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Full Length Research Paper

Evaluation of toxicity profile of leaf base extract of *Sorghum bicolor* in rat

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The toxicity profile of 70% methanolic extract of *Sorghum bicolor* leaf base widely used in ethnomedicine was evaluated in male rats treated daily for 28 days using 100 – 400 mg/kg p. o. doses. No adverse clinical signs were observed. There was no significant change in the feed intake, body weight and relative organ weight except the significant ($P < 0.05$) reduction in weight of kidneys and increase in relative weight of the testes observed at doses of 200 and 400 mg/kg respectively. No gross or histopathological changes were seen in the kidneys, heart, spleen, lungs, liver and testes. No significant effect was observed in the haematological indices (packed cell volume, heamoglobin, total and differential white blood cells), hepatic function indices (glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, direct bilirubin, total bilirubin, alkaline phosphatase, albumin, total protein) as well as renal function indices (urea and creatinine). Uric acid was however reduced significantly ($P < 0.05$). Study of effect on serum lipid profile showed no significant effect on cholesterol but a significant reduction of triglyceride at 200 mg/kg p. o. dose. The results suggest that *S. bicolor* leaf extract is relatively safe.

Key words: *Sorghum bicolor*, organ weight, pathology, haematology, serum biochemistry.

INTRODUCTION

Sorghum bicolor (Linn.) Pers. (Family: Gramineae; Poaceae) is an annual plant having its different parts widely used in traditional medicine. Ethnobotanical report shows that decoction from *S. bicolor* seed is demulcent and diuretic (Grieve, 1984). The inflorescence is astringent and haemostatic (Chiej, 1984). In Zimbabwe, the root is used for malaria, the seed for breast disease and diarrhea, while the stem is used for tubercular swellings. In India, the plant is considered anthelminthic and insecticidal, while in South Africa, in combination with *Erigeron canadense* L., it is used for eczema (Watt and Breyer-Brandwijk, 1962). Duke and Wain (1981) reported folkloric use of *S. bicolor* as anti-abortive demulcent, diuretic, emollient and remedy for cancer, epilepsy, flux and stomachache. In China, the seed husk is braised in brown sugar with a little water and applied to the chest of measles patients. The stomachic seeds are also considered beneficial in fluxes (Perry, 1980). Curacao natives drink the leaf decoction for measles, grinding the seeds with those of the calabash tree (Cresentia) for lung ailments. Venezuelans toast and pulverise the seed for diarrhoea. Brazilians decoct the seed for bronchitis, cough and other chest ailments, possibly using the ash for goiter while Arubans poultice hot oil packs of the seeds on the back of those suffering pulmonary congestion (Morton, 1981). The seed decoction was also reported as a folk medication for kidney and urinary complaints (Grieve, 1931). In Nigeria, the plant is used for purification of blood and stimulation of blood production (Okokoh, 1999). They are also used to improve the blood, body defense system as well as fertility (Perso-

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nal Communications: Ibrahim Muazzam, Plant Taxanomist and G Ali, an animal attendant, all with National Institute for Pharmaceutical Research and Development.NIPRD, Abuja, Nigeria). *S. bicolor* is one out of the four herbal components (*Piper guineenses* seeds, *Pterocarpus osun* stem, *Eugenia caryophyllum* fruit and *Sorghum bicolor* leaves) of the sickle cell drug (NIPRISAN®) developed by National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria (Wambebe et al., 2001). *S. bicolor* is also one out of the three components of Jubi Formular®, (*Parquetina nigrescens, Sorghum bicolor* and *Harungana madagascariensis*), a commercial herbal haematinic manufactured by Health Forever Products Ltd., Lagos, Nigeria (Erah et al., 2003). According to the company's claim, the product enhances packed cell volume (PCV), haemoglobin (Hb) and is therefore recommended for the support of treatment of moderate to severe anaemia as in sickle cell anaemia, cancer and HIV/AIDS. It is also recommended as nutritional supplement in stress, exhaustion and convalescent situations. It is also helpful in diabetes, hypertension, arthritis and infertility.

All these show the extensive use of *S. bicolor* in traditional medicine. According to Zhu et al. (2002), the rationale for the utilization of medicinal plants has rested largely on long-term clinical experience with little or no scientific data on their efficacy and safety. The possibility of toxicity associated with long-term low dose exposure of medicinal products has on this basis been pointed out (McNamara, 1976). This possibly explains why the need for thorough scientific investigation of herbal medicines for the validation of their folkloric usages including benefits and toxicity was emphasized (Sofowora, 1982).

Presently, the toxicological report made generally on sorghum (a common name for the numerous grasses of the genus *Sorghum*) is that it contains hydrocyanic acid, the alkaloid hordenine and sometimes accumulates toxic levels of nitrate (Morton, 1981). The danger with hydrogencyanide (HCN) was reported to be slight when grain is nearly mature while young plants and suckers are dangerous, particularly when suffering from drought. HCN is however destroyed when fodder is ensiled or cured as hay (Morton, 1981). This toxicological information did not provide lucid information about the effect of the plant parts on different body system, information that could be useful in the toxicity classification of the plant, drug development and subsequent clinical uses. The present study therefore involved the evaluation of the acute and sub-acute toxicity effects of mature dry leaf base of *S. bicolor* to provide some of this information.

MATERIALS AND METHODS

Plant preparation and extraction

The dry mature leaves of *S. bicolor* were collected from Maganawa town, Sokoto State, Nigeria between November and January, 2006. The plant was authenticated by a plant taxonomist, Mr. Ibrahim Muazzam of Herbarium Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The specimen was deposited in NIPRD's Herbarium with voucher specimen number 3815. The dark red portions of the leaves attached to the suckers of the plants were cut out from the entire leaves (the portion of the leaves especially claimed to be used ethnomedicinally). They were then pulverized in a mortar. Two hundred grams (200 g) of the pulverized sample was cold macerated successively in 5 L of 70% v/v methanol over 96 h period on a shaker (GFL D 3006 mgH, Germany) to ensure maximum extraction. The extract was then filtered using clean cotton wool. The filtrate was placed on water bath to allow evaporation of the solvents and consequent concentration of the extract for subsequent studies. A yield of 23.6% (w/w) extract was obtained.

Animals

Wistar rats (88.5 – 100.5 g) and Swiss albino mice (19.8 – 20.4 g) of both sexes were used for the studies. They were obtained from the Animal Facility Centre, Department of Pharmacology and Toxicology, NIPRD, Abuja. The experimental animals were separated for two weeks in the experimental room for acclimatization. They were housed in appropriately designed cages suitably bedded with wood shavings. The animals were maintained under normal environmental temperature (26 – 28°C) with approximately normal 12 h day and night illumination cycle. The animals were fed *ad libitum* with standard NIPRD formulated feed and had free access to water from Abuja Municipal water supply. The experimental rooms were cleaned and disinfected regularly. Soiled wood shavings were replaced often. The feed and water containers as well as the animal cages were also washed regularly.

In the experimental grouping of the animals, their age/body weight and sex of the animals were taken into consideration to achieve approximately equal conditions among the groups. The animals were identified using picric acid solution to mark unique numbers on individual animals. Small cards indicating the study number, group number, animal number and dose level were stuck to different cages for identification.

The 'Principles of laboratory animal care' (NIH Publication # 85-23, 1985) were followed in the study.

Chemicals

The chemicals used in these studies included; Methanol (Fluka Chemie, Switzerland), Chloroform (Sigma-Aldrich, Gillingham, Dorset), Formaldehyde (May and Baker, England). The kits for glutamate oxaloacetate transaminase (GOT), Glutamate pyruvic transaminase (GPT), direct and total bilirubin, alkaline phosphate, albumin, total protein, urea, creatinine, triglyceride, cholesterol and uric acid used for the serum biochemical studies were all from Randox Ltd, UK.

Acute toxicity studies

Acute toxicity studies were carried out using the modified method of (Lorke, 1983). The method estimates the dose of the extract that will kill 50% of the animal population (LD_{50}). The estimation of LD_{50} values for the crude extract was done in Swiss albino mice and Wistar rats of both sexes. The test routes were both intraperitoneal and oral.

The administration of the extract in both rats and mice was done in phases. The first phase involved the administration of widely differing doses of the extract (100, 1000, 1500 and 2000 mg/kg i.p. and p.o.) to determine the range within which toxicity lied. The

second phase dependent on the observations made in the first phase and involved the administration of more specific doses (800, 1000 and 1200 mg/kg i.p.) to new sets of experimental animals. The animals treated i.p. and p.o. were observed for 24 and 72 h, respectively, for behavioural effects such as nervousness, ataxia, excitement, alertness, dullness and death. The LD_{50} was calculated as the geometric mean of the dose that resulted in 100% lethality and that which caused no lethality at all.

Sub-acute toxicity studies

The study was carried out over a 28 day period. Adult male Wistar rats (88.5 – 100.5 g) were used. They were grouped into four (of six rats per group). Groups two, three and four in addition to feed and water received graded doses of the extract (100, 200 and 400 mg/kg orally) for 28 days while group one received only water and feed orally and served as the control. The first day of dosing was taken as day zero (D1) while the sacrifice day was taken as day twenty-eight (D28).

Mortality and clinical signs

All the animals were observed throughout the 28 days dosing period for clinical signs/behavioural changes and/or mortality patterns before and after dosing.

Effect on feed and water consumption

The amount of feed and water consumed were measured daily from the quantity of feed and water supplied the previous day and the amount remaining after 24 h.

Effect on body weight

The body weight (g) of each rat was taken before commencement of dosing (D1), once every 5 days during the dosing and on the day of sacrifice (D28).

Effect on relative organ weight

On D28, all the rats were euthanised and exsanguinated under chloroform anaesthesia. Organs such as heart, liver, lungs, spleen, kidneys and testes were surgically dissected out and weighed in grams to get the absolute organ weight. The relative organ weight (ROW) of organs for each rat was calculated.

Gross and histopathological examinations

The macroscopic/physical examination of the organs dissected out were carried out to assess the gross effect on the morphologies/ features, consistencies and appearance of the organs. The organs were then fixed in 10% formal saline for histopathological processing and examinations. The process involved the use of automatic tissue processor (Shandon Citadel model 2000). The steps involved the dehydration of the 10% formal saline-fixed tissue with ethanol, clearing with xylene, infiltration of the tissues with paraffin wax, embedding and sectioning with rotary microtome. Haematoxylin and Eosin were then used to stain the tissues and microscopic examinations were done at the magnification of X 400.

Effect on haematological indices

A portion of blood sample collected from the heart of each of the euthanised rats was dispensed into EDTA anticoagulant bottle for haematological analyses. The standard methods of Baker et al. (1985) and Jain (1986) were used to measure the haematological indices such as packed cell volume (PCV), haemoglobin (Hb), total leucocyte count and differential leucocyte count.

Effect on serum biochemistry

The remaining portion of the blood samples from the euthanized rats were dispensed into plain tubes and allowed to stand for 3 h to ensure complete clotting. The clotted blood samples were then centrifuged at 3000 rpm for 10 min. The clear sera were aspirated and stored frozen for serum biochemical analyses. Biomedical indices were determined using standard ready-to-use kits (Randox Ltd., UK) following the manufacturer's instructions. The indices determined included glutamate oxaloacetate transaminase (GOT), glutamate pyruvic transaminase (GPT), alkaline phosphatase (ALP), total proteins, albumin, total bilirubin, urea, creatinine, uric acid, total cholesterol and triglycerides.

Statistical analysis

The results of the studies were expressed as mean ± SEM. The difference between the control and treated means were analysed using one-way or two-way analysis of variance (ANOVA) as appropriate. Student t-test was applied were ANOVA showed significant difference. Statistical significance was established at $P < 0.05$.

Compliance with Good Laboratory Practice (GLP)

The studies were carried out according to Good Laboratory Practice (GLP) regulations of Organization for Economic Cooperation and Development – OECD (UNDP/World Bank/WHO, 2001).

RESULTS

Acute toxicity studies

No overt toxicity sign or death was observed in rats and mice 72 h post oral treatment with 100 – 2,000 mg/kg doses of *S. bicolor* leaf base extract. The oral median lethal dose (LD_{50}) of the extract in rats and mice was therefore ≥ 2000 mg/kg p.o. The rats treated intraperitoneally (i.p.) with the leaf base extract (100 – 2,000 mg/kg) showed no overt toxicity sign or death 24 h post treatment. However, all the rats treated with 2,000 mg/kg i.p. dose became recumbent and died within 48 h of the intraperitoneal treatment while those treated with 100 – 1,000 mg/kg i.p. doses neither showed toxicity signs nor death 72 h post i.p. treatment. For the estimation of the intraperitoneal median lethal dose (LD_{50} i.p.) in rats, assessment based on 24 h post treatment showed a median lethal dose (LD_{50}) ≥ 2,000 mg/kg i.p. since no overt toxicity sign or death was observed in i.p.-treated rats after 24 h. However, an assessment based on 48 h post i.p. treatment observation gave a calculated median lethal dose of 1,414.2 mg/kg i.p. in rats. The mice treated with doses of the extract ≤ 1,200 mg/kg i.p. showed neither toxicity signs nor death 24 h post treatment. At the dose of 1,500 mg/kg i.p., the mice were

Table 1. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) on feed intake of male rats treated for 28 days.

Treatment	Mean feed intake (g)			
	Week 1	Week 2	Week 3	Week 4
Control	57.19 ± 2.7	56.69 ± 2.6	68.81 ± 4.1	80.4 ± 4.6
100 mg/kg p.o.	60.69 ± 1.8	54.93 ± 2.7	66.49 ± 4.3	73.48 ± 3.6
200 mg/kg p.o.	51.73 ± 1.8	52.31 ± 2.2	64.36 ± 4.1	80.04 ± 3.3
400 mg/kg p.o.	51.04 ± 2.1	54.37 ± 2.1	65.54 ± 3.1	89.24 ± 3.7

n = 6.

Table 2. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) on water intake of male rats treated for 28 days.

Treatment	Mean water intake (ml)			
	Week 1	Week 2	Week 3	Week 4
Control	96.07 ± 5.1	101.79 ± 5.5	96.43 ± 6.4	85.42 ± 3.8
100 mg/kg p.o.	105.36 ± 5.8	116.00 ± 3.9*	99.93 ± 6.6	93.75 ± 5.4
200 mg/kg p.o.	99.29 ± 4.8	107.14 ± 4.0	92.86 ± 4.0	87.50 ± 3.2
400 mg/kg p.o.	101.79 ± 4.6	105.36 ± 5.8	82.14 ± 5.0	79.17 ± 5.3

* = $p<0.05$, significant increase in water intake; (Student t-test); n = 6.

calm, dull, with increased respiratory rate. At this dose, mortality of 66.7 and 100.0% occurred within 24 and 48 h of i.p. treatment respectively.

The mice treated i.p. with 2,000 mg/kg dose was calm, dull, and recumbent with increased respiratory rate. A mortality of 100.0% occurred at this dose within 24 h. The calculated intraperitoneal medial lethal dose in mice was 1,248.0 and 1,341.6 mg/kg i.p. for 24 and 48 h post treatment observations respectively.

Mortality and clinical signs

Throughout the treatment duration of 28 days, no adverse clinical sign or toxicity sign was observed in all the rats. No mortality was recorded in all the groups.

Effect on feed and water consumption

Feed were consumed variably throughout the treatment period in all the groups. Both increases and decreases in feed consumption were recorded. However, none of these changes differed significantly from the control (Table 1). Water was also taken variably with both increases and decreases in consumption recorded in all the groups throughout the 28 days treatment period. The only significant intake was the increase recorded in week 2 for 100 mg/kg p.o. treated rats (Table 2).

Effect on body weight

Increase in body weight was observed throughout the study duration in all the groups (including the control group). However, *S. bicolor* leaf base extract (100 – 400 mg/kg p.o.) did not produce any significant change in the body weight of treated rats when compared with the control (Table 3).

Effect on the relative organ weight

There were no significant changes in the relative weights of the liver, heart, spleen and lungs. However, 200 mg/kg dose produced a significant ($P< 0.05$) reduction in the relative weight of the kidneys while 400 mg/kg dose caused a significant increase in the relative weight of the testes (Table 4).

Gross and hisopathological examinations

No gross abnormality was seen in the morphologies/ features consistencies and appearances of the liver, kidney, heart, spleen, lungs and testes of the male rats treated for 28 days with *S. bicolor* leaf base extract (100 – 400 mg/kg p.o.). Histopathological examinations of organs stained with Haematoxylin and Eosin stains, viewed under the microscope at X 400 magnifications revealed that there were no abnormalities in the kidneys, heart, spleen, lungs, liver and testes of the male rats treated for 28 days with *S. bicolor* leaf extract (plates not shown).

Effect on haematological indices

The result showed both increases and decreases in the total while blood cells. The lymphocytes were dose-

Table 3. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) on body weight of male rats treated for 28 days.

Treatment	Mean body weight (g)						
	D0	D5	D10	D15	D20	D25	D28
Control	99.90±3.9	104.40±4.2	107.73±4.7	112.58±5.7	119.7±6.7	127.95±7.6	131.12±9.0
100 mg/kg p.o.	101.92±5.8	104.93±4.1	107.33±3.9	112.40±4.3	119.95±5.3	128.43±5.7	132.08±6.9
200 mg/kg p.o.	101.50±6.0	103.4±2.4	106.67±4.6	109.95±4.9	115.98±5.2	123.48±6.2	130.12±6.7
400 mg/kg p.o.	100.00±6.3	103.06±6.1	107.78±7.0	112.03±7.5	118.08±8.5	126.07±9.0	131.35±9.4

D0 – D28 = treatment days; n = 6.

Table 4. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) on relative organ weight of male rats treated for 28 days.

Treatment	Mean relative organ weight (g/100 g)					
	Liver	Kidney	Heart	Spleen	Lungs	Testes
Control	3.74 ± 0.10	0.61 ± 0.03	0.37 ± 0.03	0.47 ± 0.06	0.73 ± 0.10	1.36 ± 0.05
S. bicolor						
100 mg/kg p.o.	3.48 ± 0.10	0.60 ± 0.02	0.34 ± 0.01	0.43 ± 0.05	0.65 ± 0.03	1.44 ± 0.06
200 mg/kg p.o.	3.65 ± 0.05	0.47 ± 0.05*	0.33 ± 0.02	0.48 ± 0.03	0.68 ± 0.07	1.55 ± 0.09
400 mg/kg p.o.	3.43 ± 0.09	0.63 ± 0.02	0.33 ± 0.01	0.46 ± 0.03	0.69 ± 0.04	1.51 ± 0.03**

*=Significant reduction; **= significant increase in relative organ weight ($p<0.05$; ANOVA; Student t-test); n = 6.

Table 5. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) on haematological indices of male rats treated for 28 days.

Treatment	PVC	Hb	WBC	DLC				
				Neut.	Lymph.	Mono.	Eos.	Bas.
Control	38.00±2.2	12.72±0.7	8.45±1.2	23.33±5.0	76.67± 5.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
100 mg/kg p.o.	35.67±2.1	11.90±0.7	9.80±1.2	17.67±2.6	80.50± 3.4	0.17± 0.17	0.0 ± 0.0	0.0 ± 0.0
200 mg/kg p.o.	34.67±2.5	11.57±0.8	9.93±2.5	17.17±2.3	81.17± 3.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
400 mg/kg p.o.	34.17±1.9	11.45±0.6	8.23±0.9	16.83±3.4	83.17± 3.4	0.00 ± 0.0	0.00± 0.0	0.00± 0.0

PCV = Packed cell volume; Hb = haemoglobin; WBC = White blood cell; DLC = differential leucocyte count; Neut = neutrophils; Lymph = lymphocytes; mono = Monocytes; Eos = Eosinophils; Bas. = basophils; n = 6.

dependently increased white monocytes, eosinophils and basophils were virtually absent in all the viewed fields. A reduction was recorded for the neutrophils, haemoglobin and packed cell volume. However, none of these changes (increases or decreases) differed significantly from the control (Table 5).

Effect on hepatic function indices

Both increases and decreases were recorded for direct bilirubin, total bilirubin and albumin. Increases were recorded for GOT, GPT and alkaline phosphatase while reductions were recorded for total protein. However, these effects were neither dose-dependent nor significantly different from the control (Table 6).

Effect on renal function indices

There were both decreases and increases in all the renal function indices. However, the changes in urea and creatinine were not significantly different from the control at all the tested doses, while the doses of 100 and 200 mg/kg p.o. of the extract significantly ($P < 0.05$) reduced uric acid (Table 7).

Effect on serum lipid profile

Cholesterol had both reductions and an increase but none of these changes differed significantly from the control. On the other hand, triglyceride was generally reduced at all the tested doses. The reduction was only significant ($P < 0.05$) at the dose of 200 mg/kg p. o. (Table 8).

DISCUSSION

The fact that different substances have different toxicity levels is shown in the classification of substances into

Table 6. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) on hepatic function indices of male rats treated for 28 days.

Treatment	GOT (IU/L)	GPT (IU/L)	Direct bilirubin (mg/dl)	Total Bilirubin (mg/dl)	Alkaline phosphatase (IU/L)	Albumin (mg/dl)	Total protein (mg/dl)
Control	65.0±6.4	28.50±1.4	0.11±0.05	0.49±0.09	109.65±11.1	3.13±0.1	6.57±0.3
400 mg/kg p.o.	69.33±5.3	35.5±3.6	0.18±0.06	0.57±0.1	112.8±8.6	3.37±0.04	6.35±0.1
200 mg/kg p.o.	68.33±7.0	30.17±1.8	0.04±0.01	0.43±0.09	119.78±8.2	3.00±0.09	6.02±0.11
100 mg/kg p.o.	70.33±6.0	35.67±6.5	0.16±0.03	0.38±0.1	113.5±10.7	3.43±0.08	6.50±0.3

GOT= Glutamate oxaloacetate transaminase; GPT= Glutamate pyruvate transaminas; n = 6

Table 7. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o) on renal function indices of male rats treated for 28 days.

Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	26.43 ± 5.9	0.50 ± 0.08	5.08 ± 0.35
100 mg/kg p.o.	33.90 ± 6.4	0.40 ± 0.05	4.10 ± 0.02*
200 mg/kg p.o.	19.72 ± 5.5	0.52 ± 0.07	4.13 ± 0.4*
400 mg/kg p.o.	27.18 ± 6.5	0.45 ± 0.07	5.58 ± 0.67

*= $p<0.05$ (significant reduction; Student t-test); n = 6.

Table 8. Effect of *S. bicolor* leaf extract (100 – 400 mg/kg p.o.) on serum lipid profile of male rats treated for 28 days.

Treatment	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Control	115.07 ± 5.7	102.58 ± 9.3
100 mg/kg p.o.	106.63 ± 10.3	103.40 ± 7.3
200 mg/kg p.o.	92.37 ± 7.0*	94.52 ± 7.7
400 mg/kg p.o.	104.28 ± 9.3	101.47 ± 12.4

*= $p<0.05$ (significant reduction; Student t-test); n = 6.

very toxic, toxic, less toxic or only slightly toxic (Lorke, 1983). The revelation of the toxicity profile of plant extract intended to be used as medicine will therefore help in the determination of the extent of its safety if in use as drug. This is because, as important as it is for drugs to be efficacious, cheap and available, there is extreme need for these drugs to be safe for short and long term uses. This implies that evaluation of safety profile of a drug is paramount in the development of drugs and in their subsequent clinical uses.

In the present investigation, acute toxicity study was used to establish the median lethal dose (LD_{50}) of mice and rats treated orally and intraperitoneally. This is the dose of the extract that will kill 50% of the animal population. The median lethal dose has been shown not to be an absolute value but inherently variable biological parameters that cannot be compared to constants such as molecular weight or melting point (Oliver, 1986). It is therefore further reported that "accuracy should not be used to describe LD_{50} but precision". The precision being only relevant to the experiment for which the median lethal dose (LD_{50}) was derived and does not increase the probability that in subsequent experiments, the LD_{50} will be identical or even similar (Oliver, 1986).

It is worth noting at this point that acute toxicity (LD_{50}) test has its limitations. These were seen in the criticisms of the use of acute toxicity (LD_{50}) test as a parameter for assessing toxicity (Lorke, 1983; Klassen, 2001; Timbrel, 2002). This was buttressed by the report of Orisakwe et al. (2002) that LD_{50} does not necessarily guarantee the safety of the tested agent not withstanding its value. According to the report, rinbacin with a high LD_{50} value induced toxic effects to the rat testes. Despite these limitations however, acute toxicity study furnishes some useful in information. For instance, it helps in the selection of dose ranges that could be used for subsequent studies. The possible clinical signs induced by the substance of investigation could manifest at this level of study. It is also applied in the establishment of therapeutic index (LD_{50}/ED_{50}) of drugs and xenobiotics (Rang et al., 2001).

The present study showed that *S. bicolor* leaf base extract caused no overt toxicity sign or death in mice and rats 72 h post oral treatment. The oral LD_{50} of the extract was estimated to be ≥ 2000 mg/kg in both mice and rats. The Organization for Economic Cooperation and Development (OECD, Paris, France) recommended chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values as: very toxic, ≤ 5 mg/kg; toxic, > 5 ≤ 50 mg/kg; harmful, > 50 ≤ 500 mg/kg; and not toxic or harmful, > 500 ≤ 2,000 mg/kg (Walum, 1998). Based on this classification, the oral LD_{50} up to 2,000 mg/kg established for both mice and rats indicated relative oral safety. Lack of overt toxicity signs in these experimental animals also pointed to that fact.

On the other hand, intraperitoneal administration of the extract in mice and rats elicited toxicity signs which included calmness, dullness, increased respiration and death at doses between 1,500 – 2,000 mg/kg i.p. The intraperitoneal LD_{50} value ranges between 1,248.0 – 1,341.6 mg/kg i.p. were estimated for the mice while that of the rat was 1,414.2 mg/kg i.p. This also meant that i.p. administration of the extract was relatively safe. LD_{50} values > 1000 mg/kg are considered as safe (Lorke, 1983). However, attention should be given to the calmness, dullness and increased respiration observed (though at high doses), few minutes post treatment.

The clinical signs and mortalities observed at certain dose ranges have proven that acute toxicity study (LD_{50}) is useful after all. However, such acute toxicity data are of limited clinical application since cumulative toxic effects to occur even at very low doses. Hence, sub-acute and chronic toxicity studies are almost always invaluable in evaluating the safety profile of phytomedicines (Aniagu et al., 2005). This is probably the basis for suggestion that sub-chronic toxicity data be used to predict the hazard of long term, low-dose exposure to a particular component (McNamara, 1976). In the present investigation, sub-acute toxicity study was carried out for evaluation of long term, low dose effect of *S. bicolor* leaf base extract.

The study revealed that no adverse clinical sign or toxicity sign or death was observed throughout the treatment duration of 28 days. This is in line with the acute toxicity studies where experimental animals treated orally with the extract doses < 2000 mg/kg showed neither toxicity sign nor death. This may be an indication that long term oral administration of the extract within these low dose ranges is safe.

The non-significant effect of the extract on the feed intake showed that *S. bicolor* leaf base extract possibly did not interfere with the nutritional benefits (weight gain, stability of appetite) expected of animals that were supplied with feed and water *ad libitum* as has been earlier suggested (Aniagu et al., 2005). This was confirmed by the general but non significant increases in the body weight observed in all the treated groups throughout the study duration. When animals lose appetite (anorexia), weight loss is bound to ensue due to disturbances in carbohydrate, protein or fat metabolism (Klaassen, 2001). The general increase in body weight observed shows that the extract possibly did not induce anorexia, an effect that could have resulted in loss of body weight.

Increased organ weight (either absolute or relative) has been observed as a sensitive indicator of organ toxicity by known toxicants (Simmons et al., 1995). This is in line with the study and report on Beagle dogs where a novel lipid was shown to induce hepatocellular hypertrophy, with alanine transaminase (ALT) and aspartate transaminase (AST) being elevated, while there was a 2 – 3 fold increase in cytochrome P450 content of hepatic microsomes (Wolfang et al., 1995). This report was however disputed by the work and report on rinbacin (a herbal preparation), that there was no increase in either the absolute or relative weight of the rat liver even with increase in the biochemical parameters, which are indications of hepatotoxicity (Dioka et al., 2002). Another report on the other hand, showed an increase in liver weight of rats exposed to diisopropyl ether, without any increase in biochemical parameters (Dalbey and Feuston, 1996). These reports establish the rationale to evaluate both organ weights and their histopathology. The present study showed that *S. bicolor* leaf base extract caused no significant changes in the relative weight of the liver, heart, spleen and lungs. 200 mg/kg p.o. dose of the extract however significantly ($p < 0.05$) reduced the relative weight of the kidneys while 400 mg/kg dose caused a significant increase in the relative weight of the testes. These results could mean that the integrity of all the above organs (with exception of the kidneys and testes) were not tampered with by the extract. However, this deduction can only be possibly true if the results of the effects of the extract on relative organ weight, serum biochemical indices and histopathology of these organs are considered together. Gross pathological observation of the organs showed no gross abnormalities in the morphologies/features, consistencies and appearances of the liver, kidney, heart, spleen, lungs and testes of the male rats treated for 28 days with the extract. Histopathological examinations also revealed that there were no abnormalities in the kidneys, heart, spleen, liver, lungs and testes of the rats.

Liver cell damage is characterised by a rise in plasma enzymes such as AST, ALT, etc (Aniagu et al., 2005). Serum biochemical analyses carried out to evaluate the effect of the extract on hepatic function indices revealed that the extract caused no significant effect on direct bilirubin, total bilirubin, albumin, GOT also known as aspartate aminotransferase (AST), GPT also referred to as alanine aminotransferase (ALT), alkaline phosphatase and total protein. The absence of significant changes in the relative weight of the liver, the absence of abnormalities in the morphologies/features, consistencies and appearances of the liver observed grossly and the non-significant difference observed in the hepatic function indices suggest absence of hepatotoxicity.

The result of the effect of the extract on renal function indices showed that the extract produced no significant effect on both urea and creatinine. It however reduced uric acid significantly ($P< 0.05$) at doses of 100 and 200 mg/kg p. o. This is a reflection of the preserved renal integrity of the treated rats (Kaneko, 1989). This result also points out that therapeutic advantage can be taken of the extracts' ability to reduce uric acid in hyperuricemia, a condition that can pre-dispose to gouty arthritis, intense inflammation of soft tissues on which urate crystals are deposited (Rock et al., 1986).

Absence of abnormalities in the gross and histopathological examination of the kidney, in addition to the

observed effects on renal function indices further suggest that the excretory capability of the kidneys was not impaired.

Effect of the extract tested on serum lipid profile showed that the extract had no significant effect on cholesterol. It however reduced triglyceride significantly. In human nutrition, triglycerides are the most prevalent glycerol esters encountered. They constitute 95% of tissue storage fat and are the predominant form of glycerol esters found in plasma. Following absorption, triglycerides are resynthesized in the epithelial cells and combine with proteins to form chylomicrons which travel through the lymphatic system to the thoracic duct and eventually to the jugular vein (Stein, 1986). The significant reduction of triglyceride by the extract is indicative of the lipid-lowering potential of the extract in mixed hyperlipidaemic states (Aniagu et al., 2005). The report of Stein (1986) about the combination of triglyceride with cholesterol for eventual movement to the thoracic duct and jugular vein is indicative of clinical importance of this extract-lipid effect. This is because of the correlation existing between the serum cholesterol levels and the incidence of ischaemic and coronary heart disease such as atherosclerosis (Stamler et al., 1986; Dixit et al., 1992).

The present study has provided information on the sub-acute toxicity effect of *S. bicolor* leaf base extract. However, further toxicity studies including sub-chronic, chronic, reproductive, developmental and genetic toxicity studies as well as mutagenicity and carcinogenicity tests, effects on drug metabolizing enzymes and toxicokinetic profiling still need to be done for the complete elucidation of the safety profile of *S. bicolor* leaf base extract. However, the present acute and sub-acute toxicity studies have shown relative safety of the extract.

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