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Article

Effect of Orijin Bitters, Aqueous Extracts of *Hibiscus Sabdariffa* and *Zingiber Officinale Roscoe* on the Kidney of Adult Wistar Rats

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ABSTRACT

Orijin bitters, *Hibiscus sabdariffa* and *Zingiber officinale roscoe* have been reported to possess various medicinal properties. The aim of the study is to evaluate their effects on the kidney of adult wistar rats. Methodology**:** thirty rats were randomly divided into 6 groups, each having 5 rats. Group 1 received distilled water, group 2, 70cl/70kg/bw orijin bitters, group 3, orijin bitters and 200mg/kg/bw *Hibiscus sabdariffa* calyx aqueous extract, group 4, orijin bitters and 500mg/kg/bw *Hibiscus sabdariffa* calyx aqueous extract, group 5, orijin

bitters and 200mg/kg/bw *officinale* rhizome aqueous extract, group 6, orijin bitters and 500mg/kg/bw *Zingiber officinale* rhizome aqueous extract over a 21-day period. After administration, the rats were sacrificed and the kidney harvested for biochemical and histological analysis. Results: Orijin bitters altered renal function, indicated by elevated level of malondialdehyde and decreased level of superoxide dismutase, catalase and glutathione, along with histopathological changes such as glomerular atrophy and tubular degeneration compared to the control. The result was not statistically significant ($P < 0.05$). Conversely, aqueous extracts of *Hibiscus sabdariffa* calyx and *Zingiber officinale* rhizome exhibited antioxidant properties, reducing oxidative stress and mitigating renal damage compared to the orijin bitters group only. Although the result was not statistically significant ($P < 0.05$). Conclusion: Orijin bitters may have nephrotoxic effects, while *Hibiscus sabdariffa* and *Zingiber officinale* may offer protective benefits, due to their antioxidant properties.

Keywords: Orijin bitters, *Hibiscus sabdariffa*, *Zingiber officinale Roscoe*, Antioxidant and Histology

1. INTRODUCTION

The kidneys are bean like shaped organs, having a medial concavity, a lateral convexity, and situated retroperitoneal on the posterior abdominal wall (Soriano *et al.,* 2023). The kidney functions in the removal of metabolic waste from the blood and regulation of water and electrolyte balance in the body (Adjene *et al.*, 2013). The kidney's integrity and cellular membrane function can be impaired, when there is excessive production of reactive oxygen species (Agarwal *et al.*, 2008; Chukwuebuka *et al.*, 2020).

Diseases of the central nervous system, liver, kidney, lipid metabolism and cardiovascular are some adverse effects related alcohol consumption (Ruffle, 2014). Orijin bitters is a type of alcoholic bitters. Alcohol bitters are traditionally prepared alcoholic flavoured drinks with botanical herb infusion that is characterized by a bitter, sour, or bittersweet flavour (Johnson *et al.*, 2021). In many developing countries including Nigeria many local populaces depend solely on herbal-based alcoholic beverages as a source of medication (Odey *et al.*, 2019). Their chronic consumption of locally produced alcoholic beverages pose a potential downstream physiologically and biochemically because, while deriving pleasure and recreation there is no consideration of the potential harmful effects of chronic intake of these beverages (Odey *et al.*, 2019). Alcoholic bitters are considered as a multipurpose medicine and their usage is without consideration of their effects on organs e.g. the kidneys (Waribo *et al.*, 2021). Kidney impairment is linked to the intake of alcoholic bitters as an important contributory factor with many people, young people especially using herbal remedies for the treatment of a wide range of diseases because of the claims of their efficacies by the manufacturers (Nwachuku & Elekima, 2018; Lee *et al.*, 2021; Yuan *et al.*, 2021). Some of these alcoholic beverages, fortified with diverse kinds of herbs and plant products are alomo bitters, action bitters, orijin bitters, 1960 bitters and local gin with claims suggesting their nutritional and medical importance as they are assumed to ameliorate several ailments like waist pain, menstrual cramps, cardiovascular disorders, digestive difficulties, malaria and males infertility linked to production of spermatocytes, and causes blood purification by the kidneys, regulate blood pressure through arterial dilatation, prevent kidney stones formation, cleanse the colon of impurities and possess antitumor, antiinflammatory, antibiotic and antifungal properties (Hoffmann, 2000; Mcdonald, 2014; Odey *et al.*, 2019; Johnson *et al.*, 2021). Some content of these bitters include orange peel, quinine and cassia (Ould *et al.*, 2017).

Roselle (*Hibiscus sabdariffa*) of the Malvaceae family is a medicinal plant used in Ayurveda, Siddha and Unani systems of medicine (Danapur *et al.*, 2009). *H. sabdariffa* dried or fresh, the calyces, seeds and leaves of are either eaten raw, as herbal medicines, beverages and fermented drinks (Da-Costa-Rocha *et al.*, 2014). The traditional usage of *H. sabdariffa* includes for diuretic, cholerectic, febrifugal, antihypertensive purpose, treatment of cardiac and nerve diseases, respiratory and genital problems, external wounds and abscesses, kidney and liver disorders and high blood pressure (Leung & Foster, 1996; Wilson *et al.*, 1996; Neuwinger *et al.*, 2002; Pegu *et al.*, 2021). Roselle calyx, seeds and leaves depending on the variety and geographical area, are rich in minerals, amino acids, organic acids, carotene, vitamin C and total sugar as well as in bioactive compounds such as anthocyanin and other flavonoids, organic acid, polysaccharides, triterpenoids, steroids and alkaloids. (Muller & Franz, 1992; Pegu *et al.*, 2021). These compounds are responsible for its antioxidant, antibacterial, anti-inflammatory, hepatoprotective and anticholesterol activities (Formagio *et al.*, 2015; Islam, 2019; Da-Costa-Rocha *et al.*, 2014). The calyces contain flavonoids such as gossypetine, hibiscetine and sabdaretine, a high amount of iron and are rich in organic acids (citric acid, malic acid, tartaric acid and hibiscus protocatechuic acid) (Khafaga *et al.*, 1980; Tseng *et al.*, 1996; Margesi *et al.*, 2013)

Ginger (*Zingiber officinale* Roscoe) belongs to the Zingiberaceae family (White, 2007). The rhizome is widely used as a spice in Chinese and ayurvedic medicine for centuries (Grzanna *et al.*, 2005) and herbal component in traditional medicine because it has been found to possess bioactive compounds such as phenolic compounds, flavonoid compounds, and essential oils which are responsible for pharmacological activities as well as other compounds such as polysaccharides, amino acids, organic acids, and minerals (Motawi *et al.*, 2011; Yeh *et al.*, 2014; Prasad & Tyagi, 2015; Liu *et al.*, 2019; Styawan *et al.*, 2022). The major phenolic compound in the ginger rhizome, gingerol, consists of shogaol, paradol, zingerol, gingerones, and gingerdiones (Ali *et al.*, 2008; Srinivasan, 2017; Arablou & Aryaeian, 2018; Styawan *et al.*, 2022). The biological activities of ginger provide health advantages such as antioxidant, antiinflammation, antibacterial, antiviral, antifungal, antihyperlipidemic, antiobesity, and hepatoprotective activities marking it suitable for food and beverages (Ali *et al.*, 2008; Srinivasan, 2017; Arablou & Aryaeian, 2018; Pourmasoumi *et al.*, 2018; Hasani *et al.*, 2019; Mahboubi, 2019; Mao *et al.*, 2019; Hajimoosayi *et al.*, 2020; Styawan *et al.*, 2022). The antioxidant properties of medicinal herbs are dependent on many factors during planting and harvesting, its environmental conditions, weather, seasonal changes, geographical area, degree of ripe and growth (Škrovánková *et al.*, 2012). Traditionally, ginger is employed in treatment to help digestion, treat colic, diarrhea, nausea, bleeding disorders, rheumatism, baldness, toothache, snakebite, and respiratory conditions (Bhatt *et al.*, 2013; Sharifi-Rad *et al.*, 2017).

The aim of the study is to evaluate the effect of orijin bitters, aqueous extracts of *Hibiscus sabdariffa* calyx and *Zingiber officinale* roscoe rhizome on the kidney of adult male wistar rats.

2. MATERIALS AND METHODS

Plant Procurement and Extraction

Roselle (*Hibiscus sabdariffa*) calyces and Ginger (*Zingiber officinale Roscoe*) rhizome were purchased from a local market, in Masaka, Nasarawa State, Nigeria. Extraction were carried out at the Chemistry Department, Bingham University, Karu, Nasarawa state, Nigeria. Aqueous extracts of Roselle (*Hibiscus sabdariffa*) calyces and Ginger (*Zingiber officinale Roscoe*) rhizome were obtained by the maceration procedure. The process of maceration softens and break the plants so as to release its phytochemicals. Roselle (*Hibiscus sabdariffa*) calyces and Ginger (*Zingiber officinale Roscoe*) rhizome were washed and air

dried to remove debris. After which they were soaked in distilled water for three (3) days stirring 3 times at an interval of 6 hours. After 3 days, the extracts were filtered using a sieve, the filtrate was dehydrated in an oven and stored under room temperature.

Ethical clearance

All protocols and treatment procedures were in accordance to the Animal Care and Use Committee guidelines (National Institute of health, 2011) and as approved by the Faculty of Basic Medical Sciences Ethics Review Committee Bingham University, Karu, Nasarawa State, Nigeria. The approval number is BHUAUC/2024/012.

Experimental design

Thirty (30) adult male wistar rats (80-170g) was obtained from the animal house of the Faculty of Basic Medical Sciences, Bingham University, Karu, Nasarawa State. They were housed in well ventilated cages at room temperature in a hygienic condition under 12 hours' daylight cycle. They were maintained on a regular common rat feed and water *ad libitum*. After two (2) weeks of acclimatization, they were randomly chosen and grouped into six (6), five (5) rats in each. The experiment lasted for 21 days. The animals were fasted overnight and sacrificed after exposure to chloroform. The kidneys were excised and were fixed in 10% formalin and phosphate buffer for histological and biochemical analysis respectively. Table 1 shows the experimental design.

Table 1: Grouping and administration dosage

Groups	Treatment and daily dose
I (Control)	70cl/75kg/bw Distilled water
II	70cl/75kg/bw Orijin bitters
III	70cl/75kg/bw Orijin bitters + 200mg/kg/bw aqueous extract of (*Hibiscus sabdariffa*) calyces
IV	70cl/75kg/bw Orijin bitters + 500mg/kg/bw aqueous extract of (*Hibiscus sabdariffa*) calyces
V	70cl/75kg/bw Orijin bitters + 200mg/kg/bw aqueous extract of (*Zingiber officinale roscoe*) rhizome
VI	70cl/75kg/bw Orijin bitters + 500mg/kg/bw aqueous extract of (*Zingiber officinale roscoe*) rhizome

The administration was done orally via gavage and once daily. The administration of Orijin bitters was adopted from Johnson *et al.*, 2021.

Biochemical analysis

Animals were sacrificed and the kidneys were harvested into an organ bottle with phosphate buffer. The tissues were homogenized and centrifuged at 3000 rpm for 10 min. The supernatant obtained was utilized for the estimation of the activities of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH). Nwogueze *et al.* (2021) method was adopted.

Histological Analysis

Histological examination of the kidneys was conducted following specific procedures. The dissected kidney portions were first placed in 10% formalin within organ bottles to prevent tissue autolysis. Subsequently, the tissues were dehydrated using an automated tissue processor, progressing through 70% graded alcohol for initial dehydration and 90% alcohol for complete dehydration. They were then cleared in xylene, impregnated with paraffin wax and embedded. Thin sections, approximately five microns thick, were prepared using a rotatory microtome and mounted onto albumenized slides, left to dry on a hot plate. The slides were then dewaxed sequentially using xylene, absolute alcohol,

70% alcohol and 50% alcohol, followed by a water rinse. Finally, the sections were stained using Hematoxylin and Eosin and Periodic Acid Schiff techniques. After which the slides were observed under a microscope at 400x magnification. (Obasi & Ogugua, 2020).

Statistical Analysis

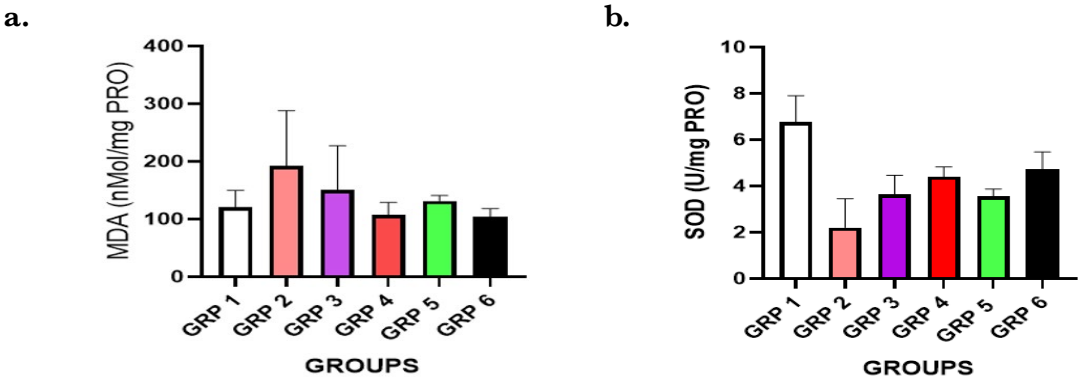
The data obtained were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to compare the result using SPSS (Statistical Package for the Social Sciences) and followed by Tukey post-hoc test using Graph pad prism. $P < 0.05$ was considered statistically significant.

3. RESULTS

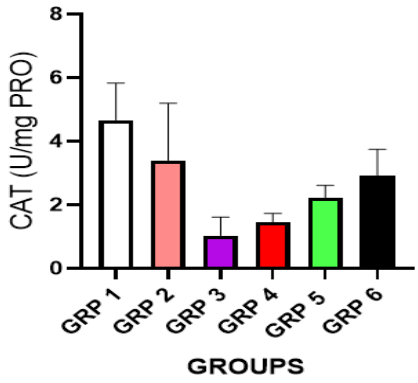
Biochemical Analysis

Following the administration of orijin bitters, aqueous extracts of *Hibiscus sabdariffa* and *Zingiber officinale roscoe*, the activities of antioxidant enzymes, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) of the kidney of adult Wistar rats were investigated (Figure 1). MDA activity was highest in group II, followed by III and V (Fig. 1a). There was increase in SOD activity in group IV and VI higher than group II but lower than group I (Fig. 1b). CAT was highest in group I, followed by group II and VI (Fig. 1c). GSH activity was highest in group 1, followed by group 6 (Fig. 1d). All the results showed no statistical significance ($P < 0.05$).

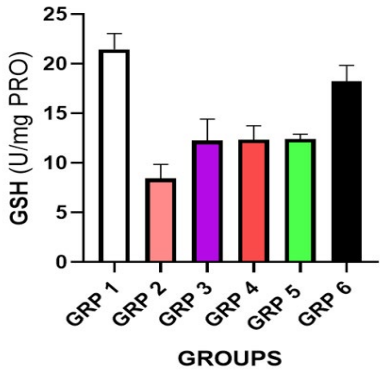
Figure 1: Biochemical activities of Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione (GSH) after administration of orijin bitters, aqueous extracts of *Hibiscus sabdariffa* and *Zingiber officinale* roscoe on the kidney of adult wistar rats



c.



d.



GRP 1 = control (distilled water), GRP 2 = 70cl/75kg/bw orijin bitters, GRP 3 = 70cl/75kg/bw orijin bitters and 200mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces, GRP4 =70cl/75kg/bw orijin bitters and 500mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces, GRP 5 = 70cl/75kg/bw and 200mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome, GRP 6 = 70cl/75kg/bw origin bitters and 500mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome. Result was presented in Mean ± SEM. $P < 0.05$ was considered statistically significant.

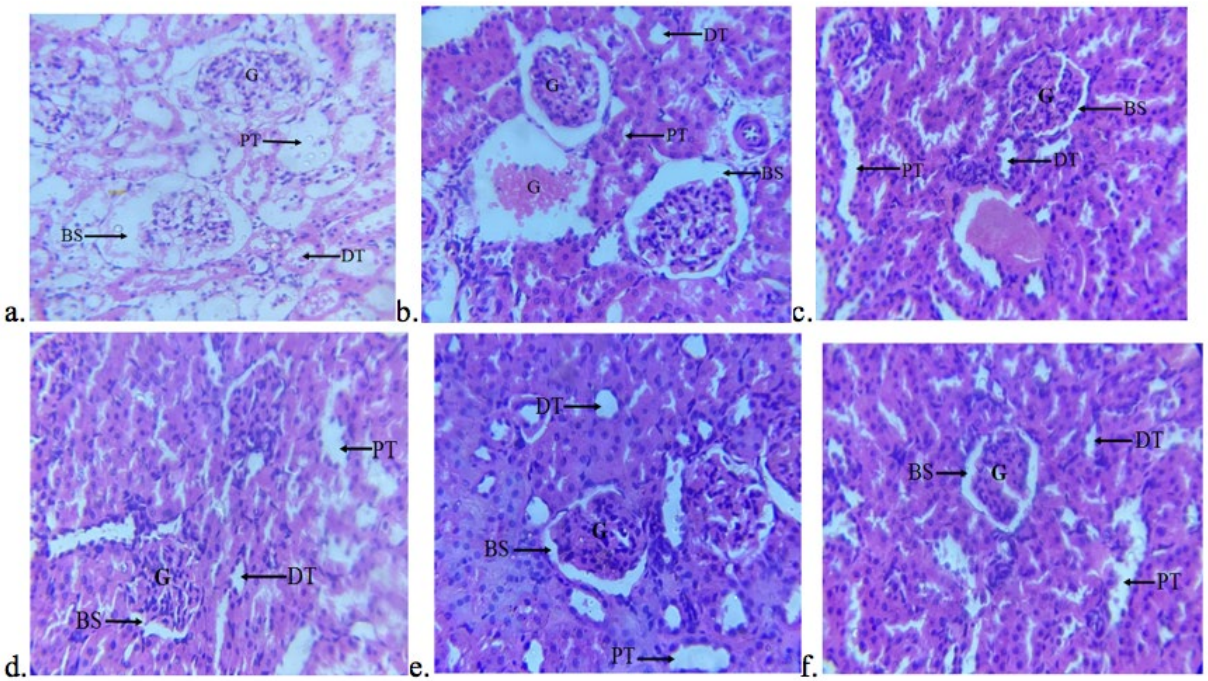
Histological Analysis

The histological analysis of the kidney was observed after staining with Haematoxylin and Eosin (H & E) and Periodic Acid Schiff (PAS) stains. Photomicrograph are presented in (Fig. 2 and 3).

Haematoxylin and Eosin (H & E) stain

Histological profile of rat kidneys for group I which received distilled water showed normal histoarchitecture (Fig. 2a). Photomicrograph for group II (Fig. 2b) which were administered 70cl/75kg/bw Orijin bitters only, showed glomerular atrophy. Group III and IV, administered and 200mg/kg/bw and 500mg/kg/bw aqueous extract aqueous extract of *Hibiscus sabdariffa* calyces together with 70cl/75kg/bw Orijin bitters, showed glomerular atrophy, narrowing of the bowman's space, alteration in the histoarchitecture of the proximal tubule and distal tubule (Fig. 2c and 2d respectively). The glomerulus appeared shrunken in group IV (Fig. 2d). The photomicrograph for group V (Fig. 2e) and VI (Fig 2f), which received 200mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome and 500mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome together with 70cl/75kg/bw Orijin bitters respectively, showed restoration of the histoarchitecture compared to groups II, III and IV.

Figure 2: Photomicrographs of Haematoxylin and Eosin (H & E) stain after administration of orijin bitters, aqueous extracts of *Hibiscus sabdariffa* and *Zingiber officinale* roscoe on the kidney of adult wistar rats.



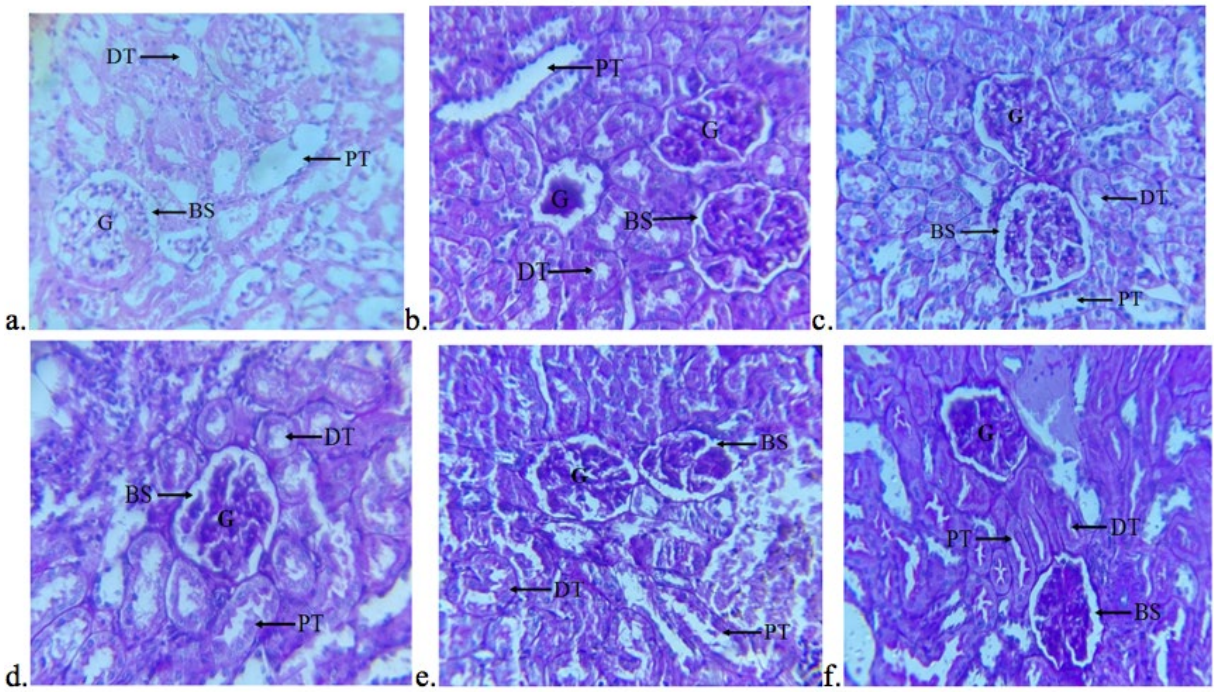
(a) Control group showed normal bowman's capsule with glomerulus, bowman space, proximal tubule and distal tubule. (b) rat kidney administered 70cl/75kg/bw orijin bitters only showed presence of glomerular atrophy. (c) rat kidney administered 70cl/75kg/bw orijin bitters and 200mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces showed presence of glomerular atrophy, narrowing of the bowman's space, alteration in the histoarchitecture of the proximal tubule and distal tubule. (d) rat kidney administered 70cl/75kg/bw orijin bitters and 500mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces showed presence of alteration in the histoarchitecture, glomerulus appeared shrunken. (e) and (f) rat kidney administered and 200mg/kg/bw and 500mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome together with 70cl/75kg/bw orijin bitters respectively showed restoration of the histoarchitecture compared to groups (a), (c) and (d). Proximal tubule, BS= Bowman capsule, DT = Distal tubule (H&E, x400).

Periodic Acid Schiff (PAS) stain

The histological profile of rat kidney for group I, which was administered distilled water showed negative PAS intensity in the bowman's capsule, proximal and distal tubules (Fig. 3a). Glycogen deposition on the kidney tissue was absent. Group II, which was administered 70cl/75kg/bw orijin bitters showed a high PAS intensity in the capsular membrane of the bowman's capsule and the basement membrane of the proximal and distal tubules with increased glycogen deposition on the kidney tissue (Fig. 2b) which indicates tissue damage. Group III, which was administered 70cl/75kg/bw orijin bitters and 200mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces showed a faint PAS intensity in the glomerulus and reduction in glycogen deposition on the kidney tissue (Fig. 3c) indicating tissue regeneration. Group IV, which was administered 70cl/75kg/bw orijin bitters and 500mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces exhibited a slight reduction in PAS intensity in the

capsular membrane of the bowman's capsule with the glomerulus and in the basement membrane of the proximal and distal tubules (Fig. 3d) when compared to group II (Fig. 3b). Slightly reduced glycogen deposits on the kidney tissue was also observed, indicating tissue regeneration. Group V, which was administered 70cl/75kg/bw orijin bitters and 200mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome showed alterations in the bowman's capsule, renal tubules and exhibited a high PAS intensity in the capsular membrane of the bowman's capsule (Fig. 3e). There was increased glycogen deposition on the kidney tissue which indicating tissue damage. Group 6, which was administered 70cl/75kg/bw orijin bitters and 500mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome exhibited a slight reduction in PAS intensity in the capsular membrane of the bowman's capsule with the glomerulus (Fig. 3f). There was reduced glycogen deposits on the kidney tissue indicating tissue regeneration.

Figure 3: Photomicrographs of Periodic Acid Schiff (PAS) stain stain after administration of orijin bitters, aqueous extracts of *Hibiscus sabdariffa* and *Zingiber officinale* roscoe on the kidney of adult wistar rats



(a) Control group kidney administered distilled water showed negative PAS intensity in the bowman's capsule and in the proximal and distal tubules indicating absence of glycogen deposits on the kidney tissue. (b) rat kidney administered 70cl/75kg/bw orijin bitters showed high PAS intensity in the capsular membrane of the bowman's capsule and the basement membrane of the proximal and distal tubules indicating increased glycogen deposits on the kidney tissue which indicate tissue damage. (c) rat kidney administered 70cl/75kg/bw orijin bitters and 200mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces showed faint PAS intensity in the glomerulus indicating reduction in glycogen deposits on the kidney tissue which indicate tissue regeneration. (d) rat kidney administered 70cl/75kg/bw orijin bitters and 500mg/kg/bw aqueous extract of *Hibiscus sabdariffa* calyces showed slight reduction in PAS intensity in the capsular membrane of the bowman's capsule with the glomerulus and in the basement membrane of the proximal and distal tubules when compared to (b)

indicating slightly reduced glycogen deposits on the kidney tissue which indicate tissue regeneration. (e) rat kidney administered 70cl/75kg/bw orijin bitters and 200mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome showed alteration in the bowman's capsule, renal tubules and a high PAS intensity in the capsular membrane of the bowman's capsule indicating increased glycogen deposits on the kidney tissue which indicate tissue damage. (f) rat kidney administered 70cl/75kg/bw orijin bitters and 500mg/kg/bw aqueous extract of *Zingiber officinale roscoe* rhizome showed slight reduction in PAS intensity in the capsular membrane of the bowman's capsule containing and the glomerulus indicating reduced glycogen deposits on the kidney tissue indicate tissue regeneration. G= Glomerulus, BS= Bowman's space, DT= Distal tubule, PT= Proximal tubule (PAS x400).

4. DISCUSSION

The present study was carried out to evaluate the effect of Orijin bitters, aqueous extracts of *Hibiscus sabdariffa* and *Zingiber officinale* roscoe on the kidneys of adult wistar rats. Numerous natural products such as fruits, vegetables, cereal grains, edible flowers, medicinal plants, and herbal infusions have been identified to possess antioxidant potential, ginger (*Zingiber officinale roscoe*) rhizome and roselle (*Hibiscus sabdariffa*) calyces inclusive (Darekar *et al.*, 2023). Natural antioxidants from plants hold great promise than synthetic antioxidants more so, they are less expensive alternatives (Anwar *et al.*, 2018).

The onset of many chronic illnesses have been associated to the overproduction of reactive oxygen species (ROS) (Darekar *et al.*, 2023) leading to oxidative stress. Oxidative stress is an imbalance occurring in an organism between free radicals produced and the levels of antioxidative systems such that the imbalance favours oxidative radicals (Sarandol *et al.*, 2007). Oxidative stress is linked to increased cellular damage (Ishii *et al.*, 2014; Nwogueze *et al.*, 2021). The antioxidant activity of a compound is its protective property to inhibit oxidative mechanisms by scavenging and neutralizing reactive oxygen and free radicals in humans, rodents and other species (Tremellen, 2008; Pegu *et al.*, 2021). Many studies have found that ginger has a high level of antioxidant activity (Ji *et al.*, 2017). Ginger's antioxidant activity is mainly linked with its polyphenolic contents, which play a role in eliminating free radicals (Lobo *et al.*, 2010; Indiarto *et al.*, 2019; Darekar *et al.*, 2023). The calyces of *H. sabdariffa* have been studied repeatedly whether in *vitro* and in *vivo* and have been shown to have positive health effects as well as contain potent antioxidants such as polyphenolic acid, flavonoids and anthocyanins (Da-Costa-Rocha *et al.*, 2014; Pegu *et al.*, 2021). Because the phenolic contents are soluble in polar solvents; the use of water is important in the extraction of anthocyanin (Khoo *et al.*, 2017). Hence the use of aqueous extract of both (*Zingiber officinale roscoe*) rhizome and roselle (*Hibiscus sabdariffa*) calyces for the study.

This study evaluated the effects of orijin bitters, aqueous extracts of *Hibiscus sabdariffa* and *Zingiber officinale* roscoe on malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels. There was increase in MDA level and decrease in SOD, CAT and GSH by orijin bitters compared to the control suggesting the potential of this alcohol bitter to result in kidney damage. Elevation of MDA levels suggests enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanism to prevent excessive free radicals (Himakar *et al.*, 2010). Uncontrolled lipid peroxidation causes disruption of membrane, lipids and cellular organelles damage and oxidative stress (Mahboob *et al.*, 2005; Rao *et al.*, 2011). The increase in kidney MDA level may be as a result of the alcohol content and possibly that the herbal content may not be sufficient to ameliorate its insult. Alcohol is known to result in tissue damage (Befrits *et al.*, 1995; Shanmugam *et al.*, 2020). Decreased GSH level in the kidney tissue is linked to lower peroxidase activity, alteration of kidney membrane integrity and hepatotoxicity (Nwogueze *et al.*, 2021).

The treatment group of both low and high doses of the aqueous extracts of ginger (*Zingiber officinale roscoe*) rhizome and roselle (*Hibiscus sabdariffa*) calyces showed positive response for MDA, SOD and GSH when compared to orijin bitters alone. Although the result was not statistically significant, this suggests the potency of both plants ability to reduce the activity of reactive oxygen species induced by orijin bitters due to their antioxidant properties (Ji *et al.*, 2017; Indiarto *et al.*, 2021; Darekar *et al.*, 2023). *H. sabdariffa* extracts have been shown to possess antioxidant activity on lipid peroxidation with the calyces having higher effect than the leaf (Ochani & D'Mello, 2009; Suhaili & Manshoor, 2022). According to Geng *et al.*, 2012, ginger extract therapeutic effect is shown in the increase in DNA repair, increase in antioxidants level, reduction of lipid peroxidase, and decrease in DNA damage in a bid to maintain the immune system of the body. Also, ginger improvement of renal function and structure by reducing lipid accumulation in kidney tissues has been shown by Xu *et al.* (2018) and Ramudu *et al.* (2011). SOD is an important antioxidant that protects the cell against oxidative damages by scavenging superoxide anion from hydrogen peroxide leading to the reduction in the toxic effects and maintaining integrity of cell membranes (Brock, 2007; Khan *et al.*, 2012; Bhatt *et al.*, 2013). Shanmugam *et al.* (2020) reported increased levels of SOD and GSH that is linked to the presence of bioactive compounds in ginger when albino rats were administered ethanolic extract of ginger alone or together with alcohol supporting the idea that ginger is protective against kidney tissue damage induced by orijin bitters. Catalase is a key component of the antioxidant defense system whose protective mechanism inhibition, enhances the sensitivity to free radical induced cellular damage by breaking down potentially harmful hydrogen peroxide in the cells to glutathione peroxidase (Young *et al.*, 2001; Bhatt *et al.*, 2013).

Histological analysis showed normal histoarchitecture for the control group for both haematoxylin and eosin (H&E), and periodic acid schiff stain (PAS). There was distortion in the histoarchitecture and high PAS intensity following the administration of orijin bitters indicating tissue damage. Alcohol treated rats induced damage to the glomeruli and renal tubules while ginger restored the damage (Shanmugam *et al.*, 2020). Oforibika & Uzor, (2020) reported minimal negative side effects on kidney of albino rats administered some herbal bitters (confam, G. winco and 1960 roots) when used at moderate dose concentrations between 10 and 21 days. The result of this study for orijin bitters however showed no corroboration. Although, low and high doses of aqueous extract of *Hibiscus Sabdariffa* calyces together with orijin bitters showed damage to the tissue for H&E, the reduced intensity of PAS stain for both groups compared to orijin bitters alone indicated tissue regeneration. Low and high dose of aqueous extract of *Zingiber officinale roscoe* rhizome together with orijin bitters resulted in tissue regeneration especially in the high dose. Studies have shown the beneficial effects of ginger on the histomorphology of the kidney with improvement in repairs of kidney damage and restoration of membrane integrity in renal tissue and structural derangement (Rehman *et al.*, 2019), cell apoptosis (Hajhosieni *et al.* 2014), and bleeding in the cortical area of the kidney (Khaki *et al.*, 2010).

5. CONCLUSION

The study concludes that Orijin bitters may have a toxic effect on the kidney as observed in the decrease of antioxidant enzymes and damage to the histoarchitecture of the kidney. Aqueous extracts of *Hibiscus sabdariffa* and *Zingiber officinale* roscoe possess the ability to eliminate the effect of Orijin bitters due to the phytochemicals and antioxidants that are found in them.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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RESUMEN

Se ha informado que Orijin bitters, Hibiscus sabdariffa y Zingiber officinale Roscoe poseen varias propiedades medicinales. El objetivo del estudio es evaluar sus efectos sobre el riñón de ratas adultas Wistar. Metodología: Treinta ratas fueron divididas aleatoriamente en 6 grupos, cada uno con 5 ratas. El grupo 1 recibió agua destilada, el grupo 2, 70 cl/70 kg/pc de Orijin bitters, el grupo 3, Orijin bitters y 200 mg/kg/pc de extracto acuoso de cáliz de Hibiscus sabdariffa, el grupo 4, Orijin bitters y 500 mg/kg/pc de extracto acuoso de cáliz de Hibiscus sabdariffa, el grupo 5, Orijin bitters y 200 mg/kg/pc de extracto acuoso del rizoma de Zingiber officinale, el grupo 6, Orijin bitters y 500 mg/kg/pc de extracto acuoso del rizoma de Zingiber officinale durante un período de 21 días. Después de la administración, las ratas fueron sacrificadas y se extrajeron los riñones para análisis bioquímico e histológico. Resultados: Orijin bitters alteró la función renal, lo que se indica por un aumento en el nivel de malondialdehído y una disminución en el nivel de superóxido dismutasa, catalasa y glutatión, junto con cambios histopatológicos como atrofia glomerular y degeneración tubular en comparación con el grupo de control. El resultado no fue estadísticamente significativo ($P < 0.05$). Por el contrario, los extractos acuosos de cáliz de Hibiscus sabdariffa y rizoma de Zingiber officinale exhibieron propiedades antioxidantes, reduciendo el estrés oxidativo y mitigando el daño renal en comparación con el grupo que solo recibió Orijin bitters. Aunque el resultado no fue estadísticamente significativo ($P < 0.05$). Conclusión: Orijin bitters puede tener efectos nefrotóxicos, mientras que Hibiscus sabdariffa y Zingiber officinale pueden ofrecer beneficios protectores debido a sus propiedades antioxidantes.

Palabras clave: Orijin bitters, Hibiscus sabdariffa, Zingiber officinale Roscoe, Antioxidante e Histología.