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Research Article

A Preliminary Oral Toxicity Studies on Pausinystalia Yohimbe

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Abstract: The oral toxicity profile of the crude aqueous stem bark extract of *Pausinystalia yohimbe* was studied in adult albino rats. The Up and Down method (AOT425statPgm version 1.0) for acute oral toxicity was carried out using a starting dose of 175 mg/kg body weight via the oral route. For the sub chronic study, 40 male albino rats weighing between 150-200 g, were grouped into three treatment groups and a control group with 10 rats in each group. A repeat dose oral toxicity study was conducted by daily oral dosing of 600 mg/kg body weight, 150 mg/kg body weight of extract dissolved in 1ml of 0.9% saline to groups 1 and 2, respectively and 30% powdered stem bark mixed with 70% rat chow (w/w) to group 3 and 1ml of 0.9% saline was administered to rats in the control group for 28 days. On day 29, blood samples for bioassay were collected by cardiac puncture under diethyl ether anesthesia. The LD_{50} estimate of the extract was calculated to be greater than 2000mg/kg body weight/oral route. The extract did not cause any significant difference in the body weight of the rats. Platelet count and White Blood Cell count (WBC) were significantly elevated across the treatment groups $p<0.05$. Total bilirubin was significantly higher $p<0.05$ in the 600 and 150mg treatment groups, while conjugated bilirubin was significantly higher in the 600 mg treatment group. Total protein was significantly lower $p<0.05$ in the 600mg treatment group. Aspartate Transaminase was significantly higher in all the treatment groups, while Alkaline phosphatase was significantly higher in the 600 and 150mg treatment groups, $p<0.05$, respectively. Alanine transaminase was significantly higher in 600 mg treatment group. Na^+ was elevated significantly, $p<0.05$. These results suggest incipient toxicity of the extract and indicate need for morphometric toxicity study.

Keywords: Oral toxicity, *pausynistalia yohimbe*, rats, treats group and extract

INTRODUCTION

As far back as 1977, the World Health Assembly (WHA) drew attention to the potential of tradition medicine, especially its human power reserve in national health care systems, urging member countries to utilize traditional medicines (Akerele, 1987; Nakajima, 1987). In 1978, the WHO highlighted the crucial role of medicinal herbs in the health care systems of many developing countries; this was reinforced in the same year by the Alma Ata International conference declaration that urged governments to give priority to utilizing traditional medicines in natural drug policies and regulations (Akerele, 1987). *Pausinystalia yohimbe* (*PY*), (Syn: *Corynanthe yohimbe*), family *Rubiaceae* is an evergreen rapidly growing tree, native to the tropical rain forest of West and Central Africa, where it is used as a medicinal plant (Bunkill, 1985). In the northern parts of Nigeria, it is referred to as 'Dan Kamaru'. The folkloric use of the stem bark includes; for the treatment of fevers, coughs and leprosy (Duke, 1985). Other medicinal uses include the dilatation of pupils, treatment of heart disease and as local anaesthetics. Its significance as a medicinal plant lies in its use as an aphrodisiac. *Yohimbine* is an alpha 2 adrenergic receptor blocker (Goldberg *et al*., 1983; Riley, 1994) and has had a long use as an aphrodisiac (Huhner, 1916; Lebeouf *et al*., 1981; Ang *et al*., 1997). Herbalists incorporate several *Pausinystalia yohimbe* quantities in their concoctions for the treatment of erectile dysfunction (Jacks *et al*., 2007). Various scholars have documented physiological and pharmacological effects of *yohimbine* (Goldberg and Robertson, 1983; Sala *et al*., 1990; Riley, 1994; Ernst and Pittler, 1998; Sharabi *et al*., 2004). *Yohimbine* has been used in the treatment of erectile dysfunction (ED) (Riley, 1994; Ernst and Pittler, 1998; Morales, 2000; Adeniyi *et al*., 2007). Hassan *et al*. (2012) reported that methanolic

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extract of *Pausinystalia yohimbe* decreased the blood glucose level in normal fasting rats but without significant hypoglycemic effects. These Folkloric use and research findings point to inherent, unraveled potentials in *Pausinystalia yohimbe* extract, other than its aphrodisiac properties. Although it is widely used as medicinal plant, information on its toxicity in man and animal models is still incomplete. Such dearth of information could have serious safety health implications especially in large populations of impoverished developing countries like Nigeria (Tharyan and Gopalakrishnan, 2006) where there is high tendency to resort to the use of these herbal remedies. This study therefore was designed to evaluate the toxicity (LD_{50} and sub chronic) of stem bark extract of *Pausinystalia yohimbe* in adult albino rats with a view to providing more information for future research/ elaborate efficacy study on the stem bark of *Pausinystalia yohimbe*.

MATERIALS AND METHODS

Collection and processing of plant materials: About 5kg of the stem bark of *Pausinystalia yohimbe* (*PY*) (*Dan Kamaru*), consisting of all tissue outside the woody part of the stem were purchased from five different herb sellers. The plants were then verified by two traditional practitioners (Alh. Usman Abubakar and Hajiya Rabi Ibrahim -the chairman, Sokoto traditional medical practitioners association and the traditional medical practitioner of College of Health Sciences, Usmanu Danfodiyo University, Sokoto, respectively). The plant materials were further identified by Dr. Mohammed Abubakar, the Taxonomist in the Department of Botany, Usmanu Danfodiyo University, Sokoto and the voucher specimen deposited in the herbarium of the department (with specimen number UDUH/ANS/0008). The stem bark was cleaned, air dried for two weeks and grounded into powder using the laboratory hammer mill. The powdered samples were stored in air tight water proof containers, protected from direct sun light and heat until required for extraction.

Preparation of aqueous crude extract: Extraction was done using 20 gm of the powdered stem bark with 200cm^3 distilled water in a soxhlet extractor assembly for six hours. The extract obtained was filtered with Whatman filter paper number 1 and concentrated to dryness using digital aeration oven preset at 50 degrees centigrade. The extract was stored in tightly covered specimen bottles, at 4 degrees Celsius in a refrigerator until use.

Preparation of animals: The experiment was conducted in accordance with the Guide for the care and use of laboratory animals, 8th edition (National Research Council, 2011). Fifty male albino rats were obtained from the laboratory animal house of the department of biological Sciences Usmanu Danfodiyo University, Sokoto, for the entire study. The rats were kept in metal cages in the metabolic laboratory with uniform temperature of 22-25 degrees centigrade, 12 hours light and 12 hours dark periodicity. The rats were fed standard rat chow (Vital Feeds, Nig. Ltd) and water *ad libitum* and allowed to acclimatize for 14 days before the procedures.

Acute oral toxicity study: Acute toxicity study was carried out *in vivo*. All solutions were prepared using 1ml of 0.9% of saline solution and administered *per os* using gastric tube. The acute oral toxicity study was conducted using the 'up and down' procedure according to OECD/OCDE test guidelines on acute oral toxicity under a computer guided statistical program AOT-425 start program version 1.0 (AOT, 2001). The respective doses used for this experiment were 175, 550 and 2000mg per kg body weight of the rats. A total of 10 male rats were randomly selected from the population of 50 male albino rats for this study. The rats were fasted for 15 h over night of rat chow, prior to dosing on each occasion. The calculated dose in 1ml was then administered orally, in a single dose using an esophageal tube. After administration of the extract, food was withheld for a further 3 hours.

Observations for pharmaco-toxic signs were made at 10, 30, 60 and 120 min and at 4 and 6 h after dosing, on the first day and daily thereafter for 14 days. Behavioral manifestations of acute oral toxicity were noted. All observations were systematically recorded with individual records being maintained for each rat.

Observations within the first 24 h constituted the short term outcome, while observations at the end of 14days constituted the long term outcome. Deaths are entered as 'X' while no deaths are entered as '0' on the computer program for determining the LD50. The entries were made for the short term and long term outcome. The body weights of the rats were recorded shortly before dosing and weekly thereafter. At the end of 14days surviving rats were humanely killed with ether anesthesia and subjected to gross necropsy (OECD, 2001). The computer program determined the direction of dosing.

Sub chronic oral toxicity study: adult male albino rats (150-200gm) were used for this study. They were randomly assigned into 4 groups, 1, 2, 3 and control, of 10 rats each. Group 1 received 600 mg/kg body weight of the extract, group 2 received 150 mg/kg body weight of the extract by gastric feeding, daily for 28days. The extract was dissolved in 1ml of 0.9% normal saline for group 1 and 2. While group 3 was fed on 30%

Table 1: Acute oral toxicity test for *PY* extract

Test animal				
Sequence	Identity	Dosemg/kg	Short term outcome	Long term outcome
1	PY 1	175	O	O
2	PY 2	550	O	O
3	PY 3	2000 *	O	O
4	PY 4	2000 *	O	O
5	PY 5	2000 *	O	O

(X = Died, O = Survived); Dose Recommendation: The main test is complete, Stopping criteria met: 3 at Limit Dose.*, Statistical Estimate based on long term outcomes: The LD_{50} is greater than 2000 mg/kg

Table 2: Haematological parameters of albino rats treated with various doses of PY for 28 days

PARAMETER	CONTROL (distilled water)	GROUP1 (600mg extract)	GROUP2 (150mg extract)	GROUP 3 (30% w/w powdered plant material with 70% rat feed)
PCV %	40.78±0.69	40.9±0.68	41.9±0.64	40.35±0.75
WBC x 10^9/l	10.12±0.56	14.92±0.82*	17.84±0.67*	14.58±0.39 *
Granulocyte x 10^9/l	4±0.2	5.7±0.3	5.±0.1	3.6±0.1
Plateletsx10^9/l	273.8±8.4	305.2±12*	311.7±5.9*	224.6±10.6 *

Values: Mean ± SEM, n = 10; *p<0.05; ANOVA

powdered extract mixed with 70% rat chow w/w. The control group received 1ml of normal saline. The rats were weighed on days 0, 7, 14, 21, 28.

On the 29th day, the rats in each group were anaesthetized with diethyl ether, blood samples for biochemical and hematological analysis were collected by cardiac puncture under diethyl ether anesthesia. Whole blood for hematological analysis; Packed Cell Volume (PCV), WBC count, Granulocyte count and Platelet count, were collected into bottles containing anti coagulant Ethylene Diamine Tetra-Acetic acid (EDTA). The hematological analyses were done immediately with the aid of the particle counter, Erma PCE-210 hematology analyzer. While samples for biochemical analysis; Liver Function Test (LFT), electrolytes, urea and creatinine were collected in plain sample bottles and allowed to coagulate. The serum for biochemical analysis was stored in refrigerator at-20°C. The methods used for the analysis followed those outlined by Burtis *et al*. (2012). The liver, spleen, kidney, testis, seminal vesicles, prostate and penis were harvested and fixed in Bouin's solution for histological examination.

Histological study: The tissues (liver, spleen, kidney, testis, seminal vesicles, prostate and penis) were stained with Haematoxylin and Eosin (H&E). Preparation of tissues for histology followed the technique outline by Culling (1974).

Statistical analysis: Results are presented as mean± SEM. Graphs were drawn using the Excel package for drawing graphs. Statistical analysis were done using a computer assisted statistical package INSTAT. One way analysis of variance (ANOVA) was done followed by a post-hoc students Newman-Keuls test. A p-value of less than 0.05 was taken as statistically significant.

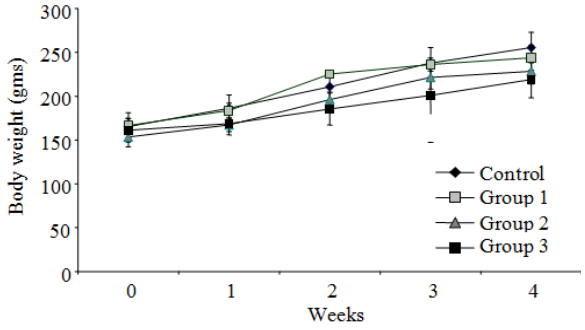


Fig. 1: Effect of *PY* on bodyweight of rats

RESULTS AND DISCUSSION

Results:

Acute toxicity study: There were no deaths recorded among the rats during the short term and long term periods of observation. The behavioral signs of toxicity observed in the rats especially at the 2000 g/kg body weight, included arching of the back, irritability, short dashes, repeated tugging of head between the hind limbs, rapid blinking of eye lids and restlessness. The LD_{50}, oral route of *PY* stem bark extract was estimated at greater than 2000 g/kg bodyweight (Table 1).

Sub chronic toxicity study: There was no significant difference ($p \leq 0.05$) in body weight of the rats (experimental and control) at the end of administration of *PY* stem bark extract to rats for 28 days (Fig. 1).

Haematological parameters: The PCV and granulocyte counts did not show any statistical difference, the WBC and platelet counts were significantly higher in the three treatment groups ($p<0.05$) (Table 2).

Biochemical parameters:

Serum bilirubin: The result shows total bilirubin significantly raised in the 1st and 2nd group while only

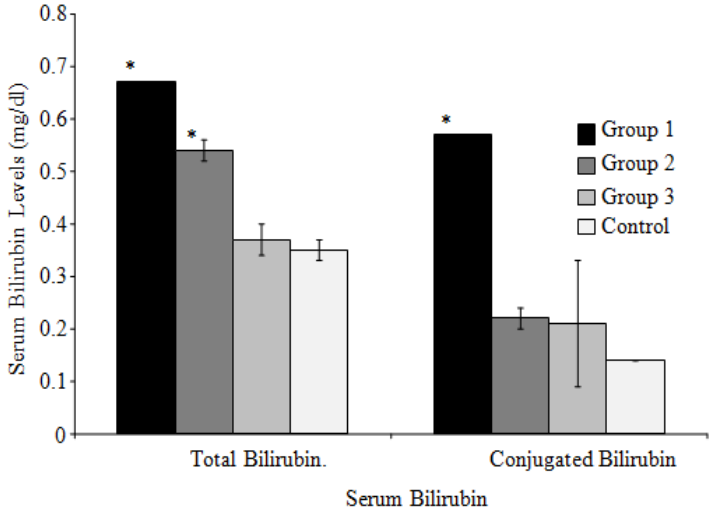


Fig. 2: Effect of oral administration of various doses of *PY* aqueous stem bark extract for 28 days, on serum bilirubin of rats

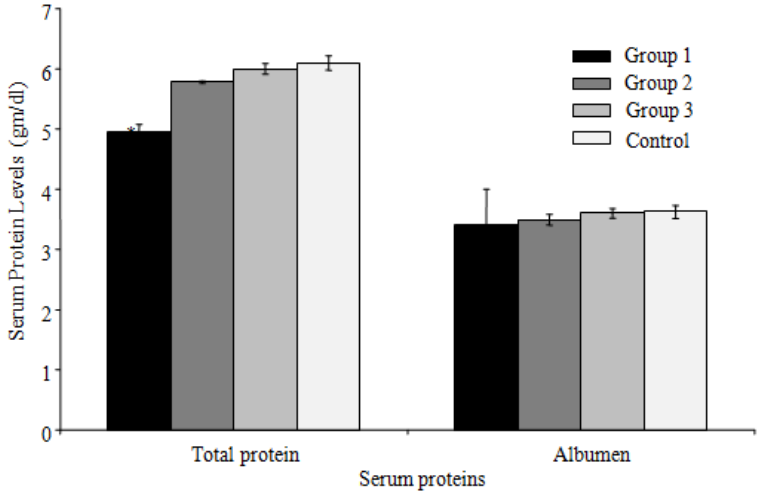


Fig. 3: Effect of oral administration of various doses of *PY* aqueous stem bark extract for 28 days, on serum proteins of rats

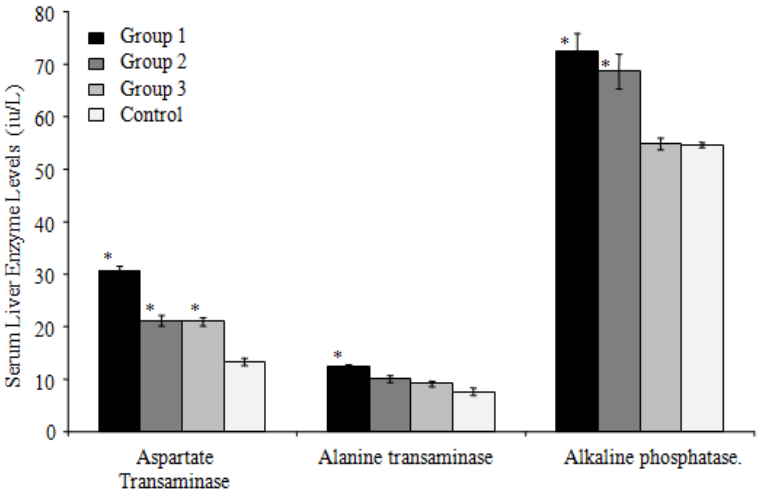
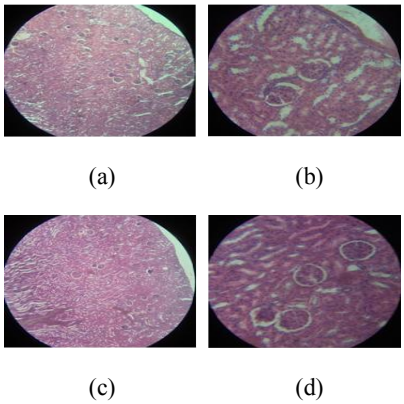


Fig. 4: Effect of oral administration of various doses of *PY* aqueous stem bark extract for 28 days, on serum liver enzymes of rats

Table 3: The effects of oral administration of various doses of *PY* aqueous stem bark extract for 28 days, on serum electrolytes of rats

Doses	No. of rats	Na^+	K^+	HCO_3^-	Urea	Creatinine
0	10	126.6±0.5	5.04±0.1	23.4±0.2	56±5.8	0.88±0.02
600	10	128.3±0.7*	4.76±0.1	24.3±0.3	58±4.7	0.78±0.04
150	10	127.1±0.7	4.89±0.1	23.8±0.5	54±5.2	0.82±0.05
30%w/w of rat chow	10	127.5±0.7	4.67±0.2	22.9±0.4	54±5.2	0.82±0.04

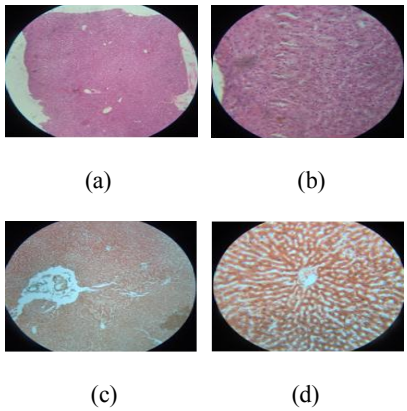
Values: Mean ± SEM; N = 10. * $p<0.05$; ANOVA; Electrolytes (mmol/dm^3) Urea and Creatinine (mg/dl)



(a) (b)

(c) (d)

Plate 1: Photomicrographs of H and E stained sections of rat kidney; No significant difference was observed between treated and control. (a): Control (x100); (b): Control (x400); (c): PY treated (x100); (d): PY treated (x100)



(a) (b)

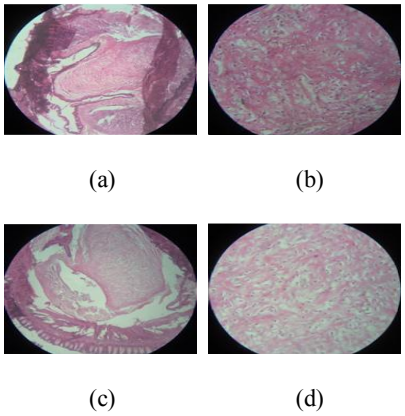
(c) (d)

Plate 2: Photomicrographs of H and E stained of rat liver. No significant histological difference was observed between treated and control. (a): Control (x100); (b): Control (x400); (c): PY treated (x100); (d): PY treated (x100)

the 1st group was significantly raised for conjugated bilirubin (Fig. 2)

Serum proteins: Total protein was significantly reduced in 1st group (600 mg/kg dose) and there was no significant change in the albumen (Fig. 3).

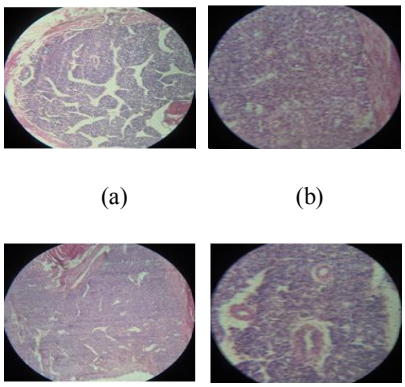
Serum liver enzymes: The aspartate transaminases were significantly raised in the treatment groups when compared with the control; the alanine transaminase was only significantly raised in the 600 mg/kg group. But the alkaline phosphatases were raised in the 600 mg and 150 mg/kg groups (Fig. 4).



(a) (b)

(c) (d)

Plate 3: Photomicrographs of H and E stained sections of rat penile cavemous tissue; No significant histological difference was observed between treated and control. (a): Control (x100); (b): Control (x400); (c): PY treated (x100); (d) : PY treated (x400)



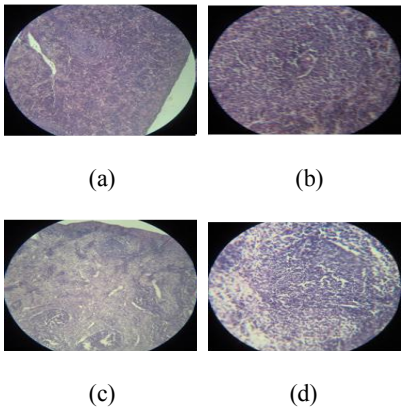
(a) (b)

(c) (d)

Plate 4: Photomicrographs of H and E stained sections of rat prostate gland. No significant histological difference was observed between control and treated. (a): Control (x100); (b): Control (x400); (c): PY treated (x100); (d): PY treated (x400)

Serum electrolytes: Of the serum electrolytes, only Na^+ showed significant elevation ($p<0.05$) at the 600mg/kg body weight dose (Table 3).

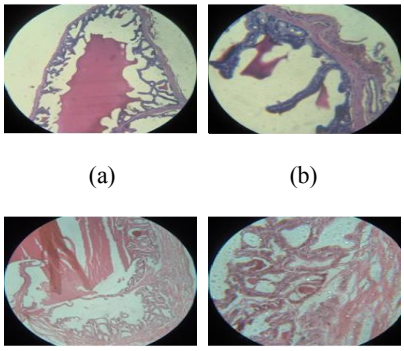
Histology: Plate 1 to 7 showed the light microscopic picture (H&E) of the kidney, liver, cavernous tissue, prostate, spleen, seminal vesicle and testis of rats treated with PY for a period of 28 days. There were no obvious histological aberrations in the treated when compared with the control.



(a) (b)

(c) (d)

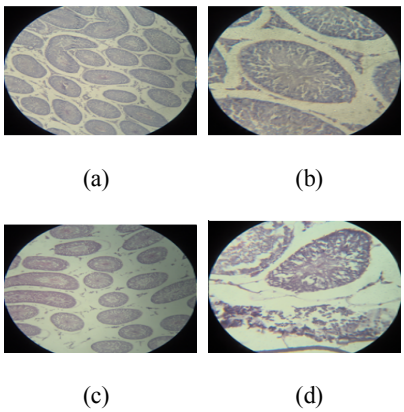
Plate 5: Photomicrograph of H and E stained sections of rat spleen. No histological difference was observed between the treated and control. (a): Control (x100); (b): Control (x400); (c): PY treated (x100); (d): PY treated (x400)



(a) (b)

(c) (d)

Plate 6: Photomicrographs of H and E stained sections of rat seminal vesicle. No signficant histological difference was observed between treated and control. (a): Control (x100); (b): Control (x400); (c): PY treated (x100); (d): PY treated (x400)



(a) (b)

(c) (d)

Plate 7: Photomicrographs of H and E stained sections of rat testis. No significant histological difference was observed between treated and control. (a): Control (x100); (b): Control (x400); (c): PY treated (x100); (d): PY treated (x400)

DISCUSSION

The Acute oral toxicity value (LD_{50}), for *Pausinystalia yohimbe (PY)* stem bark extract, obtained using albino rats from this study was above 2000mg/kg body weight as against LD_{50} of 1050mg/kg obtained in 2015 by Ijioma *et al*. (2015) in mice. The variation in LD_{50} may be attributable to variations in the bioactive substance in the same plant from different environments (Elujoba *et al*., 2005), or due to inter laboratory variations (Griffith, 1964). The Up and Down method used in this study offered the advantage of using fewer number of rats, in this case six as against the fixed dose and other conventional methods of determining oral acute toxicity LD_{50} (Lipnick *et al*., 1995). This result indicates a relative measure of safety referencing to the standard that oral acute toxicity of a substance of above 1000 mg/kg is relatively safe (Clarke and Clarke, 1977). Administration of aqueous stem bark of *PY* to male albino rats for 28days did not show any significant effect on body weight of the treated rats. The relative safety of the *PY* stem bark extract was further seen/indicated in the sub chronic 28 days study where the haematological indices (which included packed cell volume of the red blood cells, hite blood count (WBC), granulocyte count and platelet count) only showed a significant increase ($p<0.05$) of the WBC and platelet count (treatment groups) with other parameters being normal. Increase in WBC count may be associated with infection; extreme physical exercise (Baker *et al*., 1998; Mbaya *et al*., 2008) and or stimulation of the immune defense system (Kashinath, 1990; Abdulrahman *et al*., 2010) by the *PY* stem bark extract. Also the Biochemical function of the kidneys as accessed in this study, showed only Na^+ levels to be significantly high in the 600 mg/kg body weight dose, indicating a probable derangement at the tubular level leading to an increased re-absorption of sodium ions. This effect could be mediated via increased secretion of aldosterone or other mineralocorticoids or anti diuretic hormone (Tietz *et al*., 1994). Hypernatremia could be as a result of liver damage (Kleinman and Lorenz, 1989). Although the liver biochemical parameter in this study showed significant increases in total bilirubin for the 600 and 150 mg /kg body weight, it was insignificant in the 30% mixed rat chow group. And the conjugated bilirubin level was only elevated in the 600 mg/kg body weight dose group. The causes of elevated serum bilirubin levels could be pre hepatic, hepatic and post hepatic. Hepatic toxicity presents initially with elevation in the total and conjugated serum bilirubin levels which is likely to be the case in this study. While serum proteins were significantly lower in the 600 mg/kg bodyweight, the albumen level remained normal across the groups. It is likely that the liver still maintained its ability to produce albumin, needed to conjugate bilirubin (Mayne and Zilva, 1994). This

corroborates with the normal conjugated bilirubin levels in the lower dose treatment groups in this study. Aspartae (AST), Alanine (ALA) and Alkaline Phosphatase (ALP) are three liver enzymes usually employed in the assessment of the integrity of the liver cells. AST was significantly higher across the various treatment groups, while ALP was significantly higher in the 600 and 150 mg/kg body weight groups and ALT was significantly higher in the 600mg/kg body weight group, $p<0.05$ respectively. Taking these findings in the liver function tests together, they point to potential impairment of hepatocellular function and necessitates for more research into the toxicity of *PY*. This therefore calls for caution on the use of *yohimbine* in pre existing liver or kidney disease (Blumenthal and Brusse, 1998; McGuffin *et al*., 1997). The histological report showed no apparent damage to the kidney, liver, cavernous tissue, prostate, spleen, seminal vesicle and testis of rats treated with *PY* for a period of 28 days, indicating further relative safety of the plant.

CONCLUSION

In conclusion, the aqueous stem bark of *Pausinystalia yohimbe* with an LD_{50} of about 2000 mg/kg body weight, is relatively safe but there is call for caution in the consumed amount, length of period of consumption and its use in pre existing liver or kidney disease. This statement is more relevant today than it was several years ago owing to the improved patronage of traditional medicines.

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