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Research Article

Sub-chronic Oral Toxicity Assessment of Opaeyin in Albino Wistar Rats

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ABSTRACT

Opaeyin, popularly known as manpower is a formulated herbal product mostly used to manage erectile dysfunction in South Western Nigeria. However, there is no scientific evidence regarding its safety with long-term use, hence, this work evaluated 28-day sub-chronic oral toxicity in the haematological parameters and hepato-renal tissues of albino Wistar rats. In sub-chronic toxicity, twenty (20) male albino Wistar rats were assigned to 4 groups (n=5). The first group received 0.4 ml/kg of normal saline and served as control, while the 2nd ,3rd and 4th groups received graded doses of Opaeyin (0.20, 0.40 and 0.80 ml/kg respectively). The treatments were given orally and daily for 28 days. At the end of the experiment (29th day), the animals were anaesthetized to obtain blood samples and organs for haematological, biochemical and histological evaluations. There was a dose dependent decrease in haematocrit, haemoglobin and red blood cell count due to oral administration of graded doses of Opaeyin, no significant ($p < 0.05$) changes were observed in the white blood cell count. Oral administration of Opaeyin produced a dose dependent increase in the activities of serum alkaline phosphatase, aspartate aminotransferase and alanine amino transaminase. Oral administration of the high dose Opaeyin led to a significant increase ($p < 0.05$) in serum creatinine of the experimental animals. Prolonged oral administration of Opaeyin in high doses caused vascular congestion in both the hepatic and renal tissues. In conclusion, the study suggests that prolonged use of Opaeyin especially higher doses may result in haematological abnormalities and hepato-renal toxicity.

Keywords: *Opaeyin, Sub-chronic toxicity, Erectile dysfunction*

INTRODUCTION

A penile erection that is sufficient for sexual satisfaction, including satisfactory sexual performance, cannot be obtained and/or maintained in men with erectile dysfunction (ED). ED is defined as a disturbance of the arousal stage of the sexual response (Burnett *et al.*, 2018). According to Ariba *et al.* (2007), it is one of the most common sexual dysfunctions in men worldwide. ED is usually an issue of embarrassment especially among the uneducated in underdeveloped and developing countries. According to Prieto-Castro *et al.* (2020), it is not uncommon for males to either not recognise that they have a sexual disorder, play down its significance, or find it uncomfortable to discuss with their doctor. Thus only very few seek medical help particularly older men (Jannini *et al.*, 2014**).** According to Shaeer *et al.* (2003) in Nigeria, the age-adjusted prevalence rates of ED among men attending primary care clinics were found to be 57.4%. In a study carried out by Oyelade *et al.* (2016), the general prevalence of ED in South-West Nigeria was found to be 58.9 %. Of this figure, it was also reported by the authors that 79.4% of respondents had never raised the issue or sought treatment for ED from a doctor. The reason for this has been attributed to feeling ashamed and the fear of stigmatization (Peate, 2012). Most of the men suffering from erectile dysfunction rather resort to

unprescribed herbal remedies. Opaeyin, popularly known as *manpower*, is one of such herbal remedies commonly used to manage erectile dysfunction especially among the urban dwellers in Lagos, Southwest Nigeria. Opaeyin has great popularity among artisans and traders in the Lagos metropolis and it is used to enhance sexual performance.

According to Oreagba *et al*. (2011), herbal medicine is popular among urban dwellers in Lagos, Nigeria, although many appear to be unaware of its possible toxicity. It is a fact that more than 75% of the world population depends on herbal medicine for their health care need (WHO, 2002). These herbal medicines are believed to be safe because they are from natural sources. However, literature has shown that some of these herbal medicines have detrimental effects on some organs contrary to the organs of interest (Oyewo *et al*., 2013; Adeyemi and Akinwande, 2015; Patrick-Iwuanyanwu and Nkpaa, 2015).

There is however dearth of information on haematological and biochemical changes due to the administration of this herbal remedy. The purpose of this study was to ascertain the effects of the administration of graded doses of Opaeyin on various haematological and biochemical indices, as well as the histology of hepatic and renal tissues in normal Albino Wistar rats.

MATERIALS AND METHODS

The sample of Opaeyin was obtained from a traditional medicine hawker in the Mushin market in the Mushin Local Government Area of Lagos State, Nigeria. There was no evidence of official registration of this remedy. The hawkers interviewed were not willing to give out their recipes due to their oath of secrecy.

Phytochemical screening

Phytochemical analysis of Opaeyin sample was carried out using the methods of Sofowora (1999).

Acute toxicity studies

Ten male Wistar albino rats weighing between 200-220 g kept under standard laboratory conditions were used for acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guidelines 425 (OECD 2000 guidelines). The animals received a single dose of 2 ml/kg body weight of Opaeyin.

Animals were allowed to acclimatise for 5 days, prior to drug administration they were fasted overnight and administration was done by oral gavage. Food was withheld for 3-4 more hours. The animals were observed individually at least once during first 30 minutes after dosing, then periodically for the first 4 hours during the first 24 hours and then daily for a period of 14 days. Daily monitoring on the changes of skin and fur, eyes and mucus membrane respiratory rate, heart rate, blood pressure, salivation, lacrimation, perspiration, piloerection, urinary incontinence, defecation, ptosis, drowsiness, gait, any tremors and convulsion were noted.

Subchronic toxicity

A total of 20 healthy male Wistar rats were obtained from the Department of Pharmacognosy, University of Lagos. The animals weighing between 200-220g were housed in plastic cages within the animal house of the department. The experimental animals were then allowed to acclimatize for 7 days before experimentation. The procedure for the animal care was done in accordance with the University of Lagos, Animal Ethics Policy.

Twenty (20) male albino Wistar rats were assigned to 4 groups (n=5). The first group received 0.4 ml/kg of normal saline and served as control, while the 2nd, 3rd and 4th groups received graded doses of Opaeyin (0.20, 0.40 and 0.80 ml/kg bodyweight respectively). The treatments were given orally and daily for 28 days. At the end of the experiment (29th day), the animals were anaesthetized to obtain blood samples and organs for haematological, biochemical and histological evaluations.

Sample preparation

Twenty-four hours after the last administration, the animals were anaesthetized under chloroform vapour and dissected. Blood samples were collected by cardiac puncture, using sterile syringes. Blood samples for serum preparation were collected into sterile plain tubes while the samples for haematological analysis were dispensed into test tubes containing Ethylene Diamine Tetraacetic Acid (EDTA). The Mindray BC-3200 auto haematology analyzer was used for the whole blood analysis while COBAS C 311 Automated chemistry Analyzer was used for the analysis involving the serum. The procedures employed for the determination were in accordance with the manufacturer's instructions.

The kidney and liver were isolated from each animal, weighed and fixed in 10 % formalin for histological study.

Haematological investigations

The haematocrit (HCT), haemoglobin (HGB), Red Blood Cell (RBC) count, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV) Mean Corpuscular Haemoglobin (MCH) and White Blood Cell (WBC) count were determined using the Mindray BC-3200 auto haematology analyser. The procedures employed for the determination were in accordance with the manufacturer's instructions.

Biochemical investigations

The blood samples for the biochemical studies were collected in sterile tubes without anticoagulants and allowed to coagulate in order to obtain sera. The samples were left to coagulate for 30 minutes and then centrifuged at 3000 rpm for 10 minutes to get clear sera. The sera were stored frozen until required. The activities of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) alanine aminotransferase (ALT), total protein, bilirubin, albumin, urea, creatinine, Sodium, potassium chloride levels, total cholesterol, high density lipoprotein (HDL) – cholesterol, triglycerides, were also determined using the appropriate Randox kits while LDL-cholesterol was determined based on the Friedewald *et al.* (1972) formula: LDL = TC - HDL – TG / 2.17 (mmol/L). Atherogenic index of plasma (AIP) was calculated, also, using the formula log (TG/HDL-C). The protocols employed for each kit were in accordance with the manufacturer's instructions.

Histological investigation

The histological investigations were done according to the method described by Bancroft and Stevens 1990. Small pieces of dissected liver and kidney tissues were fixed in 10% buffered formalin. They were further embedded in paraffin wax, sectioned into 5-µm thick pieces using a microtome, and then stained with hematoxylin-eosin. The stained sections were observed using an Olympus light 'microscope (Olympus, Tokyo, Japan) through high resolution digital camera system.

Statistical analysis

Results were presented as mean and standard deviation. One-way analysis of variance (one-way ANOVA) was used to determine the differences between groups and where significant difference existed; Dunnett's post hoc test was employed to establish the source. Differences in means were considered significant at $P < 0.05$.

RESULTS

Phytochemical Analysis

The result of the phytochemical analysis of Opaeyin is presented in Table 1. The result reveals a total anthocyanin content of 109.91 mg/100g and reducing sugar 31.30 mg/100g. Also present are phenol, flavonoids, tannin, terpernoid, steroid, saponin, and alkaloids.

Table 1. Phytochemical constituents of Opaeyin

Phytochemicals	Quantity
Flavonoid (mg/100g)	98.82 ± 0.38
Steroid (mg/100g)	58.39 ± 1.28
Alkaloid (mg/100g)	56.55 ± 2.38
Reducing sugar (mg/100g)	31.30 ± 0.12
Tannin (mg/100g)	55.77 ± 0.32
Terpenoids (mg/100g)	51.20 ± 0.36
Saponin (mg/100g)	56.11 ± 1.12
Total Anthocyanin (mg/100g)	109.91 ± 5.13

Values are expressed as mean ± SD

Acute toxicity studies

The results of the acute toxicity studies indicated no changes of skin and fur, eyes and mucus membrane, respiratory rate, heart rate, blood pressure, salivation, lacrimation, perspiration, piloerection, urinary incontinence, defecation; also no ptosis, drowsiness, change in gait, tremors and convulsion were observed in the animals administered with 2 ml/kg body weight of Opaeyin. Since none of the stated toxic signs and symptoms or mortality was observed in the animals at the above mentioned dose, 0.2, 0.4 and 0.8 ml/ kg body weight of the herbal remedy were selected for the study.

Haematological studies

Results showing the effect of oral administration of Opaeyin on haematological parameters of Wistar albino rats are presented on Table 2. There was 14.19% decrease in haematocrit (HCT), 33.68 % decrease in haemoglobin (HGB) and up to 39.79% decrease in red blood cell (RBC) count due to oral administration of high dose (0.8 ml/kg) Opaeyin compared to the control. Oral administration of 0.8 ml/kg (high dose) Opaeyin led to a significant ($p < 0.05$) decrease in HCT and RBC count of the experimental animals which was not observed in the other experimental groups. A 25.76% decrease in the mean corpuscular haemoglobin concentration (MCHC), from 30.80 ± 0.53 g/dl (group 1) to 23.07 ± 2.32 g/dl (group 4) and up to 52.78% increase in mean cell volume (MCV) due to oral administration of high dose of Opaeyin were observed. Oral administration of Opaeyin also led to an increase in mean corpuscular haemoglobin (MCH) from 17.20 ± 0.20 pg (group 1) to 19.30.0.44 pg (group 4). Although there were changes in white blood cell (WBC) count of the experimental animals, these were neither dose dependent nor statistically ($p< 0.05$) significant.

Table 2. Some haematological effects due to oral administration of graded doses of Opaeyin

Parameters	Group 1	Group 2	Group 3	Group 4
HCT %	43.97 ± 1.43	42.53± 3.21	41.80 ±0.2	37.73 ±2.72*
HGB g/dl	13.57 ± 0.31	12.47 ± 0.76	11.7 ± 0.20	9.00 ± 1.00*
RBC X 10^{12}/L	7.64 ± 0.20	6.67 ± 0.23	6.67 ± 0.25	4.60 ± 0.09*
MCH pg	17.70 ± 0.20	17.20 ± 1.75	17.77 ± 0.45	19.30 ± 0.44
MCHC g/dl	30.80 ± 0.53	29.20 ± 0.85	29.90 ± 0.44	23.07 ± 2.32*
MCV fL	56.90 ±1.25	67.80 ± 1.47	59.67 ± 2.35	86.93 ±1.66*
WBC X 10^{9}/L	13.23 ±3.21	10.27 ± 5.95	10.53 ± 3.39	11.1 ± 3.85

Values are expressed as mean ± SD, * indicates significant ($p < 0.05$) difference from the control group

Biochemical Studies

The effect of the oral administration of graded doses of Opaeyin on biochemical parameters of Wistar albino rats is presented on Table 3.

There was a 22.78% increase in serum ALP activity due to oral administration of high dose Opaeyin. Similarly, serum AST activity increased by 19.55% due to high dose of Opaeyin. Serum ALT activity increased by up to 68.91% due high dose of the Opaeyin. Oral administration of Opaeyin produced a dose dependent increase in the activities of serum ALP, AST and ALT. Oral administration of up to 0.4ml/kg body weight of Opaeyin led to significant ($p < 0.05$) increase in the activities of these enzymes when compared to the control.

There was up to 10.81% increase in serum urea, 4.23% for serum creatinine due the oral administration of Opaeyin.

Total cholesterol decreased by up to 28.57%, LDL-cholesterol decrease by up to 16.67% while up to 37.39% decrease in HDL-cholesterol was recorded due to oral administration of high dose Opaeyin. These changes in lipoproteins were nonetheless not dose dependent.

Among the serum electrolytes, there was up to 23.99% decrease in potassium, 2.51 % decrease in chloride and 1.48% decrease in sodium due to long term oral administration of Opaeyin. These changes were however not dose dependent.

Up to 22.11% decrease in serum albumin was observed due to oral administration of Opaeyin the changes in albumin were observed to be dose dependent. Conversely, a decrease of up to 5.25% was observed for total protein but the changes were not dose dependent.

Table 3. Some biochemical effects due to oral administration of graded doses of Opaeyin

Parameters	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Chloride (mmol/L)	97.73 ± 0.47	97.5 ± 1.42	97.17 ± 1.52	95.27 ± 2.89*
Potassium (mmol/L)	5.21 ± 0.08	3.96 ± 0.11*	4.57 ± 0.01	4.23 ± 0.06*
Sodium (mmol/L)	135 ± 0.00	136 ± 0.00	137 ± 0.00	136 ± 0.00
Total Chol (mmol/L)	1.68 ± 0.07	1.60 ± 0.08	1.06 ± 0.14*	1.20 ± 0.07*
Trig (mmol/L)	0.65 ± 0.02	1.11 ± 0.05*	0.45 ± 0.05*	0.61 ± 0.02
LDL (mmol/L)	0.24 ±0.06	0.05 ± 0.28	0.19 ± 0.02	0.20 ± 0.03
AIP	-0.25 ± 0.01	0.02 ± 0.03	-0.16 ± 0.04	-0.07 ±0.04
HDL (mmol/L)	1.15 ± 0.01	1.04 ± 0.05	0.66 ± 0.01*	0.72 ± 0.05*
Total Protein(g/L)	70.5 ± 0.28	68.25 ± 0.21	66.8 ± 0.14	71.75 ± 0.07
Albumin (g/L)	39.93 ± 0.45	32.1 ± 0.1*	31.1 ± 0.1*	30.5 ± 0.1*
Bilirubin (µmol/L)	1.9 ± 0.36	1.9 ± 0.10	1.3 ± 0.06*	2.06 ± 0.25
ALP (IU/L)	211.57 ± 7.35	226.57 ±7.35	245.57 ± 0.06*	259. 77 ± 12.45*
AST(IU/L)	119.36 ± 3.20	128.1 ± 5.89	136.8 ± 2.6*	142.7 ± 7.10*
ALT(IU/L)	53.70 ± 4.1	75.9 ± 12.1	88.1 ± 12.2*	90.71 ± 4.65*
AST/ALT	2.22	1.69	1.55	1.57
Creatinine (µmol/L)	34.73 ± 0.71	35.6 ± 0.1	34.4 ± 0.1	36.2 ± 0.1*
Urea (mmol/L)	6.20 ± 0.10	6.47 ± 0.39	6.7 ± 0.00*	6.87 ± 0.11*

Values are expressed as mean ± SD, * indicates significant ($p < 0.05$) difference from the control group

Histological studies

Liver

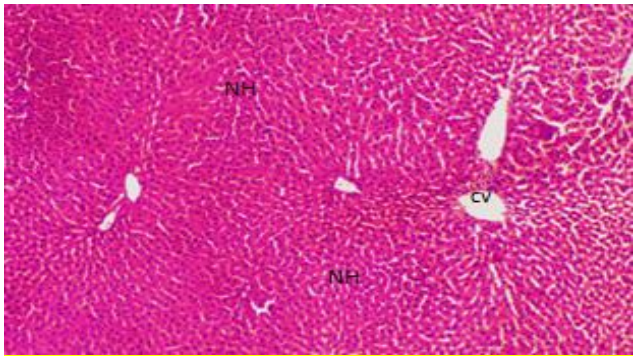

Figure 1(a) CONTROL: Hepatocyte plates are organized in parallel and radially in liver tissue histological sections. There are no obvious anomalies. Central Vein (CV) Normal Hepatocyte (NH)

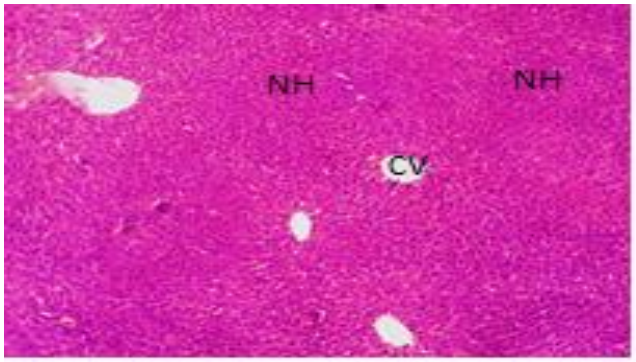

Figure 1(b) GROUP 2: Hepatocyte plates containing blood vessels are organized parallel and radially in liver tissue histological sections. There are no obvious anomalies H and E STAIN X100

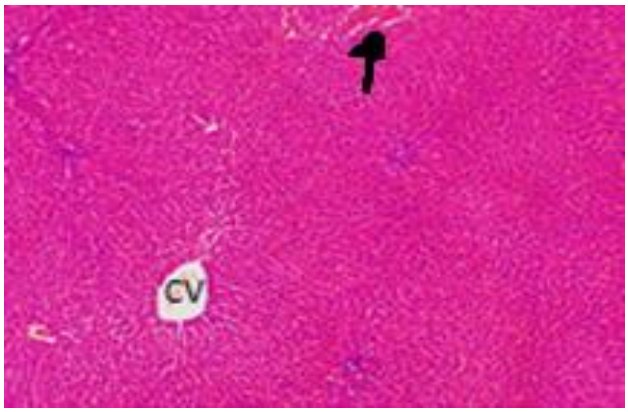

Figure 1 (c) GROUP 3: Parallel radially oriented hepatocyte plates of the liver, together with somewhat congested blood arteries (↑). Moderate vascular congestion; H and E STAIN X100

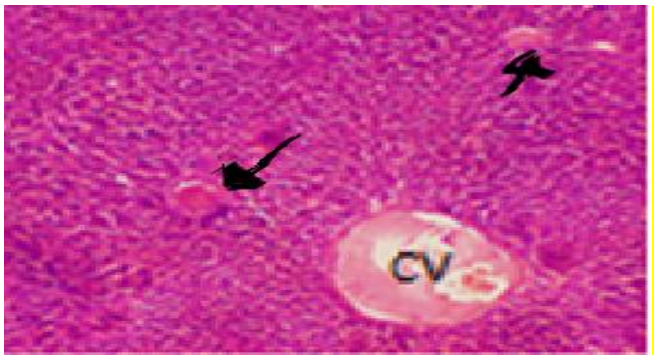

Figure 1 (d) GROUP 4: Parallel radially oriented plates of hepatocytes are seen in the liver tissue's histologic sections. Congested clusters of red blood cells are also visible. (↑) Edema and vascular congestion; H and E STAIN X100

Kidney

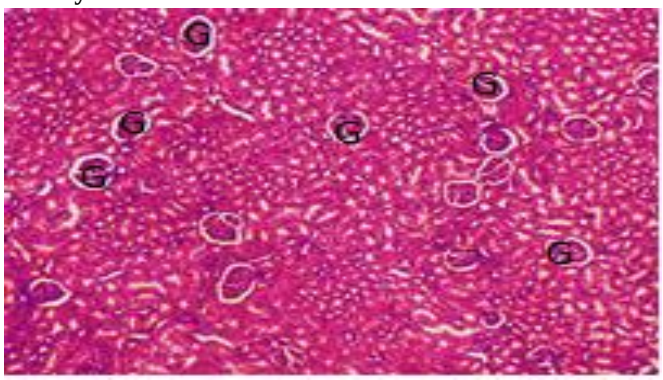

Figure 2(a) CONTROL: The glomerular tufts (G) in normocellular kidney tissue are arranged on a background of renal tubules in histologic sections of the kidney tissue. Nothing unusual is visible. H and E STAIN X100

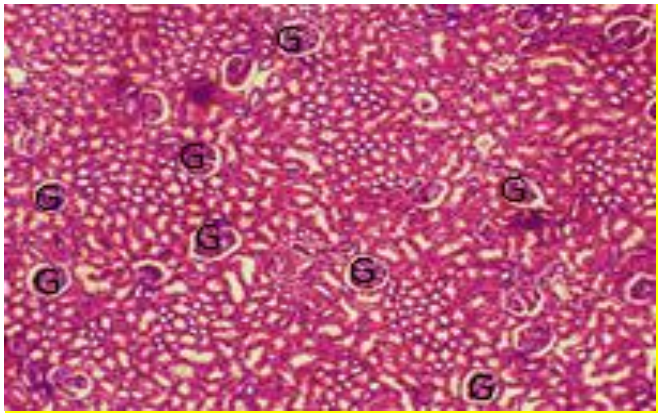

Figure 2(b) GROUP 2: Kidney tissue slices show normocellular glomerular tufts (G) arranged on a background of renal tubules. There are no obvious anomalies. H and E STAIN X100

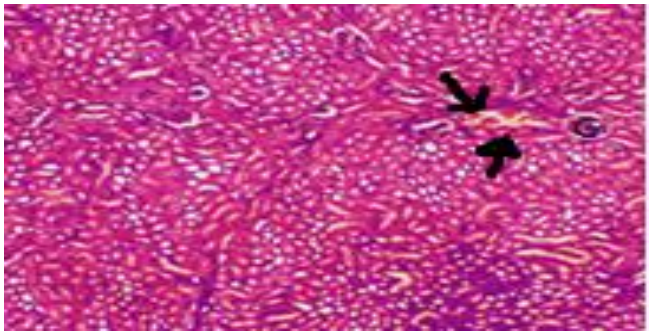

Figure 2(c) GROUP 3: Kidney tissue slices reveal normocellular glomerular tufts (G) arranged on a background of functional tubules. Vascular congestion is seen (↑). H and E STAIN X100

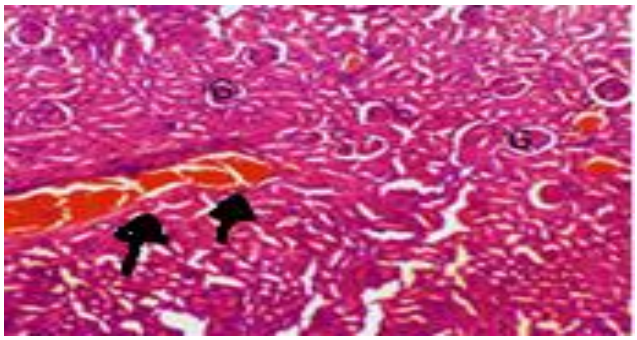

Figure 2 (d) GROUP 4: Histological sections of kidney tissue slices reveal normocellular glomerular tufts arranged on a background of functional tubules. Vascular congestion is seen (↑). Hand E STAIN X100

DISCUSSION

This study evaluated a 28-day sub-chronic oral toxicity of *Opaeyin* on the haematological parameters and hepato-renal tissues of albino *Wistar* rats. Previous reports have shown that haematological parameters provide substantial information about the state of an animal (Arika *et al.*, 2016; Seibe *et al.*, 2021). Decrease in HBG and RBC is usually an indication of RBC toxicity. Also when HCT is lower than normal, it is suggestive of anaemia, especially the aplastic anemia. Aplastic anemia is usually a result of damage involving the bone marrow tissues by certain chemicals (benzene), toxins, medications and gamma radiations; all these inhibit the enzymes involved in hemopoiesis (Young, 2018). The decreases in RBC and HCT observed may have occurred due to disruption of the RBC production by some components Opaenyi especially in high dose. A further study of the RBC indices showed a significant increase in the MCV for group 4 which was not observed in the other groups. High mean corpuscular volume (MCV) indicates macrocytosis. Macrocytic anaemia is known to be due, among other factors, to certain types of chemicals (Nagao and Hirokawa, 2017), unlike microcytic anaemia which occurs when there is low MCV level. Low MCV level is indicated in iron deficiency, it is therefore obvious that the significant ($p < 0.05$) decrease in PCV, HGB and RBC observed especially in group 4 is not due to iron deficiency but could have been elicited by drug induced toxicity occasioned by the oral administration of high dose of Opaeyin.

Given that xenobiotics are metabolized in the liver, most of these agents can attack it (Singh *et al.*, 2015). The first to alert the body of an attack on the liver is typically the liver enzymes. These enzymes are normally found in the hepatocytes. They are, however, released into the blood stream when the liver is damaged for whatever reason. The most sensitive ones, aminotransferase, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are frequently the first to be increased pointing to hepatocellular injury. AST is a cytosolic and mitochondrial isoenzyme that is present in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red blood cells. Hence, the rise in AST may also be regarded as owing to non-hepatic sources because it is not as sensitive or specific for the liver. The liver contains large levels of the cytosolic enzyme ALT. This enzyme is released into the circulation in response to hepatocellular injury, not necessarily cell death (Lala *et al.*, 2021). Alkaline phosphatase (ALP) is also an enzyme present in the liver, however with isoezymes the placenta, ileal mucosa, kidney, bone but more than 80% of the serum ALP come from the liver and bone with small amounts from the intestine (Lowe *et al.*, .2021). In the present study there were dose dependent increases in the activities of the enzymes, AST, ALT and ALP which were all significant ($p < 0.05$) due to oral administration of up to 0.4 ml/kg body weight of Opaeyin. On a further study of the rate of increase of the serum activity of these enzymes, AST had 14.6-19.55% increase in serum activity, ALP had 16.3-23.1 % increase in serum activity while ALT had 64.05 -68.91 % increase in serum activity due to the oral administration of up to 0.4ml/kg body weight of Opaeyin. According to Lala *et al.* (2021) elevations in ALT and AST in out of proportion to ALP and bilirubin denotes a hepatocellular injury; whereas an elevation in ALP and bilirubin in disproportion to ALT and AST would denote a cholestatic pattern. The ALT-predominance observed in this study therefore may be an indication that the injury is hepatocellular. This fact is further reiterated by the significant ($p< 0.05$) decrease serum albumin observed in the experimental animals. Although there were changes in the bilirubin these changes were neither dose-dependent nor significant ($p< 0.05$) reiterating that the effect of the oral administration of Opaeyin on the liver was not cholestatic (Muchova *et al.* 2011). Furthermore, histological examination of the liver tissues revealed no abnormalities in the control group and those administered with 0.2ml/kg body weight, however mild vascular congestions were

observed in those treated with 0.4ml/kg body weight Opaeyin, while in the high dose (0.8ml/kg body weight) group, slightly floccular pink fluid material common with edema and congested aggregates of red blood cells were observed. Thus, confirming the effect on the liver tissues due to oral administration of high dose of Opaeyin.

Serum creatinine and urea are parameters used for the evaluation of the state of kidney. This is because they reflect the glomerular filtration rate. The body continuously produces creatinine, a by-product of the breakdown of creatine phosphate in muscle. The kidney is the only organ that primarily removes creatinine from the blood. Blood creatinine concentration increases as a result of decreased renal clearance. As the by-product of protein metabolism and the urea cycle, urea is a nitrogen-containing molecule that is created in the liver. The kidneys eliminate about 85% of the urea, with the remaining 15% being expelled through the gastrointestinal (GI) tract (Gounden *et al.*, 2021). In cases of acute and chronic renal failure/impairment, where renal clearance is reduced, serum urea levels rise. It is recognized that certain circumstances, such as upper GI haemorrhage, dehydration, catabolic states, and high-protein diets, which are unrelated to renal disorders, might raise serum urea (Mathew, 2020). Although urea is elevated sooner in renal disease, serum creatinine is a more precise measure of renal function (Gounden *et al.*, 2021). In this study, there was a dose dependent increase in the serum urea which was significant for doses up to 0.4ml/kg body weight. However, only doses up to 0.8ml/kg body weight caused a significant increase in serum creatinine. This may be an indication that oral administration of high dose of Opaeyin had an adverse effect on the kidney.

These observations were also affirmed by the histological study of the kidney tissues. The tissues from the control group and those of the animal dosed with 0.2 ml/kg body weight Opaeyin showed no abnormalities however vascular congestion was observed in the group administered with 0.4ml/kg body weight dosage which was further pronounced with the high dose (0.8ml/kg body weight) which is similar to the observations of Amole *et al.* (2021) in their study on hydroethanolic leaf extract of *Clerodendrum polycephalum* on rats.

The lipid profile parameters are important tools in the assessment of the cardiovascular wellbeing. The oral administration of Opaeyin caused significant decrease in both total cholesterol and HDL-cholesterol but these were not dose dependent. The LDL-cholesterol was however not significantly affected by administration of this herbal remedy. This is contrary to the finding of Adeyemi and Orekoya (2014) who observed no significant changes in total cholesterol but a decrease in HDL. Atherogenic index of plasma (AIP) is one of the most reliable markers in predicting cardiovascular (CV) risk (Dobiásová, 2016). AIP values of -0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high CV risk (Dobiásová, 2016). In this study all the values are within the low risk level. Consequently, it can be conveniently presumed that oral administration of the various doses of Opaeyin used in this study will not lead to a risk of cardiovascular disease.

CONCLUSION

This study suggests that prolonged use of Opaeyin, especially at higher dose, may result in haematological abnormalities and hepato-renal toxicity.

AUTHORS' CONTRIBUTIONS

UA and NOE conceived the work and designed the work; UA did the literature search and the drafting of the manuscript. SA did the analysis. NOE did the statistical analysis and produced the final manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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