



Subchronic Toxicological Assessment of Dr Iguedo Goko Cleanser® on Lipid Profile and Serum Antioxidant Enzymes in Exposed Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GJU, JEO and JAU designed the work. Author GJU wrote the protocol and first draft of the manuscript. Authors JEO and JAU reviewed and vetted the first draft. Author DNO managed the literature searches, while author NJO effected corrections to the first draft. Author GJU performed the statistical analysis and managed the analytical cost of the study. All authors read and approved the final manuscript.

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ABSTRACT

Dr Iguedo Goko Cleanser® is a polyherbal mixture promoted as an effective herbal remedy for numerous diseases. Study aimed to evaluate the toxicity concern of the polyherbal mixture (PHM) on lipid profile and oxidative status in Wistar rats of both gender. Acute toxicity study was conducted using modified method of Lorke. Thirty Wistar rats of bother gender were randomly divided into six groups (5/group) and exposed to the polyherbal mixture for 60 days via oral gavage. Control groups (1 and 4) received 10 mL/kg distilled water, while groups 2-3 and 5-6 received 476.24 and 158.75 mg/kg body weight of Dr Iguedo Goko Cleanser® respectively. On 62nd day, animals were

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sacrificed under diethyl ether anaesthesia; blood samples were collected by cardiac puncture for biochemical analysis. PHM significantly ($p < 0.05$) increased high density lipoproteins (HDL) levels in male rats as well as high dose female rats relative to control. However, low dose female rats recorded low HDL levels relative to control. Total cholesterol, triglycerides, low density and very low density lipoprotein levels were significantly reduced in all test groups relative to controls. The low dose males (LDM) had reduced serum glutathione peroxidase (GPX) activity; while increased and decreased GPX and glutathione (GSH) activities were respectively recorded for female rats. Male rats had dose-dependent increase in malondialdehyde. The recorded reductions in serum lipids suggest that the polyherbal mixture may have hypolipidemic potentials. While the increased malondialdehyde as well as decreased GPX and GSH indicate lipid peroxidation and oxidative stress inducing potentials of the PHM. Despite the positive modulation on lipid profile, findings suggest utmost caution on chronic use as its oxidative stress inducing potentials is considerable.

Keywords: Herbal remedy; hypolipidemic; dyslipidaemia; oxidative stress; toxicity.

1. INTRODUCTION

Lipid profile is one of the diagnostic tests often requested by clinicians especially when taking care of patients with cardiovascular and metabolic disorders. In biological systems, lipids include cholesterol, triglycerides, fat soluble vitamins etc. [1]. Cholesterol is an important biomolecule that is implicated in health and diseases. For instance, it is an essential constituent of cellular membranes as well as a precursor for the synthesis of steroid hormones, bile acid and vitamin D [1]. Cholesterol is also a co-factor for numerous enzymes and provides principal or anchor-like support for membrane proteins. Among other functions, cholesterol serves as energy depot or reservoir [2]. Lipids are either mobilized from dietary sources or synthesize *de novo* and distributed in the body as lipoprotein particles. To avoid over-accumulation and abnormal deposit in biological systems, cholesterol synthesis and usage must be properly regulated. Failure to achieve this results in the abnormal deposit of cholesterol and cholesterol-rich lipoproteins in the blood vessels, especially the coronary arteries. Overtime, such deposit leads to atherosclerosis which is the major contributory risk factor for cardiovascular diseases [1]. Thus, despite the biological usefulness of cholesterol and triglycerides, an abnormal level of these lipids predisposes patients to premature cardiovascular disorders [2].

Following exposure to any substance (biological, chemical or physical agent), toxic or adverse effects can occur either at the molecular or cellular level, in specific organs, and/or the whole organism. These substances can interact with proteins, lipids and DNA [3]. For instance, biological or chemical agents can induce lipid

peroxidation and disrupt the membrane lipid bilayer arrangement, inactivating membrane bound receptors, enzymes and/or may increase tissue permeability [4]. Also, they can generate reactive oxygen (ROS) or reactive nitrogen species (RNS). These free radicals once generated attacks biological molecules such as lipids, proteins, and DNA, resulting in oxidative stress, which is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies [3, 5]. Dr Iguedo Goko Cleanser® is a polyherbal mixture licensed by the National Agency for Food and Drug Administration and Control (NAFDAC) with registration number, A7-0804L, and popularly promoted among native Nigerians to be very effective against an array of illnesses including angina pectoris, Alzheimer's disease, giardiasis, toilet infections, hypertension, diabetes, ulcer, cancer, impotence amongst others [6]. Its contents are made of five different plants (*Vernonia amygdalina*, *Cajanus cajan*, *Zingiber officinale*, *Allium sativum* and *Saccharum officinarum*) and caramel as a colouring agent. Due to poor standardization, lack of toxicological evaluation and deplorable manufacturing practices, medicinal herbs and their derivatives may potentiate a range of undesirable outcomes especially on repeated exposures. Numerous and irrefutable cases of poisoning associated with herbal remedies have been reported in the literature [7-9]. Therefore, this research was designed to determine the effect of Dr Iguedo Goko Cleanser® on essential lipids and antioxidant enzymes in exposed Wistar rats. Such findings if and when communicated effectively (after interspecies' extrapolation), will help protect public health against exposure-associated adverse health effects.

2. MATERIALS AND METHODS

2.1 Preparation of Stock Solution and Calculation of Dose

The test samples were purchased from a major distributor in Uyo Metropolis. Aliquots (5 mL) of the polyherbal mixture was measured into five weighed empty beakers and evaporated to dryness using a hot plate (Griffin, Britain) and the marc was determined. The stock concentration was determined by taking the average of the differences between the weight of the beakers and weight of marc in 5 mL of the solution (test sample) using the procedure below:

$$\begin{aligned} \text{Weight of beaker} &= A \text{ (g)} \\ \text{Weight of beaker + marc} &= B \text{ (g)} \\ \text{Weight of marc} &= B - A \text{ (g)} \\ \text{Concentration of drug used} &= \frac{\sum B - A \text{ (g)}/N}{\text{(mL)}} = X \text{ g/mL} \end{aligned}$$

The final doses administered in mL were calculated using the formula:

$$\text{Dose [mL]} = \frac{\text{Weight of Animal [kg]} \times \text{Dose [mg/kg]}}{\text{Stock concentration [mg/mL]}}$$

2.2 Experimental Animals

The animals (Wistar albino rats of both genders) were obtained from and kept at the Department of Pharmacology & Toxicology Animal House of the Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. The animals were maintained under standard environmental conditions and fed with standard Pfizer-branded rodent feed (Livestock Feed, Nigeria Ltd) and given access to water *ad libitum*. All animals were kept at room temperature in cross-ventilated rooms, without illumination at night to achieve the 12 h light/ 12 h dark period. The animals were acclimatized to the laboratory condition for at least 7 days prior to the experiment, during which they were given access to food and water *ad libitum*.

2.3 Acute Toxicity Test

The median lethal dose (LD₅₀) of the polyherbal mixture was determined intraperitoneally (ip) according to the modified method of Lorke [10] using Swiss albino mice (16 – 27 g; n = 18) fasted overnight. The animals were divided into six groups of three animals per group and intraperitoneally administered varying doses of the polyherbal mixture as shown in Table 1.

The animals were observed for cardinal signs of toxicity and mortality within 24 h. The estimated LD₅₀ was used to select the appropriate doses to be administered during the 60-day subchronic toxicity studies. The LD₅₀ was calculated using the formula:

$$LD_{50} = \sqrt{AB}$$

Where

A = maximum dose with 0% mortality and
B = minimum dose with 100% mortality.

2.4 Experimental Design

A total of 30 adult Wistar rats of both genders (15 each) were weighed and randomly allotted to six groups of five animals each and treated as shown in Table 2.

The doses were administered daily using oral gavage for 60 days of the test period [11,12]. Rats in different groups were observed closely for any behavioural changes, feeding and drinking habits, as well as body weight and general morphological changes. After the test period, the animals were euthanized under diethyl ether (Sigma, USA) anaesthesia and sacrificed. Blood samples were collected through cardiac puncture into plain sample bottles for biochemical (lipids, SOD, CAT, GSH, MDA and GPx) investigations.

Table 1. Experimental design for acute toxicity testing of the polyherbal mixture

S/N	Treatment	Dosage (mg/kg)	Observed Duration
1	Group 1	1000	24 h
2	Group 2	1200	24 h
3	Group 3	1400	24 h
4	Group 4	1600	24 h
5	Group 5	1800	24 h
6	Group 6	2000	24 h

Table 2. Experimental design

S/N	Treatment Group	Dosage	Duration
1	CM	10 mL/kg DW	60 days
2	HDM	476.24 mg/kg GC	60 days
3	LDM	158.75 mg/kg GC	60 days
4	CF	10 mL/kg DW	60 days
5	HDF	476.24 mg/kg GC	60 days
6	LDF	158.75 mg/kg GC	60 days

DW = Distilled water, GC = Goko Cleanser, CM = control males, HDM = high dose males, LDM = low dose males, CF = control females, HDF = high dose females, LDF = low dose females.

2.4.1 Biochemical analysis

Using a centrifuge (Nikon optical Co., Japan), whole blood of each sacrificed rat collected through cardiac puncture into different plain sample bottles were centrifuged at 2500 rpm for 20 min at 10 °C to obtain the serum. Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels of the treated rats were measured using standard colorimetric methods [13,14]. Low and very low-density lipoprotein (LDL and VLDL) were estimated from the formula of Friedwald et al. [15]. Superoxide dismutase (SOD) activity was estimated by the method of Marklund and Marklund [16]. Reduced glutathione (GSH) was estimated according to method earlier described by Ellman [17]. While glutathione peroxidase (GPx) and catalase (CAT) activities were estimated using Fortress Diagnostic Kits® according to standard procedures of manufacturer's protocols. MDA was estimated using Colorimetric TBARS Microplate Assay Kit® (FR40, Oxford Biomedical Research Inc., USA) according to standard procedures of manufacturer's protocols. Except otherwise stated, all biochemical investigations were done using automated analysers and Fortress Diagnostic Kits® (Fortress Diagnostic Limited, UK) according to standard procedures of manufacturer's protocols at Bridge Bio-Tech Ltd, Ilorin, Nigeria.

2.4.2 Statistical analysis

Data generated was statistically analysed using SPSS version 17. Statistical significance between the groups were analysed by means of one-way analysis of variance (ANOVA). Results were presented as Mean ± S.E.M. and values less than ($p < 0.05$) were considered significant.

2.4.3 Limitations

In this study, a 14-day reversibility assessment (where exposure of the lower animals to the

polyherbal mixture must have been discontinued and all parameters evaluated repeated for any occurrence of reversal of test effects) was not done due to set limits.

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity Test

The result of this test is presented in Table 3. From the result, 100% and 0% mortality were respectively recorded at 1800 – 2000 and 1000 – 1400mg/kg body weight of the herbal mixture. At the dose levels tested, cardinal signs of toxicity observed were decreased motor function and somnolence. There were no changes in the nature of stool, urine and eye colour in all the surviving mice. This suggests that the herbal mixture may possess some central nervous system inhibitory properties. The LD₅₀ value of the herbal mixture was estimated to be 1587.45 mg/kg body weight (mouse, i.p).

3.2 Lipid Profile Analysis

The result for the lipid profile of rats administered the polyherbal mixture is as presented in Fig. 1. Male rats has significantly ($p < 0.05$) reduced total cholesterol between the test groups and relative to control. Dose dependent reductions in total cholesterol were recorded among the female rats relative to control and male groups. There was a significant increase in high density lipoproteins (HDL) in high dose and low dose male rats relative to the control males. A significant increase and decrease was respectively recorded for high and low dose female rats relative to control females. Female rats had significantly higher HDL levels than the male rats. A dose dependent reduction in triglycerides was recorded in both male and female rats relative to controls. Female rats had significantly low triglycerides compared to the male rats. Same results were recorded for both low and very low density lipoproteins (Fig. 1).

Cholesterol, triglycerides and high density lipoproteins are considered essential constituents of the lipid fraction of the human body. Cholesterol – an unsaturated alcohol is a predominant constituent of animal cell membranes and is needed for the normal cellular physiology. It is a known precursor of various essential substances like the adrenal and gonadal steroid hormones and bile acids. On the other hand, triglycerides are fatty acid esters of glycerol. They are the main lipid component of dietary fat and animal fat depots. Together with cholesterol, triglycerides are transported in the plasma alongside the lipoproteins chiefly because of their non-polar nature. Plasma lipoproteins contain variable proportions of cholesterol, triglycerides, phospholipids and specific proteins tagged ‘apoproteins’ [18]. The plasma lipoproteins are classified majorly as chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). High density lipoproteins functions predominantly in the reverse transportation of cholesterol (LDL) from different tissues into the liver for its removal. When in abnormal concentrations, cholesterol and triglycerides attracts clinical attention. They are indicators of underlying cardiovascular and/or metabolic issues. Abnormalities in the synthesis, degradation and transport of these substances as well as their associated lipoproteins cause corresponding increases or decreases in the general circulation. The plasma cholesterol and triglyceride levels give insight as to which of the lipoproteins are increased. For example, an isolated elevation of total plasma cholesterol usually indicates elevated LDL, while isolated elevation of plasma triglycerides indicates elevated chylomicrons and VLDLs. Thus, abnormal amounts of these lipids (hypercholesterolemia vis-a-vis hyperlipidemia) are considered high risk factor for atherosclerosis and severe coronary heart disease [18].

Unusually low levels of HDL (hypolipoproteinemia) are related to

increased incidence of coronary heart disease in high-risk populations. Whereas elevation of HDL (hyperlipoproteinemia) is linked to decreased risk of coronary atherosclerosis and heightened longevity [18]. The majority of cases of elevated plasma HDL levels are genetic with either a dominant or polygenic inheritance. Nevertheless, secondary HDL elevations are related to weight loss, regular exercise, moderate use of alcohol and proper dieting amongst other factors. The recorded decreases in total cholesterol, triglycerides, VLDL, LDL as well as the increase in HDL levels is indicative of the cholesterol and triglyceride lowering effect of the polyherbal mixture. This is attributed to phytochemical constituents of the plants found in the herbal mixture. For example, *Allium sativum* (garlic) has been linked to mark reduction in blood glucose, total cholesterol, phospholipids, and triglycerides in healthy individuals as per clinical trials [19]. Earlier study suggested that the consumption of garlic might prevent cardiovascular complications in individuals with metabolic syndrome; especially as it significantly increases plasma levels of adiponectin [19]. Similarly, previous studies [20,21] have demonstrated the hypolipidemic potentials of *V. amygdalina* (bitter leaf) – another plant content of the herbal mixture. As earlier reported by Chitra et al. [22], there exist a relationship between glucose homeostasis and lipid metabolism especially in some pathological states (e.g. diabetes). Diabetes is often associated with high level of circulatory cholesterol and other lipids. The considerable reductions in serum lipids recorded in the present study suggest that the polyherbal mixture possesses hypolipidemic potentials.

3.3 Oxidative Stress Biomarkers

Experimental rats (both gender) had no significant ($p > 0.05$) differences in serum superoxide dismutase (SOD) and catalase (CAT) activities at all the doses tested. The low dose males had significantly ($p < 0.05$) lower GPX activity relative to control and high dose males.

Table 3. Acute toxicity test result of Dr Iguedo Goko Cleanser®*

Test groups	Dose (mg/kg)	Fraction of death	% Mortality
1	2000	3/3	100
2	1800	3/3	100
3	1600	2/3	67
4	1400	0/3	0
5	1200	0/3	0
6	1000	0/3	0

Route of administration: Intraperitoneally; n = 18.

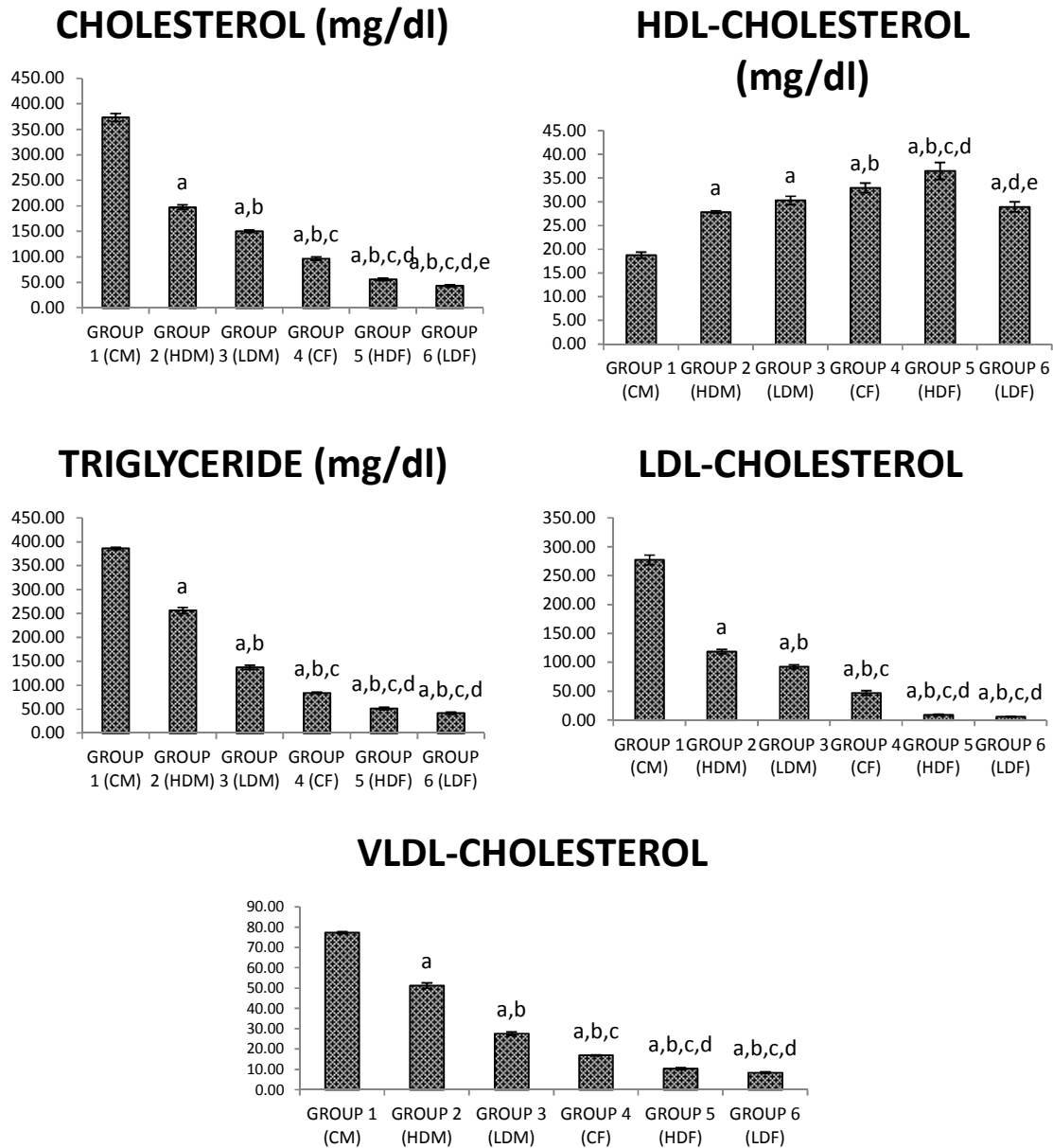


Fig. 1. Serum lipid profile of Wistar rats exposed to Dr Iguedo Goko Cleanser®
 Data presented as Mean ± Standard Error of Mean (SEM). Compared means are considered statistically significant at P=.05; a = significantly different when compared to CM (control males); b = significantly different when compared to HDM (high dose males); c = significantly different when compared to LDM (low dose males); d = significantly different when compared to CF (control females); e = significantly different when compared to HDF (high dose females); n = 5

Female rats had elevated GPX activity relative to control females but a significant decrease was recorded in comparison to male rats (Fig. 2). No significant difference in serum GSH activity was recorded for the experimental males. However, female rats had significantly reduced GSH activity relative to control and all male rats.

Significant dose dependent increase in MDA levels was recorded for the experimental male rats relative to control. While a reduced MDA level was recorded for the low dose males in comparison to the high dose males. There was no significant difference in this parameter in the female rats. However, the control females had

significantly higher MDA levels compared to control and low dose males. Low dose females had low MDA level relative to high dose males,

while high dose females had higher and lower levels respectively relative to control males and high dose males (Fig. 2).

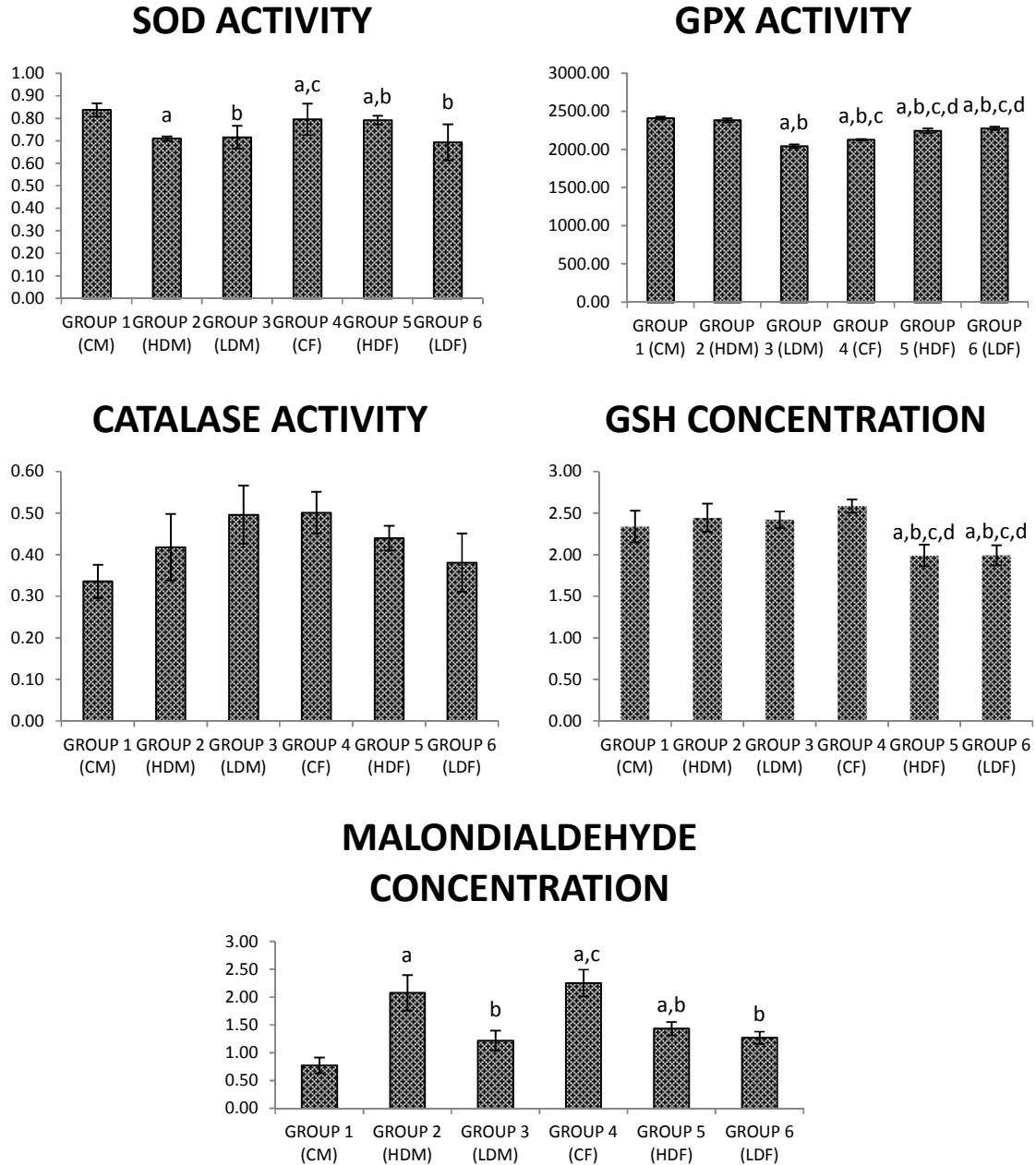


Fig. 2. Serum antioxidative enzymes of Wistar rats exposed to Dr Iguedo Goko Cleanser®
 Data presented as Mean ± Standard Error of Mean (SEM). Compared means are considered statistically significant at P=.05; a = significantly different when compared to CM (control males); b = significantly different when compared to HDM (high dose males); c = significantly different when compared to LDM (low dose males); d = significantly different when compared to CF (control females); e = significantly different when compared to HDF (high dose females); n = 5

In *in vivo* experimental models, SOD, CAT, GPX and GSH are useful and reliable markers of antioxidant status, while MDA is a sensitive and reliable marker for lipid peroxidation [23,24]. SOD plays an important role in oxygen defence metabolism by intercepting and reducing superoxide to hydrogen peroxide, which in mammals is readily reduced to water principally by CAT and GPX. GPX plays a pivotal role in minimizing the oxidative stress. Glutathione peroxidase (GPX) and glutathione-S-transferase (GST) work together with GSH to decompose H₂O₂ and other organic hydroperoxides to non-toxic products. Glutathione (GSH) is a naturally occurring tripeptide whose nucleophilic and reducing properties play a central role in metabolic pathways, as well as in the antioxidant system of most aerobic cells. It plays a major role in drug metabolism, calcium metabolism, the glutamyl cycle, cell membrane and blood platelet functions. GSH is crucial to a variety of life processes, including the detoxification of xenobiotics, maintenance of the –SH level of proteins, thiol-disulfide exchange, removal of hydroperoxides and free radicals as well as the transport of amino acid across cell membranes. Therefore, glutathione is vital for both intracellular and extracellular protection against oxidative and nitrosative stress inducers [25, 26]. This enzyme substrate is primarily converted to its reduced form by glutathione reductase (GR). Free radicals – induced lipid and protein peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations [3]. Ultimately, in humans and other mammals, reactive oxygen species (ROS) are formed in the cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes under both physiological and pathological conditions [27]. To combat these radicals, living organisms produce enzymes (e.g. catalase, superoxide dismutase, and glutathione peroxidase). According to Wang et al. [28], decrease in the activities of glutathione, glutathione peroxidase, superoxide dismutase and catalase indicate oxidative stress. Our findings revealed reductions in serum GPX and GSH activities in low dose male and female rats respectively. This suggests an overwhelming ratio of oxidant to antioxidant defence system and is indicative of oxidative stress in these groups of experimental animals.

Furthermore, lipid peroxidation is one of the frequent/major outcomes of free radical-mediated injury that directly damages cell membranes and

generates a number of secondary products including aldehydes, such as malondialdehyde (MDA) [29]. Thus, MDA is the most abundant individual aldehyde resulting from peroxidation. Consequently, increased MDA can be used as important biomarker of lipid and protein peroxidation [30] and as such, indicative of high oxidative stress [31]. The recorded significant increase in serum MDA levels in experimental male rats is indicative of lipid peroxidation. In overall, the findings of this study suggests the occurrence of oxidative stress – a series of event seen in virtually all pathological conditions e.g. infertility etc. However, the authors cannot ascertain whether or not these test effects were reversed on exposure withdrawal or discontinuation, especially as no reversibility study was carried out.

4. CONCLUSION

Findings of this study reveal that Dr Iguedo Goko Cleanser® is relatively safe on acute oral exposure with an estimated LD₅₀ of 1587.45 mg/kg body weight (mouse, i.p). It further highlights inherent abilities of the polyherbal mixture to positively modulate the lipid profile of the experimental subjects. Nevertheless, the polyherbal mixture may induce oxidative stress and lipid peroxidation especially on long term use. Contrary to the popular belief that herbal drugs are 100% natural, completely safe and devoid of any toxicity whatsoever, the present study has revealed that despite the hypolipidemic potentials of Dr Iguedo Goko Cleanser®, it may also induce oxidative stress. Therefore, the chronic (intermittent, continuous) or long-term use of Dr Iguedo Goko Cleanser® should be done with utmost caution, and wherever possible, be avoided.

DISCLAIMER

The products employed in this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products especially as the authors do not intend to use these products as an avenue for any litigation but for the advancement of scientific knowledge. Also, the research was not funded by the manufacturers of the products rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All necessary ethical considerations as regard the use of animals and humans in research were satisfactorily met. The care and use of animals was conducted in accordance with the National Institute of Health Guide for the Use of Laboratory Animals (NIH, 1996). Moreover, ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the Faculty of Pharmacy, University of Uyo, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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