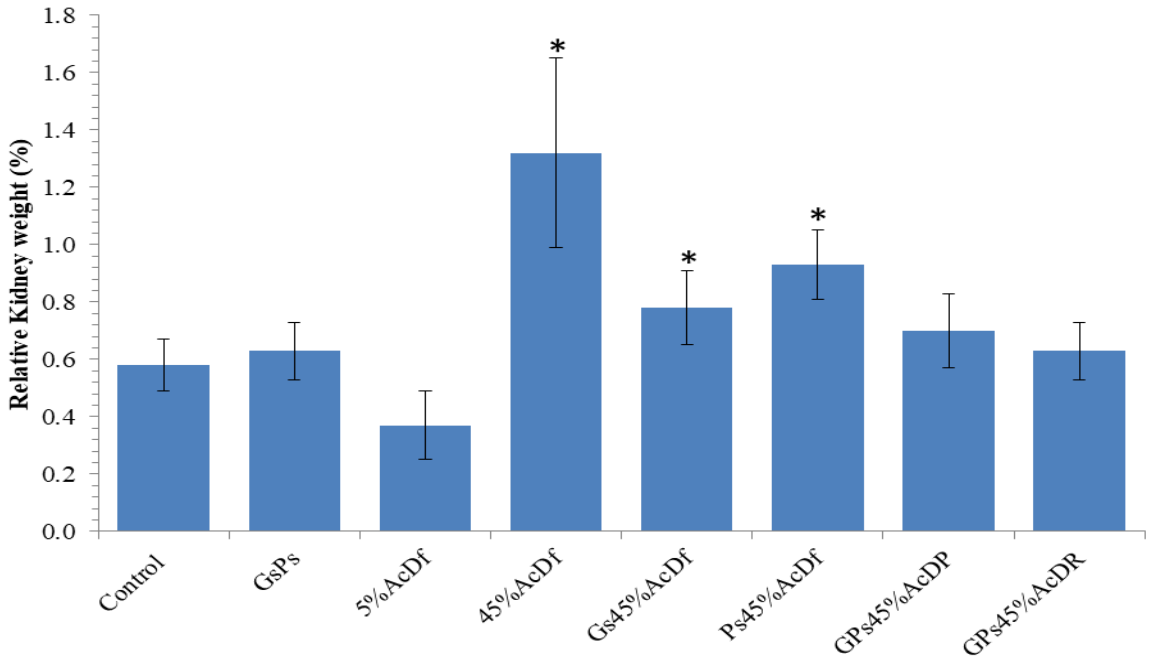
2.4 STATISTICAL ANALYSIS

Data obtained from the laboratory analysis was evaluated by One-Way Analysis Of Variance (ANOVA) and T-test using SPSS version 20.0 and GraphPad prism. The degree of variability of results was expressed as means ± standard deviation (SD). The level of significance was accepted at $P < 0.05$.

3. RESULTS

Figure 1 shows the relative organ weight (Kidney to Body weight ratio) of rats given alcohol-diclofenac combination with GSE/PSE. A significant increase in the relative kidney weight was obtained for the group administered 45% Alcohol/Diclofenac (1.32±0.33; $p < 0.05$). A significant increase in Relative kidney weight was obtained for groups given GSE with 45% Alcohol/Diclofenac (0.78±0.13; $p<0.05$) and unripe PSE with 45% Alcohol/Diclofenac (0.93±0.12; $p<0.05$) in comparison with control. 5% Alcohol/Diclofenac treatment resulted in reduced relative kidney weight and Relative liver weight (0.37±0.12; $p<0.05$). GSE combination significantly reduced the relative kidney/liver weight value observed for the 45% Alcohol + Diclofenac combination.

Figure 1. Relative organ weight (Kidney to Body weight ratio) of rats given alcohol-diclofenac combination with GSE/PSE.



Key:

*=Difference in mean values is significant versus control at 0.05 levels. Control= Animal group given food and water only; **GsPs**=GSE and PSE; **5%AcDf**=5% Alcohol and Diclofenac combination; **45%AcDf**=45% Alcohol and Diclofenac combination; **Gs45%AcDf**=GSE, 45% Alcohol and Diclofenac combination; **Ps45%AcDf** =Unripe PSE, 45% Alcohol and Diclofenac combination; **GPs45%AcDP** =GSE & Unripe PSE, 45% Alcohol and Diclofenac combination peritoneal administration; **GPs45%AcDR**= Recovery group (initially given 10mg/kg of Diclofenac concomitantly with 45% alcohol for 45 days and then discontinued. Then GSE and PSE only for 45 days).

Figures 2 to 4 show the renal function test parameters of rats given alcohol-Diclofenac combination with GSE/PSE. There was significant increase in serum potassium levels for groups administered GSE and PSE (4.9±0.4; p=0.000), 45% Alcohol and Diclofenac (5.5±0.5; p=0.000), GSE with 45% Alcohol and Diclofenac (4.4±0.3; p=0.002) and Recovery group (initially given 10mg/kg of Diclofenac concomitantly with 45% alcohol for 45 days and then discontinued. Then Grape and PSE only for 45days) (4.7±0.4; p=0.000), in comparison with the control (3.8±0.4). A significant decrease in serum potassium level was obtained for the group given 5% Alcohol and Diclofenac (3.2±0.3; p=0.013) in comparison with the control. In comparison to group treated with 45% Alcohol and Diclofenac (5.5±0.5), significantly reduced serum potassium levels was observed for group treated with Unripe PSE, with 45% Alcohol/ Diclofenac (3.4±0.2; p=0.00) and group treated with GSE & Unripe PSE with 45% Alcohol/Diclofenac by peritoneal administration (3.9±0.3; p=0.00).

Bicarbonate levels reduced significantly in rats given GSE and PSE (12.5±4.6; p=0.038), GSE with 45% Alcohol/Diclofenac (10.5±5.2; p=0.006), GSE & Unripe PSE with 45% Alcohol/ Diclofenac peritoneal administration (8.9±4.3; p=0.001) and Recovery group (11.5±3.8; p=0.016) in comparison with the control (18.3±4.3).

From Figure 3, administration of 45% Alcohol/Diclofenac increased serum sodium concentration (149.5±6.3) significantly (p=0.00) in comparison with control (134.8±1.5). The sodium level of the 45% Alcohol/Diclofenac group was also significantly increased in comparison with groups treated with; GSE and PSE (138.8±1.7; p=0.001), 5% Alcohol and Diclofenac (131.0±5.6; p=0.000), GSE with 45% Alcohol and Diclofenac (136.2±3.0; p=0.000), Unripe PSE, with 45% Alcohol and Diclofenac (136.2±1.7; p=0.000), GSE & Unripe PSE with 45% Alcohol and Diclofenac peritoneal administration (137.0±5.8; p=0.000) and Recovery group (136.5±2.1; p=0.000).

In comparison with control (76.5±7.8), significantly higher chloride levels were obtained for groups treated with 5% Alcohol and Diclofenac (103.2±13.6; p=0.000), 45% Alcohol and Diclofenac (112.2±11.8; p=0.000), GSE with 45% Alcohol/Diclofenac (102.5±5.1; p=0.000), Unripe PSE, with 45% Alcohol/Diclofenac (107.7±7.5; p=0.000), GSE & Unripe PSE with 45% Alcohol/Diclofenac by peritoneal administration (91.5±11.7; p=0.012) and Recovery group (118.8±5.3; p=0.000).

Figure 2. Plasma potassium and Bicarbonate levels of rats given alcohol-Diclofenac combination with GSE/PSE.

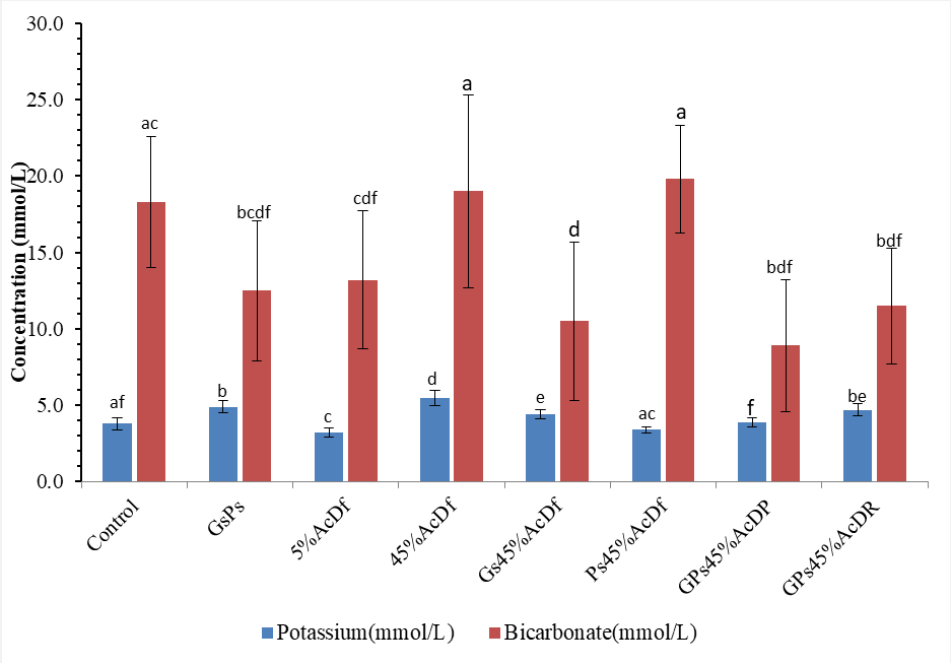


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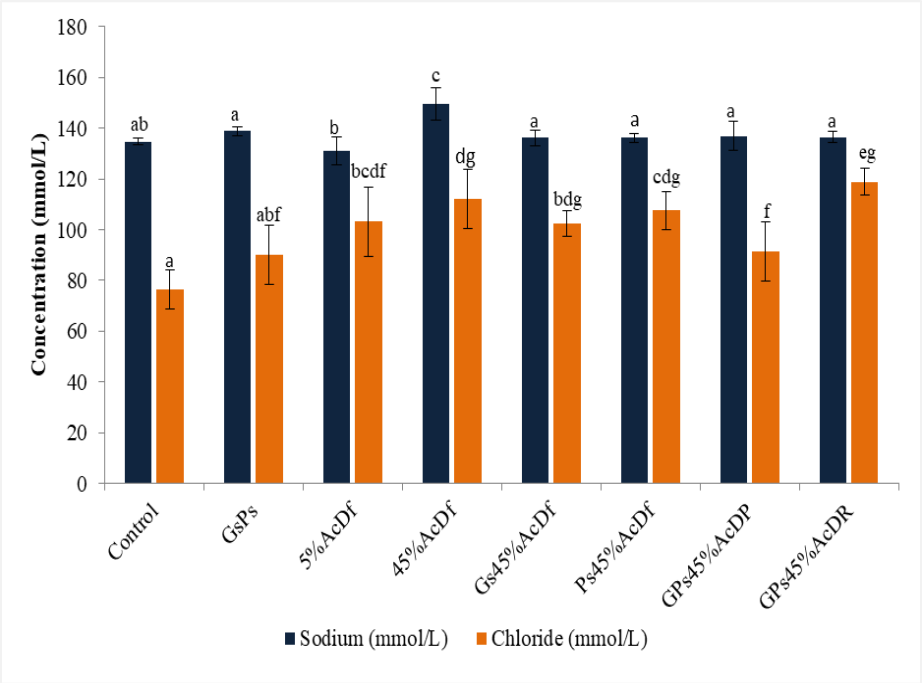
Figure 3. Plasma sodium and chloride levels of rats given alcohol-Diclofenac combination with GSE/PSE.



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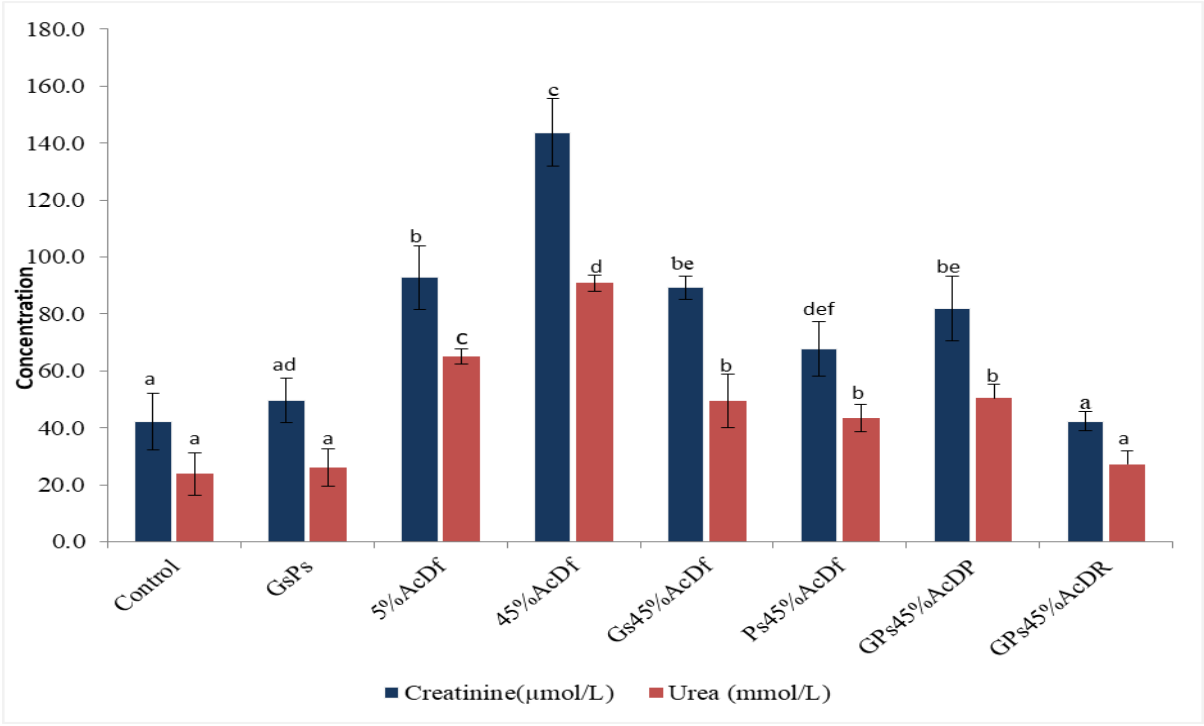
Alcohol and Diclofenac combination; **Ps45%AcDf** =Unripe PSE, 45% Alcohol and Diclofenac combination; **GPs45%AcDP** =GSE & Unripe PSE, 45% Alcohol and Diclofenac combination peritoneal administration; **GPs45%AcDR**= Recovery group (initially given 10mg/kg of Diclofenac concomitantly with 45% alcohol for 45 days and then discontinued. Then GSE and PSE only for 45 days).

As shown in Figure 4, statistical analysis of data obtained in comparison with control (42.3±9.9) showed significant increase in serum creatinine concentration for groups administered; 5% Alcohol/ Diclofenac (92.8±11.1; p=0.000), 45% Alcohol/Diclofenac (143.7±11.9; p=0.000), GSE with 45% Alcohol/Diclofenac (89.2±4.1; p=0.000), Unripe PSE, with 45% Alcohol/Diclofenac (67.7±9.7; p=0.026) and GSE & Unripe PSE with 45% Alcohol/Diclofenac by peritoneal administration (81.8±11.3; p=0.001). The recovery group had significantly lower serum creatinine levels (42.3±3.4) in comparison with the other groups than the control and group given grape and PSE (p>0.05).

Figure 4. Plasma creatinine and urea levels of rats given alcohol-Diclofenac combination with GSE/PSE.



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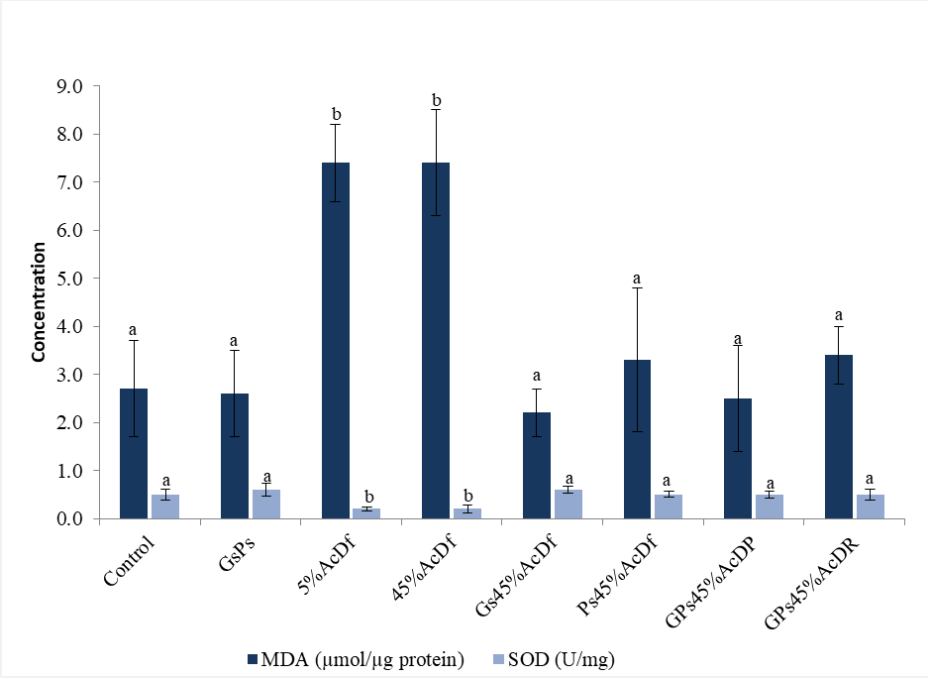
Figures 5 and 6 show the level of oxidative stress markers in kidney homogenate of rats exposed to combinations of Alcohol, Diclofenac, GSE and PSE. Kidney homogenate levels of MDA were significantly increased in groups treated with 5% Alcohol/ Diclofenac (7.4±0.8) and 45% Alcohol/ Diclofenac (7.4±1.1) in comparison with control (2.7±1.0) (p=0.000 and p=0.000 respectively) and the group treated with just GSE and PSE (2.6±0.9) (p=0.000 and p=0.000 respectively).

In comparison with control (0.5±0.11), administration of 5% Alcohol/ Diclofenac and 45% Alcohol/ along with Diclofenac significantly reduced Superoxide dismutase in kidney homogenate of rats (0.2±0.04 and 0.2±0.08) (p=0.012 and p=0.000 respectively).

Figure 5. MDA and SOD levels in kidney homogenate of rats exposed to combinations of Alcohol, Diclofenac, GSE and PSE.



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Significant decreases in kidney homogenate catalase levels of Wistar rats were recorded in groups given 5% Alcohol/Diclofenac (7.3±1.0), 45% Alcohol/Diclofenac (8.2±1.3) and Recovery group (10.9±2.3), in comparison with the control (22.7±1.9) (p=0.000 respectively) and other groups (p>0.05). Administration of 5% Alcohol/Diclofenac and 45% Alcohol/Diclofenac to Wistar rats significantly decreased GSH levels in kidney homogenate (3.4±1.7 and 3.1±1.7; p=0.000, respectively).

Figure 6. Catalase and reduced GSH levels in kidney homogenate of rats exposed to combinations of Alcohol, Diclofenac, GSE and PSE.

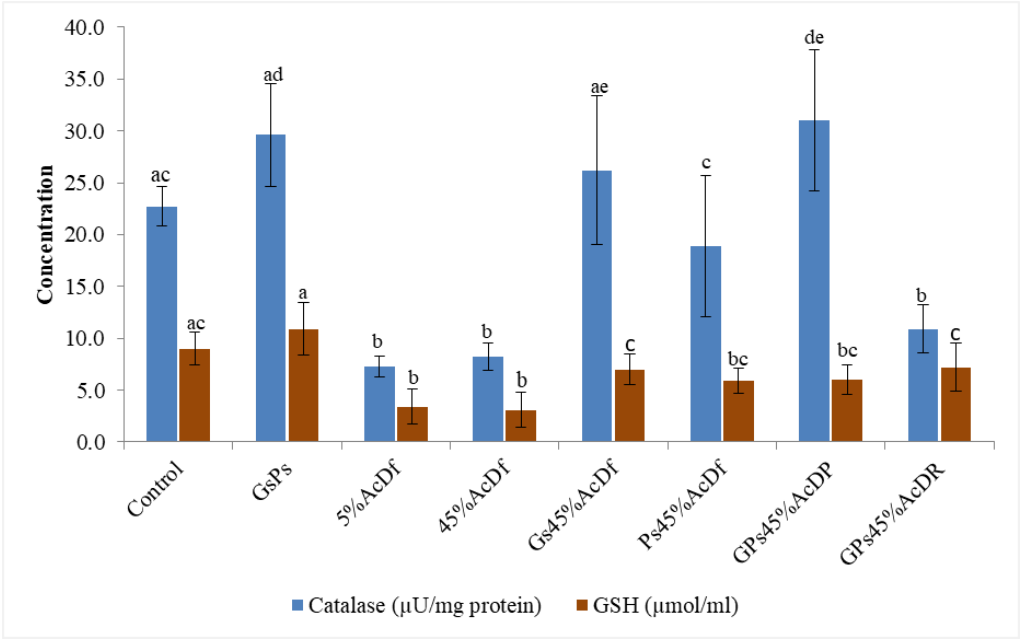

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Figure 7 shows the kidney histology of the rat group administered 5% Alcohol and Diclofenac combination, stained with H&E, X100. The photomicrograph shows moderate interstitial congestion and hemorrhage (H) in the rat kidney section.

Figure 7. Photomicrograph of rat kidney administered 5% Alcohol and 2mg/kg Diclofenac combination.

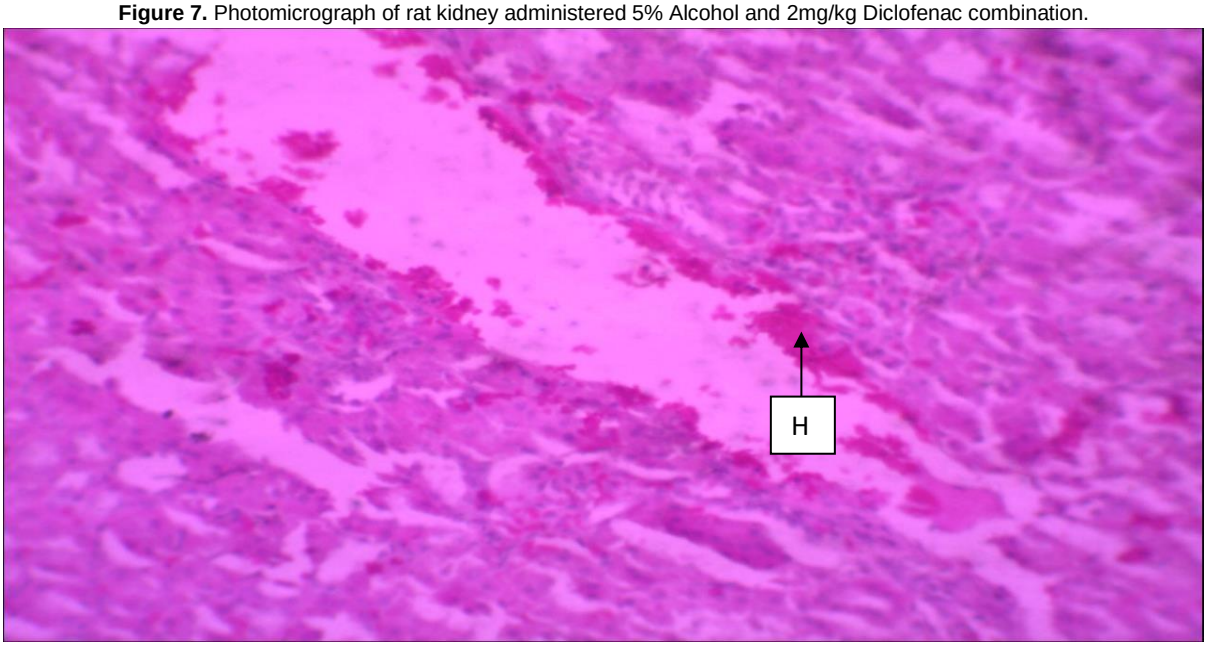


Figure 8 shows the kidney histology of the rat group administered 45% Alcohol and Diclofenac combination, stained with H&E, X400. The photomicrograph shows moderate interstitial congestion and hemorrhage (C) in the rat kidney section.

Figure 8. Photomicrograph kidney section of the rat group administered 45% Alcohol and 2mg/kg Diclofenac combination.

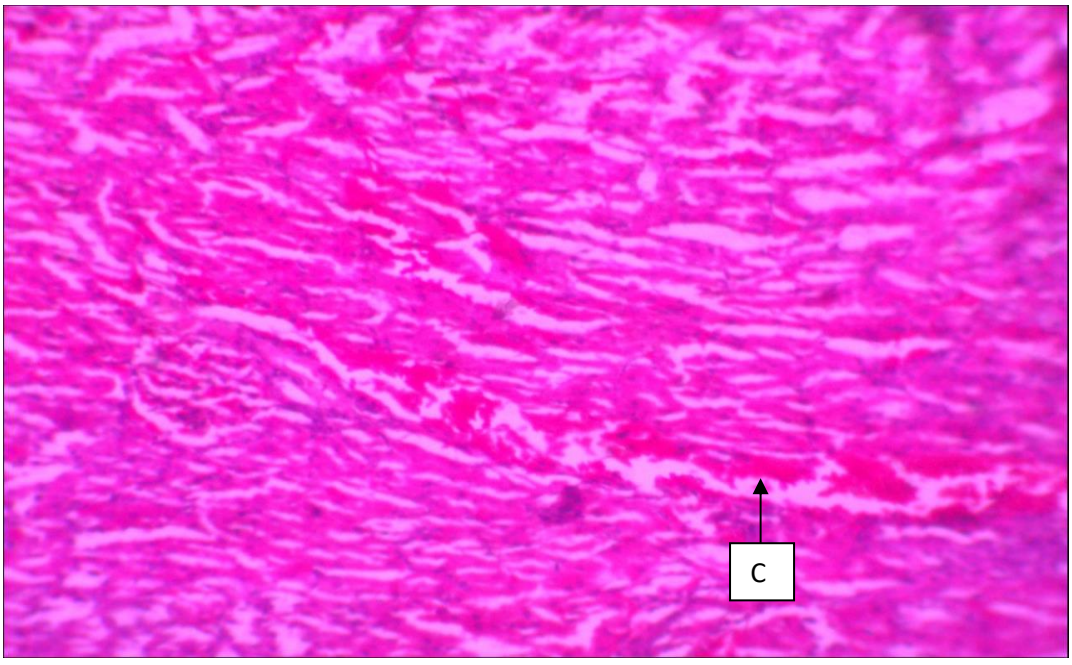


Figure 9 shows the kidney histology of the rat group administered GSE Extract, 45% Alcohol and Diclofenac combination, stained with H&E, X100. The photomicrograph shows normal histology composed of mainly renal corpuscles (C) and tubules.

Figure 9. Photomicrograph of kidney section of rat group administered 150mg/kg GSE, 45% Alcohol and 2mg/kg Diclofenac combination.

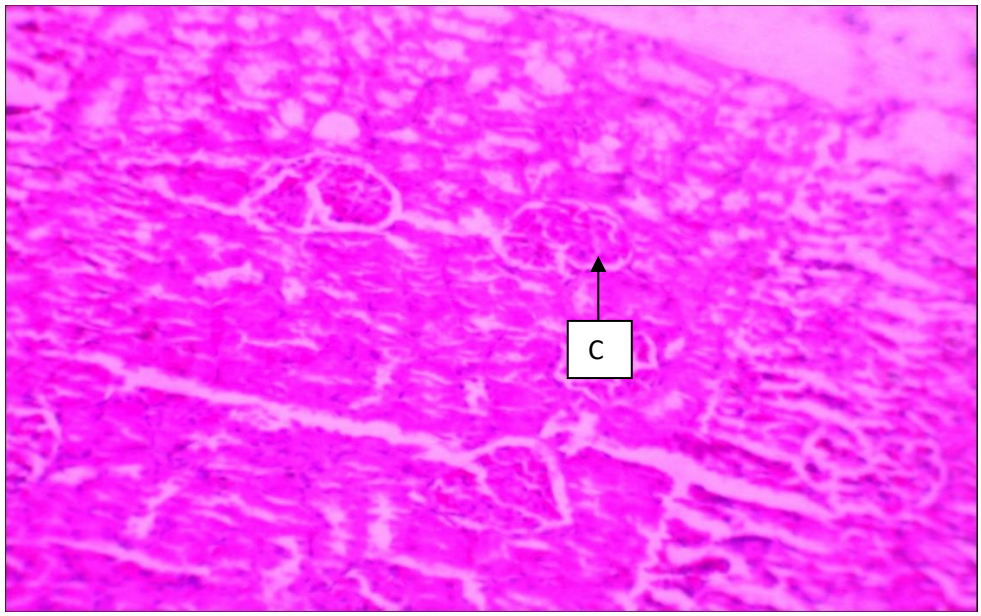


Figure 10 shows the kidney histology of the rat group administered 150mg/kg unripe PSE, 45% Alcohol and 2mg/kg Diclofenac combination, stained with H&E, X100. The photomicrograph shows normal histology composed of mainly renal corpuscles (C) and tubules.

Figure 10. Photomicrograph of kidney section of rat group administered 150mg/kg unripe PSE, 45% Alcohol and 2mg/kg Diclofenac combination.

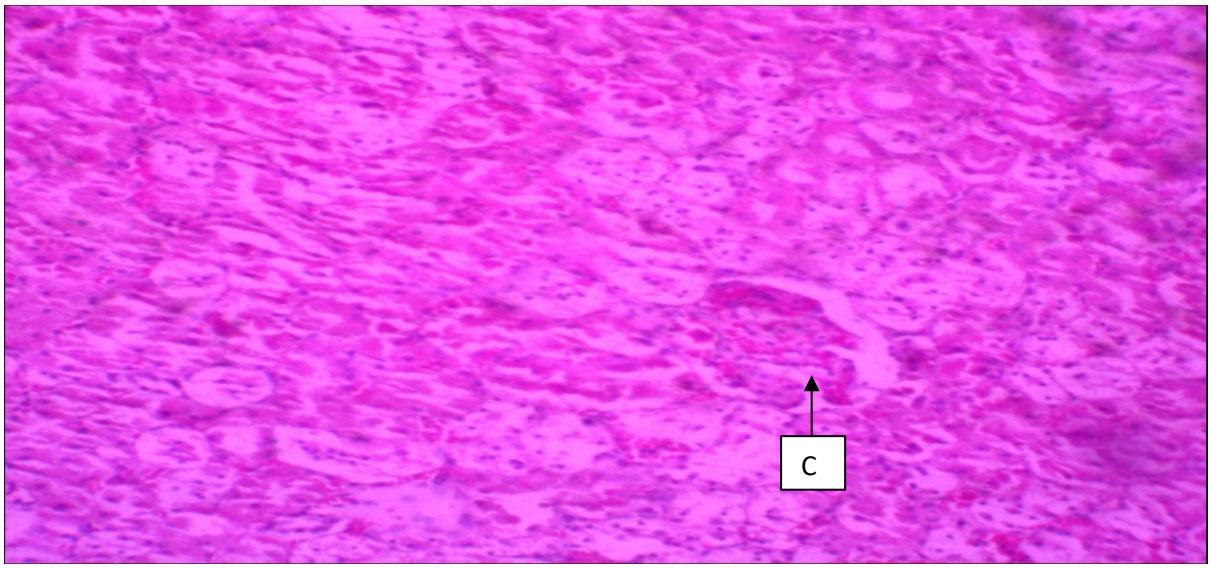


Figure 11 shows the kidney histology of the rat group treated with GSE and unripe PSE, along with 45% Alcohol and Diclofenac combination by peritoneal administration, stained with H&E, X400. The photomicrograph shows normal histology composed of mainly renal corpuscles (C).

Figure 11. Photomicrograph of kidney section of rat group treated with 150mg/kg GSE and Unripe PSE, along with 45% Alcohol and 2mg/kg Diclofenac combination by peritoneal administration.

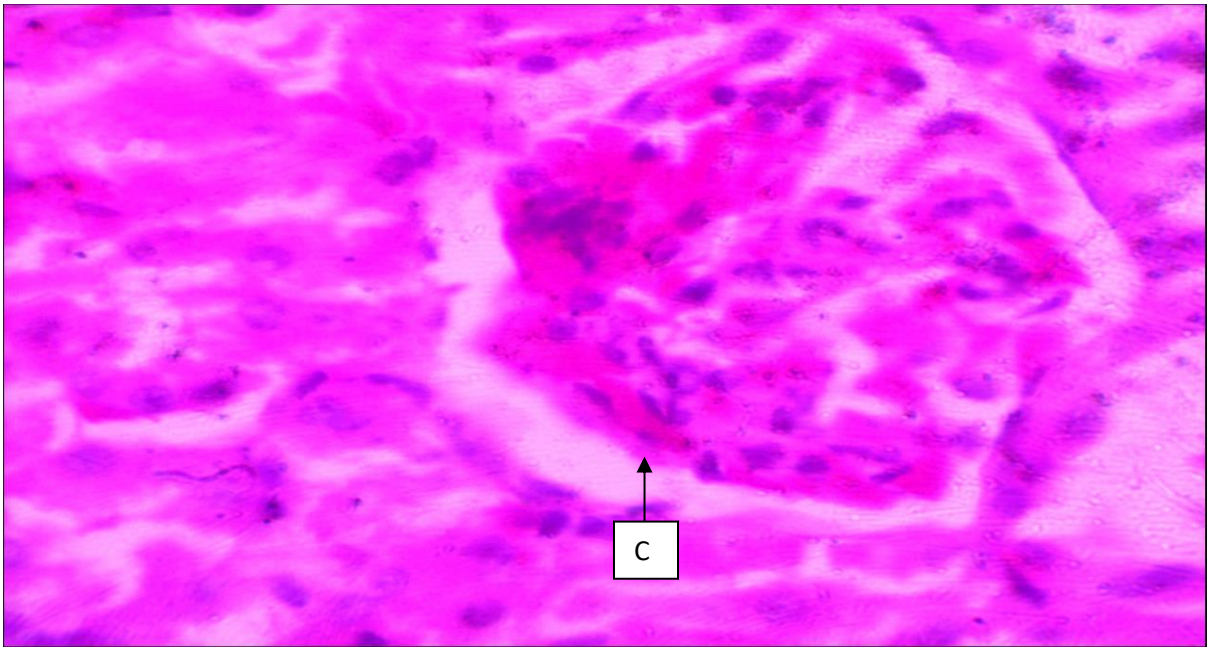
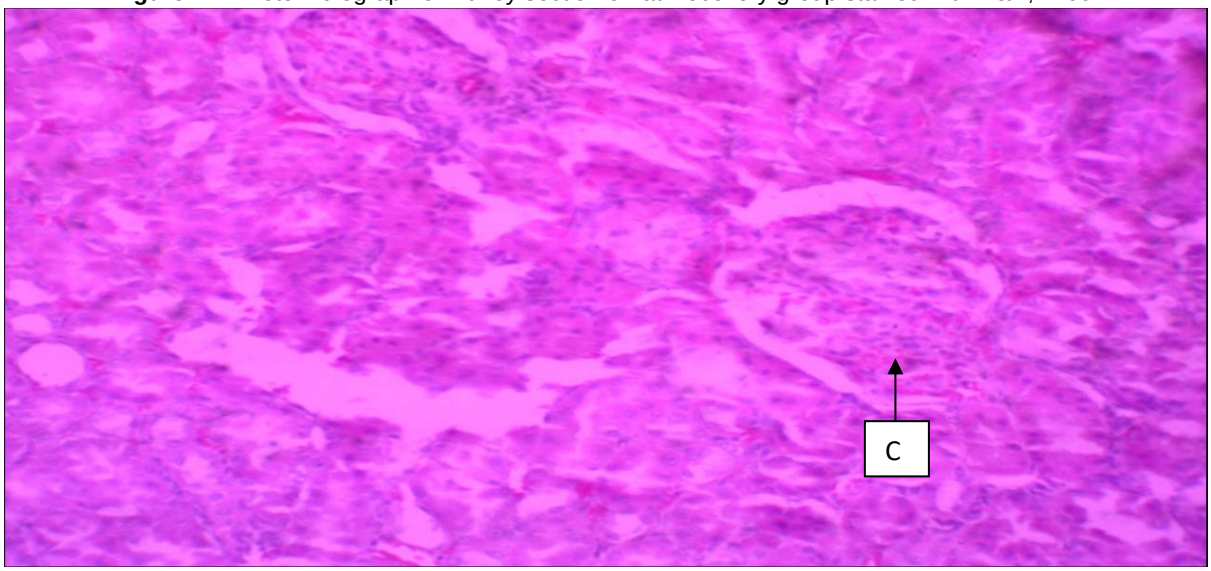


Figure 12 shows the kidney histology of the rat Recovery group which were albino rats initially given 10mg/kg of Diclofenac concomitantly with 45% alcohol for 45 days and then discontinued. Then, 150mg/kg Grape and PSEs only for 45days. The section was stained with H&E, x400. The photomicrograph shows normal histology composed of mainly renal corpuscles (C) and tubules.

Figure 12. Photomicrograph of kidney section of rat Recovery group stained with H&E, x400.



4. DISCUSSION

This study showed the effect of alcohol-drug combinations using an animal model by exposing adult albino rats to prolong administration of diclofenac and alcohol combinations. Chronic administration of Diclofenac (2mg/kg body weight) + 5% alcohol reduced relative kidney weight, although non-significantly, whereas rats given 45% alcohol + Diclofenac (2mg/kg body weight) had significantly higher relative kidney weight than the normal control rats. Hence, relative organ weight changes observed with Diclofenac (2mg/kg body weight (bw) + 5% and 45% alcohol-treated rats followed the alcohol concentration pattern, in line with the findings of Linnoila *et al*. [18] and Traynor *et al*. [19] that alcohol increases the bioavailability of drugs, such that there was an increased effect of diclofenac with the increase in alcohol concentration

which was characterized by increase relative organ weight similar to the high doses of diclofenac only. The findings of Owumi and Dim [20] revealed a significant increase in relative kidney weights in rats treated with 10mg/kg diclofenac.

The combination of GSE with 45% alcohol + Diclofenac significantly attenuated the increased relative kidney weight observed for rats treated with 45% alcohol/diclofenac combination. Studies have reported that GSE significantly reduced toxic changes in Kidney tissues [21,22]. In this study, Pawpaw (*Carica papaya*) seed did not show a significant attenuating effect on the increased relative kidney weight caused by 45% alcohol + Diclofenac combination, except in the combined form with GSE.

In the diclofenac study group, significant changes in electrolytes were recorded in rats treated with 45% alcohol and diclofenac combination for 90 days. There were increased serum potassium, sodium and chloride, urea and creatinine levels than control rats. Similarly, treatment of rats with 5% alcohol + 2mg/kg of diclofenac resulted in a significant increase in serum urea and creatinine levels. The nephrotoxicity exerted by the chronic combination of diclofenac and alcohol was further demonstrated by the histological findings of moderately severe interstitial hemorrhagic congestion. Nephrotoxicity associated with chronic use of low doses and acute use of high doses of diclofenac, characterized by elevated urea, creatinine, and histological changes, have been reported by several studies [20,23]. Diclofenac is well-documented to be safe in non-chronic use of therapeutic doses [20]. Increased bioavailability associated with combining diclofenac with alcohol has the capacity to increase nephrotoxicity, which could result in acute renal injury and nephropathy. Findings from this study suggest that renal oxidative stress from alcohol + diclofenac combined use could be the predisposing factor for renal injury reported, as elevated kidney homogenate levels of MDA, which is an important oxidative stress marker, was recorded, while there was a decrease in kidney homogenate levels of antioxidant parameters such as catalase, glutathione and superoxide dismutase which are known to help mop up free radicals and reactive oxygen species.

Dose regulation is a major factor in diclofenac-induced nephrotoxicity [23], and findings from this study showed higher oxidative stress with a higher concentration of alcohol (45% alcohol) combined with 2mg/kg of diclofenac than in 5% alcohol combination, which had a similar outcome as other experimental studies with high doses of only diclofenac [20,24]. Catalases, superoxide dismutase, like other enzymatic antioxidants, catalyze the inactivation of superoxide anion and peroxide radicals by converting them to water and oxygen [25]. The nephroprotective capacity of GSE and PSE was demonstrated by their ability to cause or induce an increase in antioxidant activities in this study. The combination of GSE and PSE significantly reduced the nephrotoxicity induced by 45% alcohol + diclofenac and increased antioxidant activities in kidney homogenate. The recovery group was initially treated with 10mg/kg of Diclofenac concomitantly with 45% alcohol for 45 days, after which it was discontinued, and then given GSE and PSE only for another 45 days, showed almost complete reversal of the induced renal injury, and the mechanism of this action could be the capacity of the GSE and PSE to suppress the actions of inflammatory cytokines and cause depletion of reactive oxygen species signaling which could suppress kidney injury and fibrosis.

5. CONCLUSION

This finding shows that recovery from a drug-alcohol-induced organ injury is more rapid when the toxic item is first eliminated. This current study disclosed novel evidence of the antioxidant effect of the GSE and PSE combination, which can be termed '*papaya-vinifera* complex' and the first to reveal the effect of '*papaya-vinifera* complex' in attenuating nephrotoxicity.

5.1 RECOMMENDATION

Our study has shown the severe nephrotoxicity associated with Alcohol and Diclofenac concomitant use using animal models, and there is a need for a study to corroborate our findings with observational studies of human subjects. There is a need for public health awareness to be heightened to prevent renal damage and other complications associated with emerging chronic alcohol-drug use, especially among young males in sub-Saharan Africa. Also, there is a need for further animal studies to understand the protective mechanism of *vinifera* and *papaya* completely.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this study.

CONFLICT OF INTEREST

None.

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REFERENCES

1. Alabi QK, Akomolafe RO. Kolaviron diminishes diclofenac-induced liver and kidney toxicity in wistar rats via suppressing inflammatory events, upregulating antioxidant defences, and improving Haematological indices. Dose Response. 2020;1-12. https://doi.org/10.1177%2F1559325819899256
2. Blower AL, Brooks A, Fenn GC. Emergency admissions for upper gastrointestinal disease and their relation to NSAID use. Aliment Pharmacol Ther. 1997; 11(2): 283–291. [PMID: 9146764] https://doi.org/10.1046/j.1365-2036.1997.d01-604.x
3. Riendeau D, Percival MD, Brideau C. Etoricoxib (MK-0663); preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. J Pharmacol Exp Ther. 2001; 296(2): 558–566 [PMID:11160644] https://pubmed.ncbi.nlm.nih.gov/11160644/
4. Sansbury LB, Millikan RC, Schroeder JC. COX-2 polymorphism, use of nonsteroidal anti-inflammatory drugs, and risk of colon cancer in African Americans (United States). Cancer Causes Control. 2006; 17(3): 257–266. [PMID: 16489533] https://doi.org/10.1007/s10552-005-0417-0
5. Madaki M, Aminu MD. The Influence of Substance Abuse on Youths' Prospects in Nigeria and the Way Forward. Adv Psychol Neurosci. 2017; 2(2-1): 15-20. https://doi.org/10.11648/j.apn.s.2017020201.13
6. WHO-ECDD. Expert Committee on Drug Dependence (ECDD) Thirty-ninth Meeting of World Health Organization (WHO); Agenda item 5.3.Tramadol Preview Report. 2017; Pp 6.
7. United Nations Office on Drugs and Crime (UNODC). Drug use survey in Nigeria 2018. Executive Summary. Pp 10.
8. Yamakoshi J, Saito M, Kataoka S, Kikuchi M. Safety evaluation of proanthocyanidin-rich extract from grape seeds. Food Chem Toxicol. 2002; 40: 599-607. [PMID: 11955665] https://doi.org/10.1016/s0278-6915(02)00006-6
9. Valli-Kanagarla NSSA, Kuppast IJ, Veerashekar T, Reddy CL. A review on benefits and uses of *Vitis vinifera* (Grape). Res Rev Biosci. 2013; 7(5): 175-180. https://www.tsijournals.com/articles/a-review-on-benefits-and-uses-of-vitis-vinifera-grape.pdf
10. Adeneye AA. Subchronic and Chronic Toxicities of African Medicinal Plants. In: Toxicological Survey of African Medicinal Plants. 2014; 99-133. https://doi.org/10.1016/B978-0-12-800018-2.00006-6
11. Naggayi M, Mukiibi N, Iliya E. The protective effects of aqueous extract of *Carica papaya* seeds in paracetamol induced nephrotoxicity in male wistar rats. Afr Health Sci. 2015; 15(2): 598–605. [PMID: 26124809] https://doi.org/10.4314/ahs.v15i2.37
12. Adeneye AA, Benebo AS. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. J Ethno-Pharmacol. 2008; 118: 318–323. [PMID:18554830] https://doi.org/10.1016/j.jep.2008.04.025
13. Cocchetto DM, Bjornsson TD. Methods for vascular access and collection of body fluids from the laboratory rat. J Pharm Sci. 1983; 72(5): 465-92. [PMID: 6345750] https://doi.org/10.1002/jps.2600720503
14. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82: 70-77. [PMID: 13650640] https://doi.org/10.1016/0003-9861(59)90090-6
15. Uchiyama M, Mihara M. Determination of malondialdehyde in tissue by thiobarbituric acid test. Anal Biochem. 1978; 86: 271-278. [PMID: 655387] https://doi.org/10.1016/0003-2697(78)90342-1
16. Weatherburn MW. Principle of Urease-Berthelot colorimetric method for Urea. Anal Chem. 1967; 39: 971-974. https://www.scirp.org/reference/referencespapers?referenceid=2063332
17. Bancroft JD, Gamble M. Theory and practice of histological techniques. 5th Edn. Edinburgh. Churchill Livingstone Pub. 2002; pp: 172-175, pp: 593-620. https://www.academia.edu/40252505/Bancrofts_Theory_and_Practice_of_Histological_Techniques
18. Linnoila M, Mattila MJ, Kitchell BS. Drug interactions with alcohol. Drugs. 1979; 18(4): 299-311. [PMID: 387374] https://doi.org/10.2165/00003495-197918040-00003
19. Traynor MJ, Brown MB, Pannala A, Beck P, Martin GP. Influence of alcohol on the release of tramadol from 24-h controlled release formulations during in vitro dissolution experiments. Drug Dev Ind Pharm. 2008; 34(8): 885-889. [PMID: 18618305] https://doi.org/10.1080/03639040801929240
20. Owumi SE, Dim UJ. Biochemical alterations in diclofenac-treated rats: Effect of selenium on oxidative stress, inflammation, and haematological Changes. Toxicol Res Appl. 2019; 3: 1-10. https://doi.org/10.1177/23978473198
21. Bao L, Zhang Z, Dai X, Ding Y, Jiang Y, Li Y. Effects of grape seed proanthocyanidin extract on renal injury in type 2 diabetic rats. Mol Med Rep. 2015; 11(1): 645-652. [PMID: 25351255] https://doi.org/10.3892/mmr.2014.2768
22. Ding Y, Li Y, Liu D, Zhang L, Wang T, Liu T, *et al*. Protective effects of Grape seed proanthocyanidins on the kidneys of Diabetic rats through the Nrf2 signalling pathway. Evidence-Based Complementary Altern Med. 2020; 20: 1-9. https://doi.org/10.1155/2020/5205903
23. Nouri A, Izak-Shirian F, Fanaei V, Dastan M, Abolfathi M, Moradi A, *et al*. Carvacrol exerts nephroprotective effect in rat model of diclofenac-induced renal injury through regulation of oxidative stress and suppression of inflammatory response. Heliyon. 2021; 7(e08358). [PMID: 34816045] https://doi.org/10.1016/j.heliyon.2021
24. Abiola TS, Adebayo OC, Babalola OO. Diclofenac-induced kidney damage in wistar rats: Involvement of antioxidant mechanisms. J Biosci Med. 2019; 7: 44-57. https://doi.org/10.4236/jbm.2019.712005
25. Tremellen K. Oxidative stress and male infertility - A clinical perspective. Human Reproductive Update. 2008; 14: 243-258. [PMID: 18281241] https://doi.org/10.1093/humupd/dmm004