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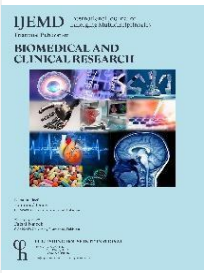

Effect of Orijin Bitters, Aqueous Extracts of Hibiscus Sabdariffa Calyx and Zingiber Officinale Roscoe Rhizome on the Liver of adult wistar rats

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ABSTRACT

BACKGROUND AND AIM: Orijin bitters, *Hibiscus sabdariffa* and *Zingiber officinale roscoe* are consumed for medicinal properties and their effects were evaluated in this study on adult wistar rats' liver.

METHODOLOGY: Thirty rats were divided into 6 groups, 5 in each. 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. After administration, the rats were sacrificed and the liver harvested for biochemical and histological analysis.

RESULTS: Aspartate aminotransferase and alkaline phosphate increased in group 2 while alanine transaminase decreased. Aspartate aminotransferase and alanine transaminase increased in most treatment groups while alkaline phosphate decreased. $P < 0.05$ was considered statistically significant. Orijin bitters distorted the liver tissue while *Hibiscus sabdariffa* and *Zingiber officinale roscoe* extracts resulted in restoration.

CONCLUSION: Orijin bitters may possess toxicity to the liver. *Hibiscus sabdariffa* calyx and *Zingiber officinale roscoe* rhizome due to antioxidants present ameliorated the effect.

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

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INTRODUCTION

The liver is the largest vital organ of the human body (about 2-3% of the total body weight), responsible for metabolism, secretion and storage as well as detoxification of drugs and xenobiotics [1,2]. The liver can be exposed to liver dysfunction, liver injury or liver damage resulting from an overload of drugs, alcohol or xenobiotics [3]. The liver is particularly susceptible to alcohol-related injury because it is the primary site of alcohol oxidation and metabolism leading to generation of potentially dangerous by-products and highly reactive molecules which contributes to alcohol-induced liver damage [4,5].

Bitters are an alcoholic preparation flavoured with botanical herb and made up of numerous groups of chemical compounds from the extracted herbs and roots, having bitter, sour or bittersweet flavour, usually dark in colour and valued for their ability to promote appetite and digestion, used as patent medicine, digestion aids and as flavouring in cocktails [6-8]. Many well-known bitter brands were once created as patent medications, but they are currently offered as digestifs, occasionally with herbal qualities, and as flavouring for cocktails [8]. Cinchona bark, gentian, orange peel, cascarilla, and cassia are a few of the most popular ingredients with majority of bitters containing both water and alcohol [8]. Alcohol is a major constituent of bitters and has been proven capable of causing disease conditions, based on different factors such as malnutrition, contaminants of viral infectious of the liver, gastric predisposition [8]. The alcoholic beverages made up of grain ethanol which is considered as a toxin when consumed excessively and can result in liver cirrhosis [9]. Brands and kinds of bitters differ greatly in their degree of alcohol content [10]. The alcohol content of Orijin bitters is 30%.

Despite the initial fears over the hygiene level of their product and their composition, some alcoholic beverages such as Alomo bitters, Action bitters, Orijin bitters, Pasa bitters, 1960 bitters, Osomo bitters still make an impact in the alcoholic beverages market [11, 12] after being fortified with different kinds of herbs and plant products even though they have unclear product descriptions, no scientific screening, and unidentified active ingredients [13]. There is claim and/or believe that alcohol bitters; are body purifiers, enhance sex energy, increase production of spermatocytes in males and improve sexual function, ensure proper fats and oils digestion and functioning of the excretory functions of the liver, reduce fat (triglycerides) and cholesterol levels, enhance haemorrhoids healing, enhance blood circulation, purification of blood by the kidneys, blood pressure regulation, prevent kidney stones formation, cleanse impurities in colon, possess aphrodisiac, antitumor, antidiabetic, antimalaria, hypolipidemic, anti-inflammatory, antibiotic and antifungal properties, and alleviate waist pain, menstrual cramps, cardiovascular disorders, digestive difficulties [8, 13-15].

Hibiscus sabdariffa also referred to as Roselle (English), Rosella (Indonesia), Karkade (Egypt, Arab, and Sudan), Asam paya (Malaysia), and Zobo (Nigeria), is a member of the Malvaceae family [5, 16-17]. It is found in tropical and subtropical areas, including Sudan, Jordan, India and Africa [18]. *Hibiscus sabdariffa* contains anthocyanin, flavonoids, phenol derivatives, organic acids, polysaccharides, triterpenoids, steroids and alkaloids, which it contains, are responsible for its antioxidant, antibacterial, anti-inflammatory, hepatoprotective and cholesterol-lowering properties [19-22]. The calyx is high in citric acid and pectin. Minerals, amino acids, organic acids, carotene, vitamin C, and total sugar in its calyx, seeds, and leaves have varying levels depending on variety and geographical area [23]. Some of the medicinal applications of *Hibiscus sabdariffa* includes; treatment of hypertension, pyrexia, liver damage

and leukaemia due to its high content of protocatechuic acid [24], prevention of cancer, low blood pressure, improving the digestive system in human and effective treatment kidney stone [25]. Traditionally the leaves, calyces, roots or seeds used for their diuretic, cholerectic, febrifugal and antihypertensive effects, to treat cardiac, nerve diseases drunkenness, respiratory problems, genital problems, liver disorders, external wounds and abscesses, to increase urination and enhance lactation in cases of poor milk production, poor letdown and maternal mortality [23, 26-28].

Ginger (*Zingiber officinale Roscoe*), a member of the Zingiberaceae family, originating from India, but is however cultivated in different countries such as Nigeria, Fiji, Taiwan and Australia [29]. It is a spice, flavouring agent, garnish, medicine, and food preservative that can be used either fresh, in a fresh paste, or dry, in a dry powder [30]. It is widely used worldwide for diverse therapeutic, medicinal, nutritional and ethnomedical properties [31]. Ginger consists of more than 60 compounds including essential oil and resin known collectively as oleoresin [32]. The essential oil composition can vary based on the geographical origin [30]. Sesquiterpene hydrocarbons are the chief constituents responsible for the characteristic aroma, gingerole is the main phenolic compound and can be degraded to shogaols, zingerone, and paradol. Zingerone and shogaols are found in small amounts in fresh ginger and in larger amounts in dried or extracted products [30]. Ginger also contains small amounts of alkaloids, tannins, carotenoids, saponins, flavonoids, steroids and cardinolides [33].

Ginger has been discovered to have biological activities such as antioxidants, anti-inflammatory, antimicrobial, antibacterial and anticancer/antitumor properties attributed to its phenolic compounds, primarily gingerols, shogaols and paradols [34-39]. Ginger is used in treating ailments such as diabetes, digestive problems like indigestion, intestinal infections, various types of food poisoning, colic, diarrhea, nausea, and vomiting related to pregnancy, travel sickness, skin burns, high blood pressure, arthritis, inflammation and pain [40-44]. The aim of the study was to evaluate the effect of orijin bitters, aqueous extracts of *Hibiscus sabdariffa* calyx and Z*ingiber officinale roscoe* rhizome on the liver of adult wistar rats.

MATERIALS AND METHODS

Plant Procurement and Extraction

Roselle (*Hibiscus sabdariffa*) calyces and Ginger (*Zingiber officinale Roscoe*) rhizome were selected and purchased from a local market, Masaka market in Nasarawa State, Nigeria. They were authenticated and extracted 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. 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 and made to stand in a room temperature for a period of three (3) days stirring 3 times at an interval of 6 hours respectively. This maceration process helped to soften and break the Roselle (*Hibiscus sabdariffa*) calyces and Ginger (*Zingiber officinale Roscoe*) rhizome so as to release its phytochemicals. After 3 days, the extracts were filtered using a sieve of about 53um. They were 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/2024/010.

Experimental design

Thirty (30) adult male wistar rats (80-205g) were purchased from and housed in well ventilated cages at room temperature in a hygienic condition under 12 hours' daylight cycle in the animal house of the Faculty of Basic Medical Sciences, Bingham University, Karu, Nasarawa State. 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 livers were excised and were fixed in 10% formalin and phosphate buffer for histological and biochemical analysis respectively. Below is the experimental design.

Table 1: Animal Grouping

Experimental Group	Administration
1 (Control)	70cl/75kg/bw distilled water only
2	70cl/75kg/bw Orijin bitters only
3	70cl/75kg/bw Orijin bitters only + 200mg/kg/bw aqueous extract of *Hibiscus sabdariffa*
4	70cl/75kg/bw Orijin bitters only + 500mg/kg/bw aqueous extract of *Hibiscus sabdariffa*
5	70cl/75kg/bw Orijin bitters only + 200mg/kg/bw aqueous extract of *Zingiber officinale Roscoe*
Group 6	70cl/75kg/bw Orijin bitters only + 500mg/kg/bw aqueous extract of *Zingiber officinale Roscoe*

All administration was done orally and once daily. Administration of Orijin bitters was adopted from [8].

Histological Analysis

The livers were fixed in 10% formalin. Following fixation, they were dehydrated using an increasing series of ethanol, cleaned with xylene, and then embedded in paraffin. Using a rotating microtome, they were cut into serial pieces that were 5 µm thick. Hematoxylin and eosin stain were used to stain the resulting sections, which were then examined under a light microscope [45, 46].

Biochemical Analysis

The harvested livers were stored in cooled phosphate buffer and then homogenized for the examination of the activity of liver enzymes; alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP). Reitman and Frankel, (1957) method was adopted to determine the activity [47].

Statistical Analysis

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.

RESULTS

Liver Function Test

The levels of Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphate (ALP) following 21 days' administration of orijin bitters, aqueous extracts of roselle (*Hibiscus sabdariffa*) calyces and ginger (*Zingiber officinale roscoe*) rhizome in adult wistar rats was measured (Table 2). Alanine transaminase was significantly ($P<0.05$) reduced in group 2 which received orijin bitters when compared to control group 1 which received distilled water. Groups 4, 5 and 6 also resulted in increase in ALT when compared to group 1. Group 3 was slightly reduced. The result was however not statistically significant ($P<0.05$). There was also increase in ALT level for all treatment groups when compared to group 2. Statistical significance ($P<0.05$) was only observed in group 4.

Aspartate aminotransferase level was increased in all treatment groups except group 4 which received orijin bitters and high dose *Hibiscus sabdariffa* calyces aqueous extract when compared to control group 1, and in all treatment groups except group 3 and group 4 when compared to control group 2. There however, was no statistical significance ($P<0.05$).

Alkaline phosphate level increased in group 2, slightly decreased in groups 3 and 4, and decreased in group 5 and 6 when compared to group 1. The differences were only statistically significant ($P<0.05$) in group 2. In all treatment groups when compared with group 2 showed decrease in ALP. However, statistical significance ($P<0.05$) was only noted in group 5 and 6.

Table 2: Result for liver function test following administration of orijin bitters, aqueous extracts of roselle (*Hibiscus sabdariffa*) calyces and ginger (*Zingiber officinale roscoe*) rhizome

GROUPS	ALT Mean ± SEM	AST Mean ± SEM	ALP Mean ± SEM
1	6.67 ± 2.91	10.67 ± 1.20	8.73 ± 0.50
2	2.67 ± 1.67[a]	15.00 ± 4.61	12.87 ± 3.25[a]
3	6.33 ± 2.33	13.00 ± 4.73	8.33 ± 3.37
4	12.33 ±1.20[b]	8.00 ± 1.00	8.37 ± 1.34
5	7.67 ± 1.20	16.33 ± 2.03	5.43 ± 0.62 [b]
6	7.00 ± 0.95	20.00 ± 4.04	5.43 ± 1.82 [b]

Aspartate Aminotransferase (AST), Alanine transaminase (ALT) and Alkaline Phosphate (ALP). ANOVA followed by Tukey post-hoc test; Result is expressed as mean ± standard error of mean. P < 0.05

[a] Means significantly different from GRP 1, [b] significantly different from GRP 2

Histological analysis

The photomicrographs obtained after histological analysis of the liver are shown in Figure 1a-f. The photomicrograph of the liver sections of the wistar rats showed normal cytoarchitecture of the liver in the control (Fig. 1a). The characteristic appearance of hepatocytes, central vein, sinusoids and the portal area is shown. The group that received orijin bitters alone (Fig. 1b) showed distortions in the cytoarchitecture of the hepatocytes, sinusoids were enlarged and blood was present in the central vein. The group administered orijin bitters and 200mg/kg/bw of aqueous extract of roselle (*Hibiscus sabdariffa*) calyx showed distortion of the cytoarchitecture however when compared to orijin bitters alone it appeared to be better indicating restoration (Fig. 1c). The recuperation was better in the group administered orijin bitters and 500mg/kg/bw of aqueous extract of roselle (*Hibiscus sabdariffa*) calyx (Fig. 1d). The groups which received 200mg/kg/bw and 500mg/kg/bw of aqueous extract of ginger (*Zingiber officinale*) rhizome) together with orijin bitters (Fig. 1e and Fig. 1f respectively) although presented with blood in the central vein, both cytoarchitecture appeared better than group that received only orijin bitters.

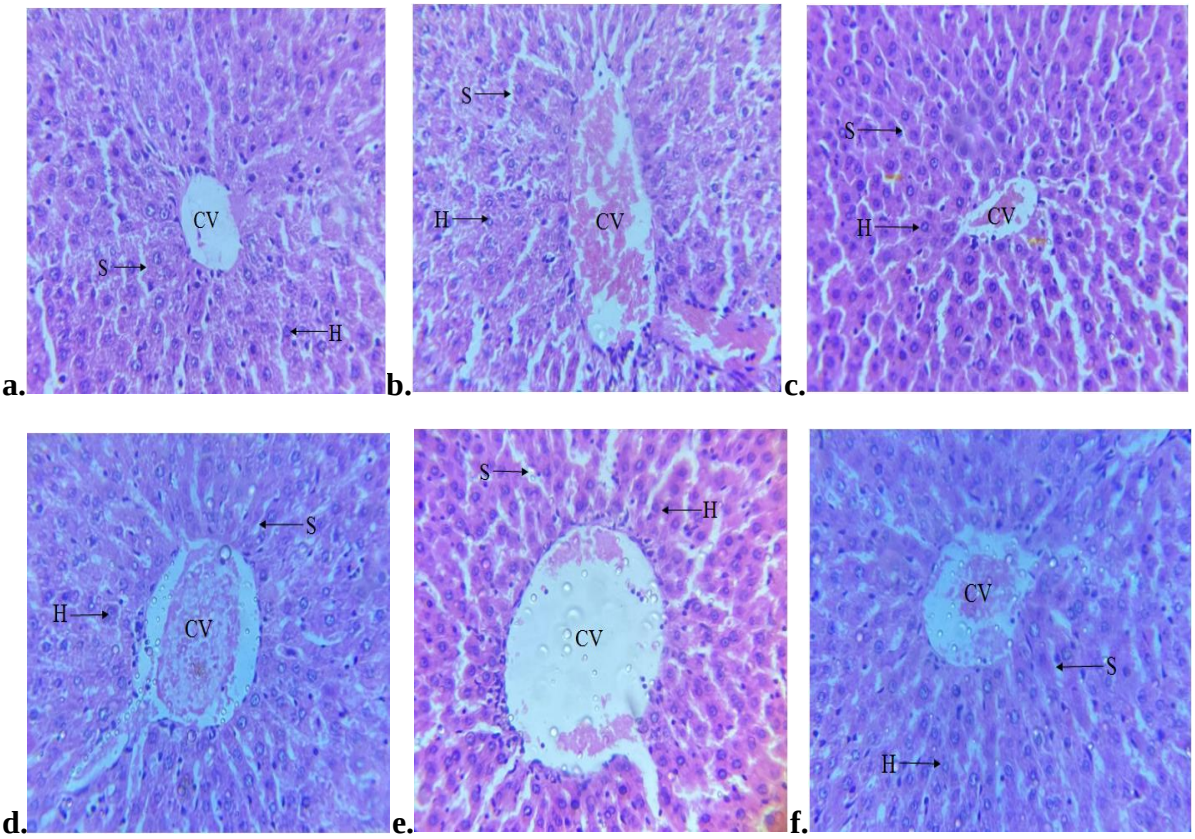

Figure 1: Photomicrograph of rat liver stained with haematoxylin and eosin stain following administration of Orijin bitters, aqueous extract of roselle (*Hibiscus sabdariffa*) calyx and aqueous extract of ginger (*Zingiber officinale*) rhizome.

a. Control group (distilled water only) showed normal radiating hepatocytes with central vein and sinusoids. b. Group 2 rat (orijin bitters only) showed distortion of hepatocytes, enlarged sinusoids and central vein containing blood. c. Group 3 (orijin bitters and 200mg/kg/bw of aqueous extract of roselle (*Hibiscus sabdariffa*) calyx) showed slight distorting of hepatocytes and sinusoids and reduced blood in central vein. The cytoarchitecture appeared better arranged in group 2. d. Group 4 (orijin bitters and 500mg/kg/bw of aqueous extract of roselle (*Hibiscus*

sabdariffa) calyx) showed better cytoarchitecture appearance than in group 2 and 3. e. Group 5 (orijin bitters and 200mg/kg/bw of aqueous extract of ginger (*Zingiber officinale*) rhizome) showed distortion of cytoarchitecture with central vein containing small pocket of blood. f. group 6 (orijin bitters and aqueous extract of ginger (*Zingiber officinale*) rhizome) showed normal radiating hepatocyes with sinusoids. Central vein contains blood however, general cytoarchitecture appeared better arranged than in group 2 and 5. CV= Central vein, S= Sinusoid, H= Hepatocyte (H&E, x400).

Discussion

Humans use plants for food traditional medicine preparation because they contain chemical constituents that are beneficial [48]. There is claim/or believe that alcohol bitters have positive effect to the body due to the herbal constituent found in them [8, 13]. *Hibiscus sabdariffa* contains phytochemical elements such as anthocyanins, flavonoids, and phenol derivatives that have unique effect on illness and is used to treat a variety of illnesses, including intestinal issues, blood impurities, stomach ailments and hypertension [20, 49]. Ginger is valued for its aromatic, culinary, and medicinal properties [50]. Ginger is used in a variety of food and beverage applications due to its bioactive compounds [51]. This study evaluated the effect of orijin bitters, aqueous extracts of roselle calyces (*Hibiscus sabdariffa*) and ginger (*Zingiber officinale roscoe*) rhizome on the liver of adult wistar rats.

The enzymatic activity of Alanine transaminase (ALT) and aspartate aminotransferases (AST) and alkaline phosphatase (ALP) were studied to evaluate the of orijin bitters, aqueous extracts of roselle calyces (*Hibiscus sabdariffa*) and ginger (*Zingiber officinale roscoe*) rhizome effect liver. Liver enzymes levels when increased are indicative of cellular functions and integrity compromise, hepatocellular damage, necrosis, altered membrane permeability and cholestasis [52-54]. Increased AST activity is a specific marker for liver damage and disease, increased ALT is considered as a marker for liver indicative of degeneration, liver fat accumulation [55] and related to hepatic insulin sensitivity [56] and increased ALP level indicates tubular degeneration and necrosis. AST and ALP increase in the group administered orijin bitters alone suggests its possible toxicity on the liver although, there was decrease in ALT. Reports from other studies showed increase in AST and ALT when 2.7 ml/kg bodyweight of herbal bitters were administered to albino rats [11], AST, ALT and ALP increased on exposure of commonly consumed alcoholic beverages, orijin bitters inclusive, to male wistar rats, resulting in hepatotoxicity [13], significant increase in levels of AST and ALP following administration of fijik herbal bitters with significantly decreased ALT level [7], significant increase in AST and ALT when a major constituent of action bitters *Tetrapleura tetraptera* were administered in rats [57]. Nwachuku and Elekima (2018) reported non-significant increase in ALP and AST suggesting it was as a result of exerted pressure on the liver due to the 40% alcoholic content of action bitters that was however, not strong enough to induce adverse effect on hepatocytes at a dose of 0.68ml/kg bodyweight for 30 days [10]. Groups treated with orijin bitters together with extracts of *Hibiscus sabdariffa* calyces, had reduced levels of AST and ALP, suggesting the extracts potential to ameliorate the effect of orijin bitters alcoholic content. *Hibiscus sabdariffa* calyces are rich in vitamin C, anthocyanin and flavonoids which are natural potent antioxidants known to scavenge free radicals [58-60], hence the reduction in the liver damage may be as a result of these phytochemicals. Groups treated with orijin bitters together with extracts of *Zingiber Officinale* rhizomes decreased the level of ALP. Water-ethanol based ginger extraction produced oleoresin and essential oils which contain many phenolic compounds that have functional and pharmacological properties such as antioxidants,

antihyperglycemic, antimicrobial, anticarcinogenic, anti-inflammatory, immunomodulatory, antilipidemic antitumor, and antimutagenic [61-63]. These properties possibly brought about the decreases of ALP, indicating that ginger ameliorated the effect of the alcohol in orijin bitters. Motawi *et al*. (2011) also reported hepatoprotective activity of ginger in rats against carbon tetrachloride where AST, ALT ALP, GGT and total bilirubin significantly decreased [64].

The histology of liver consists of its fundamental structural and functional unit, the hepatic lobule which possesses a hexagonal form approximately the size of a sesame seed and is characterized by the arrangement of liver cells such as the hepatocytes which are the predominant cells, and kupffer cells which functions as macrophages [2]. The liver lobule is also comprised of the portal triad, central vein, liver sinusoids, bile canaliculi, and the space of Disse, which denotes the narrow gap between sinusoids and hepatocytes [2]. The hepatocytes, encompass the sinusoidal capillaries in significant quantities and are accountable for the major hepatic functions; the synthesis and storage functions and the filtering of blood from the portal vein [2]. Hepatocytes are layered in hexagonal plates on top of one another to form the liver. Hepatocytes in each plate radiate outward from a central vein. As they spread towards the periphery, the hepatocytes are organized in strips, and the hepatic sinusoids drain into the central vein [65]. The photomicrographs of rat which received only distilled showed normal cytoarchitecture while that of orijin bitters showed alterations indication possible damage to the liver tissue. This may be as a result of the presence of alcohol found in the drink. The administration of low and high dose of aqueous extract of roselle (*Hibiscus sabdariffa*) calyx together with orijin bitters was able result in recuperation of liver tissue damage by orijin bitters. The histological morphology of groups that received low and high of aqueous extract of ginger (*Zingiber officinale*) rhizome together with orijin bitters together indicated recuperation of the cytoarchitecture. Roselle (*Hibiscus sabdariffa*) and ginger (*Zingiber officinale*) have both been shown to possess phytochemicals and antioxidant properties that are able to protect against tissue damage [20, 33, 65]. The protective effect of both aqueous extracts were dose dependent.

CONCLUSION

The study concludes that orijin bitters may possess the ability to cause toxicity to the liver possibly due to the alcohol content present in it. Aqueous extracts of roselle calyces (*Hibiscus sabdariffa*) calyx and ginger (*Zingiber officinale roscoe*) rhizome possess phytochemicals that may be able to reduce the effects of toxicity caused by orijin bitters either alone or together with the herbal contents in the orijin bitters.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

SID conceived the idea of the research, monitored and shared in the laboratory analysis. GD and SDG shared in the treatment of animals and histological analysis. AA advised on methodology as well as proofreading.

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