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Repeated Exposure of Herbal Concoction Imprints Toxicological Implications through Redox Imbalance in Male Wistar Rats

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Authors' contributions

This study was carried out through the collaboration of all authors. Authors JOF, JAB and OTA developed the proposal and approved by all authors. The biochemical data were collected by authors JOF, ORL, IAO and TMO while histological were by authors OTA and AAA. The data were analysed by authors JOF, JAB and AAA. The first and final drafts were developed by authors JOF, JAB and OTA. All authors read and approved the final draft and responsible for the manuscript content.

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ABSTRACT

Aim: A Large percentage of the Nigerian populace consumes herb concoction prepared by local vendors without due regard to quality control such as toxicity and validity testing. The present study assessed the toxicological effects of continuous intake of some locally prepare herbs in male Wistar rat.

Materials and Methods: The animals (n=50) were exposed to 2.5 ml/kg b.w of herbal concoctions sourced from ten (10) different vendors for four (4) weeks. Control animals (n=5) received distilled water for the same period. Plasma AST, ALT, urea, creatinine and erythrocyte SOD and lipid peroxidation were evaluated. Hepatic reduced glutathione, glutathione peroxidase, lipid peroxidation were also assessed spectrophotometrically. Histological changes in hepatocytes were examined. ANOVA followed by Tukey's test was used to analyze the results with p= 0.05 considered significant.

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Results: Plasma transaminases and urea significantly reduced while increase (p = 0.05) was observed in the level of creatinine in most of the treated groups relative to the control group. Some Herbal concoctions induced a significant decrease (p = 0.05) in liver GSH concentration and GPx activities while some induced non-significant reductions in SOD activities. Significant increase (p< 0.001) in the levels of erythrocyte and liver malondialdehyde was the hallmark of the observations in the treated groups. Hepatic histopathology revealed that there were disruptions of hepatic architectures, congestion as well as infiltration of mononuclear cells.
Conclusion: This study indicates that the continuous intake of locally formulated herbal concoction disrupts erythrocyte and hepatic redox balances and hepatic architecture and functions.

Keywords: Antioxidants; lipid peroxidation; oxidative stress and histopathology.

1. INTRODUCTION

The use of herbal products as means of medications predates written human history [1]. Archaeological evidence indicates that the use of medicinal plants, dated at least to about 60,000 years ago [2]. Meanwhile, the use and search for drugs of therapeutic importance from plant sources is increasing [3]. According to WHO, about 80% of Africans made use of herbs to treat both major and minor ailments [4] and knowledge about this traditional medicinal practice is transmitted from generation to generations [5]. Specifically, the medicinal value of these plants is inherent in some of the chemical substances synthesized by these plants [6]. As observed by Elujoba [7], millions of people consume these products without regulatory agency validity of the acclaimed medicinal and appraisal of toxic potential. In addition, it is evident that the identification of the medicinal values of most of these herbal products is basically by instinct, intuition or by trial and error.

Toxicological problems associated with the use of herbal medicines are complex and include, but not limited to cardiotoxicity, nephrotoxicity, carcinogenicity and even mortality [8,9]. Heavy metal contaminants like Arsenic (As), Cadmium (Cd), Lead (Pb) etc can be a risk factor that contributes to the toxicity of herbal concoction [10-12]. Similarly, herbs from farm lands that are polluted with herbicides, pesticides, microbial contaminants, toxic substances and adulterants could also be toxic to human [13]. Lack of scientific knowledge of the local vendors who formulate the concoctions and their warped business orientations that do not give due regard to the safety of consumers of their products are another major limiting factors.

Toxicity of some of these products, however, may result from the generation of reactive oxygen species (ROS), which can covalently bind DNA and protein [14,15]. A very good number of human diseases have been linked to oxidative stress as the free radical damages cells by initiating chain reactions such as lipid peroxidation [16]. Damage to DNA can result into mutation and possibly cancer [17]. Similarly, damage to protein can as well leads to inhibition of enzyme, denaturation and degradation of important protein [18].

However, as observed by Johns [19] and Fugh-Berman [20], beyond verification of herbal drug efficacy, it is necessary to subject herbal concoctions to sound toxicity testing so as to have a clear understanding of the collateral systemic consequences of such concoctions. Therefore, the effective and safe use of herbal products should be a top research priority. The present study beamed searchlight on the effect of some selected water – based and alcohol – based herbal concoction claimed to have antimalarial effect produced by vendors in Ogbomoso, South-Western Nigeria on lipid peroxidation, antioxidant system, liver and renal functions.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used were of the analytical grade obtained from the British Drug House (BDH) Chemicals Limited, Poole, England and Sigma-Aldrich, Missouri, U. S. A.

2.2 Herbal Products

Already prepared herbal concoctions claimed by the respective vendors to possess antimalarial potentials were purchased from 10 different sources in the five Local Government Areas of Ogbomoso (Ogbomoso North, Ogbomoso South, Orire, Surulere and Ogo Oluwa Local

Government areas), South-western Nigeria. The herbal products obtained from sources I, II, III, IV and V were water based, while those obtained from sources VI, VII, VIII, IX and X were alcohol based. Some of the claimed constituents of the herbal preparations include; *Enantia chlorantha, Mangifera indica* leaves, *Morinda lucida, Aframomum melegueta, Allium sativum, Amomum subulatum, Azadirachta indica, Sphenocentrum jollyanum, Nauclea latifolia, Cymbopogon citratus*

2.3 Animals and Treatment

Experimental protocols were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee and approved (LAU/FBS/170014) by the Animal Ethical Committee of the Faculty of Basic Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Fifty-five male Wistar rats (bred in the College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria) with a mean body weight of 135 g were used for the experiments. They were housed in an animal room with a normal controlled temperature (22 ± 2°C) and a regular 12 h light–dark cycle (06:00–18:00 h). They were allowed 14 days to acclimatize before commencement of the study. The animals were maintained on a standard pellet diet. Animals were divided into 10 groups of 5 animals each. Group I served as control and received distilled water through oral intubation for 4 weeks, the remaining 10 groups were exposed to 2.5 ml/kg body weight (b.w) water and alcohol based herbal concoction (5 groups each) obtained from vendors I-X for 4 weeks through oral gavage. This dose of herbal drugs was chosen based on the average dose recommended by the vendors for an adult human of 70 kg b.w. At the end of the exposure, blood was collected from the animals into heparinised tubes by cardiac puncture under light anesthesia after an overnight fast. Liver was also removed from the animals for biochemical analyses and histopathology. The blood samples were centrifuge at 5,000 rpm for 5 min to separate plasma and red blood cells. All samples were stored at −20°C until analyzed.

2.4 Enzyme Assay

ALT and AST activities were assayed in the plasma by using the reagent kits according to the method of Reitman and Frankel [21]. Creatinine concentration was determined as described by Bartel et al. [22] while urea concentration was determined by Urease-Berthelot method. Superoxide dismutase (SOD) was evaluated using Fortress diagnostic kit which employed Arthur method [23]. Briefly, xanthine and xanthine oxidase is used to generate free radicals which in turn react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (I.N.T) to form a red formazan dye. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of I.N.T under the conditions of the assay. Glutathione peroxidase activity was evaluated using the method of Rotruck and co-workers [24]. The method described by Beutler et al. [25] was employed in evaluating the level of reduced glutathione in the tissue homogenates. Lipid peroxidation was evaluated by measuring thiobarbituric reactive oxygen species (TBARS) in form of MDA using the method described by Varshney and Kale [26].

2.5 Histopathology

Harvested livers were rinsed in phosphate buffered saline, blotted dry on filter paper and weighed. Sections of the liver were fixed in 10% p-formaldehyde processed and embedded in paraffin. The formalin fixed, paraffin embedded tissue was further sectioned and layered onto glass slide which was stained with haematoxylin-eosin dye and finally read under microscope as previously described by Hochegger et al. [27]

2.6 Statistical Evaluation

Results are expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by Tukey's test was used to analyze the results with $p < 0.05$ considered significant.

3. RESULTS

Figs. 1 and 2 show the effects of the herbal products on alanine and amino transferases. As shown (Fig. 1), herbal concoctions from sources III, VIII, and IX induced significant reduction in plasma alanine aminotransferase activities. Similarly, herbal drugs from sources IV, V (water – based), VIII, IX and X (alcohol – based) significantly reduced the activities of plasma aspartate aminotransferases (Fig. 2). The herbal concoctions from other sources produced no significant effect on the activity of the enzyme.

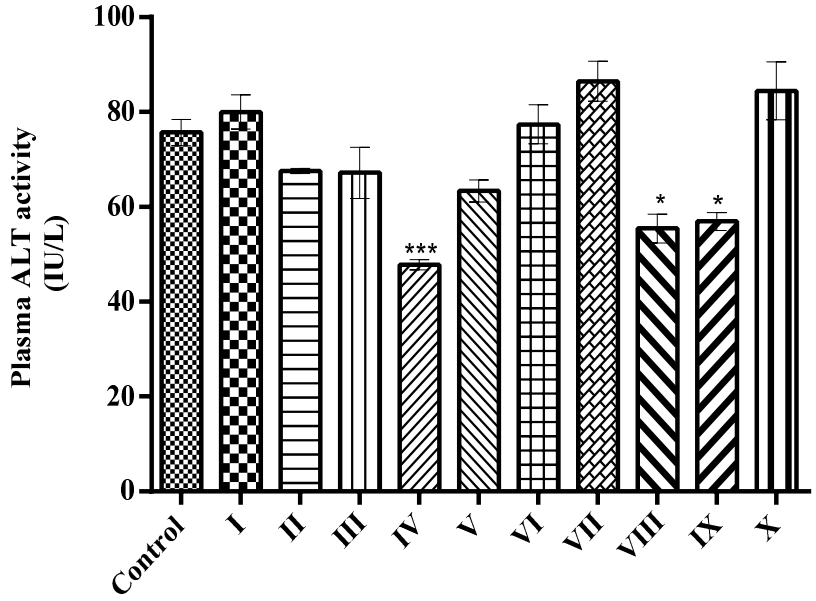

Fig. 1: The effects of repeated exposure of different herbal concoctions on Plasma ALT activities in Male Wistar rat.
Each bar repesents Mean ± SEM of five animals in each group while * and *** denote significant level at P=0.05 and P<0.001 respectively.

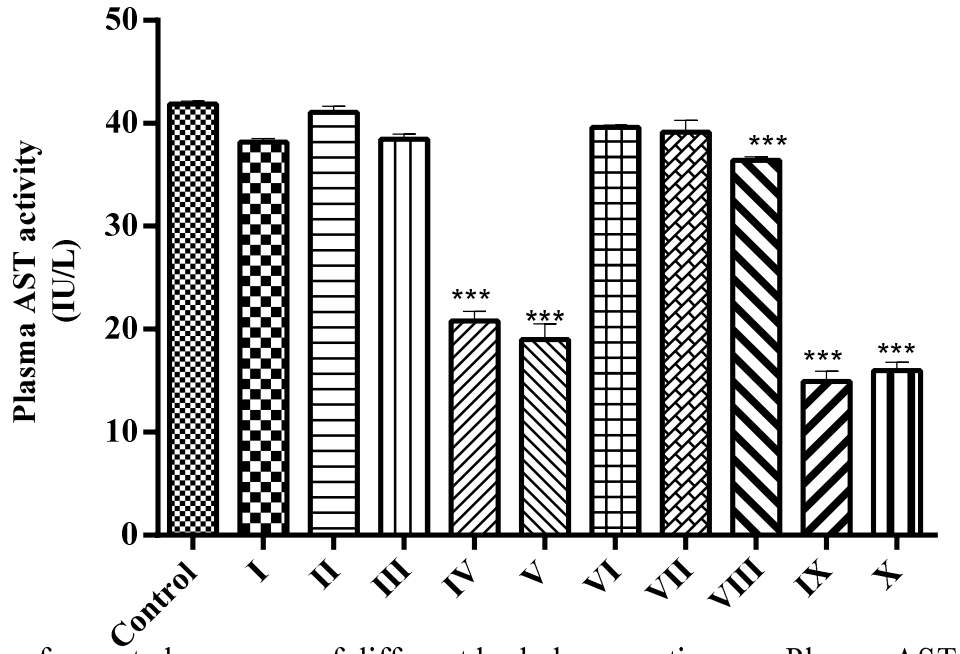

Fig. 2: The effects of repeated exposure of different herbal concoctions on Plasma AST activities in Male Wistar rat. Each bar repesents Mean ± SEM of five animals in each group while *** denotes significant level at P<0.001.

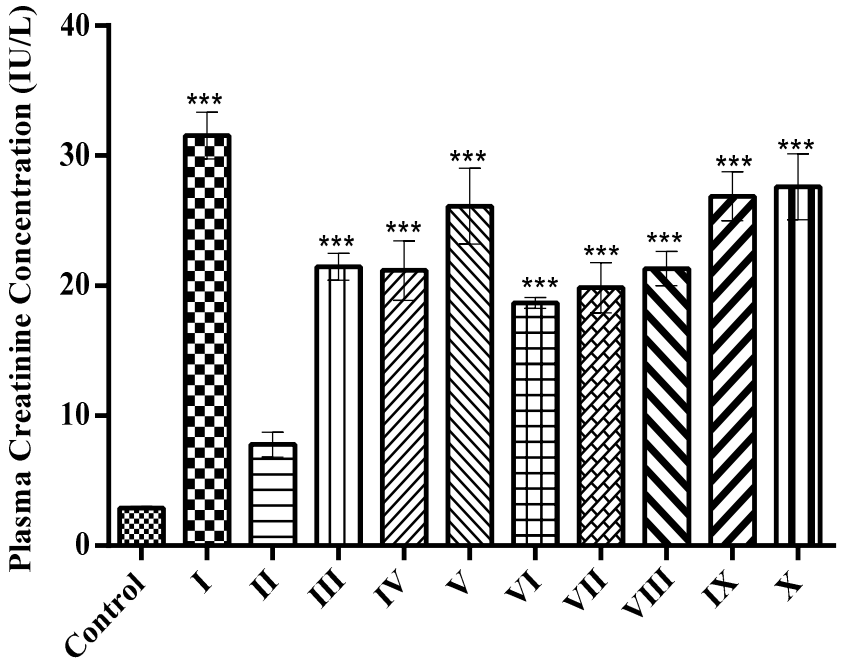

Fig. 3: The effects of repeated exposure of different herbal concoctions on Plasma creatinine concentration in Male Wistar rat. Each bar repesents Mean ± SEM of five animals in each group while *** denotes significant level at P<0.001.

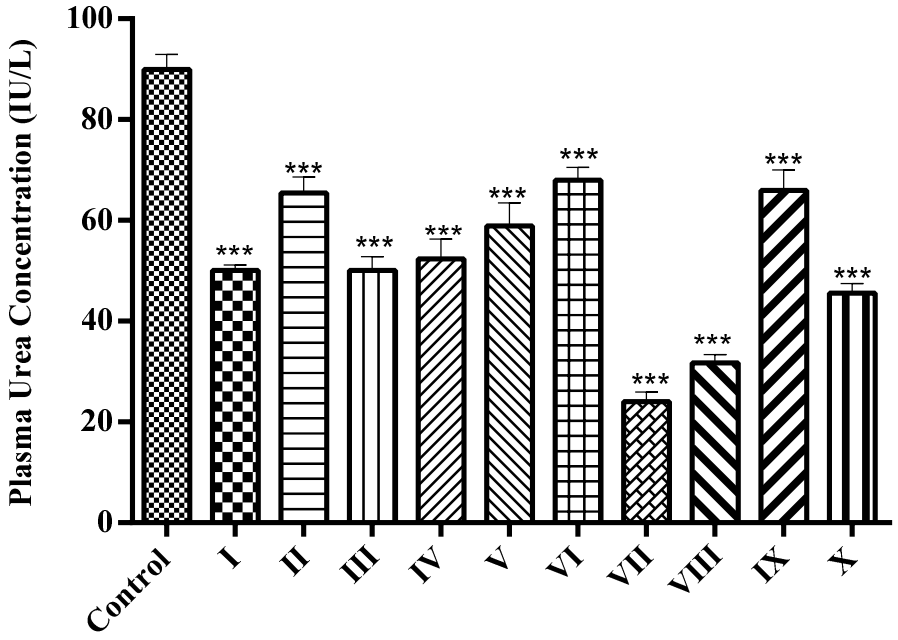

Fig. 4: The effects of repeated exposure of different herbal concoctions on Plasma Urea concentration in Male Wistar rat. Each bar repesents Mean ± SEM of five animals in each group while *** denotes significant level at P<0.001.

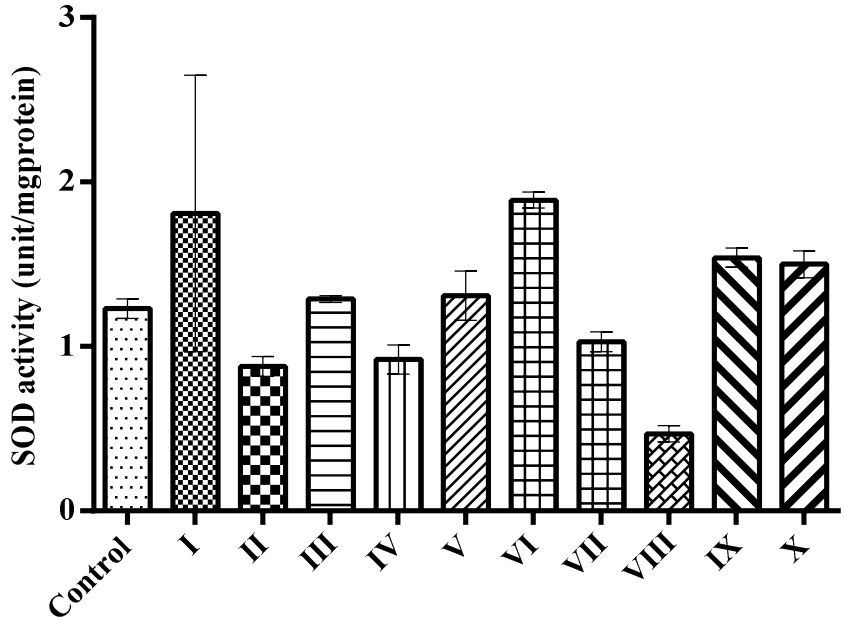

Fig. 5: The effects of repeated exposure of different herbal concoctions on SOD activities in Male Wistar rat.
Each bar repesents Mean ± SEM of five animals in each group.

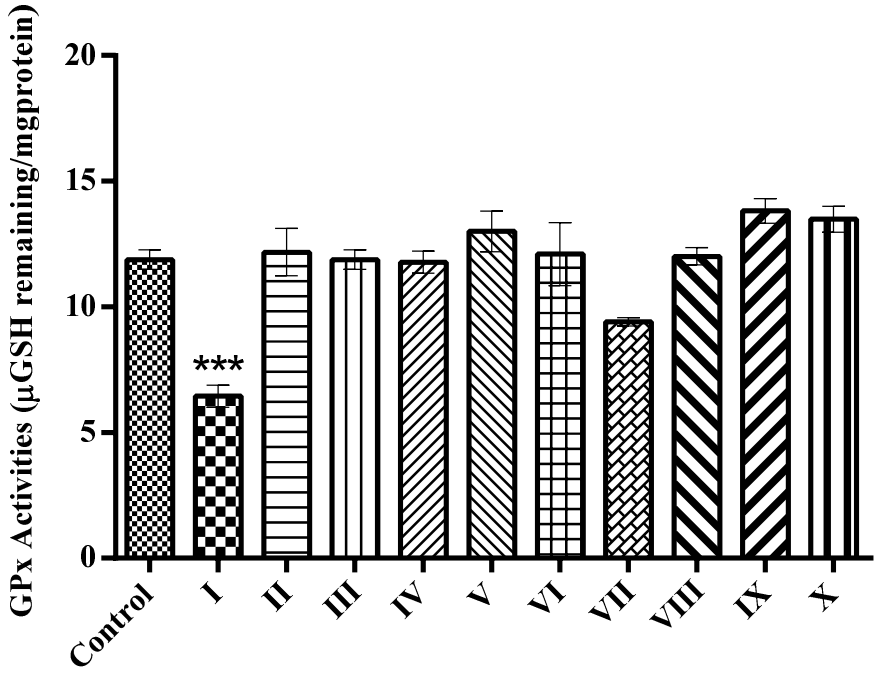

Fig. 6: The effects of repeated exposure of different herbal concoctions on GPx activities in Male Wistar rat. Each bar repesents Mean ± SEM of five animals in each group while *** denotes significant level at P<0.001.

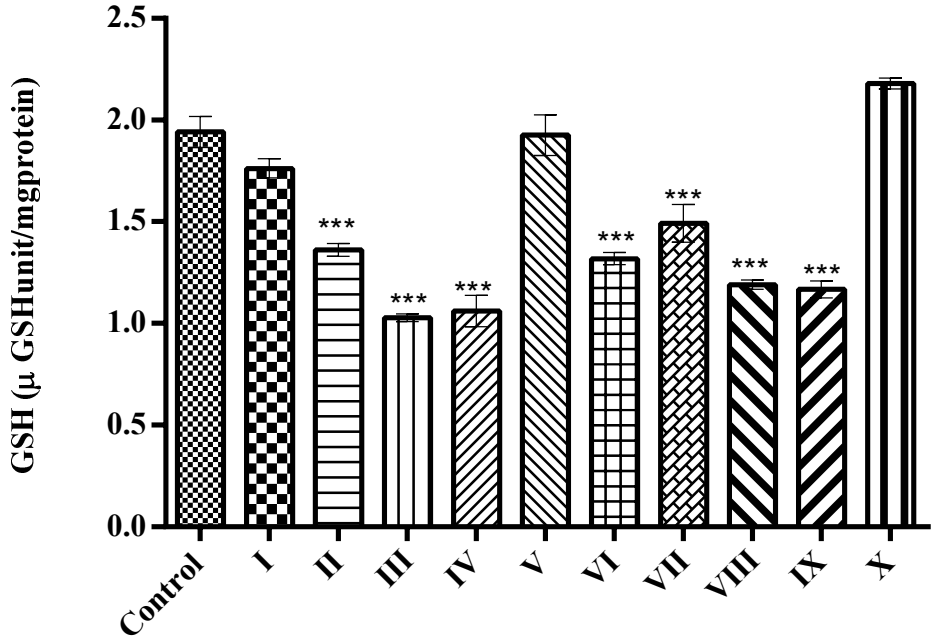

Fig. 7: The effects of repeated exposure of different herbal concoctions on Hepatic GSH levels in Male Wistar rat. Each bar repesents Mean ± SEM of five animals in each group while *** denotes significant level at $P<0.001$.

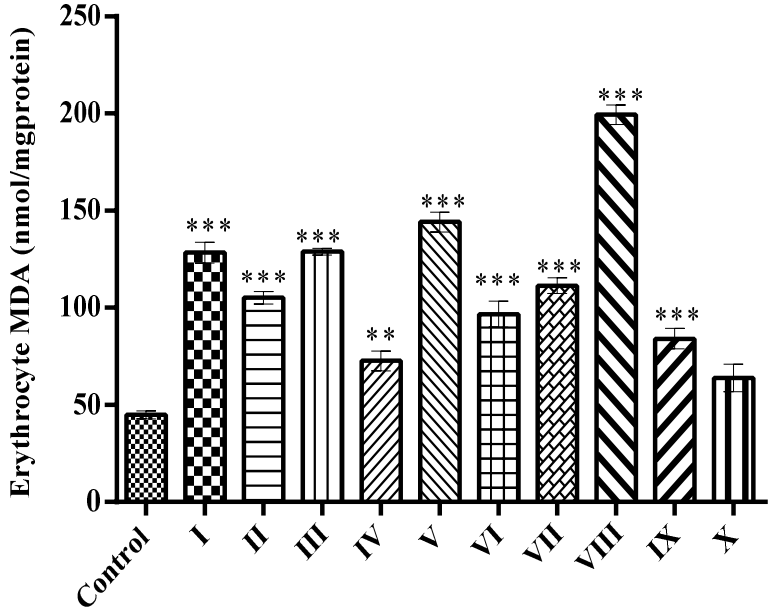

Fig. 8: The effects of repeated exposure of different herbal concoctions on Erythrocyte MDA levels in Male Wistar rat.
Each bar repesents Mean ± SEM of five animals in each group while ** and *** denote significant level at P<0.01 and P<0.001 respectively.

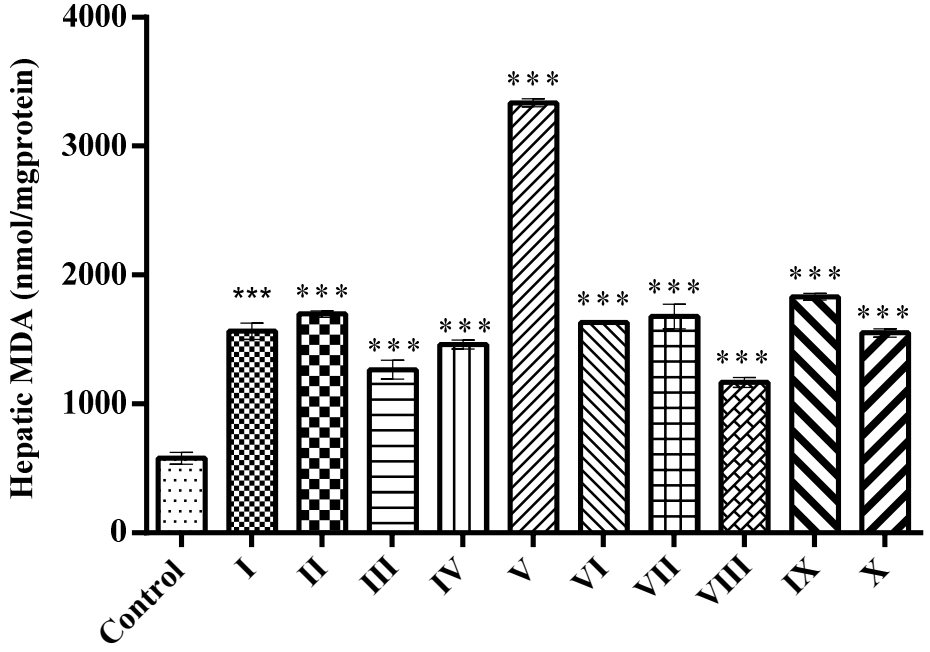

Fig. 9: The effects of repeated exposure of different herbal concoctions on Hepatic MDA levels in Male Wistar rat. Each bar repesents Mean ± SEM of five animals in each group while*** denotes significant level at P<0.001.

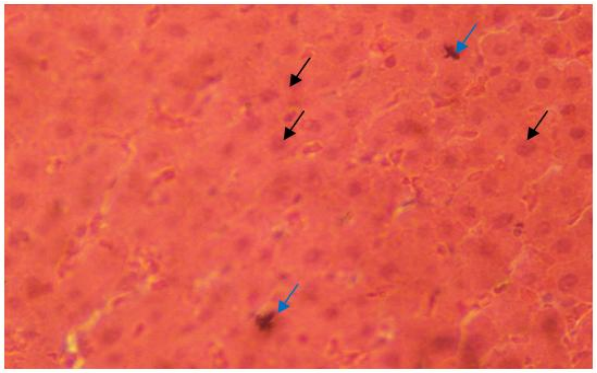

Plate 1. Photomicrograph of control animal. The plate demonstrating normal histoarchitecture showing distribution of Kupfer cells (blue arrow) and hepatocytes (black arrow)

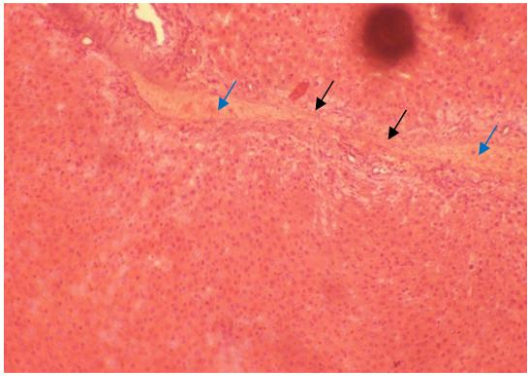

Plate 2. Photomicrograph of group I animal. The plate showed moderate portal congestion (black arrow), with fibroplasia of the periportal region (blue arrow).

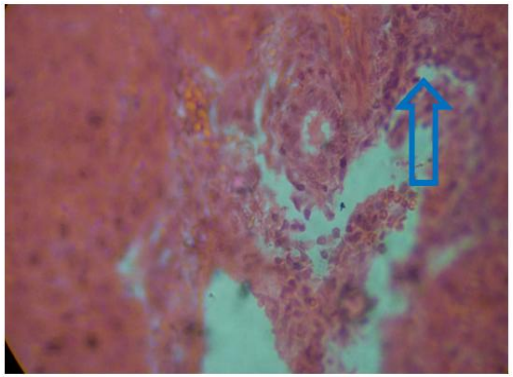

Plate 3. Photomicrograph of group II animal. The plate showed mild periportal hepatic necrosis (blue arrow), portal cellular infiltration by mononuclear cells).

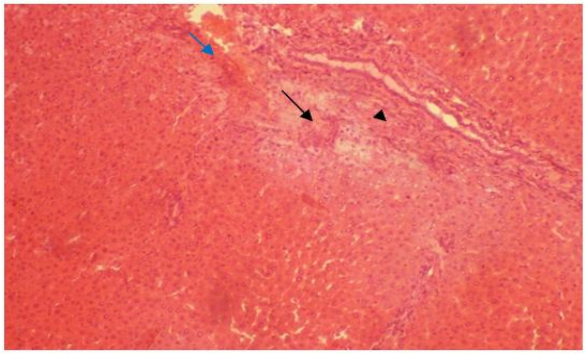

Plate 4. Photomicrograph of group III animal. The plate indicated mild hepatic necrosis (periportal) (blue arrow) and fibrosis (black arrow), with mild portal congestion.

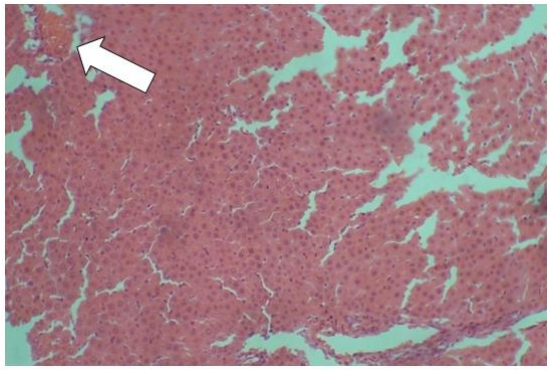

Plate 5. Photomicrograph of group IV. The plate showed a very mild portal cellular infiltration by mononuclear cells.

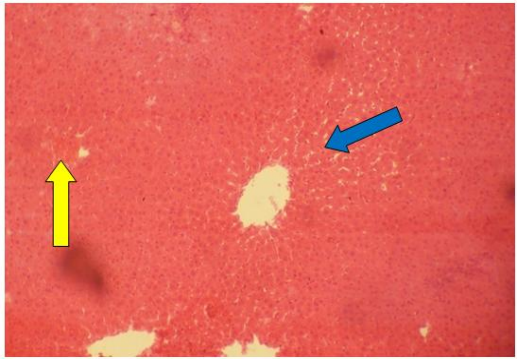

Plate 6. Photomicrograph of group V animal. The plate showed mild diffuse portal blue arrow and central venous congestion (yellow arrow).

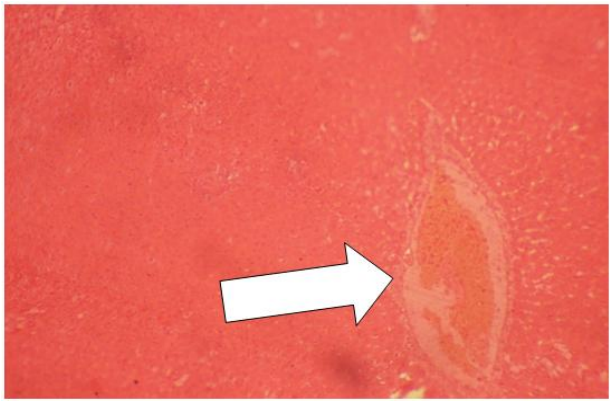

Plate 7. Photomicrograph of group VI animal. The plate showed a severe central venous congestion.

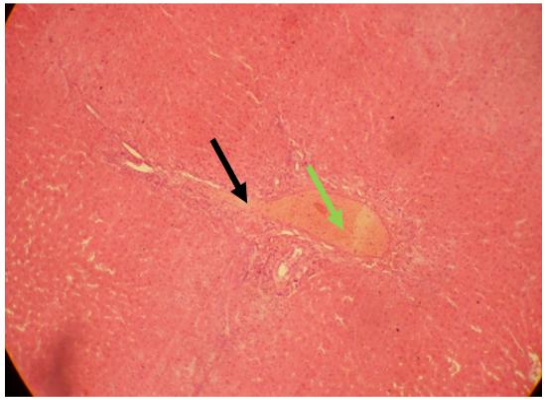

Plate 8. Photomicrograph of group VI animal. The plate indicated portal congestion (green arrow) and periportal cellular infiltration by mononuclear cells (black arrow).

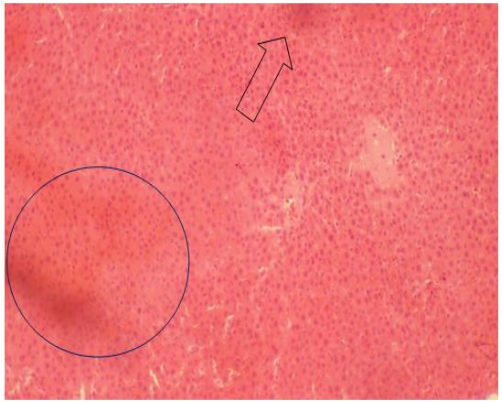

Plate 9. Photomicrograph of group VII animal. The plate indicated moderate sinusoidal congestion.

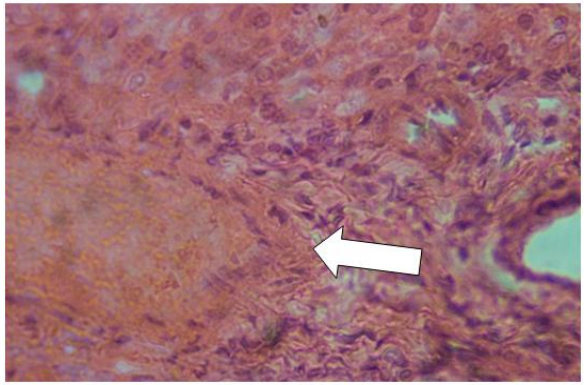

Plate 10. Photomicrograph of Group VIII animal. The plate indicated moderate portal congestion, with fibroplasia of the periportal region.

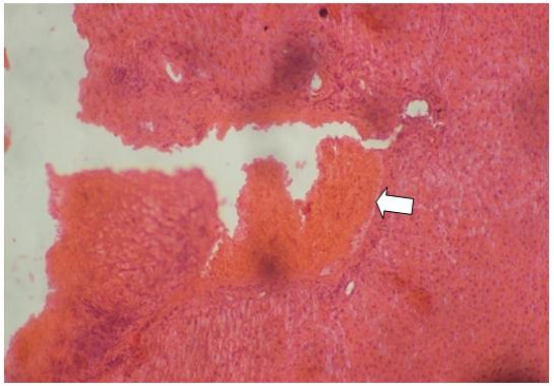

Plate 11. Photomicrograph of Group IX animal. The plate showed severe portal and sinusoidal congestion.

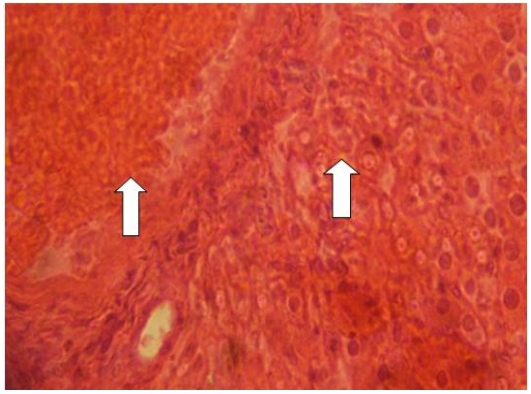

Plate 12. Photomicrograph of Group X animal. The plate showed mild diffuse portal and central venous congestion.

The effects of herbal concoction on plasma creatinine and urea concentrations are shown in Figs. 3 and 4 respectively. As shown in Fig 3, herbs from all the sources significantly up-regulate plasma creatinine levels. Herbs from sources I and II induced significant increase levels of plasma creatinine relative to the control group. On the other hand, herbs from all the ten sources caused a significant decrease of the plasma urea concentration (Fig. 4.)

Fig. 5 depicts the effects of the herbal concoctions on the activity of erythrocyte superoxide dismutase. Herbal products obtained from sources II and IV (water-based) as well as sources VII and VIII (alcohol-based) induced non-significant reduction in the SOD activity of the exposed animals. Source VIII induced 63% reduction in SOD activity, which is the highest reduction when compared with the control. As shown in Fig. 6, herbal concoction from source I significantly reduced the activity of hepatic glutathione peroxidase enzyme. The reduction in the activity was found to be 69%.

Fig 7 shows significant reduction in GSH levels of all the herbs exposed groups except source V and X with no significant effect. Significant elevations of erythrocyte MDA levels were observed in all the exposed groups as depicted in Fig. 8. The most significant elevation of MDA level with about 4 – folds increase was induced by herbal concoction from source VII. Similarly, the concoction from all the ten sources significantly increased hepatic MDA levels (Fig 9). Herbal concoction from source V (water-based) increased the hepatic MDA level by about 6–folds when compared with the control animals, while source VIII (alcohol-based) increased the indicator of lipid peroxidation by about 2 – folds.

4. DISCUSSION

Consumption of herbal products is a very common practice to treat a specific ailment, some use it as a prophylactic while some are inordinate consumer of the concoction most especially the alcoholic based. The youth command the large percentage of the abuser of the concoctions because of affordability and easy access. In addition, the acclaimed benefit adduce to some of the concoction is another factor that entices the army of youth to outrageous consumption of the product. Interestingly, the local manufacturer, the end user and all the three tiers of governments alike pay no serious attention to the toxicological potential and health consequence of these products. In the present work, rats were exposed to the dose of concoction relative to an average weight of 70 kg man as espoused by the vendors. The dose adopted in the study will go a long way to correlate our findings to human toxicological implications of locally made herbal products as well as predict likely health concerns.

Plasma ALT and AST are important biomarkers for the assessment of liver damage [28,29]. This is important as the hepatocytes are the major targets for chemically induced injuries. The present findings indicated that continuous exposure of rats to herbal concoction for four weeks resulted in a significant decrease of the two plasma transaminases activities. The reduced plasma AST and ALT activities is an indication that the herbal concoction might have suppressed or inactivate the synthesis of these very important hepatic enzymes with concomitant consequence on the oxidation of amino acids and energy metabolism. This finding is in agreement with the previous work of Malomo et al [30].

Urea is the principal end products of protein catabolism [31], while creatinine is the last variable of non – protein nitrogenous blood constituents [32]. The reduced plasma levels of these metabolites corroborated our earlier observation that exposure to the herbal concoction might have suppressed or inactivate the synthesis of transaminases required for metabolism of proteins hence the depletion of product of protein metabolism in circulation.

Oxidative stress results when free radical produced overwhelmed antioxidant defense system. Meanwhile, oxidative stress has been implicated in the damage of biological macromolecules as well as disruption of normal metabolism and physiology. Consequently, oxidative stress contributes to the onset of health disorders such as cancer, diabetes and other life threatening conditions. Therefore, aerobic living systems must therefore maintain a delicate balance between the rate of generation of free radicals and removal [33].

Superoxide dismutase (SOD) catalyzes the dismutation of superoxide anion to hydrogen peroxide so as to prevent the formation of reactive hydroxyl radical [34]. The reduction of SOD activities as a result of exposure to herbal concoctions indicates that continuous exposure to some of these locally prepared herbal drugs

can likely deprive the body of the very important functions of SOD and allow superoxide anion to either attack macromolecules directly or react with nitric oxide to form peroxinitrate, a more virulent radical.

GSH is presently one of the most studied antioxidant tripeptide molecules. It is a major source of thiol groups in the extracellular compartment and ubiquitously present in all the aerobic organisms. It plays a very important role in catalysis, metabolism as well as transport [35] and serves as a cofactor for GPx [36]. The reduction in the levels of GSH concentrations observed in the present study is an indication of oxidative stress. This is in agreement with previous studies by Kidd [37] and Mascolo et al. [38] who noted that depletion of hepatic GSH is associated with liver damage and enhanced chemical toxicity.

GPx is an important tetrameric selenoprotein enzyme localized in the cytosol and mitochondrial. It catalyzes the reduction of both organic and inorganic hydroperoxides to water in aerobic organisms at the expense of reduced glutathione as cofactor [39,40]. The herbal concoction from source I only showed decrease GPx activity and also no significant difference in GSH level when compared with the control group. This shows that decreased GPx activity is responsible for non-utilization of GSH, the enzyme co-factor in herbal concoction I treated group. The reduced GPx activity may allow H_2O_2 to attack important macromolecules and disrupt vital enzyme activity which can lead to oxidative stress induced ailment.

Lipid peroxidation is one of the major outcomes of free radicals induce cell membrane disruption, a result of total let down of antioxidant defense system. It is a measure of membrane damage and alteration in the structure and function. It causes changes in membrane fluidity, permeability, ion transport alteration and inhibition of metabolic processes [41]. Increased level of lipid peroxidation is a biomarker of toxicity and it may imply oxidation of polyunsaturated fatty acid (FUFA) present in circulation [42]. This study reveals significant increase in the level of malondialdehyde (MDA) following exposure to water – and alcohol – based herbal concoctions. The observed MDA levels elevations in the erythrocyte and hepatocyte may be due to possible anti-nutrients inherent in the plant or heavy metals contents of the plants or solvents used for the preparation of herbal concoctions. This elevated MDA levels suggests oxidative stress induction and failure of antioxidant defense system which is potential for tissue damage. The observed decrease activity of SOD and GSH concentration corroborate the increase lipid peroxidation. This indicates that reduced activities of the antioxidant enzymes lead to increase oxidative stress, which manifested in the lipid peroxidation elevation.

Hepatic histological examination of rats exposed to locally prepare herbal concoctions relative to the untreated control group shows varying degree of disruption of hepatic architecture such as portal congestion, fibrosis, periportal hepatic necrosis as well as infiltration of mononuclear cells while the hepatocyte of control group appears to be cellularly and structurally normal. The observed hepatic architectural manifestation corroborates the induced increase lipid peroxidation and the lower antioxidant activities observed in this study.

5. CONCLUSION

The finding shows that the continuous intake of locally made herbs may induce negative effects on the hepatic structure and function, antioxidant redox system and lipid peroxidation in the erythrocytes and hepatocytes with severe consequence. Regulatory agencies may wish to look into the subject matter.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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