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EFFECTS OF ETHANOLIC /POTASH EXTRACT OF *SORGHUM BICOLOR* LEAF SHEATH ON SERUM ELECTROLYTES, LIVER AND KIDNEY INDICATIVE ON ALBINO RATS

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ABSTRACT
Toxicity profile on serum electrolytes, hepatic and renal function indices on rats administered with mixture of ethanol/potash extract of *sorghum bicolor* leaf sheath were investigated. The ethanol/potash extract was dried and re-suspended in distilled water and the following quantities were administered orally to the albino rat per kg body weight: 1000, 2000 and 3000mg; while 0.9% normal saline was served to the control group. The blood was collected through cardiac puncture under chloroform anesthesia after 14 days. There after, the sera were analyzed for sodium (Na^+), potassium, (K^+),chloride (Cl^-) ion, urea, creatinine, total protein, total bilirubin and cholesterol levels. Serum sodium levels, (176-297 mmol/L) were significantly ($p<0.05$) increased following extract treatments, while potassium ion decreased (6.54-4.00mmol/L). The extract increased chloride ion concentration (95.8-99.0mmol/L). The serum value for creatinine (0.08-0.32mmol/L) and urea (3.16-38mmol/L) increased progressively; while cholesterol (0.96-0.19mmol/L) and total protein (54.13-17.27g/L) levels, decreased significantly ($p<0.05$) when compared to the control values. No significant change was observed in the value obtained for bilirubin except at dose level of 3000mg/kg body weight. There was a progressive decrease in body weight after 14days administration of the extract. The result showed that the mixture of ethanol/potash extract of *sorghum bicolor* leaf sheath has been observed in rats under the conditions of this study to be toxic.

Keyword: *Sorghum bicolor,* ethanol/potash, electrolytes, kidney, liver.

INTRODUCTION
Historically plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made large contribution to human health and well being. Despite the achievements recorded in drug discovery and development from plant sources, phytomedicine continues to be highly valuable for developing synthetic drugs (Adeneye and Benebo, 2007).
According to the World Health Organization (WHO), about 80% of the world's population depends wholly, or partly on plant derived pharmaceuticals (WHO, 1996). Thus, the need for toxicity evaluation of these medicinal plants, cannot be underscored (Jarnovska *et al.,* 2003), such research could advance the utilization of these indigenous herbal medicine for orthodox treatment (Parek and Chanda, 2006) or to serve as new dietary supplements which consist of minerals that may be used for the treatment of organ related diseases.
Sorghum is cultivated across the world in the warmer climatic areas. It is quantitatively the world's fifth largest most important cereal grain *(*Taylor *et al.,* 2001*)*. The grain stalk, leaves and leaf sheath contribute significantly in nutrition and medicine. The black-purple leaf sheath of *sorghum bicolor* draws out the plants incredible health benefit in the area of anaemia, heart, kidney and liver disease joint pain and improving immune system. Avwioro *et al*.,(2009), in their study on the Biochemical observations in wistar albino rat fed with dye extracted from *sorghum bicolor* leaf reported that the serum sodium, potassium, and urea increased progressively with increased in concentration of the extract, while the bilirubin levels did not change remaining at <17mmol/l. Nwinyi *et al*., (2009*)* also reported on the toxicity profile of methanolic extract of sorghum leaf sheath. It was found that there were no significant effect observed in total bilirubin and total protein as well as in creatnine, urea and cholesterol levels. Akande *et al.,*(2010) using aquoues extract of *sorghum bicolor* leaf sheath on rats also reported that the total protein concentration of the tested rat was not significantly altered compared with the control rat, Adebayo *et al.,* (2001) on prolonged administration of extract of Khaya Senegalese reported a significant increase in serum sodium ion and no significant difference in serum potassium ion concentration when compared to control.
Considering the importance of *sorghum bicolor* leaf sheath in heart, immune system, kidney and liver disease, and the fact that blood is an index of physiological and pathological status as well as the source of nutrient to the cells and tissues in the body, and the role that some biochemical parameters such as serum electrolytes, urea, cholesterol, total protein and bilirubin play in these organs, it becomes necessary to investigate the effect of

ethanol/potash extract (which is another method of consumption locally) of *Sorghum bicolor* leaf sheath on these biochemicals.

MATERIALS AND METHODS

Plant preparation and extraction

The dry matured leaves of *S.bicolar* were purchased from herb sellers at Mile 3 Market, Port Harcourt, Rivers State, Nigeria and identified by the Plant taxonomist in the Biological Science Department of Rivers State University of Science and Technology, Port Harcourt, Rivers State, Nigeria.

The study was carried out between September-December, 2011. The sample was oven dried at 60^0C for 18hrs and subsequently grounded into powder. Eight hundred grams of the powdered leaf sheath was exhaustively extracted (WHO, 1996) over 48 hr with 600ml ethanol mixed with 0.05g of potash. The extract was concentrated in vacuo and further placed in a water bath to allow evaporation of the solvents and consequent concentration of the extract for subsequent studies. A yield of 5.29% extract was obtained.

Animal collection and toxicity study

Wistar albino rats (125-160g) of both sexes were used for the studies. They were obtained from the animal room, Department of Biochemistry, University of Port Harcourt, Choba, Rivers State, Nigeria. The animals were kept in rat cages in four groups of five each, and fed with dry pellet feed and water ad libitum and all test animals were acclimatized for two week under normal environmental temperature of 26-28^0C. The principle of laboratory animal care were followed in this study (Cimos, 1985). The dried extract was administered at 0.5% per kg body weight in doses of 1000, 2000 and 3000mg/kg body weight over a period of 140days. The group 4 rat served as control and received only 0.9% of normal saline.

Blood collection

After 14 days of extract administration the rats were fasted overnight only distilled water was made available ad libitum. Blood samples were collected through cardiac puncture under chloroform anesthesia using a 5ml syringe into ethylene Diamine Tetra Acetic Acid (EDTA) treated bottles. The blood sample was centrifuged at 4000rpm for 20mins in order to separate the serum from plasma. The clean sera were aspirated and stored frozen for serum biochemical analysis. Serum electrolyses (Na^+, K^+ Cl^-), urea, total protein, cholestural and bilirubin were determined using standard ready-to-use kits (Randox Ltd, UK) following the manufacturers instructions.

STATISTICAL ANALYSIS

All data were analyzed using the analysis of variance. When analysis of variance revealed a significant effect, means were separated using Duncan's new multiple range test (Wahua, 1999).

RESULTS

Serum electrolytes.

Table 1 below showed effects of ethanol/potash extract of sorghum bicolor leaf sheath on sodium, potassium and chloride ions after 14 days administration. The values obtained ranged from 168-297mmol/l, 7.22-4.00mmol/l and 91.6-99.0mmol/L for sodium, potassium and chloride ions respectively. The concentration of sodium ion (Na^+) and chloride ions (Cl^-) increased when compared with the control. The increase was concentration dependent; while the potassium ion concentration decreased in all the tested animals. The animals treated with 3000mg/Kg extract had the least value for potassium ion concentration.

Table 1: Effects of Ethanol/potash extract of *S.bicolor* leaf sheath on serum electrolytes.

Extract treatment	Electrolytes (mmol/L)		
(mg/kg)	Sodium (Na^+)	Potassium (K^+)	Chloride (Cl^-)
1000 (group 1)	176^c ±12.6	6.54^a ±2.70	95.8^b ±4.6
2000 (group 2)	199^b ±10.2	5.18^{ab} ±1.01	96.0^b ±2.4
3000 (group 3)	297^a ±10.5	4.00^c ±0.97	99.0^a ± 2.1
Control (group 4)	168^c ± 4.1	7.22^a ± 1.23	91.6^c ±1.2

Values are means ±SD of five replicate determinations. Means in the same column not followed by the same superscript differ significantly ($p \leq 0.05$).

Hepatic and renal function indices

Effects of ethanol/potash extract of *S.bicolor* leaf sheath on albino rat after 14 days administration are shown in Table 2. There was a significant ($P<0.05$) increase in the concentrations of serum creatinine and serum urea when compared with the control. Thus, the levels of creatinine and urea were dose dependent. There was a significant

increase in the total protein and cholesterol levels of the control group compared to the tested animals. At 3000mg extract dose, the total protein and cholesterol, drastically dropped from 54.13g/l to 17.27g/l and from 0.96mmol/L to 0.19mmol/L. However, the bilirubin level (0.02-0.27mmol/l) of the animals treated with 3000mg extract, significantly ($P<0.05$) increased. There were no significant differences noticed in the bibirubin levels of animals treated with 1000mg/kg, 2000mg/kg and control (those administered with 0.9% normal saline).

Table 2: Effects of ethanol/potash extracts of *S.bicolor* leaf sheath on serum total protein, cholesterol and bilirubin.

	Extract treatment (mg/kg)			
Biochemical	**1000**	**2000**	**3000**	**Control**
Urea (mmol/L)	$3.16^{c} \pm 0.27$	$4.10^{b} \pm 1.37$	$6.38^{a} \pm 0.82$	$2.30^{c} \pm 0.4$
Creatimine (mmol/L)	$0.08^{c} \pm 0.01$	$0.12^{b} \pm 0.02$	$0.32^{a} \pm 0.04$	$0.05^{c} \pm 0.01$
Total protein (g/L)	$54.13^{b} \pm 2.37$	$26.77^{c} \pm 9.4$	$17.27^{d} \pm 1.1$	$67.24^{a} \pm 2.01$
Cholesterol (mmol/L)	$0.96^{b} \pm 0.12$	$0.55^{c} \pm 0.09$	$0.19^{d} \pm 0.07$	$1.68^{a} \pm 0.23$
Total Bilirubin (mmol/L)	$0.02^{b} \pm 0.0$	0.0^{b} 6±0.01	$0.27^{a} \pm 0.0$	$0.02^{b} \pm 0.0$

Values are means ± SD of fire replicate determinations. Means on the same row not followed by same superscript differ significantly ($P<0.05$).

Body weight of the rats treated with the extract after 14 days are shown in Table 3. The test animals decreased in weight, when compared to the original values. Values in parenthesis are the weight before extract administration. However, the group 4 (control) rats showed slight increase in body weight.

Table 3: Effects of ethanol/potash extract of the leaf sheath of *Sorghum bicolor* on the body weight of albino rats after 14 days of administration.

Extract dose (mg/kg)	**Rat Weight (g)**				
	Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**
1000 (group 1)	152	140	122	120	125
	*(160)	(150)	(160)	(125)	(125)
2000 (group 2)	135	130	120	110	100
	*(150)	(150)	(125)	(125)	(125)
3000 (group 3)	105	90	90	70	102
	*(150)	(150)	(125)	(125)	(125)
Control (group 4)	155	127	152	130	129
	*(150)	(125)	(150)	(125)	(125)

DISCUSSION

The combined ethanol/potash extract of *Sorghum bicolor* leaf sheath administered to rats resulted in significant ($P<0.05$) increased sodium ion concentration in the blood. The increased sodium ion concentration could be that the *sorghum bicolor* leaf sheath have properties that induce the depressant or sedative effect, which may have affected glomoerula filtrations and thereby decreasing the excretion of substances in the urine including sodium ion. Also, the increased Na^{+} concentration could be an indication of dehydration. (Odutula, 1992; Abdulrahman *et al.*, 2007). Although in this present study, the animals were not diarrheic, hence dehydration may not explain the presence of high sodium ion in the blood. Furthermore, elemental analysis of this sample showed high level of sodium, this could be associated to high serum sodium ion (Ogunka-Nnoka, and Horsfall, 2011).

The decreased serum potassium ion concentration observed in this study correlates well with increased sodium ion concentration. Potassium ion is a major cation of the intracellular fluid and only about 10% of the total body potassium is found extracellular. It has been observed that the intracellular potassium ion can not be measured; therefore, serum potassium concentration is not a good measure of a total body potassium because the bulk of potassium resides within the cells (Odutula, 1992, Tietz *et al.*, 2006). The decrease in potassium ion and increase in sodium ion supports the report of Ogunka-Nnoka *et al.,*(2012) on liver and kidney damages when ethanol/potash extract of *sorghum bicolor* leaf sheath was administered to the rat. Abdulraham *et al.,* (2007) also observed similar effect on sodium and potassium ion when albino rat was administered with aqueous extract of root bark of *vitex doniana.* The increased serum chloride ion concentration on rats treated with extract doses of 2000mg kg^{-1} and 3000 $mgkg^{-1}$ may be due to improper functioning of the kidney; since it has been reported that elevation of chloride ion

could be associated with kidney disease and sometimes in over activity of the parathyroid glands (http://www.medicinet.com).

The administration of this extract from *S.bicolor* leaf sheath was observed to induce increased serum creatimine and urea levels. It has been observed that a wide variety of renal disease with different permutation of glomerular, tabular, interstitial, or vascular damage can cause an increase in serum urea and creatinine (Roct *et al.,* 1998), urea being the product of protein metabolism that should be excreted through urine. This present study agrees with the report of Abdulraham *et al.,* 2007 and the report of Rabo, (1998), who observed increase in serum urea levels in rats treated with extract of *Butryrospermum paradoxum*.

Furthermore, total protein concentration and cholesterol levels significantly ($p \leq 0.05$) decreased. However total bilirubin level increased. The measurable reduction in total protein concentration suggest a toxic response to the extract administered. The toxicants in the extract may have affected the amino acid and protein synthesis in albino rats. The decrease observed might also be due to protein sparing action at different level of doses. The decreased cholesterol level observed with the increase in dose level indicates a tendency of the extract to lower the level of cholesterol in the peripheral tissues of the treated animals or it may be due to decreased cholesterol absorption from gastrointestinal tract. The present result conforms with the hypocholestrolaemic effect of other plant extracts such as *vitex domiana* (Abdulrahamm, *et al.,*2007), *Amaranthus cadalus and Amaranthus spinosa* (Arowolo *et al.,*1989). These plants induced hypocholesterolaemia in treated animals. The total bilirubin at dose levels of 1000-2000 mg/kg body weight do not change significantly when compared with the control. However, a significant difference was noticed at dose level of 3000mg/kg which is an indication that there was red blood cell lysis. This report does not agree with the report of Nwinyi *et al.,*(2009). The researchers reported no significant change in the bilirubin level of rats treated with dye extract from *Sorghum bicolor* leaf sheath. The decrease in body weight of the rats after 14days treatment further confirms that the extract had adverse effect on the rats.

This study therefore discourages the use of ethanol/potash combination for extraction as practiced by some groups in Nigeria.

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