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Evaluation of Haematinic Activity and Subchronic Toxicity of *Sphenocentrum jollyanum* (Menispermaceae) Seed Oil

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Research Article

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ABSTRACT

Aim: The haematinic activity and subchronic toxicity of *Sphenocentrum jollyanum* (Menispermaceae) seed oil was evaluated and compared with the control.
Materials and Methods: In acute toxicity study the animals tolerated up to 16 g/kg body weight (bw) of the extract in 2 % Tween 80 solution administered orally after 24 hrs fast. Another set of mice (6 per group) fasted for 24 hrs were administered with the extract intra-peritoneal (IP) at different doses (250, 500, 1000, 2000 mg/kg bw) until 100% mortality was achieved. In subchronic toxicity study, 300, 600 and 1200 mg/kg bw of the extract in 2 % Tween 80 were administered on the animals for 120 days.
Results: In acute toxicity study, the extract was found to be non toxic when it was administered orally for up to 16 g/kg bw within 24 hrs. Subchronic toxicity test showed no mortality after 120 days of oral administration. The animals showed appreciable increase in feeding habit and water intake. Increase in body and vital organs weights occurred while tissue histology showed no abnormal features. The liver function profile showed no significant difference ($p \geq 0.05$) compared to the control except for the albumin that increased markedly. The extract led to significant increase ($p < 0.05$) in RBC. The packed cell volume (PCV) and haemoglobin count (Hb) increased with increase in dose. On the other hand, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and white blood cells (WBC), did not vary markedly. Similarly, WBC differentials did not record appreciable difference compared to the control.
Conclusion: The result showed that SJ seed oil possessed haematinic and hepato-

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protective property thereby justifying its therapeutic use in traditional medicine.

Keywords: Sphenocentrum jollyanum; seed oil; haematinic activity; toxicity;

1. INTRODUCTION

The knowledge of medicinal plants and their roles in the treatment of diseases is as old as man. They constitute a predominant mode of managing health problems in developing countries and mostly among the rural populace (Sandhu and Heinrich, 2005; Gupta et al., 2005).

Herbal medicine is generally believed to be more effective and having fewer side effects compared to synthetic medicines. More often, this has led to indiscriminate use without appropriate dose resulting in abuse. The incidence of adverse effects of these herbal remedies and sometimes life-threatening conditions has been reported among various ethnic groups (Elvin-Lewis, 2001; Chan, 2003). This has made it imperative to ascertain the toxicity profile of medicinal plants even though they have been used for ages to enable for scientific documentation on their safety/risk potentials.

Anaemia is a disease that is posing serious challenge to human health particularly in the tropical region where malaria is endemic. The incidence of anaemia is further aggravated due to poor nutrition status of the populace. This disease, characterized by a reduction in circulating red blood cell, reduction in concentrating haemoglobin and packed cell volume per unit of the peripheral blood (Oma, 1991; Aguwa, 1996), appeared to be more prevalent among children. In many developing countries, treatment of anaemia with herb/herbal formulation rich in haematinic property is very popular. This constitutes a predominant mode of managing the health condition because of its rich plant source that is cheap and readily available.

Sphenocentrum jollyanum (SJ) Pierre (Menispermaceae) is a rain forest plant, an under growth of dense forest which grows naturally along the west coast sub region of Africa. The plant is deep rooted with few branches. It bears fruit that is yellowish in colour when ripe and contains a single large oval shaped seed. SJ has been shown to display a wide spectrum of biological and pharmacological activities. Its medicinal importance was first highlighted by Dalziel (Dalziel, 1955) in which it was noted that the leaves decoctions were used as vermifuge. It is reputed for use in dressing wounds particularly chronic wounds, feverish conditions, cough as well as being an aphrodisiac (Dalziel, 1955; Iwu, 1993). Studies have shown the seed to possess significant antipyretic and analgesic activities (Dalziel, 1955; Muko et al., 1998). Investigations have also revealed that the seed exhibited significant anti-oxidant (Nia et al., 2004) and anti-inflammatory (Moody et al., 2006) properties. The leave (Mbaka et al., 2010) and the root (Mbaka et al., 2010) of SJ have equally been shown to possess haematinic property.

In view of the many health benefits of SJ seed, there was apparent need to carry out an assessment of its haematinic activity and also, to conduct comprehensive investigation on its toxicity to highlight any hidden toxic activity. This study was therefore designed to investigate the effect of sub-chronic administration of seed extract of SJ on biochemical and haematological parameters using albino rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

The dried seeds of *Sphenocentrum jollyanum* were purchased from Ibode market in Ibadan, Oyo State, Nigeria. They were authenticated by a taxonomist, Dr. O. A. Ugbogu, of the Forestry Research Institute of Nigeria (FRIN), Ibadan where voucher specimen has been deposited in the herbarium (FHI/108203).

2.1.1 Preparation of the petroleum ether extract of the seed of S. jollyanum

The dried seeds were subjected to size reduction to a coarse powder with electric grinder. The seed powder, 1.24 kg, was extracted in petroleum ether (60-80 ^{o}C) in three cycles using Soxhlet extractor. The crude petroleum ether extract of the seed was filtered with Whatman filter paper No. 4 and the filtrate concentrated *in vacuo* 30^{o}C to obtain 142 g residue which gave a percentage yield of 11.5 % w/w. The residue was stored in an air tight bottle kept in a refrigerator at 4 ^{o}C till used.

2.2 Animals

Healthy male albino rats weighing between 145 and 160 g and mice (80-100g) were obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Lagos, Nigeria. They were housed in polypropylene cages at room temperature (21±2.8 ^{0}C) with 12 h dark and 12 h light circle. They were fed with standard rodent chow from livestock feeds PLC, Lagos and water *ad libitum*. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animal Research (ILAR) guidelines on the use and care of animals, in experimental studies (ILAR, 1996).

2.3 Acute Toxicity

Mice randomly grouped (8 per group) received by gavages different doses of 0.5, 1, 2, 4, 8 and 16 g/kg body weight (bw) of the extract administered in 2 % Tween 80 solution after food was withdrawn for 24 hrs. Another set of mice (6 per group) fasted for 24 hrs were administered with the extract intra-peritoneal (IP) at different doses (250, 500, 1000, 2000mg/kg bw) until 100% mortality was achieved. The animals were closely observed for the first 4 hrs; and then hourly for 12 hrs, followed by 6 hourly for 56 hrs giving a total of 72 hrs observations (Shar et al., 1997; Burger et al., 2005). Death or changes in general behaviour and other physiological activities were examined. The LD_{50} of the extract was determined through graph with probit on the Y-axis against the log dose on the X- axis.

2.4 Chronic Toxicity Tests

Twenty four male rats were randomly allotted to the control and the extract treated groups. The doses used were 300, 600 and 1200 mg/kg bw of the extract in 2% Tween 80. The doses were administered daily for 120 days by gastric probe with repeated weighing of the rats while the control group received distilled water. The animals were observed closely for behavioral changes and changes in body weight and mortality. The animals were sacrificed under mild diethyl ether anaesthesia after 24 hrs of fast and the vital organs (heart, lungs, liver, kidney and testes) were excised for the histopathological study while blood sample

collected from the heart of the rats was used for the biochemical studies. The weight of each vital organs isolated were standardized for 100 g bw of each rat.

2.5 Tissue Histology

The organs-liver, heart, kidney and testes- were fixed in Bouin's fluid for seven days before embedding in paraffin wax. A section of each organ tissue at 5µm was stained with Haematoxylin and Eosin (H and E). Each section was examined under light microscope at high power magnification for changes in organ architecture and photomicrographs were taken.

2.6 Biochemical Studies

2.6.1 Effects on blood glucose and lipid

The fasting blood glucose was determined from plasma by glucose oxidase method (Trinder, 1969). Total cholesterol (TC), high density lipoprotein-cholesterol (HDL-cholesterol) and triglyceride (TG) were determined from serum by modified enzymatic method from Sigma Diagnostics (Wasan et al., 2001). Low density lipoprotein cholesterol (LDL cholesterol) was calculated from the primary measurements using the empirical equation of Friedewald et al. (1972) (LDL-Chol) = (Total chol) – (HDL chol) – (Triglyceride)/5.

2.6.2 Effect on some blood parameters

The effect of the extract on certain biochemical parameters were examined and compared with those of the control group. The blood samples collected in heparinized bottles were centrifuged at 5000 rpm for 10 minutes to obtain clear plasma and used for the following investigations of total, direct and indirect bilirubin using the technique of Jandrassik and Grof (Koch and Doumas, 1982); alanine amino transferase (ALT) and aspatate amino transferase (AST) were measured using enzymatic method (Horder and Sampson, 1991); alkaline phosphatase (ALP) was analyzed according to the method of Kind Kind, 1954); total protein (TP) concentration was determined by Biuret method (Doumas, 1975); albumin was determined based on its reaction with bromocresol green (Spencer and Prince, 1971); urea was determined according to Urease-Berthelot method (Weatherburn, 1967) and plasma creatinine was estimated using Jaffe reaction (Perone et al., 1992).

2.7 Effects on Haematological Parameters

Diethyl ether was used to anaesthetize the animals before blood samples were collected through heart puncture into EDTA tubes for analysis of haematological parameters. The blood samples were analyzed for red blood cells (RBC) by haemocytometic method (Dacie and Lewis, 1984); the haemoglobin (Hb) content was by Cyanmethaemoglobin (Drabkin) method (Dacie and Lewis, 1984); packed cell volume (PCV) was according to Ekaidem et al., 2006) while white blood cells (WBC) and its differentials (neutrophil, eosinophil, basophil, lymphocyte and monocyte) were as described by Dacie and Lewis (1984).

2.8 Statistical Analysis

All values were expressed as mean ± standard error of mean and the statistical significance between treated and control groups were analyzed using Student's t-test. $P<0.05$ was considered significant.

3. RESULTS

The acute toxicity study showed that the animals fed by gastric gavages tolerated up to 16 g/kg bw of the extract. The intra-peritoneal (IP) administration produced dose dependent mortality with a median acute toxicity (LD_{50}) of the seed extract at 1432.0 mg/kg bw (Table 1).

Table 1. Acute toxicity determination of SJ petroleum ether seed extract (intraperitoneal route) at dose range of 250-2000mg/kg bw.

Group	Dose (mg/kg bw)	Log Dose	24h Mortality	% Mortality	Probit	Probit (Approx)
1	250mg	2.40	1/8	12.5	3.8497	3.8
2	500mg	2.70	2/8	25	4.3255	4.3
3	1000mg	3.00	3/8	37.5	4.6814	4.7
4	2000mg	3.30	5/8	62.5	5.3186	5.3

There was significant increase in weight of the rats after 120 days of extract administration with no abnormal gross changes observed ($p \geq .05$). The weight gain compared to the initial body weight of the animals is summarized in Fig. 1. The harvested organs showed no colour change; however, marginal weight increase occurred particularly in the liver, kidney and testis that showed dose effect (Table 2).

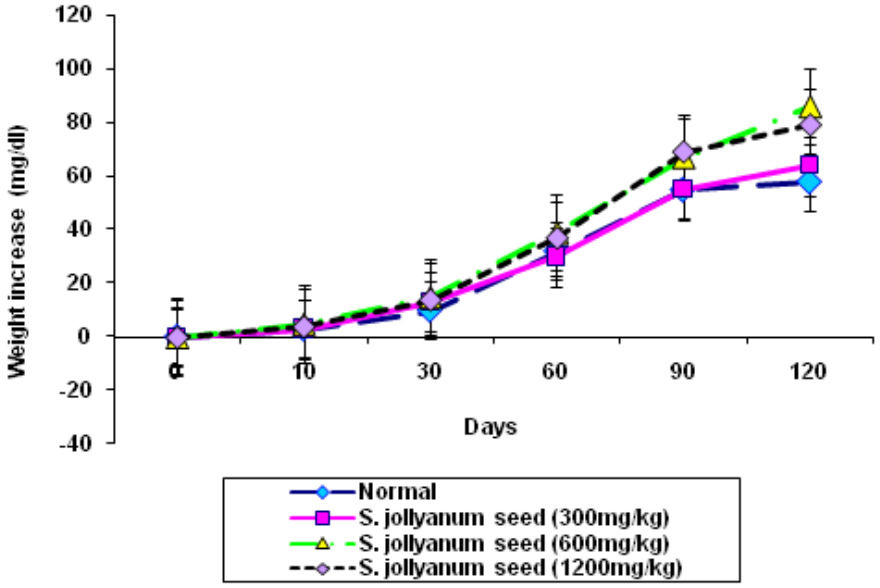

Fig. 1. Weight increase in the control and treated animals

Table 2. Data on the organ weight (per 100g body weight) in rats after sub-chronic treatment with SJ seed extract

Treatment	Mean organ weight per 100g body weight				
	Heart	Lung	Liver	Kidney	Testes
Control	0.4±0.3	1.6±0.2	7.9±0.7	1.2±0.1	3.6±0.5
300mg/kg	0.4±0.1	2.3±0.2	8.1±0.9	1.3±0.2	3.7±0.4
600mg/kg	0.4±0.3	2.2±0.2	9.0±0.6	1.4±0.9	4.1±0.5
1200mg/kg	0.5±1.1	2.2 ±0.1	9.7±0.4	1.6±0.2	4.4±0.7

*Mean ± SEM, (n=5) *p<0.05 vs. control group.*

The histological sections of the untreated testicular tissue (Figure 2) showed different sections of seminiferous tubules with the interstitium interspacing. The basement zone is a thick area comprising compactly arranged differentiating primitive cells (spermatogonia) with the spermatozoa forming a cluster at the lumina.

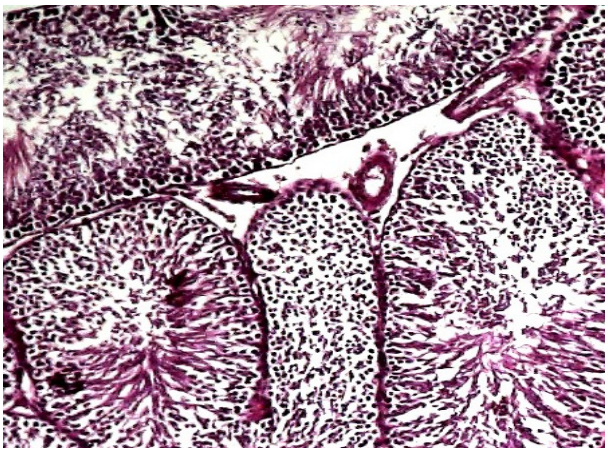

Fig. 2. The cross section of testes of the control group showing seminiferous tubules interspaced by interstitium. (H and E stain) mag. x100.

The tissue morphology of the extract treated group (Figure 3) showed no changes such as scanty spermatocytes or spermatozoa that could be caused by pathological changes at the germinal epithelium. The interstitium equally showed no lesion.

The normal hepatic tissue (Figure 4) showed the portal tracts situated at the periphery of poorly marked hepatic lobule and from the lobular margin, the hepatocytes interspaced by hepatic sinusoids form radial arrangement. At the middle of each lobule is the central vein. In Figure 5, the extract treated, the tissue histology showed normal appearance.

The kidney of the control group (Figure 6) showed cortical area of renal tissue with glomerular apparatus forming a rounded mass encircled by Bowman's space. The extract treated (Fig. 7) equally showed normal appearance.

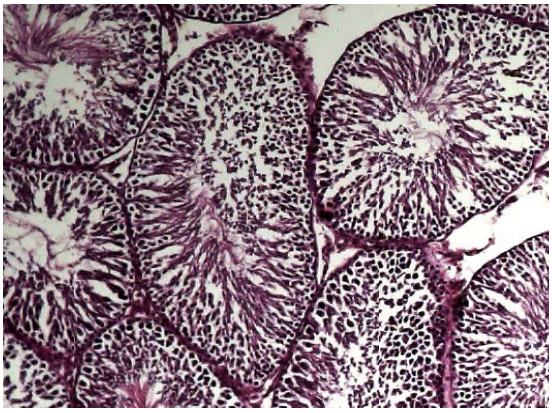

Fig. 3. The cross section of seminiferous tubules of testis treated with 1200mg/kg of the seed extract. (H and E stain) mag. x100.

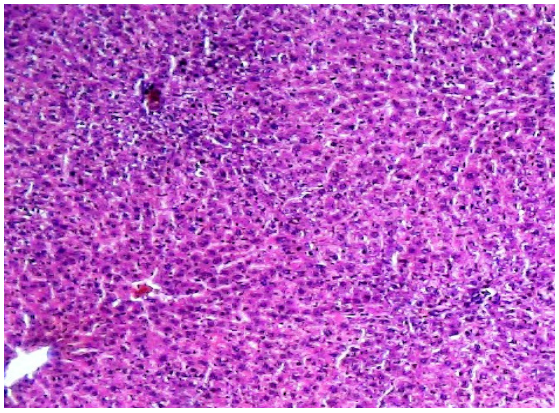

Fig. 4. The photomicrograph of hepatic tissue of the control group showing the central vein (V). (H and E stain) mag. x100.

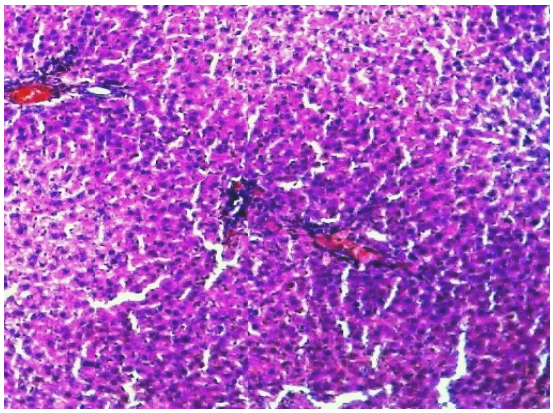

Fig. 5. Histology of the cross section of hepatic tissue treated with 1200mg/kg of the extract. (H and E stain) mag. x100.

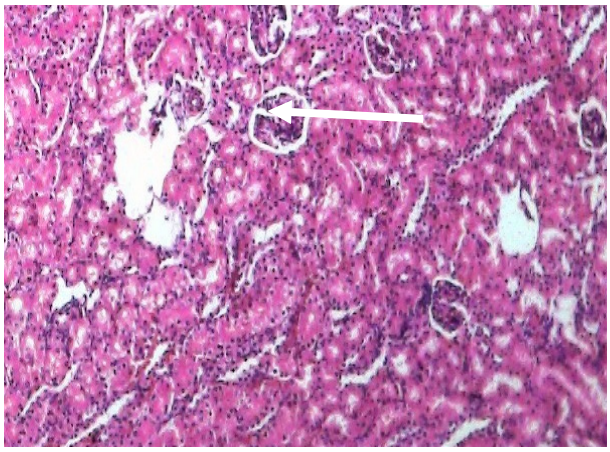

Fig. 6. The histology of a cross section of renal tissue of the control group showing renal corpuscles (arrowed) with Bowman's space surrounding it. (H and E stain) mag. x100.

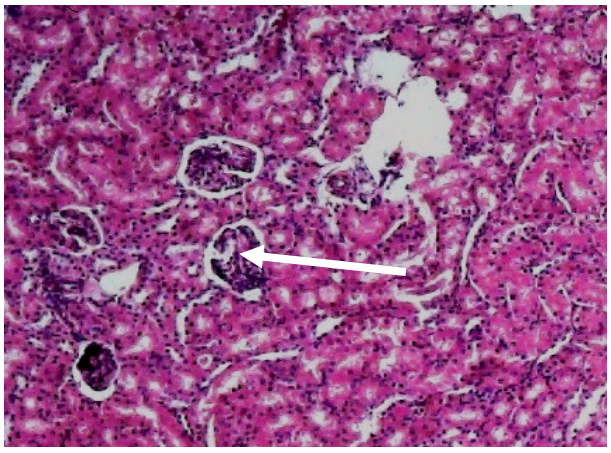

Fig. 7. The cross section of renal tissue of the extract treated. Arrow indicates the renal corpuscles showing normal appearance. (H and E stain) mag. x100.

Table 3 shows results of the biochemical studies. The lipid profile showed marginal changes, TC decreased considerably with dose while TG showed a slight increase at a dose of 1200 mg/kg bw, HDL-cholesterol increased with increase in dose whereas LDL-cholesterol showed progressive decrease with increase in dose of the extract.

The mean values of liver function profile as summarized in Table 3 showed no significant difference ($p \geq 0.05$) compared to the control with the exception of albumin that increased markedly in the extract treated group. AST, ALT and creatinine were comparably the same with the control while urea showed fluctuation in values.

Table 3. Blood chemistry values of rats in sub-chronic treatment with SJ seed extract

Parameter	Treatment (mg/kg)			
	Control	300	600	1200
Total protein (mg/dl)	50.2±0.5	51.1±3.0	59.9±1.5*	54.9±0.8
Albumin (mg/dl)	36.5±1.8	50.1±1.1*	53.6±5.2*	52.4±3.4*
Total bilirubin (mg/dl)	1.4±0.2	1.4±0.8	1.4±0.0	1.4±0.9
Direct bilirubin (mg/dl)	0.1±0.0	0.1±0.1	0.1±0.0	0.2±0.1
Indirect bilirubin (mg/dl)	1.3±0.8	1.1±02	1.3±0.0	1.7±0.1
AST (iμ/L)	55.6±14.8	57.5±5.5	55.4±9.4	56.9±14.0
ALT (iμ/L)	15.6±2.0	15.1±0.3	16.7±2.3	16.0±0.4
Alkaline phosphatase (iμ/L)	139.5±2.8	139.0±2.0	143.5±2.8	142.9±3.0
Urea (mg/dl)	94.5±4.1	92.4±3.9	94.4±7.1	93.8.0±6.7
Creatinine (mg/dl)	1.5±0.1	1.5±1.1	1.5±0.3	1.5±4.1
Glucose (mg/dl)	67.0±2.4	64.8±2.4	63.2±4.1	66.3±7.0
Total cholesterol (TC) (mg/dl)	86.3±1.3	79.4±6.1	69.0±1.4	64.6±3.5
Triglycerides (TG) (mg/dl)	55.7±6.1	50.5±5.3	47.3±4.5	52.2±7.8
LDL cholesterol (mg/dl)	123.2±0.3	104.2±4.4	97.7±3.1	97.1±1,9
HDL-cholesterol (mg/dl)	21.2±0.3	22.3±0.4	25.1±1.3	29.1±3.6

*Mean ± SEM, (n=5) *p<0.05 vs. control. .AST: Aspartate aminotransferase ALT: Alanine aminotransferase HDL-cholesterol: High density lipoprotein*

Table 4. Haematological values of rats in sub- chronic treatment with SJ seed extract

Treatment	RBC ($x10^6$)	Hb (g/dl)	PCV (%)	WBC ($x10^3$)	MCV (fl)	MCH (%)	MCHC (%)
Control	4.6±0.4	13.0±0.6	45.0±1.2	4.2±0.1	8.2±1.0	3.0±0.2	30.1±0.4
300mg/kg	6.8±0.1*	16.6±1.0*	52.2±2.2*	4.2±1.2	7.1±0.8	2.5±0.2	28.6±2.1
600mg/kg	6.9±0.4*	17.0±0.5*	59.7±1.9*	4.1±0.5	6.7±0.3	2.5±0.1	28.5±0.5
1200mg/kg	6.9±3.2*	17.7±1.4*	61.2±2.1	4.2±0.1	6.6±1.8	2.3±0.2	28.7±2.1

*Mean ± SEM, (n=5) *p<0.05 vs. control group.*
RBC: Red blood cells Hb: haemoglobin; PCV: Packed cell volume; WBC: White blood cells; MCV: Mean corpuscular haemoglobin; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

Table 4 showed the effect of the extract on haematological parameters of the animals. Administration of the seed extract led to significant increase ($p < 0.05$) in RBC. The PCV and Hb increased with increase in dose. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and WBC, on the other hand, did not vary markedly. Similarly, WBC differentials (Table 5) did not record appreciable difference compared to the control.

Table 5. Quantitative data on WBC differentials in rats after sub-chronic treatment with SJ seed extract

Treatment	Neutrophil %	Lymphocyte %	Eosinophil %	Eosinophil %	Monocyte %
Control	53.1±4.0	30.1±4.0	10.5±3.4	1.7±1.1	8.0±1.1
300mg/kg	50.2±2.1	33.6±5.1	10.7±0.2	1.5±1.2	9.3±2.4
600mg/kg	50.2±2.2	36.3±0.3	10.5±2.0	1.0±0.1	9.4±2.3
1200mg/kg	49.2±0.5	32.2±3.3	10.2±1.2	1.4±1.3	9.2±4.2

*Mean ± SEM, (n=5) *p<0.05 vs. control.*

4. DISCUSSION

The toxicity of the seed oil extract of SJ was investigated. In this study, the acute and chronic toxicity studies revealed no undesirable effect. The oral toxicity study demonstrated a high safety margin for the seed. The animals tolerated up to 13 g/kg bw orally which exceeded 2 g/kg oral dose bench mark (WHO, 1966).

Oral administration of the extract at the graded doses of 300, 600 and 1200 mg/kg bw respectively, for four months, produced periodic body weight gain which might suggest that the extract was non toxic. The animals showed no behavioural change. By visual observation the extract appeared to have stimulated more appetite for eating and water consumption which was evident by increased food and water intake. Likewise, there was no gross abnormality on the vital organs examined except for slight increase in organ weight of the treated animals.

In lipid profile study, the total cholesterol, triglyceride and LDL-cholesterol exhibited marginal decrease in the treated group. It has been observed that increase in lipid level particularly LDL-cholesterol is predictive for coronary events such as atherosclerosis and coronary heart disease (Blake et al., 2002). On the other hand, HDL- cholesterol known as the “good cholesterol” increased appreciably. The seed oil of the plant could therefore be said to have beneficial effect on the plasma lipid profile of the animals.

In this study, the two major liver marker enzymes, AST and ALT showed no increase in plasma level in the treated compared to the control. In toxic environment, the activities of the two enzymes in the blood stream are known to increase significantly (Solomon et al., 1993; Crook, 2006). It was therefore obvious that the extract was not toxic to the liver at the doses administered which to a large extent also buttressed a high safety margin for its oral consumption. Furthermore, the histological study in which tissue morphology showed no abnormal features on the hepatic tissue and the tissues of other organs examined was another indication that the extract may be relatively safe.

The results of creatinine and urea as examined by clinical blood chemistry showed no difference compared to the control. This invariably suggested that the function of renal system was not compromised. The marked increase in albumin level in the treated compared to the control might be attributable to adaptogenic activity. By initiating a rise in albumin level the extract may have prevented oxidative damage to the liver. Increases in albumin and total protein have been reported to have hepato-protective effect (Oyagbemi et al., 2008).

The assessment of hematological parameter showed that the seed oil affected the rate of RBC production. There was significant increase in total RBC count, PCV and haemoglobin

concentration in dose dependent manner. This indicated that the oil extract does have the potential to stimulate erythropoietin release in the kidney known to enhance RBC production (erythropoiesis) (Polenakovic and Sikole, 1996; Sanchez-Elsner et al., 2004). This finding was in congruous with the report on the leave (Mbaka et al., 2010) and root ((Mbaka et al., 2010) which obviously alluded to the belief that different parts of SJ plant enhances erythropoiesis and therefore could be used as haematinic agent.

The RBC indices, MCV, MCH and MCHC showed marginal decrease in the extract treated compared to the control. The importance of calculated blood indices in anaemia diagnosis has been reported (Agbor et al., 1999). The non significant difference on the RBC indices suggested that the extract did not affect a change in the average size of red blood cells. By extension, it did not induce anaemia. There was also, notably no change in WBC count which is known to rise as body defense in response to toxic environment (Ngogan, 2005). On the other hand, lymphocyte, the main effector's cell of the immune system (Mc Knight et al., 1999; Teguia et al., 2007) recorded fluctuation suggesting that the extract might not have exerted challenge on the immune system of the animals.

5. CONCLUSION

The use of SJ seed oil at the doses employed had no obvious deleterious effect which to a large extent provided more information on the therapeutic safety of the herbal drug. The extract has been observed to possess haematinic and hepato-protective property. It could equally be said to have beneficial effect on the plasma lipid profile.

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