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Original Article

Toxicity assessment of a polyherbal drug on haematological parameters, brain and spleen histoarchitecture in exposed Wistar rats

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ABSTRACT

Introduction: We investigated the subchronic toxicity concerns of a polyherbal formulation (PHF; Dr Iguedo Goko Cleanser®) on haematological indices, brain and spleen histomorphology in exposed Wistar rats of both sexes.

Methods: Thirty Wistar rats randomly allotted to six groups (5/group) were experimentally exposed to PHF via the oral route for 60 days as follows: control groups (1 and 4; given 5ml/kg distilled water); groups 2, 5 (476.24mg/kg) and 3, 6 (158.75mg/kg) body weight PHF, respectively. On 62nd day, animals were euthanized using carbon dioxide and sacrificed; spleen and brain tissues were evis-cerated, weighed and fixed in 10% buffered-formalin for histopathological assessment.

Results: Our results showed significant increase in platelet in all experimental rats relative to control. Low dosed (158.75mg/kg) male rats recorded a significant increase in WBC relative to control. Also increased were MCV and MCH in male rats. High dosed female rats had increased RBC and MCV. Neutrophils and lymphocyte differentials were respectively decreased and increased in experimental groups relative to control. Histopathology of the spleen and brain tissues revealed degrees of pathologies like abnormal cytostructure of lymphoid follicle, degenerated T-lymphocytes, numerous organic deposits, degenerating neural cells, proliferating astrocytes, widely scattered blood vessel amongst others.

Conclusion: Our findings revealed exposure-associated toxic effects of the quasi-drug formulation on blood parameters and histostructure of the spleen and brain. Findings suggest utmost caution on long-term use of the polyherbal formulation and avoidance whenever possible.

Keywords:
Drug compounding
Herbal medicine
Toxicology
Gliosis
Haematology

Introduction

The use of medicinal herbs for wellness is indigenous to the traditional medicine practiced by numerous ethnic groups around the world (Pan et al., 2014). Herbal medicine is currently gaining popularity as a result of its long history that predates orthodox medicine and general per-

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ception that herbs are naturally safe. An estimated 80% of the world's population especially in the third-world countries copiously exploit herbal medicine for their primary health concerns (Ekor, 2014). An example of such herbal preparation is Dr Iguedo Goko Cleanser® which is popularly promoted among native Nigerians as potent remedy for an array of ailments (Solaade, 2015). It comprises of five medicinal plants: bitter leaf, garlic, ginger, sugarcane and pigeon pea (Udom et al., 2020a) and is marketed across the country without regulation from national and local health regulatory authorities. The polyherbal formulation (PHF) has been reported to be a very good source of total carotenoids and vitamin C (Ejoh et al., 2005; Aregheore, 2012), as such may contain intrinsic biological actives responsible for stimulating haematopoeitins synthesis and/or release. Also reported are the probable potent hypolipidemic properties in rat models (Udom, 2021).

Of recent, herbal remedies have become increasingly popular as dietary supplements for the prevention of diseases and as alternative or complementary adjunct in the management and/or treatment of diseases. Despite the fact that herbal medicines are often thought to be safer than orthodox medicines due to their higher tolerance and natural sources, numerous side effects and untoward reactions associated with the use herbal medicines have been reported (Abebe, 2002; Shiel, 2014). Majorly, some of the reported side effects and noxious drug reactions are caused by the inherent biologically active secondary or intermediate metabolites found in these products; nonetheless, many are due to poor qualities of the products themselves, which are attributed to heavy metals and polycyclic aromatic hydrocarbons contamination, contamination with microorganisms, pesticides, chemicals amongst others as well as inadequate quality assurance methods. To be regarded as ideal, herbal products ought to be safe, not fickle, and made available in appropriate dose forms and packages (Firenzuoli et al., 2005). Although the toxicity concerns of Dr Iguedo Goko Cleanser® on vital organs like the liver (Udom et al, 2020b), kidneys (Udom et al., 2020a) as well as the endocrine (Udom et al., 2020c) and reproductive systems, and antioxidant enzymes (Udom et al., 2020d) have been previously studied and reported, there is dearth of information on its toxicological evaluation on blood components, neurological system and splenic functions. Therefore, this research was designed to determine the 60-days exposure-associated outcomes of Dr Iguedo Goko Cleanser® on blood parameters and histomorphology of the brain and spleen tissues in exposed Wistar rats of both sexes. It is thought that such findings will provide a rationale for the safety of this product vis-à-vis help protect public health against exposure-associated adverse health effects.

Material and methods

Preparation of stock solution

The polyherbal mixture was purchased from a major distributor in Uyo metropolis, Nigeria, and the stock concentration was determined as earlier reported by Udom et al. (2020a).

Experimental animals

Thirty healthy Wistar rats of both sexes (140-180g) were purchased from the Animal Unit, Department of Pharmacology & Toxicology, Faculty of Pharmacy, "details omitted for double-blind reviewing". Animals were kept in standard environmental conditions, fed Pfizer-branded rodent chow (Livestock Feed, Nigeria Ltd) and allowed free access to water *ad libitum*. To achieve the 12h light/ 12h dark period, the Wistar rats were kept at room temperature (23±2ºC) in cleaned/disinfected polypropylene cages with sterile paddy husk as bedding and without illumination at night. Prior to the onset of the study, experimental subjects were acclimatized to the laboratory conditions for at least seven days during which they had access to food and water at will. To minimize potential differences that may arise during the course of the experiment, experimental animals within the same weight range where caged together, marked with picric acid either as head, back, tail, plain and right ear for easy identification during drug administration. On the basis of their position on the rack, the six cages used were numbered accordingly. Animal handling and care was conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The protocol of the research was approved by the Experimental Ethics Committee of the Faculty of Pharmacy, University of Uyo, Nigeria under the reference number UU/EEC/2020/01.

Experimental design

Thirty healthy adult Wistar rats of both sexes (15 each; 140-180g) divided at random into six groups (five

TABLE 1: Experimental design

S/N	Treatment Group	Dosage	Duration
1	Control (males)	5 ml/kg DW	60 days
2	High dosage (males)	476.24 mg/kg GC	60 days
3	Low dosage (males)	158.75 mg/kg GC	60 days
4	Control (females)	5 ml/kg DW	60 days
5	High dosage (females)	476.24 mg/kg GC	60 days
6	Low dosage (females)	158.75 mg/kg GC	60 days

DW = Distilled water, GC = Goko Cleanser,

rats per group in 3 categories each for both sexes) were treated as shown in Table 1. Random numbers were generated using Microsoft Excel (standard = RAND() function). The small sample size was selected following the 3Rs (reduction, refining and replacement) principle as regards the use of animals in biomedical research. The doses were chosen based on the value of a previously determined LD_{50} (Udom et al., 2020a; Udom et al., 2020d) and administered daily between 8–9AM using oral gavage for 60 days (Yemitan and Adeyemi, 2004). Wistar rats in different groups were monitored closely for changes in behavioural patterns, body weight and loss of fur, change of eye, urine and stool colouration. Post 60-days test duration (precisely on the 62nd day), the animals were euthanized with carbon dioxide (Sigma, USA), and blood samples were collected into sample bottles for haematological analysis. The spleen and brain tissues were eviscerated from each euthanized animals for histopathological assessment.

Haematological analysis

Blood samples were collected from each carbon dioxide euthanized Wistar rat via cardiac puncture into different ethylene diamine tetra-acetic acid (EDTA)-coated sample bottles and analysed for all haematological indices (RBC, HGB, PCV, WBC, and differentials – neutrophils, eosinophils, basophils, lymphocytes and monocytes). This analysis was done at the University of Uyo Teaching Hospital using an automated Haematology analyser in accordance with manufacturer's protocols (Sysmex Haematology-Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Japan).

Histopathological examination

From each euthanized rats, the brain and spleen were eviscerated, freed from adventitia, blotted dry with tissue paper, weighed with a sensitive balance, sectioned and fixed in 10% buffered formalin. Fixed tissue sections were thereafter dehydrated with alcohol, cleared with xylene, infiltrated and mounted with paraffin wax, sectioned, rehydrated, stained with haematoxylin and eosin and mounted with coverslips for histopathological assays under a light microscope at a magnification of ×100. In order to reduce bias, the pathologist was not informed of the test agent (polyherbal formulation) and it administered doses (Yemitan et al., 2015).

Data analysis

Data obtained from this study were statistical analysed using SPSS software (version 17), and significance between groups was determine using one-way analysis of variance (ANOVA), followed by LSD as a post-hoc test. Results were presented as mean±SEM with values less than ($P< 0.05$) considered significant.

Results

Haematological parameters

Male rats had significant ($P<0.05$) increase in platelet and mean corpuscular volume (MCV) compared to control. Also recorded was a significant increase in MCH in male rats that were administered 476.24mg/kg body weight PHF (Table 2). A significant increase in red blood cell count and MCV were recorded for female rats exposed to 476.24mg/kg body weight PHF, while significant increase in platelet count was recorded for all experimental female rats relative to the control (Table 3). Experimental male rats given 158.75mg/kg body weight PHF recorded a significant increase in WBC relative to control. Significant decrease in neutrophils differential was observed in both male (Table 4) and female rats (Table 5). WBC was significantly increased in high dosed females but decreased in low dosed female rates (Table 5). Also recorded were significant increase in lymphocyte differential for both male (Table 4) and female rats

TABLE 2: Haematological indices of exposed male Wistar rats

Treatment	RBC ($x10^{12}$/L)	HB (g/dl)	PCV (%)	MCV (Fl)	MCH (Pg)	MCHC (g/dl)	PLT ($x10^{9}$/L)
Control	8.76 ± 0.41	17.63 ± 0.63	45.85 ± 1.00	52.50 ± 1.02	19.65 ± 0.56	37.48 ± 0.39	951.25 ± 11.32
476.24 mg/kg	8.74 ± 0.27	16.18 ± 0.33	42.88 ± 0.51	49.15 ± 1.00[a]	18.53 ± 0.26[a]	37.70 ± 0.32	1142.50 ± 15.33[a]
158.75 mg/kg	8.81 ± 0.16	16.58 ± 0.32	44.08 ± 0.88	50.03 ± 0.41[a]	18.80 ± 0.14	37.60 ± 0.13	1166.00 ± 34.05[a]

Data presented as mean±SEM. Compared means are considered statistically significant at $P<0.05$; a= significantly different when compared to control males. RBC: red blood cells; HB: haemoglobin; PCV: pack cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; PLT: platelets (n=5).

TABLE 3: Haematological indices of exposed female Wistar rats

Treatment	RBC ($x10^{12}$/L)	HB (g/dl)	PCV (%)	MCV (Fl)	MCH (Pg)	MCHC (g/dl)	PLT ($x10^{9}$/L)
Control	7.63 ± 0.31	15.70 ± 0.92	41.97 ± 2.09	54.43 ± 0.74	20.40 ± 0.44	37.43 ± 0.34	995.75 ± 31.74
476.24 mg/kg	8.67 ± 0.33[a]	16.88 ± 0.66	44.65 ± 1.61	51.55 ± 0.99[a]	19.48 ± 0.40	37.80 ± 0.34	1233.75 ± 15.16[a]
158.75 mg/kg	8.24 ± 0.29	16.60 ± 0.38	43.20 ± 1.13	52.48 ± 0.63	20.15 ± 0.30	38.45 ± 0.23	1170.50 ± 27.77[ab]

Data presented as mean±SEM. Compared means are considered statistically significant at $P<0.05$; a= significantly different when compared to control females; b= significantly different relative to 476.24 mg/kg. RBC: red blood cells; HB: haemoglobin; PCV: pack cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; PLT: platelets (n=5).

TABLE 4: Differential white blood cell count of exposed male Wistar rats

Treatment	WBC ($x10^{9}$/L)	NEU (%)	LYM (%)	EOSIN (%)	BASO (%)	MONO (%)
Control	12.38 ± 0.63	17.80 ± 1.08	66.43 ± 2.03	14.60 ± 0.95	0.00 ± 0.00	0.00 ± 0.00
476.24 mg/kg	11.20 ± 0.39	11.18 ± 0.51[a]	72.70 ± 0.88[a]	15.65 ± 0.05	0.00 ± 0.00	16.60 ± 0.80[a]
158.75 mg/kg	14.43 ± 0.19[ab]	8.70 ± 1.06[ab]	77.78 ± 2.09[ab]	12.40 ± 0.40[ab]	0.00 ± 0.00	14.03 ± 1.34[a]

Data presented as mean±SEM. Compared means are considered statistically significant at $P<0.05$; a= significantly different when compared to control males; b= significantly different relative to 476.24 mg/kg. WBC: white blood cells; NEU: neutrophils; LYM: lymphocytes; EOSIN: eosinophils; BASO: basophils; MONO: monocytes (n=5).

TABLE 5: Differential white blood cell count of exposed female Wistar rats

Treatment	WBC ($x10^{9}$/L)	NEU (%)	LYM (%)	EOSIN (%)	BASO (%)	MONO (%)
Control	13.23 ± 0.33	9.40 ± 0.36	76.88 ± 1.26	13.80 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
476.24 mg/kg	15.55 ± 0.28[a]	8.05 ± 0.60	83.83 ± 0.59[a]	12.48 ± 0.30	0.00 ± 0.00	0.00 ± 0.00
158.75 mg/kg	9.58 ± 0.28[a]	6.20 ± 0.51[a]	78.85 ± 1.63[ab]	10.20 ± 0.20[a]	0.00 ± 0.00	9.75 ± 0.05[ab]

Data presented as mean±SEM. Compared means are considered statistically significant at $P<0.05$; a= significantly different when compared to control females; b= significantly different relative to 476.24 mg/kg; WBC: white blood cells; NEU: neutrophils; LYM: lymphocytes; EOSIN: eosinophils; BASO: basophils; MONO: monocytes (n=5).

(Table 5). Finally, significant increases and decreases were recorded for monocyte differential among the experimental rats (Table 4 and 5).

Histopathological assessment

Brain

Histological examination of the cerebral cortex of rats (both sexes) in the control groups presented preserved/normal histoarchitecture of the cerebral cortex with well-arranged molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal layer, polymorphic layer, neurons and blood vessels. High and low dosed males presented some forms of pathologies such as cerebral cortex with areas of degenerating neural cells, proliferating astrocytes, widely scattered blood vessel. While the brain tissues of both high and low dosed treated females showed cerebral cortex with areas of degenerating neural cells, presence of macrovesicular steatosis, widely scattered blood vessel and scantily stained pyramidal cells (Fig. 1).

Spleen

Histological examination of the spleen of rats (both sexes) in the control groups presented normal and pre-

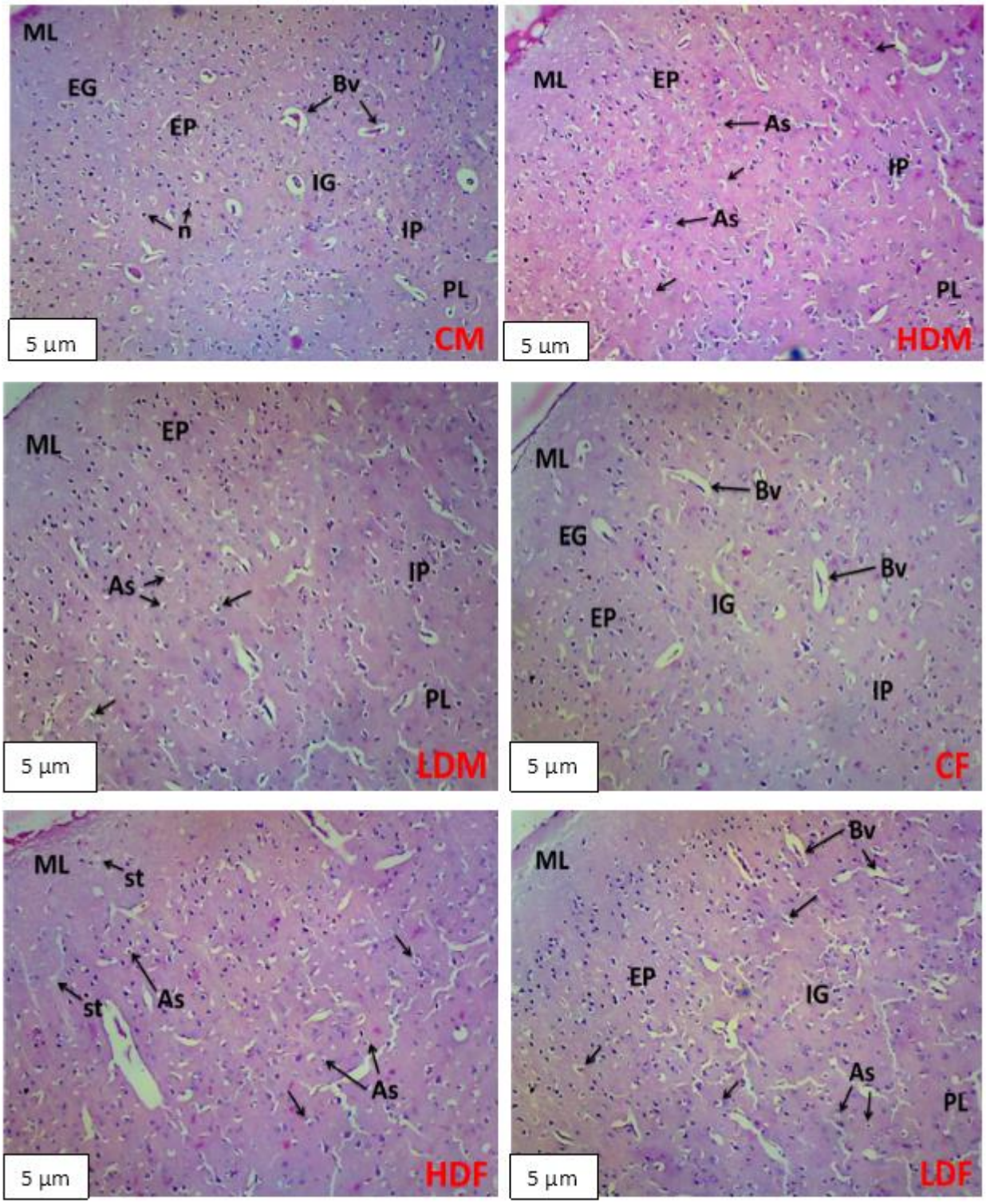

FIGURE 1. Typical transverse brain (cerebral cortex) sections from exposed Wistar rats of both sexes showing controls (CM; CF) with normal histoarchitecture of the cerebral cortex with well-arranged molecular layer (ML), external granular layer (EG), external pyramidal layer (EP), internal granular layer (IG), internal pyramidal layer (IP), polymorphic layer (PL), neurons (n) and blood vessels (Bv). The polyherbal mixture-exposed groups showed distorted cerebral cortex with areas of degenerating neural cells (arrows), proliferating astrocytes (As) and widely scattered blood vessel, presence of macro vesicular steatosis (st) and widely scattered blood vessel ×100 magnification. HDM: high dosed males; LDM: low dosed males; HDF: high dosed females; LDF: low dosed females (n=5).

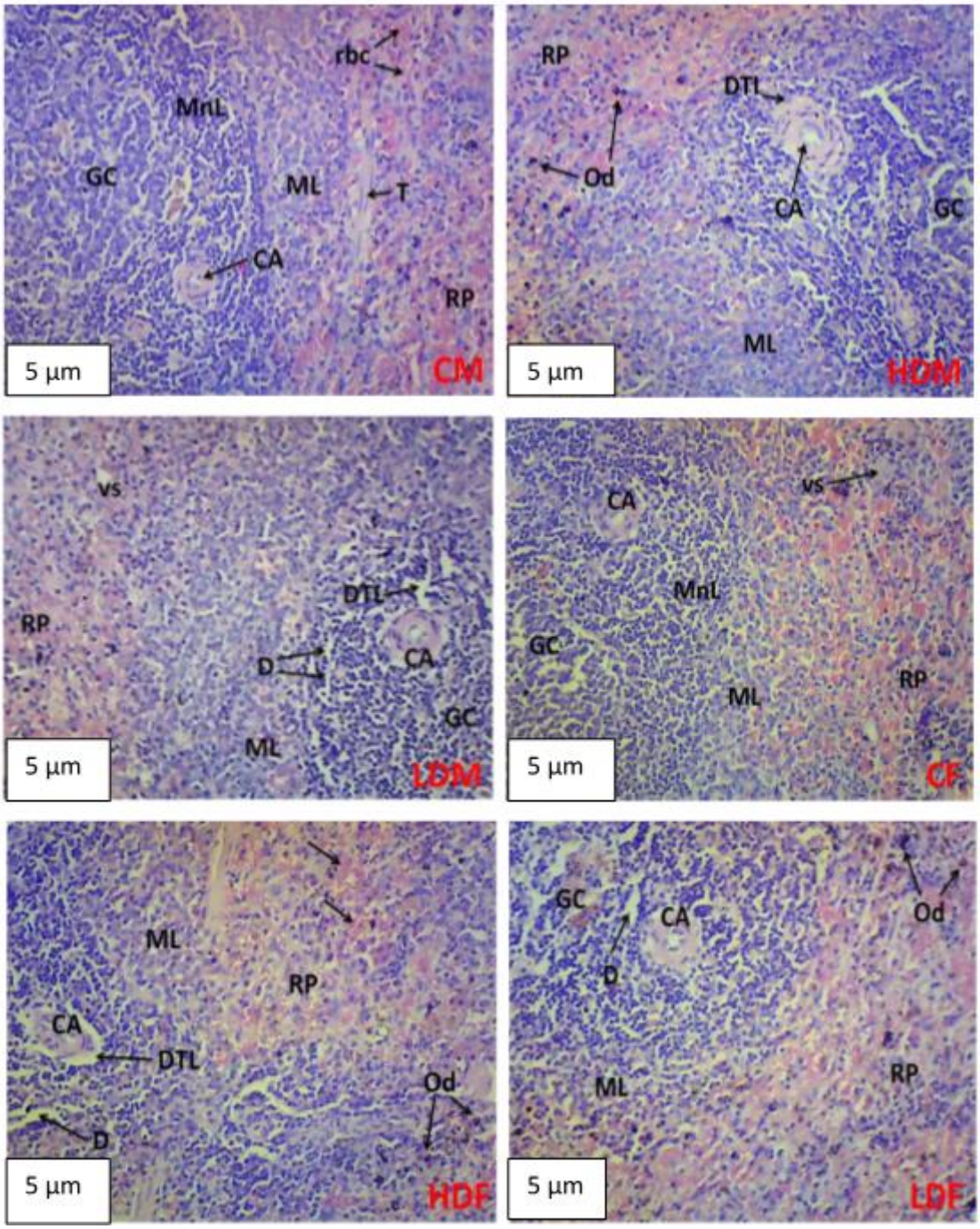

FIGURE 2. Typical spleen sections from exposed Wistar rats of both sexes showing controls (CM; CF) with normal histoarchitecture of lymphoid follicle with the germinal centre (GC), central arteriole (CA) well surrounded with the lymphoid cells, mantle layer (zone; MnL), marginal layer (zone; ML), area of the red pulp showing the invaginating trabeculae (T) and red blood cells (rbc). The polyherbal mixture-exposed groups showed abnormal histostructure of the lymphoid follicle with low stained germinal centre, central arteriole, area of degenerated T-lymphocytes (DTl), numerous organic deposits (Od), low stained marginal layer (zone), area of the red pulp (RP) showing low stained/scanty red blood cells, low stained red blood cells (arrow), area of degenerated B – lymphocytes and T – lymphocytes, and a large venous sinus ×100 magnification. HDM: high dosed males; LDM: low dosed males; HDF: high dosed females; LDF: low dosed females (n=5).

served histoarchitecture of the lymphoid follicle with the germinal centre, central arteriole well surrounded with lymphoid cells, mantle layer and marginal layers (zone) as well as the area of red pulp showing the invaginating trabeculae and red blood cells. The spleen of the high dosed males showed abnormal histostructure of the lymphoid follicle with low stained germinal centre, central arteriole, area of degenerated T- lymphocytes, numerous

organic deposits, low stained marginal layer (zone) as well as area of the red pulp showing low stained and scanty red blood cells. While that of the low dosed males showed abnormal histostructure of the lymphoid follicle with low stained germinal centre, central arteriole, area of degenerated B – lymphocytes and T – lymphocytes, numerous organic deposits, low stained marginal layer (zone) as well as area of the red pulp red blood cells and a large venous sinus. High dosed females presented with abnormal histostructure of the lymphoid follicle with low stained germinal centre, central arteriole with degenerations of surrounding area of B – lymphocytes and T – lymphocytes, degenerating and low stained marginal layer (zone), area of the red pulp with low stained and scanty red blood cells as well as numerous organic deposits. Low dosed females presented with abnormal cytostructure of the lymphoid follicle with germinal centre with areas of cellular degenerations, central arteriole with surrounding degenerated cells, marginal layer (zone), area of the red pulp showing red blood cells and numerous organic deposits (Fig. 2).

Discussion

Haematological indices are of utmost importance in health and disease. For example, haemoglobin, the protein found in the erythrocytes is required for tissue oxygenation, and thus, sufficient haemoglobin level must be maintained (Yu, 2019). When markedly reduced, anaemia occurs, however, elevated levels above normal are secondary to erythrocytosis. Physiologically, the mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration otherwise referred to as 'red cell indices' defines the size and haemoglobin content of the erythrocytes (Maner and Moosavi, 2021). Pathologically, the classification of anaemia is varied. It can be with regards to the disease aetiology as in the case of haemolytic and haemorrhagic anaemia etc.; erythropoietic response such as hypoproliferative anaemia, or cell morphology as in the case of normocytic, macrocytic or microcytic-hypochromic anaemia (s) (Maner and Moosavi, 2021). Therefore, the 'red cell indices' are invaluable for the morphologic classification of anaemia. The recorded significant difference in the red cell indices does not suggest anaemia rather; it defines the size and haemoglobin content of their respective red blood cells. The significant increase in RBC (HDF only) and platelet likely suggest that the polyherbal mixture may contain intrinsic biological actives responsible for stimulating haematopoeitins (erythropoietin, thrombopoetin) synthesis and/or release. This might be attributed to phytonutrients found in the polyherbal mixture. For example, green/edible vegetables are rich natural sources of ascorbic acid (Marcus, 2001), carotenoids and folates (Hoffbrand, 2001). Evidence suggest that vitamin C is required for the formation and maintenance of body tissues not excluding blood, blood vessels and bone (Osilesi et al., 1997). Bitter leaf (*V. amygdalina*) – one of the medicinal herbs contents of Dr Iguedo Goko Cleanser® has been reported to be a very good source of total carotenoids and vitamin C (Ejoh et al., 2005; Aregheore, 2012). It is therefore thought that the presence of these essential substances in the polyherbal mixture may be responsible for the observed effect. WBCs are classified into granulocytes, lymphocytes and monocytes. Of these granulocytes, three varieties namely neutrophils (or polymorphonuclear granulocytes), basophils and eosinophils are identified. The recorded significant decrease in neutrophils and monocyte differentials in the experimental rats suggest that the polyherbal mixture may be selectively toxic to the leucocyte lineages.

The histopathology of brain revealed an array of pathologies such as degenerating neural cells, proliferating astrocytes (HDM only), scantily stained pyramidal cells and presence of macrovesicular steatosis (HDF only). Degeneration of neural cells is characterized by a steady decline of neuronal structure or function, and is otherwise known as neuronal necrosis or neurodegeneration, and as such, disorders like amyotrophic lateral sclerosis, Huntington's disease, Parkinsonism, and Alzheimer's disease are all due to neurodegenerative processes. The brain contains certain cell types called the astrocytes which are implicated both in physiological and pathological conditions. During the critical phase of brain development, it is essential for astrocytes and neural progenitor cells to proliferate. In fact, the inhibition of such proliferation at that critical window may lead to microencephaly (Guizzetti et al., 2011). In mature brains, however, astrocytes are not expected to undergo functional proliferation. This can only occur due to or after an injury to the brain as in the case of reactive astrogliosis or in the case of brain tumours (Guizzetti et al., 2011). Astrogliosis is the anomalous increase in the number of astrocytes most likely caused by the destruc-

tion of nearby neurons secondary to CNS trauma, infection, autoimmune disorders, cerebrovascular accident, ischemia and neurodegenerative disease. Physiologically, astrocytes are essential in energy provision, regulation of blood flow, electrolytes and transmitter's homeostasis, extracellular fluid homeostasis, regulation of synaptic function as well as synaptic remodelling (Gordon et al., 2007). Proliferation of the astrocytes is seen in all forms of CNS injury and disease, indicating that astrocytes proliferation is usually at par with neuronal dysfunction (Salami et al., 2008). This explains the observed degeneration of the neural cells *vis-à-vis* the proliferating astrocytes in the high dose males as revealed by the histopathological assessment. The experimental rats recruited in this study were screened, quarantined and tagged as healthy and mature, therefore, the observed astrocytes proliferation may not be physiological but pathological probably due to exposure to the polyherbal mixture. It is strongly suspected that exposure to the polyherbal mixture may have induced CNS injury in male rats that received 476.24mg/kg body weight of Dr Iguedo Goko Cleanser®. Anatomically, the amygdala, hippocampus and cerebral cortex as well as many other areas of the brain are densely populated with pyramidal cells or neurons. They are the primary excitatory units of the corticospinal tract of the mammalian prefrontal cortex. These neurons are critical components of the circuit known for vision-guided motor function in the corticospinal tract (Salami et al., 2008). In the prefrontal cortex, the pyramidal cells are actively involved in cognition functions. Postero-anteriorly, the complexity of these cells escalates. The pyramidal neurons are thought to be involved in the processing of several types of input especially as the prefrontal cortex receives and processes sensory input from other sections of the brain. Also, they are believed to be involved in the recognition of complex objects within the visual processing sections of the cerebral cortex (Elston, 2003). From the foregoing, it may be logical to infer that the female rats that received 476.24mg/kg body weight of Dr Iguedo Goko Cleanser® might have lost some cognitive function and/or perhaps suppressed/inhibited pyramidal cells related functions. The presence of macrovesicular steatosis in the high dose female rats as well as pathologies observed in the experimental rats of both sexes are regarded as possible toxic outcomes following exposure to Dr Iguedo Goko Cleanser®. It is worthy of note that sex/gender, interspecies and individual differences are considerable factors that determines the susceptibility to toxicity following exposure to toxicants, and females are generally considered to be more susceptible to toxic outcomes than the males due to hormonal interplay and/or other factors. Steatosis is known to be an abnormal retention of fat within a cell or an organ. Though commonly known to affect the liver (hepatic steatosis or fatty liver disease), steatosis can be seen in other organs as well. Macrovesicular steatosis is characterised by the large fat accumulation such that the nucleus is displaced from the cytoplasm. While this may not be harmful in mild cases, large accumulation disrupts cellular constituents and functions, and in severe cases the cell may even burst. The pathogenesis of this condition is multidimensional as no single mechanism exists (Wilson et al., 2015). Associated risk factors include diabetes mellitus, anoxia, hypertension, sleep apnea, obesity, protein malnutrition and cell toxins.

Whenever any of the histostructure of the spleen is distorted especially the red pulp, there is a greater likelihood that some toxic outcome (e.g. decreased spleen weight due to scanty red blood cells) will ensue (Udom et al., 2020c). The histopathology of the spleen at high and low doses (i.e. 476.24 and 158.75mg/kg body weight) of the polyherbal mixture revealed some degree of pathologies such as abnormal lymphoid follicle, numerous organic deposits, low stained marginal layer, low stained germinal layer with degenerating B and T lymphocytes, low stained red pulp with scanty red blood cells as well as cellular degenerations in both male and female rats. The spleen is a secondary lymphoid organ just like the mucosa associated lymphoid tissue, lymph nodes, Peyer's patches and tonsils. It is the body's largest lymphatic organ and as such is the site where the lymphocytes are activated. The white pulp or germinal layer of the spleen is known to contain lymphoid aggregations, but mostly lymphocytes and macrophages. So, the abnormal lymphoid follicle revealed by the histopathological examination underscores the fact that the experimental rats may have been immunologically compromised following exposure to the polyherbal mixture. From a toxicological viewpoint, the low stained areas indicate sparse cellular orientation, while the organic deposit could be seen as accumulated debris. Accumulation of cellular debris impairs cellular function leading to fatigue and cellular death. These deaths account for

or explain the sparse cellular arrangement or low stained areas revealed by the histopathological assessment. Degeneration is best understood medically to mean tissue or organ deterioration in which functions are diminished or structures are significantly impaired or compromised. Thus, the degeneration of the B and T lymphocytes is indicative that both the humoral and T-cell mediated immunity may have been suppressed to some extent. Therefore, the spleen is considered a susceptible organ to the toxic concerns of the polyherbal mixture. Our findings corroborate that of Udom et al. (2020c) who reported a decrease in the weight of the spleen in female rats compared to their male counterparts and attributed such to alterations and/or damage to any of the histostructure especially the red pulp.

Conclusion

In conclusion, findings of the study necessitate utmost caution and where necessary avoidance of long-term use of the polyherbal product, especially as the observed toxicities in the organs here presented are not negligible. Also imperative is the formulations and implementation of policies as reards the necessity for toxicological evaluations of herbal products and other quasi-drug fomulations that freely circulate in the Nigerian market. If this is eagerly pursued and achieved, public health will not only be protected from exposure-associated untoward effects of these substances, but appreciatively perked up.

Conflict of interest

The authors declare that no competing interest exist. It should be noted that the products used in this study are common in our field of study and in our country. There is no conflict of interest between the authors and the product's manufacturer, especially since the authors do not intend to use these products as a vehicle for litigation but rather to advance scientific knowledge.

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References

Abebe W. Herbal medication: potential for adverse interactions with analgesic drugs. J Clin Pharmacol Ther 2002; 27(6): 391-401. https://doi.org/10.1046/j.1365-2710.2002.00444.x

Aregheore EM. Nutritive value and inherent anti-nutritive factors in four indigenous edible leafy vegetables in human nutrition in Nigeria: a review. J Food Resource Sci 2012; 1: 1-14. https://dx.doi.org/10.3923/jfrs.2012.1.14

Ejoh RA, Tanya AN, Djuikwo NA, Mbofung CM. Effect of processing and preservation methods on vitamin C and total carotenoid level of some Vernonia (bitter leaf) species. Afr J Food Agric Nutr Dev 2005; 5: 105-117. https://doi.org/10.18697/ajfand.9.1525

Ekor M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol, 4, 177. https://doi.org/10.3389/fphar.2013.00177

Elston GN. Cortex, cognition and the cell: new insights into the pyramidal neuron and prefrontal function. Cerebral cortex 2003; 13(11): 1124-1138. https://doi.org/10.1093/cercor/bhg093

Firenzuoli F, Gori L, Galapai C. Adverse reaction to an adrenergic herbal extracts (Citrus aurantium). Phytomedicine 2005; 12(3): 247-248. https://doi.org/10.1016/j.phymed.2004.02.005

Gordon GRJ, Mulligan SJ, Madvicar BA. Astrocyte control of the cerebrovasculature. Glia 2007; 55(12): 1214-1221. https://doi.org/10.1002/glia.20543

Guizzetti M, Kavanagh TJ, Costa LG. Measurements of astrocyte proliferation. Methods Mol Biol 2011; 758: 349-359. https://doi.org/10.1007/978-1-61779-170-3_24

Hoffbrand AV. Megaloblastic anaemia, In: Hoffband AV, Lewis SM, Tuddenham EGD, editors. Postgraduate haematology. 4th ed. New York: Oxford University Press, 2001.

Maner BS, Moosavi L. Mean corpuscular volume. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021. PMID: 31424859. https://www.ncbi.nlm.nih.gov/books/NBK545275/ [Accessed on July 23 2022].

Marcus R, Coulston AM. Water-soluble vitamins: the vitamin b complex and ascorbic acid, In: Hardman JG, Limbird LE, editors. Goodman and Gilman's the pharmacological basis of therapeutics. 10th ed. New York: McGraw-Hill Medical Publishing Division, 2001.

National Research Council. Guide for the care and use of laboratory animals. Washington DC: National Academies, 2011.

Osilesi O, Adeiyi A, Ogunyemi EO, Fakunle JB. The glycaemic response to selected fruits and vegetables in Nigerian diabetics. Afr J Med Pharm Sci 1997; 1: 1-6.

Pan SY, Litscher G, Gao SH, Zhou SF, Yu ZL, Chen HQ, et al. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. Evid Based Complement Alternat Med. 2014; 2014: 525340. https://doi.org/10.1155/2014/525340

Salami I, Friel KM, Martin JH. Pyramidal tract stimulation restores normal corticospinal tract connections and visuomotor skill after early postnatal motor cortex activity blockade. J Neurosci 2008; 28(29): 7426-7434. https://doi.org/10.1523/jneurosci.1078-08.2008

Shiel WC. Herbs toxicities and drug interactions. In: Stöppler MC, editors. [Document on the internet], 2014. https://www.medicinenet.com/script/main/art.asp?articlekey=7506 [Accessed on September 15 2021]

Solaade A. Assorted herbal mixtures: Herbs or poison? [Document on the internet], 2015. https://parroteye.blogspot.com/2 015/01/herbs-or-poison.html [Accessed on September 15 2021]

Udom GJ. Toxicological evaluation and health risk assessment a polyherbal mixture (Dr Iguedo Goko Cleanser®) sold in Uyo and its environs [PhD Thesis]. Uyo, Nigeria: University of Uyo; 2021.

Udom GJ, Okokon JE, Udobang JA, Obot DN, Asuquo IE. Nephrotoxicity assessment of Dr Iguedo Goko Cleanser® in exposed Wistar rats. Asian J Res Med Pharm Sci 2020a; 9(1): 30-40. https://doi.org/10.9734/ajrimps/2020/v9i130144

Udom GJ, Okokon JE, Udobang JA, Onyeukwu NJ, Obot DN, Asuquo IE. Hepatotoxicity assessment of a polyherbal mixture in exposed Wistar rats. Asian J Res Reports Hepatol 2020b; 2(1): 14-22. https://doi.org/10.9734/ajrimps/2020/v9i130144

Udom GJ, Udobang JA, Obot DN, Onyeukwu NJ, Asuquo IE, Okokon JE. Toxicological assessment of a polyherbal mixture on organ weights and hormonal profile in exposed Wistar rats. Asian J Res Reports Endocrinol 2020c; 3(2): 29-36.

Udom GJ, Okokon JE, Udobang JA, Obot DN, Onyeukwu NJ. Subchronic toxicological assessment of Dr Iguedo Goko Cleanser® on lipid profile and serum antioxidant enzymes in exposed Wistar rats. Asian J Biol 2020d; 9(4): 16-25. https://doi.org/10.9734/ajob/2020/v9i430093

Wilson CH, Ali ES, Serimgeour N, Martin AM, Hua J, Tallis GA, Rychkov GY, Baritt GJ. Steatotis inhibits liver cell store-operated Ca2+ entry and reduces ER Ca^{2+} through a protein kinase C-dependent mechanism. Biochem 2015; 466(2): 379-390. https://doi.org/10.1042/bj20140881

Yemitan OK, Adeyemi OO, Izegbu MC. Toxicological and reversibility assessment of Dalbergia saxatilis root extracts on body and organ weights, hepatic functions and peroxidation in rats. Eur J Med Plants 2015; 11(4): 1-13. https://doi.org/10.9734/EJMP/2016/21479

Yemitan OK, Adeyemi OO. Toxicity studies of the aqueous root extract of Lecaniodiscus cupanioides. Nig J Health Biomed Sci 2004; 3: 20-23. https://doi.org/10.4314/njhbs.v3i1.11501

Yu ASL. Haematologic aspects of kidney disease. In: Yu ASL, Chertow GM, Lucyckx V, editors. Brenner and Rector's The Kidney. 11th ed. Philadelphia: Elsevier, 2019.