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Research Article

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Toxicological Evaluation of a Polyherbal Stem Cell Remedy (PSCR): Haematological, Biochemical, Reproductive, and Histopathological Perspectives

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Abstract

Herbal remedy remains a fundamental alternative to conventional medicine. However, the proliferation of herbal remedies in our society calls for a scientific evaluation of their safety profile. This study evaluated the safety profile of a Polyherbal Stem Cell Remedy (PSCR) using a preclinical model in rodents. A total of 24 adult Wistar rats were randomized into four (4) groups of six (6) animals each. Group 1 served as control (10 ml/kg of vehicle) while groups 2, 3, and 4 received 2.07 ml/kg (half-therapeutic dose), 4.13 ml/kg (therapeutic dose), and 8.26 ml/kg (double-therapeutic dose) of PSCR for a period of 30 days. Body weight, behavioral changes, feed, and water intake were measured. Animals were euthanized on the 31st day, and samples were collected for haematology, biochemical, semen, testosterone, and histological analyses using standard methods. The PSCR did not produce remarkable changes ($^{ns}p>0.05$) in body weight, behavior, feed-intake, water-intake, organs weight, antioxidant, and glucose, semen count, morphology, testosterone, semen quality, haematology parameters, except statistically significant ($^{*}p<0.05$) increase in chloride, alkaline phosphatase (ALP) level (at high dose), super oxide dismutase (SOD) and decrease in sluggish spermatozoa and WBC (at high dose). Kidney, pancreas, and testes histology showed normal architecture, while hepatic and cardiac tissues showed mild changes at high dose. The study suggests that the PSCR is safe at the recommended dose, but monitoring hepatic and electrolyte levels of subjects using the PSCR above the recommended dose and duration is necessary for precautionary measures.

Keywords: Stem cell; Herbal formulation; Polyherbal; Preclinical studies; Safety profile.

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1. Introduction

Herbal remedies, the main streams of traditional medicine, are relied upon by over 80% of humanity for the treatment and management of several diseases due to their availability, acceptability, accessibility, and affordability compared to conventional drugs [1, 2].

In our contemporary society, stem cell supplements and polyherbal formulations are gaining ground due to their assumed safety, tolerability, and potential in curbing several diseases and rejuvenating adult stem cells [3]. *In vitro* studies revealed that pharmacologically active substances from plants enhance adult stem cell proliferation and also inhibit the proliferation of cancer cells [4].

In drug safety assessment, all substances are considered toxic depending on the dose [5]. Undesirable side effects have been reported in herbal medicine. This necessitates their safety evaluation, an essential aspect of standardization [6]. It should be noted that the fact that herbal remedies are claimed to be safe does not eliminate the possibility of side effects until thorough scientific studies have been carried out on them [7].

Although safety studies on some herbal-stem cell and herbal remedies have been carried out among which include; Bon-santé cleanser® polyherbal [8], NawaTab Polyherbal Formulation [9], Standardized Polyherbal Preparation POL-6 [10], KOJU®- A Nigerian Polyherbal formulation [11], polyherbal nootropic formulation [12], STC-30 stem cell remedy [13], Polyherbal formulations for malaria [14], among several others, no study has been documented on this plant-based stem cell remedy, being used among the populace for various diseases.

This PSCR is a multi-cure stem cell dietary supplement made from 100% plant stem cells, with ingredients locally sourced from Nigeria and China [15]. It is a blood and immunity booster. It cleanses, energizes, rejuvenates, repairs, and replaces dead cells. From recorded testimonies, it can cure multiple diseases, hence the name 'multi-cure'. It has numerous health benefits against several diseases including; Cataract, cervical cancer, cardio-vascular disease, anaemia/sickle cell, diabetes 1 and 2, fibroids, glaucoma, STIs, hypertension, impotence, kidney and liver health, menopause problem, low immunity, mouth ulcer/bleeding gum, ovarian tumour, stroke, piles, syphilis, prostate cancer, ulcer, vaginal yeast infection, unhealthy skin, staphylococcus, toothache/tooth cavity, rheumatism, urinary tract infection, male infertility, high cholesterol, hemorrhage, inflammation of colon, gall bladder disease, arthritis, bad breath, eczema, female infertility, fracture, low sperm count, leprosy, erectile dysfunction, memory loss, aging and wrinkled face, stress and anxiety, insomnia, mental and brain health, CD_4 count, allergy and inflammation, respiratory disorders, gastrointestinal disorders, and warts. It also cleans the blood and promotes colon health. The PSCR also functions as a blood builder and immune system booster. It also acts as a stamina enhancer and cleanses the body. It is also said to help in the rebuilding of damaged tissues and cells. Its key ingredients include: *Dialium guineense*, *Boscia angustifolia*, Cocoa species, *Khaya grandifoliola*, Sorghum bicolor, with ethanol-water as the solvent/preservative. The recommended adult dose is 20 mL twice daily (i.e., 40 mL daily) for 30 days. In some cases, it is taken for a longer duration in patients who have no positive treatment outcome within 30 days [16].

2. Materials and Methods

2.1. Chemicals and reagents

Formaldehyde, Ethanol, Sodium chloride (NaCl), Chloroform, and Acetic acid. Total cholesterol, triglyceride, HDL-Cholesterol, Alanine aminotransferase, Aspartate aminotransferase, Alkaline phosphatase, Total protein, Albumin, Sodium, Potassium, Chloride, Bicarbonate, Urea, Creatinine, kits from RANDOX, TECO, SPECTRUM, Agape, companies. Acuben testosterone Eliser kits.

2.2. Experimental animals care

Albino rats of the Wistar strain of average weight (200.66 ± 8.75 g) were procured from the Animal Facility of the Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegu University (COOU). Animals were kept under standard laboratory conditions and were fed with standard pellets, vital grower feed, and clean tap water *ad libitum* throughout the experimental period. Handling and care of Laboratory animals complied with the Faculty of Pharmaceutical Sciences, COOU animal research ethics committee (approval number, PHACOOU/AREC/2025/001) in line with the National Institute of Health Guidelines for care and use of laboratory animals (Pub No. 85-23, revised 1985).

2.3. Sample procurement

The PSCR was procured from a distributor outlet in Anambra State, Nigeria, and was preserved according to the manufacturer's instructions.

2.4. Repeated toxicity studies

A total of 24 adult Wistar rats were randomized into four (4) groups of six (6) animals each as follows;
Group 1: Control, received 10 ml/kg of water
Group 2: Low dose, received half the therapeutic dose (2.07 ml/kg)
Group 3: Medium dose, received therapeutic dose (4.13 ml/kg)
Group 4: High dose, received double therapeutic dose (8.26 ml/kg)

2.5. Dosage selection and dilution of PSCR

Based on the recommended therapeutic adult dose of 20 ml twice daily (that is 40 ml daily) for 30 days, animal equivalent dose was extrapolated from human dose using the method described by Nair and Jacob [17], where the therapeutic adult dose (40 ml/60 kg = 0.67 ml/kg) was multiplied by a factor of 6.2 (0.67 x 6.2/ kg = 4.13 ml/ kg) and served as medium dose. Half of the therapeutic adult dose (4.13/2 = 2.07 ml/kg) and double therapeutic adult dose (4.13 x 2 = 8.26 ml/kg) served as low and high doses, respectively. Dilution was done with water (vehicle) to obtain a uniform volume of 10 ml/kg, but with varying concentrations according to the Organization for Economic Cooperation and Development (OECD) guidelines [18]. Vehicle and test herbal remedy were administered orally with the aid of an oral-gastric tube/drenching device. Body weight, feed, and water intake were measured during the study. Animals were observed daily for signs of behavioural toxicity such as salivation, tremor, convulsion, lethargy, diarrhoea, alterations in eyes, skin, and mucous membrane.

2.6. Body weight, feed, and water intake determination

Before the drug was administered, the body weights of all the animals were recorded on day zero using a top digital weighing balance. The herbal remedy was administered to the rats with respect to their body weights. The animals were weighed weekly, and the doses were adjusted accordingly based on their new weights. For water intake, 500 ml of water was measured on day zero using a measuring cylinder and given to each group of animals. The following day, the remaining water was measured to estimate the amount of water taken by the animals. For feed intake, 200 g of rat chow was measured using the weighing balance and given to each group of animals on day zero. The feed intake was recorded by measuring the quantity of feed remaining in the feed troughs.

2.7. Samples collection and analyses:

On the 31st day, animals were fasted overnight and were sacrificed under anesthesia. A smaller portion of blood samples obtained via venipuncture was delivered into an EDTA tube for the assessment of hematological parameters using the i-Hema automated analyzer (Medlere Ltd, UK). A larger volume of blood sample was delivered into a plain tube, and the serum was used for the determination of biochemical parameters. The method of Roy, which uses phosphate thymolpthalein monophosphate, was used for the determination of plasma alkaline phosphatase (ALP) [19]. Alanine aminotransferases (ALT) and aspartate transaminases (AST) were determined using the method of Reitman and Frankel [20]. The buffered kinetic Jaffe reaction method was used for the determination of serum creatinine [21]. Urea was determined using the method of Weatherburn (urea in serum is hydrolyzed to ammonia in the presence of urease, and Berthelot's reaction measures the ammonia) [22]. The bromcresol green method of Doumas was used in the determination of serum albumin [23]. The method of Tietz [24] was used for the determination of total protein. Serum chloride determination was based on the modified colorimetric method of Skeggs and Hochstrasser [25]. Sodium level in plasma was determined using the method of Maruna [26]. Potassium level was determined using the method of Tietz [27]. The complex metric procedure of Gitelman [28] was used in the determination of calcium level in serum. The method of Trinder [29], which replaced chemical saponification with enzymatic saponification, was used for the determination of plasma cholesterol. The colorimetric method of Tietz [30], which involves enzymatic hydrolysis with lipase, was used for the

determination of plasma triglycerides. The method developed by the National Institute of Health Consensus Development Conference Statement was employed in the determination of HDL-Cholesterol in plasma [31]. LDL-cholesterol was calculated from triglyceride, total cholesterol, and HDL-cholesterol using the method of Friedewald *et al.* (1972) [32]. Superoxide dismutase (SOD) activity, which measures the enzyme's ability to catalyze the dismutation of superoxide radicals, was determined using the method of Misra and Fridovich [33]. Lipid peroxidation in plasma was estimated colorimetrically, using malondialdehyde (MDA) as an oxidative stress marker, by the method of Gutteridge and Wilkins [34]. Catalase activity was determined using the method of Cohen [35]. Reduced glutathione concentration was measured in plasma by calculating the content of non-protein sulfhydryl groups [36]. Semen motility, count, and morphology were carried out using the method described by Cheesbrough [37]. Testosterone assay was carried out using Accubind ELISA Microwells test kit [38]. Organs (liver, kidney, heart, testes, and pancreas) were isolated, weighed, and fixed in 10% Neutral Buffered Formalin (NBF) for histopathological analyses using the standard method described by Walsh *et al.* [39].

2.8. Method of Data Analysis

Results obtained were presented as mean ± Standard deviation (SD) of sample replicates. The raw data was subjected to one-way analysis of variance (ANOVA) followed by post-dunnet's test using the Statistical Package for the Social Sciences (SPSS-23). Statistical significance was established when $p<0.05$.

3. Results and Discussion:

From the literature, the safety profile of the individual constituents in this PSCR has been documented. Among such, toxicological reports on *Dialium guineense* revealed non-nephrotoxic [40], non-cardiotoxic [41], non-haematotoxic [42] and non-hepatotoxic effects following 28-day administration of its ethanol and aqueous stem bark extract up to 200 to 5000 mg/kg [43]. Toxicological reports on *Boscia angustifolia* revealed histopathological injury on the liver and kidney at higher doses (839.29 and 1342.86 mg/kg), and the authors recommended that it should be used with caution and in lower doses [44]. Toxicological reports on Cocoa species revealed that administration of 240 and 500 mg/kg of Theobroma cacao extract produced impaired hepatic function [45]. On the other hand, unfermented Theobroma cacao at 200, 400, and 600 mg/kg caused little or no toxicological effect on albino rats [46]. Exposure of rats to cocoa-containing diets did not produce deleterious impacts on liver and kidney function [47]. Toxicological reports on *Khaya grandifoliola* revealed no toxicity at 16 mg/kg on haematological, biochemical, and histopathological parameters following 28 days of repeated administration in rats and mice. However, the authors recommended caution to be exercised at higher doses and long duration [48]. Administration of 100, 200, and 500 mg/kg of *Khaya grandifoliola* for 7 and 21 days caused significant hypoglycaemic, hypoproteinaemic, and hypocholesterolaemic effects, and a significant reduction in liver protein content and glutathione (GSH) levels [49]. Toxicological reports on Sorghum bicolor leaf base (100 -400 mg/kg for 28 days) showed it was relatively safe on the liver, kidney, and lipid profile of Wistar rats [50]. Contrary findings by Ajiboye et al. [51] revealed a deleterious impact on the liver, kidney, and brain tissues following repeated exposure to 125 and 250 mg/kg doses of Sorghum bicolor to Wistar rats. Another study by Senou et al. [52] revealed no deleterious effect on the liver, kidney, and hematological profile following acute administration and observation for 14 days.

While the above studies have established the safety profile of the aforementioned individual components of this PSCR, there is an absence of safety data on it as a composite polyherbal remedy, which prompted this study to investigate its toxicity profile.

4.1 Effects of PSCR on body weight, organ weight, behavior, feed, and water intake

From **Table 1**, there was a progressive increase in body weight from week 0 through week 4. There was no statistically significant alteration ($^{ns}p>0.05$) in body weight gain of the test groups when compared with the control group. There was no statistically significant alteration ($^{ns}p>0.05$) in liver, kidney, heart, pancreas, testes, spleen, and lung weight of test groups when compared with the control group.

Table 1. Effects of PSCR on body weight, organ weight, feed, and water intake.

Parameter	Control (A)	Low dose (B)	Medium dose (C)	High dose (D)
Body weight, week 0 (g)	201.00 ± 22.31	209.00 ± 17.12	188.50 ± 10.95	204.17 ± 18.86
Body weight, week 1 (g)	230.83 ± 33.33	223.67 ± 18.40	212.33 ± 9.09	209.67 ± 18.45
Body weight, week 2 (g)	234.50 ± 24.24	236.50 ± 19.71	210.33 ± 13.03	232.67 ±18.80
Body weight, week 3 (g)	246.50 ± 26.43	242.50 ± 22.69	220.17 ± 14.11	242.17 ± 20.01
Body weight, week 4 (g)	256.33 ± 28.43	255.83 ± 25.28	231.17 ± 14.11	262.17 ± 21.85
Final body weight (g)	247.33 ± 30.32	245.33 ± 24.69	224.33 ± 14.56	252.33 ± 21.45
Weight gain (%)	21.59 ± 1.10	18.14 ± 3.35^{ns}	18.38 ± 3.63^{ns}	22.16 ± 2.14^{ns}
Liver weight (%)	3.22 ± 0.18	3.01 ± 0.10^{ns}	3.10 ± 0.14^{ns}	3.12 ± 0.18^{ns}
Kidney weight (%)	0.64 ± 0.35	0.61 ± 0.06^{ns}	0.62 ± 0.04^{ns}	0.62 ± 0.06^{ns}
Heart weight (%)	0.39 ± 0.04	0.41 ± 0.06^{ns}	0.46 ± 0.06^{ns}	0.46 ± 0.06^{ns}
Lung (%)	0.56 ± 0.08	0.69 ± 0.25^{ns}	0.59 ± 0.09^{ns}	0.53 ± 0.05^{ns}
Testes (%)	1.23 ± 0.23	1.25 ± .0.12^{ns}	1.13 ± 0.17^{ns}	1.20 ± 0.19^{ns}
Spleen (%)	0.33 ± 0.04	0.32 ± 0.06^{ns}	0.33 ± 0.07^{ns}	0.31 ± 0.04^{ns}
Stomach (%)	1.12 ± 0.25	0.95 ± 0.19^{ns}	0.99 ± 0.22^{ns}	0.95 ± 0.09^{ns}
Pancrease (%)	0.23 ± 0.08	0.21 ± 0.08^{ns}	0.27 ± 0.10^{ns}	0.20 ± 0.07^{ns}
Total feed-intake	4595	4501	4624	4590
Total water-intake	7140	6595	6525	6935
Behavioral changes and death	None	None	None	None

Values are presented as mean ± standard deviation (n=6). ^{ns}P>0.05: Not statistically significantly different from the control group. Control (10 ml/kg of vehicle). **Low dose** (2.07 ml/kg, half-therapeutic dose of PSCR). **Medium dose** (4.13 ml/kg, therapeutic dose of PSCR). **High dose** (8.26 ml/kg, double-therapeutic dose of PSCR).

Feed and water intake did not vary much among groups, and were not dose-dependent when compared with those in the control group. The behavior of animals in the test groups was normal and similar when compared to the control group. There was no obvious sign of toxicity and death among the test and control groups.

Body weight and organ weights, as well as feed and water intakes, are indices of toxicity, especially when appetite regulation, growth, and metabolic function are considered. Non-remarkable alterations in body weight (especially percentage weight gain), feed, and water intake in **Table 1** suggest that the PSCR lacks the potential to cause appetite suppression, nutrient malabsorption, and obesity. Non-significant changes in organ weights are suggestive of non-hypertrophy or non-hyperplasia or absence of remarkable damage to the functionality of vital organs [53].

4.2 Effects of PSCR on electrolytes, renal function, liver function, lipid profile, and oxidative stress parameters

From **Table 2**, there was no statistically significant alteration ($p > 0.05$) in sodium, potassium, and calcium levels of the test groups when compared with the control group. However, there was a statistically significant increase ($^{*}p<0.05$) in the chloride level of the high dose when compared to the control group.

There was no statistically significant alteration ($^{ns}p>0.05$) in the urea and creatinine levels of the test groups when compared with the control group. There was no statistically significant alteration ($^{ns}p>0.05$) in ALT, AST, total protein, and albumin (except ALP) levels of test groups when compared with the control group. There was no statistically significant alteration ($^{ns}p>0.05$) in total cholesterol, HDL-cholesterol, or LDL-cholesterol, except for the triglyceride levels of the test groups when compared with the control group. There was no statistically significant alteration ($^{ns}p>0.05$) in MDA, catalase, SOD, total cholesterol, HDL-cholesterol, LDL-cholesterol, except for triglyceride levels that were statistically reduced when compared with the control group. There was no statistically significant alteration ($^{ns}p>0.05$) in serum glucose of the test groups when compared with the control group.

Table 2. Effects of PSCR on electrolytes, renal function, liver function, lipid profile, and oxidative stress parameters.

Parameter	Control (A)	Low dose (B)	Medium dose (C)	High dose (D)
ELECTROLYTES				
Sodium (mEq/L)	170.60 ± 5.65	165.74 ± 4.36^{ns}	164.82 ± 11.03^{ns}	163.19 ± 3.37^{ns}
Potassium (mEq/L)	3.34 ± 2.61	4.31 ± 1.90^{ns}	3.40 ± 2.63^{ns}	5.60 ± 3.78^{ns}
Chloride (mEq/L)	97.31 ± 8.32	92.43 ± 9.16^{ns}	107.41 ± 13.30^{ns}	123.91 ± 26.12*
Calcium (mg/dl)	4.35 ± 0.46	4.16 ± 0.28^{ns}	4.17 ± 0.95^{ns}	5.63 ± 1.40^{ns}
RENAL BIOMARKERS				
Urea (mg/dl)	37.50 ± 5.27	43.75 ± 8.64^{ns}	37.50 ± 8.33^{ns}	33.35 ± 9.50^{ns}
Creatinine (mg/dl)	0.89 ± 0.18	0.81 ± 0.07^{ns}	0.84 ± 0.16^{ns}	0.84 ± 0.07^{ns}
LIVER BIOMARKERS				
ALT (U/L)	3.71 ± 0.78^{ns}	3.25 ± 0.78^{ns}	4.61 ± 1.33^{ns}	4.08 ± 0.79^{ns}
AST (U/L)	13.36 ± 2.73	11.32 ± 1.48^{ns}	13.53 ± 2.87^{ns}	9.98 ± 1.96^{ns}
ALP (IU/L)	6.98 ± 2.97	7.72 ± 1.60^{ns}	20.06 ± 9.08*	11.78 ± 5.05^{ns}
Total protein (g/dl)	7.88 ± 0.66	8.07 ± 0.46^{ns}	8.19 ± 0.43^{ns}	8.19 ± 0.43^{ns}
Albumin (mg/dl)	4.56 ± 0.84	4.57 ± 0.22^{ns}	4.52 ± 0.27^{ns}	4.79 ± 0.16^{ns}
LIPID PROFILE				
Total cholesterol (mg/dl)	160.42 ± 29.96	135.42 ± 21.53^{ns}	154.17 ± 40.05^{ns}	164.58 ± 38.26^{ns}
Triglyceride (mg/dl)	142.51 ± 8.48	131.88 ± 4.00*	128.02 ± 4.99*	134.30 ± 6.26^{ns}
HDL-Cholesterol (mg/dl)	70.84 ± 4.27	70.32 ± 3.28^{ns}	75.00 ± 15.31^{ns}	80.21 ± 20.89^{ns}
LDL-Cholesterol (mg/dl)	61.08 ± 27.75	38.73 ± 20.85^{ns}	53.56 ± 27.29^{ns}	57.52 ± 34.96^{ns}
OXIDATIVE STRESS PARAMETERS				
SOD(U/mL)	1.55 ± 1.39	2.83 ± 0.51*	2.56 ± 0.27*	2.78 ± 0.40*
Catalase (ukat)	0.96 ± 0.70	1.24 ± 0.46^{ns}	1.15 ± 0.42^{ns}	1.11 ± 0.56^{ns}
MDA (nmol/mL)	3.99 ± 0.91	4.15 ± 0.15^{ns}	3.99 ± 0.19^{ns}	3.98 ± 0.13^{ns}
GSH (µg/ml)	40.03 ± 15.84	62.00 ± 22.62^{ns}	52.71 ± 12.32^{ns}	41.76 ± 23.38^{ns}
		SERUM GLUCOSE		
Serum glucose (mg/dl)	69.39 ± 38.76	48.26 ± 30.85^{ns}	71.02 ± 30.07^{ns}	107.08 ± 46.98^{ns}

Values are presented as mean ± standard deviation (n=6). ^{ns}P>0.05: Not statistically significantly different from the control group. *P<0.05: Statistically significantly different from the control group. Control (10 ml/kg of vehicle). **Low dose** (2.07 ml/kg, half-therapeutic dose of PSCR). **Medium dose** (4.13 ml/kg, therapeutic dose of PSCR). **High dose** (8.26 ml/kg, double-therapeutic dose of PSCR).

From **Table 2**, most of the biochemical parameter values of animals that received the PSCR did not show significant alterations when compared to the control, except for chloride, triglyceride, ALP, lipid profile, and superoxide dismutase. The liver and kidney are target organs of toxicity, which prompted their selection for this assessment. While the liver metabolizes foreign/toxic agents, the kidneys excrete/eliminate waste products. A significant elevation in renal and hepatic biomarkers, in correlation with their histology report, helps to monitor the extent of xenobiotics on them [54]. Electrolytes such as sodium, potassium, and calcium play a crucial role in regulating the kidneys and heart effectively. Thus, alteration in the level of these electrolytes may indicate improper hydration, irregularity in osmotic pressure, and acid-base balance in the kidney. Although low chloride is associated with extensive burn, intestinal obstruction, vomiting, and nephritis, on the other hand, high serum chloride is common with dehydration and connective heart valve alteration [55]. Non-statistically significant alterations in urea and creatinine levels, as well as normal kidney histo-architecture (**Fig.1**), suggest the non-deleterious impact of the PSCR on the kidney. However, a statistically significant increase in chloride level at the high dose suggests that the test animals have adapted due to electrolyte imbalance when the PSCR is taken beyond the recommended dose and duration.

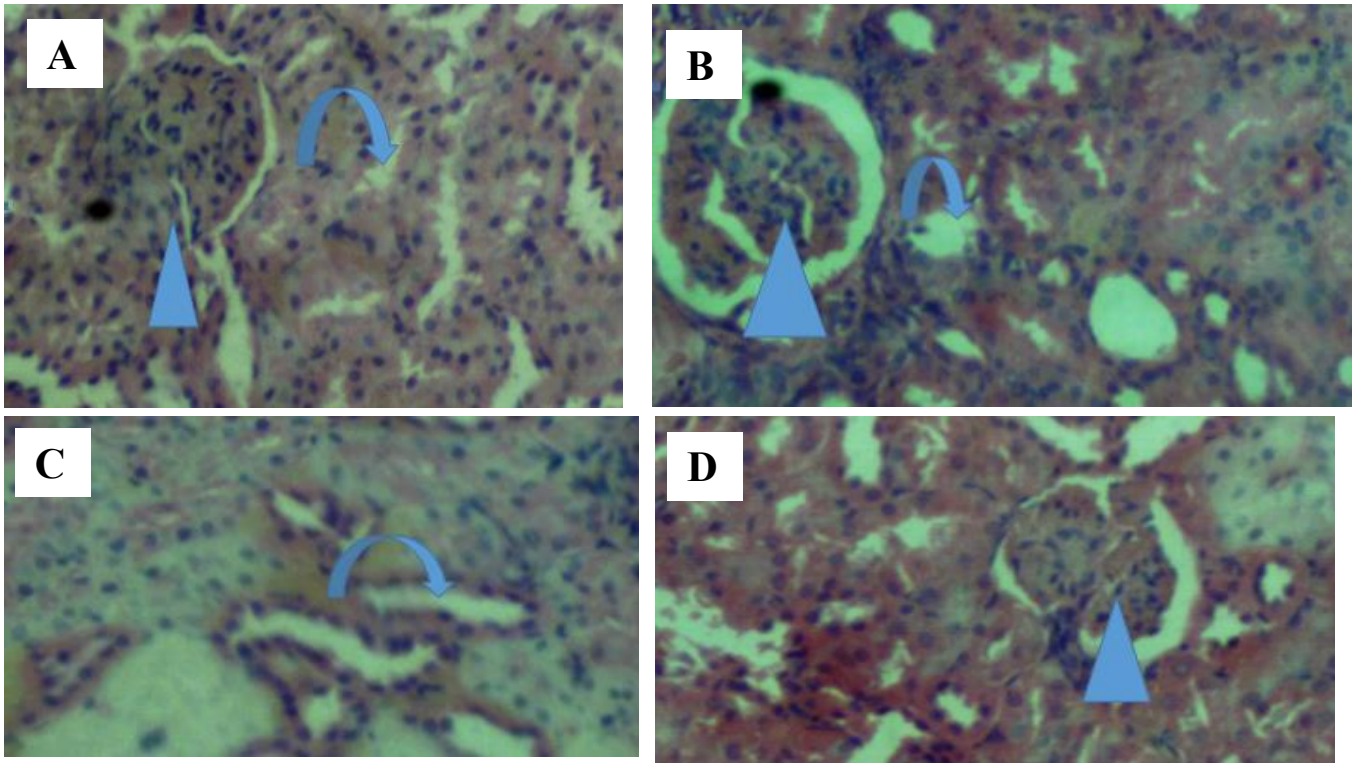

Figure 1. Section of kidney tissue of albino rat shows histology consistent with normal kidney morphology. The Renal capsules (arrow head) and tubules (curved arrow) are normal with no sign of injury (H& E X400). Plages A,B,C and D showing Control, Low, Medium and High doses (respectively) of herbal remedy.

ALT, AST, and ALP are widely used as sensitive markers to evaluate toxic manifestations of liver toxicity. While ALT is a cytoplasmic enzyme present in the liver, AST is also found in the heart, skeletal muscles, as well as in the liver. Non-significant changes in ALT and AST, total protein, and albumin at three doses of the herbal formulation suggest the absence of hepatocellular damage. An exception to this was a significant elevation in ALP level, which is suggestive of hepatic congestion, as substantiated by high-dose liver histopathology (**Fig. 2**).

Lipid profile is a biomarker of cardiovascular disease. From the study, non-statistically significant alterations in total cholesterol, HDL-cholesterol, and LDL-cholesterol, and a statistically significant reduction in triglycerides, suggest that the tested doses and duration of the PSCR do not produce lipid dysfunction. Mild congestion in cardiac muscles observed at high dose (**Fig. 3**) suggests a non-deleterious impact of the PSCR on the heart function. Non-statistically significant alteration in serum glucose level suggests the ineffectiveness of the herbal remedy to cause metabolic disorders concerning blood glucose regulation. This is substantiated by the pancreas of animals in test groups showing consistent architecture with normal pancreas histology (**Fig. 4**).

oxidative stress parameters help to monitor the potential of xenobiotics to cause oxidative damage. SOD is a unique antioxidant enzyme that inhibits superoxide radicals and prevents tissue damage [56]. Statistically significant elevation in SOD and non-statistically significant alteration in catalase, MDA, and GSH suggest that intake of PSCR at the experimented doses and duration has no deleterious impact on the antioxidant system. Higher SOD level also suggests better defense against oxidative stress mediated by free radicals [57].

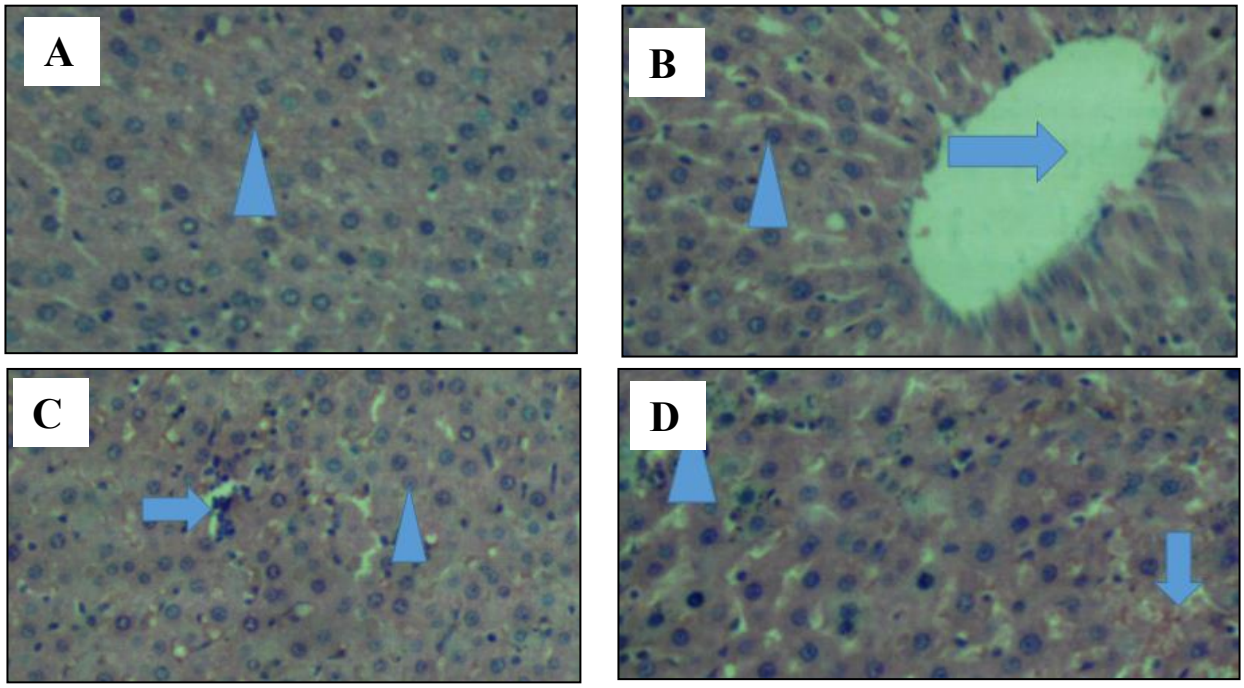

Figure 2. Photomicrograph of lilver section showing morpholotgy consistent with normal liver histology. The hepatocytes (arrow head) are normal with no obvious sign of injury (H&E X400). Photomicrograph of liver section in group D shows morphology with modrate diffuse infiltration of inflammatory cells (arrow head) and mild degenerative changes (arrow) (H & E X400). Plates A, B, C and D showing Control, Low, Medium and High doses (respectively) of herbal remedy.

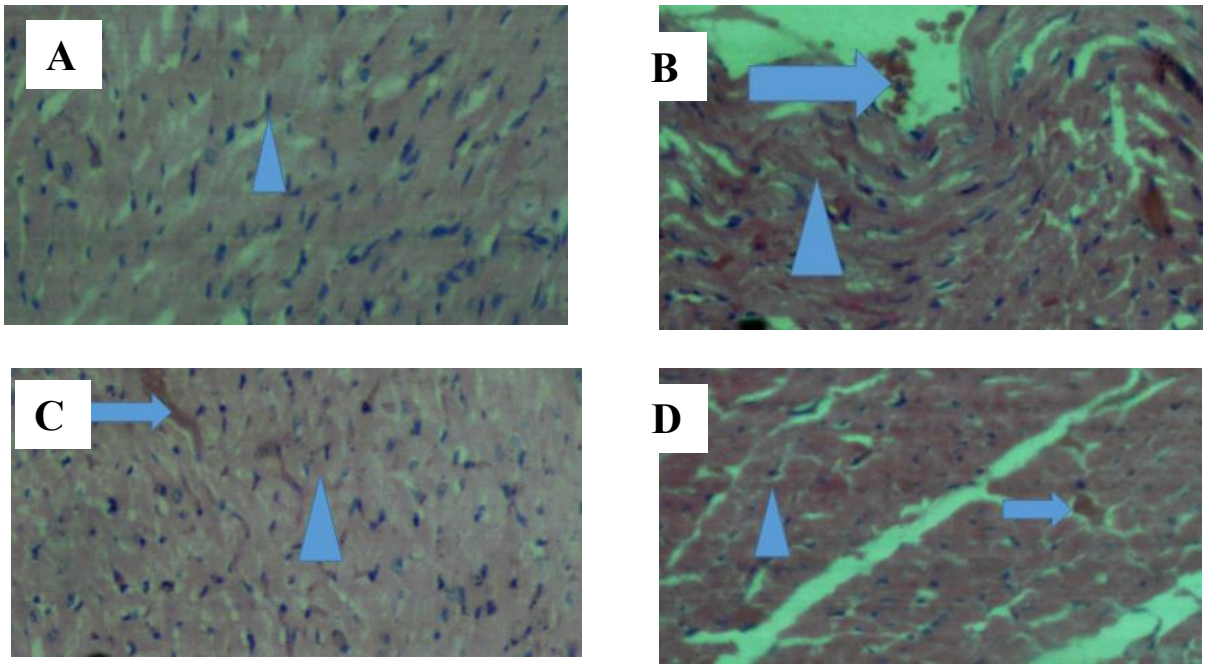

Figure 3. A section of heart tissue of shows normal morphology consistent with heart histology; the cardiac muscles, fibres and cells (arrowhead) appear normal with no area of muscular injury or cellular alteration. However, the cardiac muscles, fibres and cells (arrowhead) of high dose (D) appear normal but with mild sign of congestion (arrow) (x400 H&E). Plates **A, B**, **C**, and **D** showing Control, Low, Medium and High doses (respectively) of herbal remedy.

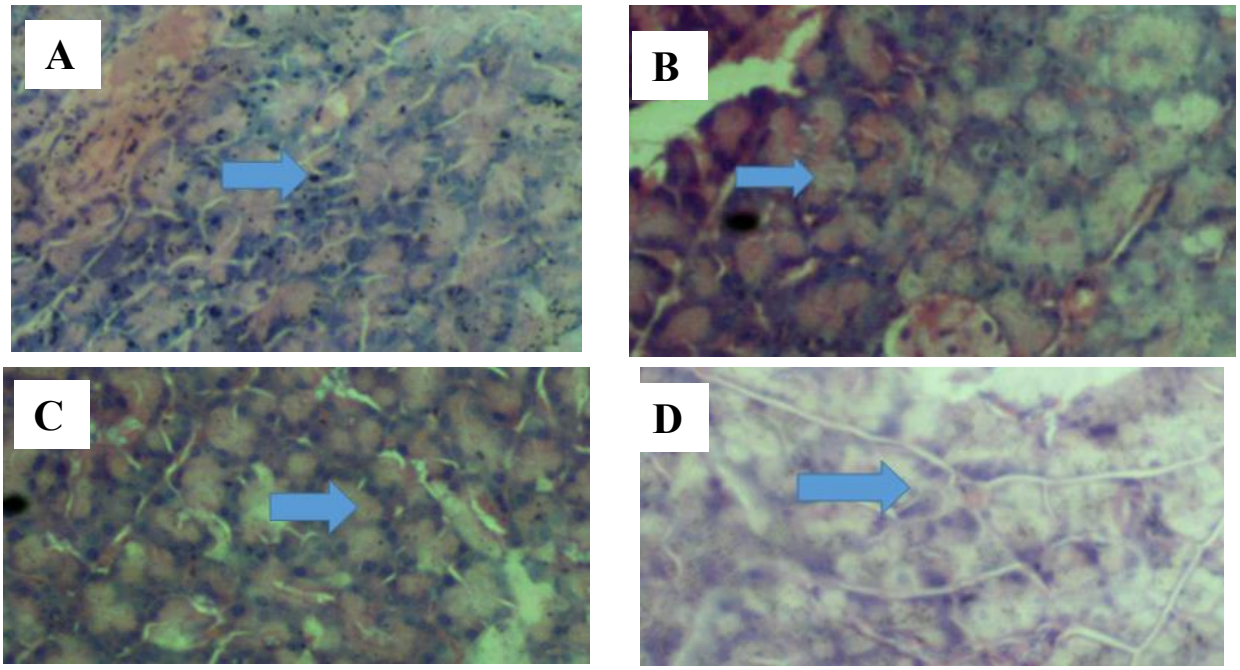

Figure. 4. Photomicrograph of pancreas of albino shows architecture consistent with normal pancreas histology. The islets of langerhans with the beta cells (arrow) show normal histology (H&E X 400). Plates **A, B**, **C**, and **D** showing Control, Low, Medium and High doses (respectively) of herbal remedy.

4.3. Effects of PSCR on semen and serum testosterone

There was no statistically significant alteration ($^{ns}p>0.05$) in semen count and morphology of the test groups when compared with the control group. However, there was a statistically significant reduction ($p<0.05$) in the number of sluggish spermatozoa of the test groups (low and high doses) when compared with the control group. The testosterone level of animals exposed to test doses of the herbal remedy was not statistically significantly different from that of the control group (**Table 3**).

In this study, semen quality and testosterone level were also assessed to evaluate the impact of PSCR on male reproductive function. From **table 3**, non-statistically significant alteration ($^{ns}p>0.05$) in semen count and morphology, significant reduction ($^{*}p<0.05$) in the number of sluggish spermatozoa of test groups (low and high doses), as well as non-significant changes in testosterone level of animals, suggest the non-deleterious impact of LHR on semen function and male reproductive performance. This is corroborated by normal testicular histoarchitecture, characterized by the absence of distortion (**Fig. 5**).

4.4. Effects of PSCR on haematology parameters

From **Table 4**, There was no statistically significant alteration ($p > 0.05$) in hematological parameters, except for WBC, which was statistically significantly reduced ($^{*}p < 0.05$) when compared with the control group.

Measurement of haematological parameters helps to understand the impact of xenobiotics on blood physiology [58]. From this present study, non-statistically significant alterations in haematological parameters (except for a significant reduction in WBC at high dose) suggest that the PSCR has no deleterious impact on red blood and platelet function at the experimented doses and duration. WBCs play a key role in the immune system by fighting against infections and other foreign substances. While the level of WBC has been reported to be elevated during infections and toxic substances [59, 60], a significant reduction in WBC at high dose suggests that the PSCR did not exhibit toxic effects on the immune system that could pose an infectious risk when administered at double the therapeutic dose.

It is worthy of note that herbal remedies with two or multiple medicinal plants/ingredients could exert

antagonistic, synergistic, or non-toxic effects as a result of the effects of some components to nullify the toxicity of other components [61, 62]. Thus, the biochemical, haematological, and histological changes observed could be attributed to the various constituents in the herbal formulation.

Conclusion:

The fact that herbal formulations are natural does not negate the need for safety caution, and blindly considering them to be safe without thorough scientific consideration. The present study evaluated the toxicity profile of a PSCR using established standard guidelines, and it revealed non-remarkable changes in various parameters at therapeutic doses, except for minor changes at a high dose (double of the therapeutic dose). Having reviewed the results of this present study, we conclude that the PSCR did not elicit adverse effects on vital organs or reproductive and haematological parameters at therapeutic doses in Wistar rats. However, alterations observed at higher doses suggest the need for precautionary measures, including monitoring of hepatic and electrolyte levels in subjects using the PSCR above the recommended doses and duration.

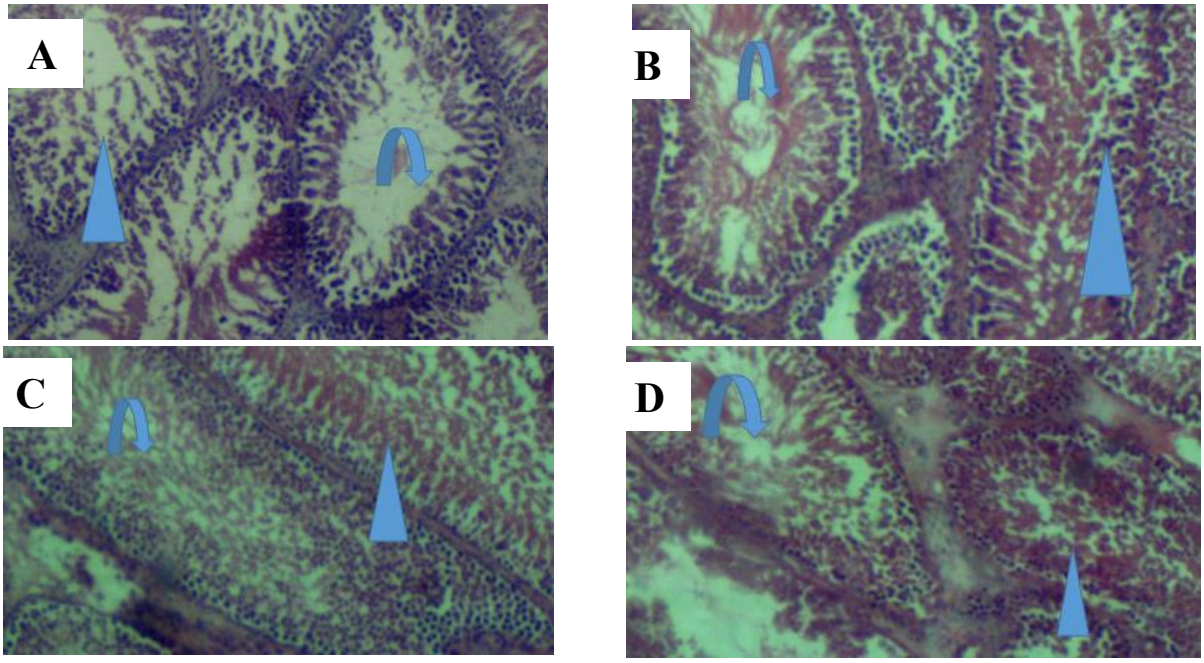

Fig. 5: Photomicrograph of testes sections show morphology consistent with testicular histology. The ductus epididimys (curved arrow) and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (H&Ex400). Plates **A, B**, **C**, and **D** showing Control, Low, Medium and High doses (respectively) of herbal remedy.

Table 3. Effects of PSCR on semen and serum testosterone.

Parameter	Control (A)	Low dose (B)	Medium dose (C)	High dose (D)
Non-motile (%)	25.83 ± 23.33	7.17 ± 2.04^{ns}	13.17 ± 5.49^{ns}	16.83 ± 13.53^{ns}
Sluggish (%)	17.00 ± 3.46	3.83 ± 2.40*	11.33 ± 9.09^{ns}	8.33 ± 4.08*
Motile (%)	57.17 ± 25.06	80.00 ± 22.29^{ns}	69.50 ± 17.25^{ns}	76.17 ± 15.01^{ns}
Count	37.67 ± 20.77	31.67 ± 9.83^{ns}	26.50 ± 11.59^{ns}	30.50 ± 12.14^{ns}
Single head (%)	91.33 ± 4.72	96.33 ± 2.07^{ns}	89.83 ± 8.08^{ns}	96.17 ± 3.31^{ns}
Double head (%)	8.67 ± 4.72	3.67 ± 2.07^{ns}	10.17 ± 8.08^{ns}	8.33 ± 11.11^{ns}
Normal morphology (%)	88.50 ± 7.82	96.00 ± 1.55^{ns}	89.83 ± 11.10^{ns}	93.00 ± 4.38^{ns}
Abnormal morphology (%)	11.50 ± 7.82	4.17 ± 1.33^{ns}	10.17 ± 11.09^{ns}	7.00 ± 4.38^{ns}
Testosterone (ng/dl)	4.86 ± 1.36	4.33 ± 1.41^{ns}	4.69 ± 1.45^{ns}	4.70 ± 1.08^{ns}

Values are presented as mean ± standard deviation (n=6). ^{ns}P>0.05: Not statistically significantly different from the control group. *P<0.05: Statistically significantly different from the control group. Control (10 ml/kg of vehicle). **Low dose** (2.07 ml/kg, half-therapeutic dose of PSCR). **Medium dose** (4.13 ml/kg, therapeutic dose of PSCR). **High dose** (8.26 ml/kg, double-therapeutic dose of PSCR).

Table 4. Effects of PSCR on haematology parameters

Parameter	Control (A)	Low dose (B)	Medium dose (C)	High dose (D)
WBC **(10^9/L)**	17.72 ± 4.34	13.01 ± 1.35^{ns}	13.91 ± 5.19^{ns}	11.59 ±1.35*
Lymphocytes (%)	69.78 ± 31.24	86.93 ± 3.31^{ns}	86.68 ± 3.82^{ns}	88.40 ±2.99^{ns}
MID (%)	8.38 ± 2.73	7.15 ± 1.37^{ns}	6.83 ± 1.46^{ns}	6.32 ± 1.49^{ns}
GRAN (%)	8.50 ± 5.98	5.92 ± 1.97^{ns}	5.48 ± 2.26^{ns}	5.28 ± 1.51^{ns}
RBC **(10^{12}/L)**	8.73 ± 0.80	8.67 ± 0.87^{ns}	8.17 ± 1.19^{ns}	9.06 ± 0.94^{ns}
HGB (g/dl)	15.03 ± 1.80	14.62 ± 1.44^{ns}	13.52 ± 2.09^{ns}	15.62 ± 1.77^{ns}
HCT (%)	44.33 ± 4.21	43.58 ± 4.24 \$^{ns}$	40.62 ± 5.65^{ns}	48.28 ± 3.98^{ns}
MCV (fL)	50.92 ± 2.56	50.28 ± 0.93^{ns}	49.82 ± 0.79^{ns}	52.83 ± 1.42^{ns}
MCH (pg)	17.03 ± 0.87	16.82 ± 0.38^{ns}	16.52 ± 0.33^{ns}	17.17 ± 0.34^{ns}
MCHC (g/dl)	33.53 ± 0.88	30.15 ± 8.23^{ns}	33.25 ± 0.86^{ns}	32.62 ± 1.31^{ns}
RDW-CV (%)	15.25 ± 1.34	14.57 ± 1.53^{ns}	15.13 ± 0.96^{ns}	14.98 ± 1.35^{ns}
RDW-SD (fL)	28.45 ± 3.12	28.02 ± 1.49^{ns}	27.65 ± 2.31^{ns}	29.97 ± 2.31^{ns}
PLT (10^9/L)	599.33 ± 57.20	602.33 ± 77.44^{ns}	613.00 ± 105.32^{ns}	618.50 ± 99.98^{ns}
MPV (fL)	6.43 ± 0.25	6.33 ± 0.27^{ns}	6.23 ± 0.22^{ns}	6.50 ± 0.33^{ns}
PDW (fL)	13.33 ± 0.63	13.08 ± 0.63^{ns}	12.97 ± 0.67^{ns}	13.00 ± 0.55^{ns}
PCT (%)	0.38 ± 0.03	0.38 ± 0.05^{ns}	0.38 ± 0.06^{ns}	0.40 ± 0.07^{ns}
PLCR (%)	1.89 ± 0.20	1.81 ± 0.20^{ns}	1.74 ± 0.20^{ns}	1.93 ± 0.23^{ns}
PLCC (10^9/L)	88.83 ± 5.12	86.17 ± 12.16^{ns}	84.33 ± 14.07^{ns}	94.67 ± 20.46^{ns}

Values are presented as mean ± standard deviation (n=6). ^{ns}P>0.05: Not statistically significantly different from the control group. *P<0.05: Statistically significantly different from the control group. Control (10 ml/kg of vehicle). **Low dose** (2.07 ml/kg, half-therapeutic dose of PSCR). **Medium dose** (4.13 ml/kg, therapeutic dose of PSCR). **High dose** (8.26 ml/kg, double-therapeutic dose of PSCR).

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Conflict of interest

No conflict of interest exists among other institutions/agencies.

Data availability

Upon request, the dataset used in this study will be made available via the corresponding author.

Authors Contributions

E.O.E conceived the research, N.U.M., E.S.I., E.A.N. T.C.A., C.F.C., O.C.E., C.E.O., E.J.A., A.U.O., G.O.U., E.U.O and E.O.E carried out the bench work. E.O.E conducted the data analysis and wrote the manuscript draft. All authors revised and approved the final manuscript for publications.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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