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Original Research Article

Acute and Repeated Toxicity Assessment of a Stem Cell Polyherbal Remedy (PHR)

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Abstract:

Several polyherbal remedies (PHR) are used in the treatment of various diseases, but there is dearth of information about their safety. This study assessed the toxicological impact of a PHR on haematological, biochemical and organs histology. Acute toxicity test was done using the Up and Down procedure. In repeated toxicity test, the PHR was administered to the test animals intraperitoneally at low dose (258.3 mg/kg), medium dose (516.6 mg/kg) and high dose (1033.3 mg/kg). The control group received the vehicle (5 ml/kg). At the end of administration, animals were fasted overnight, sacrificed and samples were collected for haematological, biochemical and histological analyses using standard methods. Acute toxicity test revealed no death at 5000 mg/kg (an indication that LD_{50}>5000 mg/kg). There was no significant alteration ($p>0.05$) in body weight and organs weight, except significant ($p<0.05$) increase in liver and kidney weight at medium dose. There was a significant reduction in hemoglobin at the three dose levels, and significant increase in platelet count at medium and high doses. The PHR posed no deleterious effects on lipid profile, antioxidant parameters, electrolytes, blood glucose, body weight, and histology of the liver, heart, pancreas, spleen, testes, and lung. Only slight distortion in kidney histology was observed at high dose when compared to control group. The results of the study revealed that the PHR is safe following single dose administration, but double of therapeutic dose, as well as repeated administration above the 2 weeks may cause platelet aggregation.

Keywords: Hepatic, Haematology, Histopathology, Herbal remedy, Renal, safety.

Introduction

Herbal medicine, one of the aspects of complementary medicine is an integral component of African traditional medicine, which remains one of the most popular medical systems in use today.This is substantiated by significant rise in the use of herbal remedies in the treatment of various ailments by approximately 80% of the world's population, which also serves as sources of lead compounds in the development of new drugs [1]. Besides the popularity of herbal medicine is significant rise in the use of polyherbal remedies in the last few decades, where some herbal products being marketed in most countries lack reliable safety scientific evidence [2].Although some herbal medicines have been claimed to be highly safe and without adverse effect, it is important to give relevance to safety principle proclaimed by Paracelsus that "all drugs are poisons, there is none that is not a poison, but the dose differentiates a poison from a remedy" [3], which calls for safety assessment of virtually every drug during pre-clinical studies to identify any potential toxicities, and also predicts the most relevant toxicities to be monitored in clinical trials.Without doubt, variety of poorly controlled factors such as raw herb quality, processing methods, potential adulteration when several herbs are combined have stimulated scientific curiosity and interest about safety of herbal preparations. Also, some PHRs are not subjected to review by the Food and Drug Administration (FDA) and other regulatory bodies in some countries [4].This PHR is a stem cell therapy that consists of 13 organic ingredients including; Astaxanthin, Goji berries (Lycium

fruits), Kiwi Fruit, Salmon Ovary Peptide, Afa (Aphanizomenonflos-aquae) extract, Soursop Juice powder, Pomegranate (Punicagranatum L.), Bilberry (*Vaccinium myrtillus*) and Bee Propolis, apple extract, Aquamin, Ashwagandha (Withaniasomnifera, fam. Solanaceae) and Collagen. These ingredients provide high quality nutrition's to the body daily needs, to prevent any damage or diseases. It is useful against inflammatory conditions, infertility, rheumatism, diabetes, hypertension, insomnia, parasitic infections, cancer, oxidative stress underlying the pathogenesis of numerous diseases, among other diseases. Some of the constituents are enriched with vitamins, minerals, and bioactive molecules [5,6]. The PHR consists of 14 sachets of 2.5 g each, which is administered sublingually daily for 14 days. Some social media distributors recommend it should be taken for about two months for effective result to be achieved.

Despite the use this PHR by the populace, there is no information concerning its safety profile. The repeated usage of this PHR further justifies the need to determine its safety profile on various vital organs using animal model. Owing to the fact that some PHR can adversely affect the liver, kidney, heamatology, heart, antioxidant functions and other vital organs, it is crucial to evaluate the effects of this PHR.

Materials and Methods

Materials used in the study include, beakers, measuring cylinders, dissecting kit, micro pipette, Digital weighing balance, Table centrifuge, Spectrophotometer, Chemicals were of analytical grades, and reagents were procured from Teco diagnostic, and randox. The herbal remedy was procured from open market in Onitsha, Anambra State.

Experimental animals

Wistar rats of either sex used for this study were gotten from the animal house facility of the Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus. The animals were allowed to acclimatize for 1 week in the experimental section of the animal facility. They were given water and feed *ad libtum*, and their cages were cleaned every two days by changing the beddings made of rice husk. They were subjected to 12 hours light/darkness cycle. The principles of laboratory animal care were also observed in this study with reference number, PHACOOU/AREC/2022/007 approved by animal research ethics committee, Faculty of Pharmaceutical Science, Chukwuemeka Odumegwu Ojukwu University, Igbariam.

Acute toxicity test

Using the Up and Down procedure as described by Erhirhie et al. [7], six animals were grouped into three; control group comprising of one male and one female rat, and test group comprising of two males and two females rats. A single dose of 5000 mg/kg of the PHR was administered to the test groups while the control group received the vehicle, 5 ml/kg using intraperitoneal route (because the PHR could not be given to the animals sublingually). After administration, observation was carried out for the first 4 hours, 24 hours, 48 hours and daily over 7 days for mortality, behavioral changes, and signs of toxicity.

Repeated Toxicity Test

The sub-acute toxicity test, also classified under the repeated toxicity test is defined as adverse effects occurring after multiple or continuous exposure between 14 and 28 days [8].

Animals were divided into four (4) groups of seven (7) animals each. Control received the vehicle (5 ml/kg) while the test groups received low dose (258.3 mg/kg, half therapeutic dose), medium dose (516.6 mg/kg, therapeutic dose), and high dose (1033.2 mg/kg, double of therapeutic dose). The animal equivalent dose (AED) was derived by multiplying human dose (2500 mg/kg/60 kg/day = 41.67 mg/kg/day) by 6.2 (41.67 mg x 6.2 = 258.33 mg/kg/day) [9]. This study, which was initially designed to last for 21 days was ended on day 19th due to observation of death between day 15th and day 18th at medium and high doses. Thus, after the last administration on day 19th, animals were fasted overnight with free access to water, and were euthanatized with chloroform anesthesia in a closed chamber and were dissected.

With the aid of 5 ml syringe, blood samples were collected from the inferior vena cava and delivered into EDTA and plain tubes for haematology and biochemical analyses respectively. Organs were fixed with 10% formalin solution for histopathological analysis using standards method described by Walsh et al.[10] Midray BC- 2000 Auto Hematology analyzer was used for determination of haematological parameters.

Serum was used for determination of biochemical parameters including; alanine aminotransferase (ALT), aspertate aminotransferase (AST), alkaline phosphatase (ALP), urea, creatinine, total protein, albumin, sodium, potassium and chloride using standard procedures with the commercial kits procured from Randox Laboratories Limited, Country Atrium, United Kingdom and Teco diagnostics, California U.S.A [11]. Weekly blood glucose was determined using Acu-answer glucometer.

Statistical analysis

Results were presented as mean ± standard deviation. Comparison of mean values was carried out using one way analysis of variance (ANOVA) followed by post-hoc dunnet's test. $P<0.05$ was considered to be statistically significant, and $P>0.05$ was considered to be statistically non-significant. Statistical Package for Social Science (SPSS-20, for windows) was the software used for data analyses.

Results

Acute toxicity study revealed no signs of toxicity and death following single intraperitoneal administration of the PHR at 5000 mg/kg. Thus, the LD_{50} was estimated to be >5000 mg/kg.

From table 1, there was statistically significant reduction ($^{*}P<0.05$) in the hemoglobin and a non-significant reductions in red blood cell of the low, medium and high dose compared to the control group. There was no statistically significant difference ($^{ns}P>0.05$) in the WBC, RBC, HCT and MCV of low, medium and high dose compared to the control group.

From table 2, there was statistically significant reduction ($^{*}P<0.05$) in the mean corpuscular hemoglobin of low and medium dose treatment groups compared to the control group. There was no statistically significant difference ($^{ns}P>0.05$) in the MCH of the high dose treatment group as well as the in the MCHC, RDW-CV, RDW-SV of the low, medium and high dose treatment group when compared to the control group. From table 3, there was statistically significant increase ($^{*}P<0.05$) in the platelet (PLT) of the medium and high doses when compared to the control group. There was no statistically significant difference ($^{ns}P>0.05$) in the PLT of low dose treatment group as well as in the MPV and PDW of the low, medium and high dose treatment group when compared to the control group. From table 4, there was a statistically significant increase in ALP of high dose when compared to control group. AST and ALT values in low, medium and high dose were not statistically significantly ($^{ns}p>0.05$) different from control groups. From table 4, administration of the PHR increased ALP level except ALT and AST enzymes levels.

Table 5 depicts the renal function parameters of the animals administered with three doses of the PHR. The result showed a statistically significant decrease in the creatinine level at medium dose only while there was no significant difference ($^{ns}p>0.05$) in the serum urea, total protein, and albumin when compared to the control group.Table 6 shows no significant difference ($^{ns}p>0.05$) in the level of the electrolytes; potassium and sodium from the various treatment groups when compared to the control group.

Although there was no significant difference in the chloride level of low dose and high dose treatment groups, there was a significant difference in the chloride level only at medium dose.

Table 1: Effect of PHR on hematological parameters of Wistar rats

Group	WBC (10^9/L)	HGB (g/dL)	RBC (10^{12}/l)	HCT (%)	MCV (fL)
Control	17.00 ± 3.78	12.95 ± 0.55	6.88 ± 0.11	36.22 ± 1.17	52.75 ± 1.79
Low dose	19.66 ± 2.89^{ns}	11.44 ± 1.50*	6.50 ± 0.59^{ns}	32.24 ± 4.83^{ns}	50.20 ± 2.60^{ns}
Medium dose	18.54 ± 0.48^{ns}	11.54 ± 0.32*	6.56 ± 0.33^{ns}	32.86 ± 0.92^{ns}	50.18 ± 2.29^{ns}
High dose	20.60 ± 0.44^{ns}	11.20 ± 0.618*	6.43 ± 0.48^{ns}	31.83 ± 1.86^{ns}	49.67 ± 3.47^{ns}

Values were presented as mean ± standard deviation, n=6, 5, 5 and 3 in control, low, medium and high doses respectively. $^{ns}P>0.05$: Not statistically significantly different from the control group $^{*}P<0.05$: Statistically significantly different from the control. WBC (White blood cell), HGB (Hemoglobin), RBC (Red blood cell), HCT (Hematocrit), MCV (Mean corpuscular volume).

Table 2: Effect of PHR on hematological parameters of Wistar rats

Group	MCH (pg)	MCHC (g/dL)	RDW CV (%)	RDW SD (fL)
Control	18.77 ± 0.66	35.72 ± 1.50	15.37 ± 0.85	28.45 ± 2.02
Low dose	17.52 ± 0.88*	35.02 ± 0.36^{ns}	16.28 ± 1.31^{ns}	28.56 ± 3.25^{ns}
Medium	17.54 ± 0.75*	35.10 ± 0.61^{ns}	17.12 ± 0.47^{ns}	29.98 ± 0.67^{ns}
High dose	17.40 ± 0.53^{ns}	35.17 ± 0.39^{ns}	14.90 ± 2.93^{ns}	29.93 ± 2.00^{ns}

Values were presented as mean ± standard deviation, n=5 n=6, 5, 5 and 3 in control, low, medium and high doses respectively. $^{ns}P>0.05$: Not statistically significantly different from the control group.$^{*}P<0.05$: Statistically different from the control group. MCH (Mean corpuscular hemoglobin), MCHC (Mean corpuscular hemoglobin concentration), RDW-CV (red cell distribution width - coefficient of variation), RDW-SD (red cell distribution width - standard deviation).

Table 3: Effect of PHR on hematological parameters of Wistar rat

Group	PLT (10^9/L)	MPV (fL)	PDW (fL)	PCT (%)
Control	521.00 ± 61.48	8.33 ± 0.15	14.45 ± 0.23	0.43 ± 0.05
Low dose	641.60 ± 81.72^{ns}	8.48 ± 0.13^{ns}	14.58 ± 0.08^{ns}	0.54 ± 0.07*
Medium dose	668.20 ± 102.03*	8.60 ± 0.43^{ns}	14.42 ± 0.27^{ns}	0.57 ± 0.08*
High dose	774.33 ± 62.66*	8.40 ± 0.26^{ns}	14.50 ± 0.10^{ns}	0.65 ± 0.51*

Values were presented as mean ± standard deviation, n=6, 5, 5 and 3 in control, low, medium and high doses respectively. ^{ns}P>0.05: Not statistically significantly different from the control group.*P<0.05: Statistically different from the control group. PLAT (Platelet), MPV (Mean platelet volume), PCT (Plateletcrit).

Table 4: Effect of PHR on liver enzymes of Wistar rats

Group	AST (U/L)	ALT (U/L)	ALP (IU/L)	Total protein (g/dl)
Control	41.07 ±5.23	7.71 ±2.73	57.20 ±11.74	6.70 ± 0.56
Low dose	40.18 ±9.37^{ns}	7.17 ±3.50^{ns}	73.81 ±31.43^{ns}	7.06 ± 0.87^{ns}
Medium dose	43.19 ±9.85^{ns}	6.72 ±2.97^{ns}	104.35 ±37.74^{ns}	6.34 ± 0.51^{ns}
High dose	44.33 ±0.53^{ns}	8.21 ±1.78^{ns}	126.75 ±38.71*	6.83 ± 0.71^{ns}

Values were presented as mean ± standard deviation, n=6, 5, 5 and 3 in control, low, medium and high doses respectively. ^{ns}P>0.05; not statistically significantly different from control group. *P<0.05; statistically significant difference from control group. AST (Aspertate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase).

Table 5: Effect of PHR on renal function and electrolytes on Wistar rats

Group	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)	Chloride (mEq/L)	Potassium (mEq/L)	Sodium (mEq/L)
Control	28.00 ± 7.50	2.10 ± 0.15	3.42 ± 0.19	68.64 ± 16.73	7.55 ± 1.60	121.68 ± 19.82
Low dose	26.90 ± 6.31^{ns}	1.66 ± 0.50^{ns}	3.00 ± 0.60^{ns}	85.69 ± 9.10^{ns}	10.83 ± 0.64^{ns}	115.44 ± 27.74^{ns}
Medium dose	27.63 ± 6.17^{ns}	1.40 ± 0.53*	3.10 ± 0.47^{ns}	90.97 ± 6.61*	8.33 ± 4.43^{ns}	99.44 ± 43.63^{ns}
High dose	37.50 ± 8.60^{ns}	1.50 ± 0.21^{ns}	3.30 ± 0.36^{ns}	76.85 ± 14.29^{ns}	8.66 ± 1.66^{ns}	104.17 ± 16.32^{ns}

Values were presented as mean ± standard deviation. n=6, 5, 5 and 3 in control, low, medium and high doses respectively. ^{ns}P>0.05; not statistically significantly different from control group. *p<0.05: Creatinine was statistically significantly different from control group.

Table 6: Effect of PHR on blood glucose of Wistar rats

	Blood glucose (mg/dl)			
Group	Week 0	Week 1	Week 2	Week 3
Control	97.50 ± 7.99	103.00 ± 10.31	96.50 ± 27.81	72.16 ± 11.75
Low dose	97.40 ± 6.84	101.00 ± 9.61^{ns}	93.20 ± 8.04^{ns}	73.60 ± 12.17^{ns}
Medium dose	100.00 ± 10.97	105.40 ± 5.12^{ns}	90.40 ± 14.50^{ns}	74.20 ± 38.46^{ns}
High dose	91.66 ± 16.04	101.66 ± 21.93^{ns}	115.00 ± 7.81^{ns}	84.66 ± 4.72^{ns}

Values were presented as mean ± standard deviation. n=6, 5, 5 and 3 in control, low, medium and high doses respectively. ^{ns}P>0.05: Blood glucose not statistically significantly different from control group.

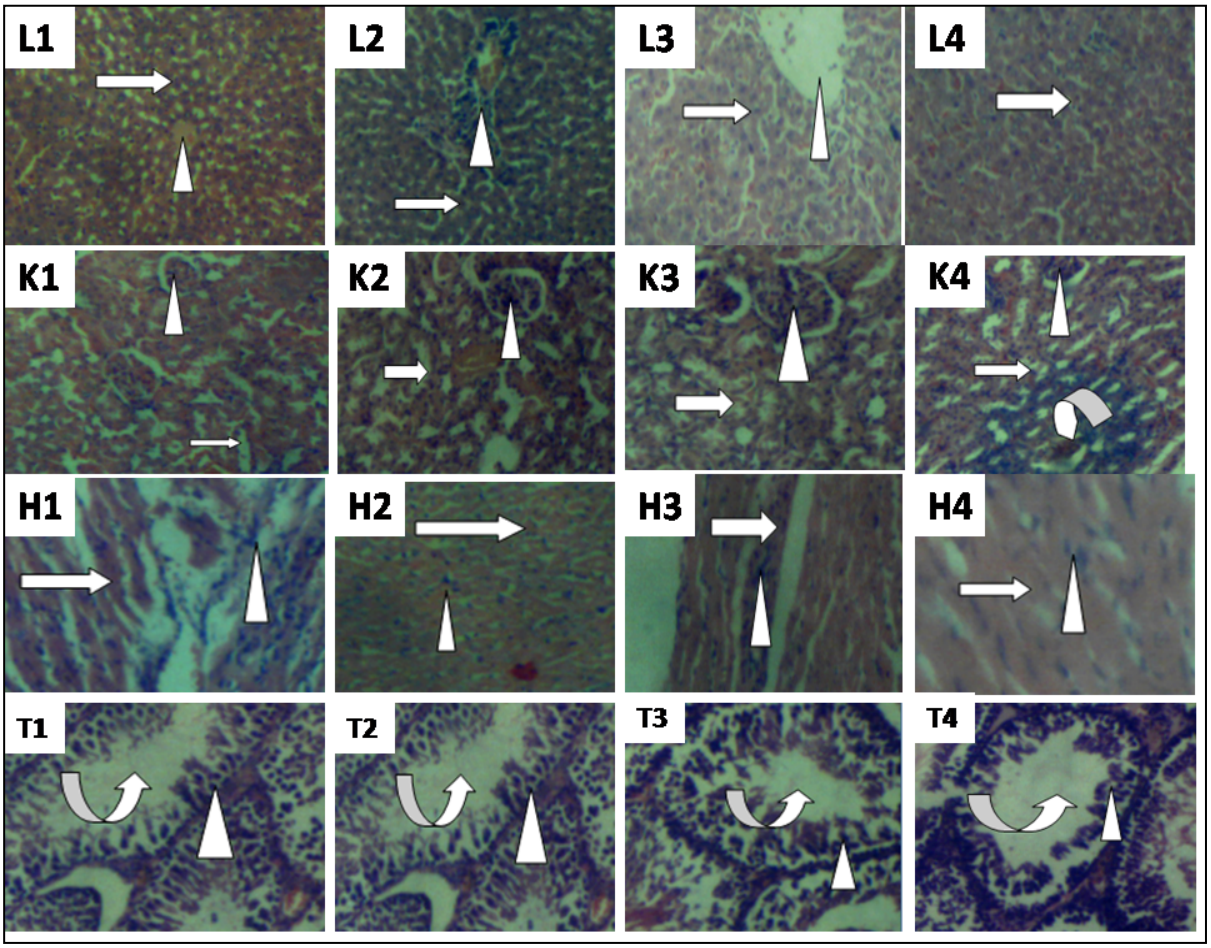

Figure 1: Histology results for Liver (L1, L2, L3, and L4), Kidney (K1, K2, K3, and K4), Heart (H1, H2, H3 and H4) and Testes (T1, T2, T3, and T4)

Liver (L1, L2, L3, L4): Photomicrograph of liver tissue of control, low, medium and high doses respectively showing morphology consistent with normal liver histology. The central vein, (arrowhead) and hepatocytes (arrow) are normal with no obvious sign of injury (H&E X 100).

Kidney (K1, K2, K3, K4): Photomicrograph of Kidney tissue of control, low, medium and high doses respectively showing kidney histology consistent with normal morphology. The Renal capsules (arrowhead) and the tubules (arrow) are normal with no sign of injury (H&E, x100). K4 showed normal morphology but with a focal unremarkable lymphocytic infiltration (curved arrow). The Renal capsules (arrowhead) and the tubules (arrow) are normal with no sign of injury (H&E, x100).

Heart (H1, H2, H3, H4): Photomicrograph of heart tissue of control, low, medium and high dose respectively showing normal morphology consistent with heart histology; the cardiac muscles (arrow), fibres and cells (arrowhead) appear normal with no area of muscular injury or cellular alteration (x100 H&E).

Testes (T1, T2, T3, T4):

Photomicrograph of control, low, medium and high dose respectively testes showing morphology consistent with normal testicular histology. The ductus epididimys (curved arrow) and the connective tissue (CN) are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (H&Ex100).

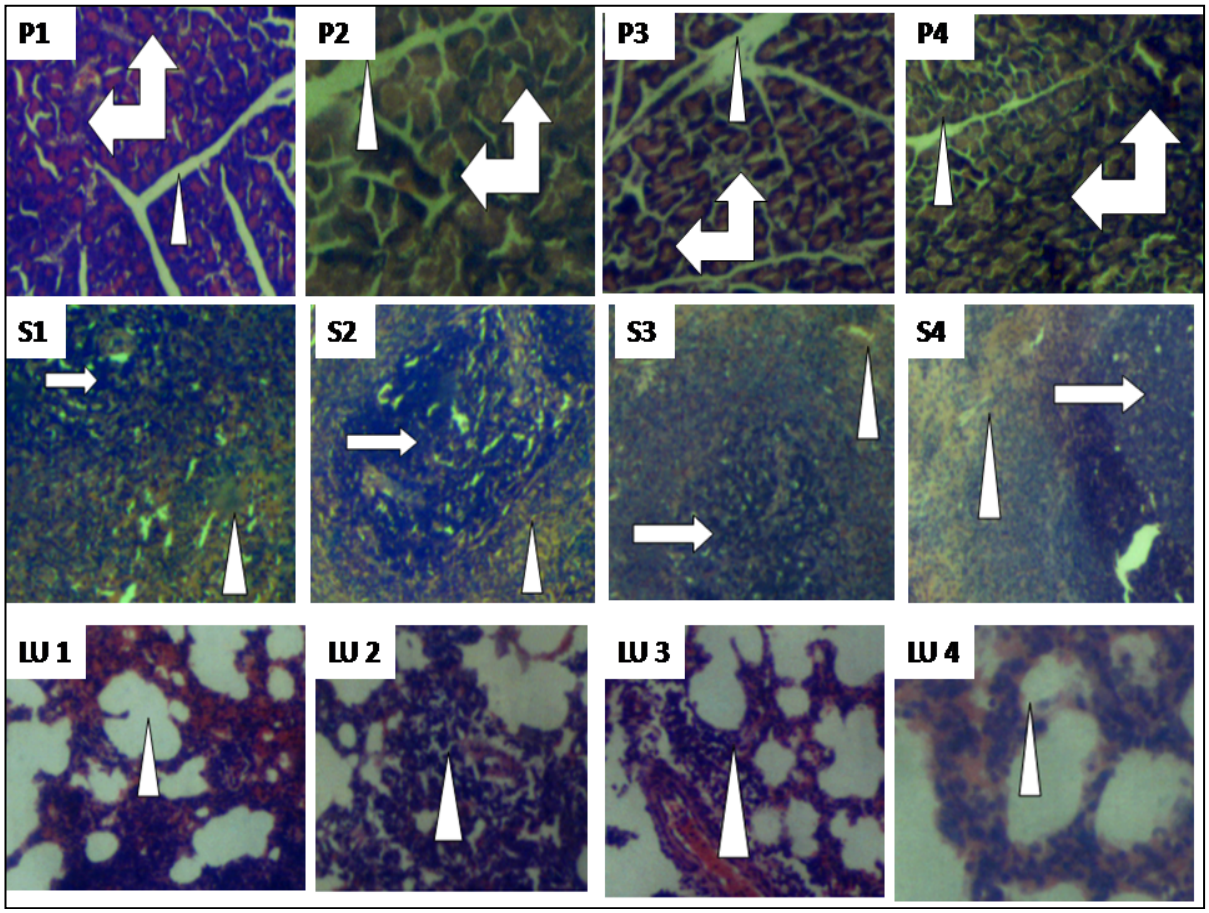

Figure 2: Histology results for Pancreas (P1, P2, P3 and P4), Spleen (S1, S2, S3 and S4) and Lungs (LU1, LU2, LU3 and LU4)

Pancreas (P1, P2, P3, P4): Photomicrograph of pancreas of control, low, medium and high doses respectively showing architecture consistent with normal pancreas histology. The islets of langerhans with the beta cells (double arrow) and intralobular duct (arrowhead) show normal histology with no sign of injury (arrow) (H&E X 100).

Spleen (S1, S2, S3, S4): Photomicrographs of spleen of control, low, medium and high doses respectively showing architecture consistent normal spleen histology, the red pulp (arrow) and white pulp (arrowhead) appear normal with no sign of injury (H&E X 100).

Lung (Lu1, Lu2, Lu3, Lu4): Photomicrograph of lung of control, low, medium and high doses respectively showing normal lung parenchyma, the alveolar spaces containing clustered and single alveolar macrophages (H&E x 100).

Discussion

Preclinical toxicity study involving use of experimental animals is fundamental to clinical trials of potential drugs.

Choice of Wistar rats used in this present study is due to the fact that function of cells and organs are basically the same in animals (rodents) and humans. Thus, animals respond to drugs in a similar way as humans do [8, 12]. Acute toxicity test with LD_{50}>5000 mg/kg suggests that the PHR is safe (practically non-toxic based on LD_{50} classification of range [7,13]. The acute toxicity test result of the PHR is in conformity with results obtained from some of the individual components of the PHR such as Pomegranate fruit extract (LD_{50}>5000 mg/kg) [14], astaxanthin-rich microalgae biomass (LD_{50}>12,000 mg/kg) [15], Withania somnifera extract standardized for Withaferin A (LD_{50}> 2000 mg/kg) [14], *Annona muricata* (Soursop) (LD_{50}> 5000 mg/kg) [15] and *Vaccinium myrtillus* L, fructus siccus- Bilberry extract (LD_{50}> 2000 mg/kg) [17]. Higher LD_{50} values of some of the individual components substantiate the practically non-toxic category of the PHR.

In course of the administration, deaths were recorded in the medium and high doses between days 16th and 18th during the repeated toxicity study, which led to termination of the study on the 19th day. Hematological profile is an important biomarker of systemic toxicity [18]. From the result of this study (Tables 1, 2 and 3), hemoglobin, Mean corpuscular hemoglobin (MCH) and platelet were the three hematological parameters that showed

significant variations when compared with the control group. Non-significant decrease in red blood cells and significant decrease in hemoglobin may have resulted from the suppression of circulating hormone, erythropoietin (a glycoprotein which stimulates the process of erythropoiesis). Significant increase in the platelet count (Table 3) suggests the ability of medium and high doses of the PHR to increase clotting capacity of the blood which may lead to thrombotic event. Studies have shown that secondary thrombocytosis can be linked to iron deficiency [19]. The rise in platelets count seen may also suggest that the drug have a stimulatory effect on thrombopoietin, and may be useful in the control of excessive bleeding (haemophilia). Non-significant alteration in white blood cell count suggests that the PHR does not adversely affect the immune function.

Elevated plasma concentrations of liver enzymes are frequently utilized as indicators of liver toxicity [20]. In relation to the liver histology, the histoarchitecture of the liver tissues in control, low dose, medium dose and high doses (Fig. 1, Liver, L1, L2, L3, and L4) showed consistency as normal hepatocytes, which suggests that the PHR may not be associated with liver damage. Significant elevation in ALP at high dose may be attributed increase in bone cell activity, but not liver toxicity. Interestingly, some ingredients contained in the PHR like Propolis and bee pollen extracts were found to restore biochemical parameters and lessen the negative effects of D-glucose on the liver and kidneys [21]. Studies have also shown that bilberry extract shielded rat liver microsomes from UV-induced oxidative damage and apolipoprotein B degradation [22]. Ashwagandha root extract was also mentioned as possibly playing a hepatoprotective role in most reports due to its free radical scavenging ability [23].

It is also necessary to determine electrolyte parameters (such as Sodium, Na+, Potassium, K+ and Chlorine, Cl-.) as part of toxicity assessment of kidney function. The kidney regulates the concentration of the electrolytes which have important roles in the homeostasis [23]. From renal function parameters result of table 5, the statistically significant decrease in the serum creatinine level at medium dose only, may not be attributed to kidney toxicity effects of the PHR, becausekidney histology results (Fig. 1. kidney (K1, K2, K3, and K4) showed normal morphology, normal renal capsules, mascular densa and renal tubules with no sign of injury, as well as non-significant alterations in other renal biomarkers, urea, total protein, and albumin when compared to the control group. This suggests that the herbal remedy at low and medium doses is safe on the kidney. The group treated with high dose (Fig. 1. kidney (K4) showed kidney tissue consistent with normal morphology but with a focal unremarkable lymphocytic infiltration, suggesting that double of the therapeutic dose may cause minimal renal damage. According to Moneim et al. [24] pomegranate, one of the components of the PHR caused significant increase in urea and a significant decrease in creatinine. According to Popovic et al. [25], anthocyanin's from the bilberry extract dramatically reduced the nephrotoxic effects caused by an acute CCl_4 exposure. From our study (Table 5), there was no significant alteration in the level of sodium and potassium in test groups when compared to the control. However, elevation in serum chloride level was only noticed in the medium group, and was not dose dependent, suggesting that it is not toxicity related.

The heart histology results in Fig. 1: Heart (H1, H2, H3 and H4), which showed no disruption in heart architecture in low (H2), medium (H3) and high (H4) doses compared to control (H1) substantiates the fact that the PHR may not produce deleterious effect on the cardiovascular system, which lipid profile also serve as one of the biomarkers. Astaxanthin as a constituent of the PHR has been reported to inhibit low-density lipoprotein (LDL) oxidation and to increase high-density lipoprotein (HDL)-cholesterol and adiponectin levels in clinical studies [26].

Table 6 shows no statistically significant difference ($^{ns}p>0.05$) in blood glucose levels of the animals administered with the PHR when compared to the control group in weeks, 1, 2 and 3. From table 6, there were no significant effects of the PHR on blood glucose. This is substantiated by the pancrease histology results (Fig. 2: Pancreas (P1, P2, P3 and P4) which revealed consistency in the morphology of the pancreas with no evident signs of injury in low, medium and high dose when compared to control group, which suggests that the herbal remedy posed no deleterious effect on the pancreatic function in relation to blood glucose regulation. Apple extract, one of the components of the PHR has been reported to reduce post-prandial glucose [27].

Histological analyses of other organs including testes (Fig. 1: Testes, T1, T2, T3, and T4) spleen (Fig 2: Spleen, S1, S2, S3 and S4) and lung (Fig. 2. Lungs, LU1, LU2, LU3 and LU4) showed no deviation/changes observed in low, medium and high doses of the PHR when compared to control group, suggesting that the herbal formulation did not pose any deleterious effect on the functionality of the testes, spleen and lung.

It is also important to note the deaths which were recorded in the medium and high dose test animals between days 16^{th} and 18^{th} during the sub-acute toxicity test may be attributed to the high bioaccumulation of the PHR in the animal system,

which calls for caution not to use the herbal remedy above 14-days, and also above the recommended daily dose. Interaction of individual constituents present in the polyherbal remedy could be responsible for the effects observed in the study.

Conclusion

This work evaluated the safety profile of a PHR, which is used among the populace for various ailments. Having carefully carried out this study, the PHR is safe following single dose exposure. When administered in the right therapeutic daily dose and for 14 days, it was suggested to be safe. However, repeated use beyond 14-days, and at double therapeutic dose should be discouraged, as such may pose risk to platelet function. In the future, it is not advisable to extend administration duration beyond the 14-19 days at the examined dose levels, as such will lead to death of the experimental animals.

Authors contributions:

EOE conceived and designed the study. E.O.E, C.I.O., C.V.O., E.O.O., P.T.O., L.N.O., V.C.O. and T.S.N. carry out the bench work and data analysis. T.C.A. assisted in supervising the bench work. E.O.E and T.C.A. drafted the manuscript. All authors revised and approved the final manuscript.

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