Original Research Article

Toxicological Assessment of a Polyherbal Mixture on Organ Weights and Hormonal Profile in Exposed Wistar Rats

ABSTRACT

Hormonal imbalance and hypertrophy of internal organs are sensitive indices of toxicity which may be multifactorial (including exposure to certain foods, drugs and herbal remedies), and are implicated in certain conditions such as infertility amongst others. Dr Iguedo Goko Cleanser® is a polyherbal mixture (PHM) often described by promoters as 'super effective' for the treatment of various ailments. The present study was aimed to evaluate the exposure-related effects of PHM on hormonal profile and weight of internal organs in Wistar rats of both gender. Thirty Wistar rats (both genders) randomly allotted to six groups (5 in each group) were exposed orally to PHM for 60 days. Control groups (1 and 4; 10 mL/kg distilled water), groups 2-3 and 5-6 received PHM (476.24; 158.75 mg/kg) respectively. On 62nd day, animals were euthanized under diethyl ether anaesthesia and sacrificed. Blood samples were collected for hormonal analysis and vital organs were eviscerated for macroscopic examinations. PHM significantly (*P*=.05) decreased spleen weight in female subjects relative to control. However, female rats had higher spleen weights compared to male rats. HDF (high dose females) had increased brain weight relative to control groups. Female rats had dose-dependent increased in progesterone relative to control. Female rats had higher progesterone concentration relative to males. Female rats recorded increased estradiol compared to male rats. However, this was reduced in HDF relative to control; low dose females had elevated estradiol compared to control and HDF. Results of the present day study strongly suggest caution and good judgement as regards the prolonged use of the polyherbal mixture especially as the probable hypertrophy of the brain observed in experimental female rats are not negligible.

Keywords: Herbal remedy, hypertrophy, hormonal imbalance, toxicity.

1. INTRODUCTION

Hormones are chemical messengers synthesized and secreted by endocrine and neuroendocrine glands. They are known to be very critical to bodily functions and homeostasis. Thus, imbalance in this messenger system distorts the body's normal physiology. Naturally, hormones are known to fluctuate in concentration at certain or particular points in living organisms, especially mammals. In humans, hormonal imbalances are frequently experienced during puberty, menstruation, pregnancy and aging. When not functioning properly, the endocrine glands precipitate hormonal imbalance. For example, hyposecretion and hypersecretion of certain hormones has been implicated in certain disease conditions (e.g. infertility, diabetes, goitres, Addison's disease amongst others). Nonetheless, certain medical conditions, environmental factors and lifestyles exert powerful impact on some if not all endocrine glands [1]. In reproductive biology, imbalance in sex hormones or their releasing factors is fundamental to the onset of infertility both in males and females. Basically, significantly low sex hormones due to abnormality of the gonads (hypogonadism) and perhaps the anterior pituitary gland leads to oligospermia, azoospermia, anovulation and amenorrhea in males and females respectively. Phytoestrogens, toxins, pollutants, and endocrine disruptors (certain heavy metals, herbicides and pesticides) amongst other factors causes hormonal imbalance [1].

In experimental toxicology, the comparison of organ weights (control versus test groups) has long been accepted and conventionally used in the evaluation of test article's toxicity. Hypertrophies of internal organs are sensitive indices of toxicity [2] which may be multifactorial, including exposure to certain foods, drugs, herbal remedies and lifestyle habits. As earlier reported by Ping et al. [3], hypertrophy of organs is a first-hand indication of toxicity of a chemical or biological substance. 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 well over 50 disease conditions [4]. 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. According to Shad *et al*. [5], medicinal plants are subjected to heavy metal contamination during growth, development and processing chiefly due contamination of agricultural soil and irrigation water by anthropogenic activities of man [6]. Therefore, due to poor quality assurance/control, lack of toxicological evaluation and good manufacturing practices, plants of medicinal values and their derivatives may be contaminated with toxic heavy metals and other impurities that may put the body's normal physiology at risk especially on repeated exposures. Thus, this research was designed to determine the effect of Dr Iguedo Goko Cleanser®) on hormones and weights of internal organs in exposed Wistar rats. Such findings will proffer basis for informed decision and protect public health against exposure-associated toxic outcomes.

2. MATERIAL AND METHODS

2.1. Preparation of Stock Solution and Calculation of Dose

After being purchased from a major distributor in Uyo Metropolis, Nigeria, the stock concentration and doses of the polyherbal mixture to be administered to the experimental animals were determined as suggested by Udom et al. [7].

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, 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 (LD_{50}) of the polyherbal mixture was determined as earlier reported by Udom et al. [7].

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 1.

Table 1. 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.

The doses were administered daily using oral gavage for 60 days of the test period [8, 9]. 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. Internal organs were eviscerated, weighed and examined macroscopically. Blood samples were collected through cardiac puncture into plain sample bottles for hormonal (FSH, LH, testosterone, estradiol and progesterone) investigations.

2.4.1. Qualitative Organ Weight Measurement

At the end of the study, the weight of vital organs (brain, liver, kidneys, spleen, testes and ovaries) eviscerated from each euthanized animal were measured using a sensitive weighing balance (Ohaus, USA). The organs were harvested, blotted with tissue paper, weighed fresh on a sensitive balance and observed for any gross morphological changes. Each weighed organ was equated to 100 g body weight of the control and treated rats using the formula:

$$SW = \frac{OW\ (g) \times 100\ (g)}{BW\ (g)}$$

where: SW, OW and BW are the standardized organ weight, organ weight and body weight respectively.

2.4.2. Hormonal Assay

The serum concentrations of the following hormones were determined in rats exposed to the polyherbal mixture using Calbiotech, Inc ELISA kit® according to standard procedures of manufacturer's protocols.

Follicle Stimulating Hormone (FSH) was assayed based on the solid phase direct sandwich ELISA method. Briefly, the samples and diluted anti-FSH-HRP conjugate were added to wells coated with

monoclonal antibody (MAb) to FSH beta subunit. FSH in the serum binds to anti-FSH MAb in the well and then anti-FSH-HRP second antibody then binds to FSH. Unbound protein and HRP conjugate were washed off by wash buffer. Upon the addition of the substrate, the intensity of colour was proportional to the concentration of FSH in the samples. Descriptively, the desired numbers of coated strips were placed into the holder and 50 µL of FSH standards, control and samples (sera) were added. To each well, 100 µL of enzyme conjugate was added and incubated for 30 min at room temperature, after which liquid was removed from all the wells. The wells were washed three times with 300 µL of 1X wash buffer and blotted on absorbent paper towels. Then 100 µL of TMB substrate was added to all the wells and incubated for 10 min at room temperature, after which 50 µL of stop solution was added. The plate was gently shaken to mix the solution and the absorbance was read 15 min later on ELISA Reader at 450 nm.

Luteinizing Hormone: Again, the desired numbers of coated strips were placed into the holder and 25 µL of LH standards, control and samples (sera) were added. To each well, 100 µL of conjugate reagent was added and mixed thoroughly by placing the plate on a plate shaker at 600rpm for 30 s and incubated for 60 min at room temperature, after which liquid was removed from all the wells. The wells were washed three times with 300 µL of 1X wash buffer and blotted on absorbent paper towels. Then 100 µL of TMB substrate was added to all the wells and incubated for 15 min at room temperature, after which 50 µL of stop solution was added. The plate was gently shaken to mix the solution and the absorbance was read 15 min later on ELISA Reader at 450 nm.

Progesterone: With the desired numbers of coated strips placed into the holder, 10 µL of progesterone standards, control and samples (sera) were added. To each well, 200 µL of progesterone enzyme conjugate was added and incubated for 60 min at room temperature, after which liquid was removed from all the wells. The wells were washed three times with 300 mL of 1X wash buffer and blotted on absorbent paper towels. Then 100 µL of TMB substrate was added to all the wells and incubated for 15 min at room temperature, after which 50 µL of stop solution was added. The plate was gently shaken to mix the solution and the absorbance was read 15 min later on ELISA Reader at 450 nm.

Estradiol: This was assayed with the E2 ELISA kit. Briefly, it is based on the principle of competitive binding between E2 in the test specimen and E2 enzyme conjugate for a constant amount of anti-estradiol polyclonal antibody. In the incubation, anti-E2 antibody coated wells are incubated with E2 standards, controls, samples, and E2 enzyme conjugate at room temperature for 60 min. During the incubation, a fixed amount of HRP-labelled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. The unbound E2 peroxidase conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 30 min, resulting in the development of a blue colour. The colour development was stopped with the addition of stop solution, and the absorbance was measured at 450 nm. Descriptively, the desired numbers of coated strips or wells were placed into the holder and 25 µL of standards, control and test samples were dispensed into appropriate wells. Then 100 µL of working solution of Estradiol enzyme conjugate was dispensed into each well of conjugate reagent and mixed thoroughly by placing the plate on a plate shaker at 600rpm for 10 – 20 s and incubated for 60 min at room temperature, after which liquid was removed from all the wells. The wells were washed trice with 300 µL of 1X wash buffer and blotted on absorbent paper towels. Then 100 µL of TMB substrate was added to all the wells, mixed gently for 10 s and incubated for 30 min at room temperature, after which 50 µL of stop solution was respectively added. The plate was gently shaken for 30 s to mix the solution until the blue colouration completely changes to yellow and the absorbance was read 15 min later with a microplate reader at 450 nm.

Testosterone: Descriptively, the desired numbers of coated strips or wells were placed into the holder and 25 µL of standards, control and test samples were dispensed into appropriate wells. Then 100 µL of working testosterone-enzyme conjugate reagent was dispensed into each well and mixed thoroughly by swirling the microplate gently for 20 -30 s and incubated for 60 min at room temperature, then liquid was

removed from all the wells. The wells were washed three times with 300 µL of 1X wash buffer and blotted on absorbent paper towels. Then 100 µL of TMB substrate was added to each well and incubated for 15 min at room temperature, after which 50 µL of stop solution was respectively added and gently mixed for 15 – 20 s. The absorbance was read 15 min later on ELISA Reader at 450 nm.

2.4.3. 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*=.05) were considered significant.

2.4.4. Limitations

As reported by Udom et al. [7], reversibility studies on the polyherbal mixture using the model described above was not carried out due to set limits.

3. RESULT AND DISCUSSION

3.1. Acute Toxicity Test

According to Udom et al. [7], the LD_{50} value of the polyherbal mixture was estimated to be 1587.45 mg/kg body weight (mouse, i.p).

3.2. Qualitative Organ Weight Measurement

There was no significant increase or decrease in the weights of the liver, kidneys, testes, ovaries, spleen (males only) and brain (males only) at all doses tested. However, significant (*P*=.05) decrease in the weight of spleen was recorded for HDF (high dose females) and LDF (low dose females) in comparison to CF (control females) (Table 2). The weights of the spleen of female rats were significantly higher compared to male rats. Also, significant increase in the weight of the brain was recorded for HDF in comparison to the control groups (Table 2). 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.

Table 2. Qualitative organ weight per 100 g body weight of rats exposed to the polyherbal mixture

Grouping	Liver	Kidney	Spleen	Testis	Ovary	Brain
CM	3.35 ± 0.07	0.59 ± 0.03	0.32 ± 0.01	1.29 ± 0.10		0.83 ± 0.07
HDM	3.29 ± 0.09	0.63 ± 0.02	0.33 ± 0.02	0.97 ± 0.23		0.86 ± 0.09
LDM	3.11 ± 0.05	0.59 ± 0.01	0.32 ± 0.02	1.02 ± 0.16		0.82 ± 0.07
CF	3.46 ± 0.06^{c}	0.62 ± 0.01	0.58 ± 0.09^{abc}		0.12 ± 0.01	0.94 ± 0.03^{ac}
HDF	3.23 ± 0.10	0.63 ± 0.04	0.33 ± 0.04^{d}		0.13 ± 0.01	1.00 ± 0.03^{ac}
LDF	3.44 ± 0.16	0.64 ± 0.02	0.33 ± 0.04^{d}		0.14 ± 0.01	1.02 ± 0.04

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.

Generally, significant changes in internal organ weights are considered sensitive indices of toxicity after exposure to toxic substance [2]. The recorded increase in the weight of the brain observed in high dose female rats could indicate hypertrophy or accumulation of fatty deposits or droplets. Also, the recorded decrease in spleen weight observed in female rats relative to experimental males could be seen as a possible toxic outcome following prolonged exposure to the polyherbal mixture. This is believed to be so as changes in spleen and thymus weights are considered to be sensitive indicators of immunotoxicity (either immune stimulation or immunosuppression), stress and physiologic perturbations [10]. Swirski et al. [11] reported that the red pulp of the spleen forms a reservoir that holds more than half of the body's monocytes. Owing to their function as inflammatory mediators, monocytes migrate from the spleen to injured tissues or sites. Physiologically, the spleen is the activity hub for the mononuclear phagocyte system, thus blood cells becomes depleted due to mass recruitment to inflamed sites, so the spleen which filters as well as stores blood now contains significantly few blood cells, and as a result the weight of the spleen decreases. Toxicologically, alterations and/or damage to any of the histostructure especially the red pulp would likely contribute to a decreased spleen weight due to scanty red blood cells.

3.3. Hormonal Profile Analysis

The experimental subjects had no significant differences in the concentrations of FSH at all doses tested. The high dose males had significant (P=.05) increase in testosterone concentration compared to the control males, while that of low dose males was significantly decreased when compared to high dose males. No significant difference in testosterone concentration was recorded in the female subjects. However, the female rats had significantly low testosterone concentration compared to the male rats (Fig. 1). Low dose males had significantly high progesterone concentration relative to control and high dose males. Also, there was a dose-dependent increase in progesterone in female rats relative to control. In comparison to male rats, female rats had significantly higher progesterone concentration. There was no significant difference in estradiol concentration in male rats. The experimental female subjects recorded significant increase in estradiol when compared to their male counterparts. However, high dose females had reduced estradiol concentration relative to control; while low dose females recorded an elevated level compared to control and high dose groups. Low dose males had a significantly increased LH concentration relative to control. There was no significant difference among the female rats; rather they had decreased concentration when compared to the high and low dose male groups (Fig. 1).

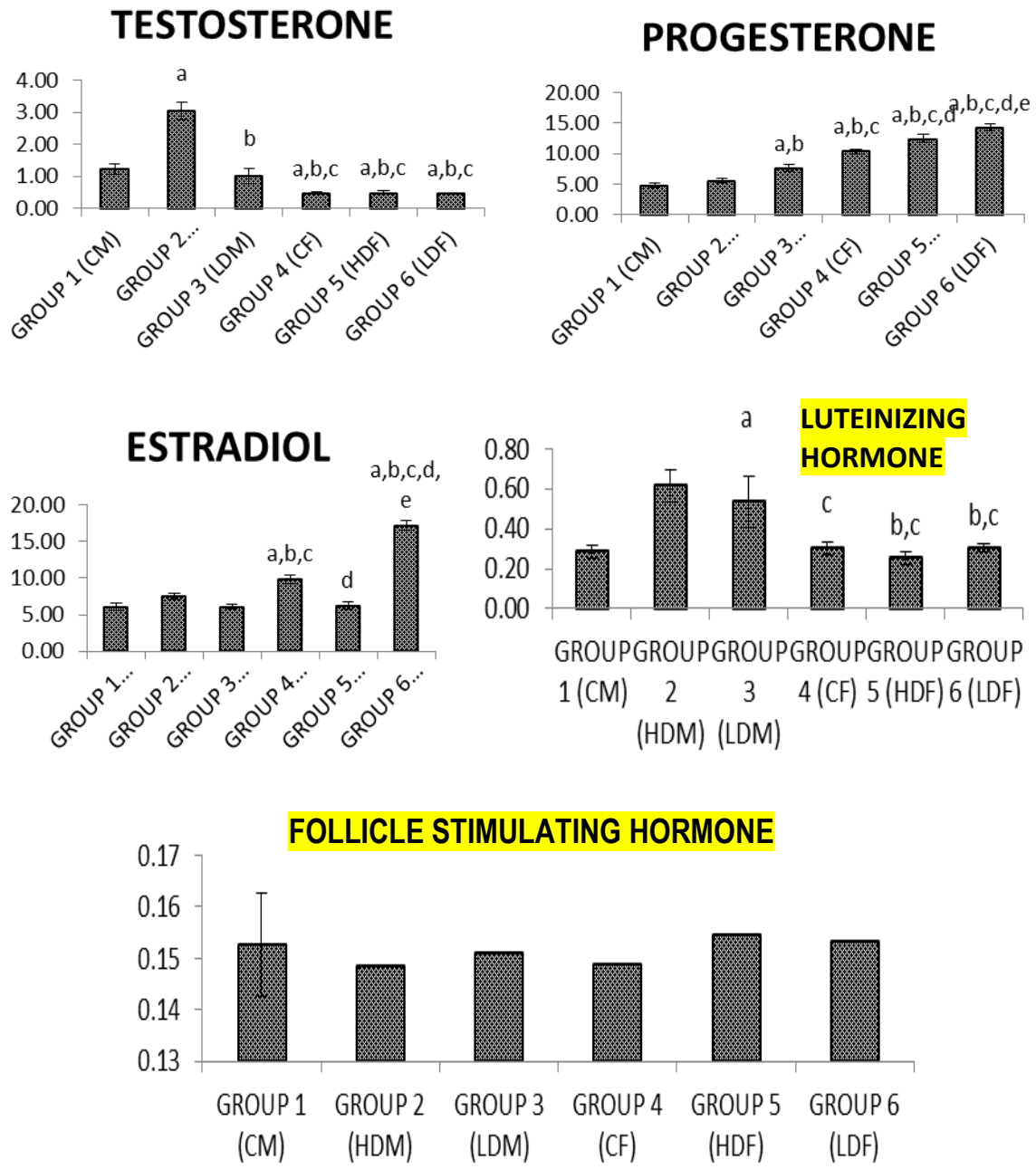

Fig. 1. Serum hormonal concentrations of Wistar rats exposed to the polyherbal mixture

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.

Hormones are chemical messengers with huge clinical importance, thus hormonal imbalance is seen in various disease states such as primary ovarian failure, infertility, testicular failure, functional disorders, etc. [12]. Follicle-stimulating hormone (FSH) is a glycoprotein produced by the anterior pituitary gland. Together with luteinizing hormone (LH), FSH controls reproductive function and the serum levels of testosterone and estradiol, which stimulate secondary sexual characteristics in both male and females. In the females, FSH stimulates follicular growth, prepares ovarian follicles for action by LH and enhances the LH induced release of oestrogen. FSH levels are elevated after menopause, castration and in premature ovarian failure. Although there are significant exceptions, however, ovarian failure is indicated when random FSH concentrations exceed 40 IU/ml. In the males, FSH stimulates seminiferous tubule and testicular growth and is involved in the early stages of spermatogenesis. Oligospermic males usually have elevated FSH levels. While testicular tumours generally depress serum FSH concentrations, the levels of LH are usually elevated. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis.

Estradiol (E2) is the most potent natural oestrogen, produced mainly by the ovary, placenta, and in smaller amounts by the adrenal cortex, and the male testes. Estradiol is secreted into the blood stream where 98% is bound to sex hormone binding globulin (SHBG). Estrogenic activity is impacted through estradiol-receptor complexes which trigger the appropriate response at the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and to a lesser extent the liver and skin. In non-pregnant women with normal menstrual cycles, estradiol secretion follows a cyclic, biphasic pattern with the highest concentration found immediately prior to ovulation. During pregnancy, maternal serum estradiol levels increase considerably, to well above the pre-ovulatory peak levels and high levels are sustained throughout pregnancy. Serum estradiol is considered a valuable index in evaluating a variety of menstrual dysfunctions such as precocious or delayed puberty in girls, primary and secondary amenorrhea as well as menopause [12]. Estradiol levels have been reported to be increased in patients with feminizing syndromes, gynaecomastia and testicular tumours. In cases of infertility, serum estradiol measurements are useful for monitoring induction of ovulation following treatment. The significant differences in concentrations of the hormones analysed in this study do not indicate any pathology, especially as they were within normal limits.

4. CONCLUSION

Findings of this study highlight the probable hypertrophy of the brain tissue in female rats especially on prolonged duration of exposure to the polyherbal mixture. Though exposure to the polyherbal mixture had no toxic outcomes on the hormonal profile of the experimental subjects, however, caution and good judgement should be exercised by the consumers of the polyherbal mixture and wherever possible usage over a prolonged duration should be avoided.

CONSENT

It is not applicable.

DISCLAIMER

It should be noted that the products employed in this research are common products in our area of research and country. There is absolutely no conflict of interest whatsoever between the authors and manufacturer 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 received no funding from any external body (the manufacturers of the test substance inclusive) rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The research was ethically approved by the Experimental Ethics Committee on Animal Use of the Faculty of Pharmacy, University of Uyo, Nigeria and was conducted in accordance with the National Institute of Health Guide for the Use of Laboratory Animals (NIH, 1996).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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