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Research Article
Safety Concern Following Sub-acute Exposure to the Popular Food Additives, *Sorghum bicolor* and Saltpetre, Either Separately or in Combination in Sprague-Dawley Rats

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Abstract

Background and Objective: *Sorghum bicolor* (SB) and saltpeter (Sp) are food additives commonly used by food vendors of West African countries. Despite widespread use, there is still paucity of information on their safety, especially when used in combination. This present study investigated the sub-acute toxicity potential of aqueous extracts of SB and Sp when administered separately or in combination. **Materials and Methods:** About forty Sprague-Dawley rats of both sexes weighing 150-200 g were assigned into ten groups of four rats per group. SB or Sp was administered in three different doses (100, 300 or 1000 mg kg^{-1}) to six different groups of rats. Three other groups received a combination of both SB and Sp at each of the dose levels while control rats received distilled water (10 mL kg^{-1}). Extracts were administered once daily by gavage for 14 days after which rats were sacrificed by decapitation. Blood and some vital organs were obtained and processed for analyses. Significant differences were determined using a one-way ANOVA with Newman Keul's *post hoc* test. **Results:** Sub-acute exposure to SB and Sp significantly ($p<0.05$) elevated activities of aspartate aminotransferase and alkaline phosphatase when administered separately or in combination. Blood glucose was significantly ($p<0.05$) elevated by SB and Sp combination at 300 and 1000 mg kg^{-1}. Furthermore, exposure to SB, Sp or a combination of both induced tubular necrosis, dilatation, congestion, glomerular hypertrophy, infiltration by inflammatory cells or atrophy of glomerulus with increased capsular space. Similarly, exposure to separate doses of SB and Sp induced hepatic necrosis, congestion and dilatation of sinusoids and central vein and in addition, induced fatty changes when combined. Mild congestion of cardiac tissues, splenic necrosis and sinusoidal dilatation as well as oedema and infiltration of lung with inflammatory cells were also observed. **Conclusion:** Exposure to SB and Sp whether separately or in combination may be harmful to health and their continuous use as food additives should be of public health concern.

Key words: *Sorghum bicolor*, saltpetre, aqueous extracts, sub-acute toxicity, histopathology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food additives are substances with little or no nutritive values but are deliberately added to food substances to impart desired characteristics such as colour, taste, softness, preservation among others[1,2]. Some additives are manufactured from natural sources such as corn, beet and soybean, while others are artificial[3]. Although, there were reports suggesting health or other benefits of some of the known food additives, some others, on the other hand, have been linked with cancer, heart disease, obesity, digestive and neurological problems[4].

Sorghum bicolor is one of the frequently used additives by food vendors of West African countries. It is a cultivated tropical cereal grass belonging to the family *Poaceae* (Gramineae) and is commonly known as sorghum. It is most extensively cultivated in the drier Northern Guinea, Sudan Savannah and Greenland of Africa, plains of India and the great plains of United States of America[5]. Sorghum has a unique tolerance to drought and adaptation to dry tropical and subtropical ecosystems throughout the world and was considered a subsistence crop as a result[6]. Sorghum is an important source of nutraceuticals such as antioxidants, phenolics and cholesterol-lowering waxes[7]. The anthocyanins are the major class of flavonoids studied in sorghum. Sorghums have high concentration of 3-Deoxyanthocyanins (i.e., luteolinidin and apigenidin) that give stable pigments at high pH[8]. Anthocyanins vary in colour from red, pink to blue and violet. These characteristics suggested that *Sorghum bicolor* leaf extracts may be suitable raw materials for colouring foods[9]. Sorghum also contains phenolic compounds, plant secondary metabolites which serve as antioxidants and are useful in the prevention of free radical mediated diseases[10]. Current research trials indicated that it may reduce DNA oxidative damage; inhibit the growth of human leukemia cells and induce these cells to differentiate, inhibit cancer cell signal transduction and induce apoptosis, act as an anti-inflammatory, anti-spasmodic or spasmolytic as well as reduce the risk of ovarian and prostate cancers[11]. Also, antiradical activity of its flour extract has been reported[12]. Haemopoietic effect of aqueous extract of the leaf sheath of *Sorghum bicolor* in albino rats has also been reported[13]. Again, sorghum leaf sheath extract has been reported to improve haematological parameters in rats fed with iron deficient diet. The report further established that the extract was nontoxic to the liver and also the integrity of the kidney was maintained after *Sorghum bicolor* administration which confirms the overall safety of the extract upon consumption[14]. This has also been confirmed in a recent study which indicated that *Sorghum bicolor* aqueous extract did not exhibit cytotoxicity *in vitro* in the larvae toxicity test and *in vivo* in acute oral toxicity test[15]. However, other studies suggest that a dye of *Sorghum bicolor* leaf sheath was capable of causing varying degrees of congestion in the heart, kidney and lungs[16]. Ethanol/potash extract of *Sorghum bicolor* has also been shown to produce severe intra-hepatic cell damage as evident by marked elevation in levels of liver enzymes in rats. Histopathological examination revealed lesions in the morphological features of the liver and kidney[17].

Saltpetre, as it is popularly known, is also called "Chinese snow" by the Arabs and "kaun" by Ghanaians[18]. It is an ionic salt of potassium (K^+) and nitrate (NO_3^-) ions[19] and commonly used as food additive. It occurs as a mineral nitrate and a natural solid source of nitrogen. Conversion of nitrates into nitrites by the microbiome of the GIT can cause cancer and the heating of proteins in the presence of nitrate creates nitrosamine which is carcinogenic. It is therefore, believed that high content of saltpetre in food can have deleterious effects, even though the symptoms of poisoning are not physically evident after exposure to small quantities over a short period of time. The effects were significantly damaging when an individual was chronically exposed to it[20]. Prominent symptoms of potassium nitrate poisoning are known to be irregular breathing, convulsions, rapid heartbeat, dizziness, coma and death in severe cases[21]. It is also known that nitrate interferes with the conversion of carotene to vitamin A and/or with the absorption of carotene and vitamin A following consumption[22].

The growing concern over the increasing use of food additives to preserve and enhance food products has made it necessary to adequately characterize the potential harmful effects and determine the safety of these agents. Considering the controversies surrounding the safety of food additives like *Sorghum bicolor* and saltpetre, the present study evaluated the safety or toxicity potential of these additives when administered for 14 days either separately or in combination. Possible changes in biochemical and haematological functions as well as possible alteration in tissue/organ histology were explored in this study.

MATERIALS AND METHODS

Collection and preparation of test compounds: The study was conducted at the Department of Biomedical Sciences, University of Cape Coast, Cape Coast, Ghana, in December, 2016. Leaf sheaths of *S. bicolor* were purchased from the University of Cape Coast (UCC) market, Cape Coast, Ghana. The plant specimen was identified and authenticated at the

herbarium of the School of Biological Sciences, University of Cape Coast and a voucher specimen was deposited in the herbarium of the university. The leaf sheaths were air dried for 3 weeks, pulverized, soaked in distilled water and allowed to stand for 3 days with intermittent vigorous shaking. The mixture was then filtered and the filtrate evaporated on boiling water in a water bath and later placed in a desiccator containing activated silica gel to produce a solid mass which was again pulverized into fine powder. Powdered saltpetre was purchased from the university market in Cape Coast, Ghana and the chemical composition ascertained in the Department of Chemistry of the School of Physical Sciences, University of Cape Coast, Ghana. Subsequently, appropriate concentrations of the extract of *S. bicolor* and the saltpetre were prepared by dissolving in distilled water and doses administered to experimental animals in mg kg^{-1} body weight.

Animals: Sprague-Dawley rats weighing 150-200 g of both sexes were obtained from the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra and kept in stainless steel cages (37×47×18 cm^3) with wood shavings as bedding within the animal house facility of the School of Biological Sciences, University of Cape Coast, Ghana. The animals were maintained under laboratory conditions (temperature: 24-25°C, relative humidity: 60-70%, 12 h light/dark cycle) and fed with normal commercial pelleted diet (Agricare, Kumasi, Ghana) and water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for 7 days before start of experiments. All procedures and animal handling techniques employed in this study were in accordance with the National Institute of Care Guidelines (1985 revised in 1996).

Sub-acute toxicity test: Forty rats were weighed, assigned to ten groups of four rats per group and treated as follows:

Group 1-3: *Sorghum bicolor* (100, 300 and 1000 mg kg^{-1}, respectively)
Group 4-6: Saltpetre (100,300 and 1000 mg kg^{-1}, respectively)
Group 7: *Sorghum bicolor* (100 mg kg^{-1})+Saltpetre (100 mg kg^{-1})
Group 8: *Sorghum bicolor* (300 mg kg^{-1})+Saltpetre (300 mg kg^{-1})
Group 9: *Sorghum bicolor* (1000 mg kg^{-1})+Saltpetre (1000 mg kg^{-1})
Group 10: Control (distilled water, 10 mL kg^{-1})

Treatment was given once daily for 14 days at the same time, 1600 GMT. Rats were sacrificed by decapitation after an overnight fast and blood samples obtained for biochemical and haematological assessment. Vital organs (liver, kidney, heart, spleen and lung) were harvested and prepared for histopathological assessment.

Measurement of haematological parameters: Blood collected into EDTA-treated sample tubes were used for the assay of haematological parameters. All assays were done within 2 h of the sample collection. Red blood cell (RBC) count was estimated using the haemocytometer. Haemoglobin (Hb) levels were measured colorimetrically by the oxyhaemoglobin methods using Reichert's haemoglobinometer.

Biochemical parameters: Blood samples collected into gel separator tubes were processed by centrifuging for 5 min at 4400 rpm to ensure separation of plasma. Plasma sample which was used for biochemical analysis was stored at -20°C until ready for use. Total protein, cholesterol, triglycerides, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) were determined using Randox® ransel biochemistry kits and were carried out using spectrophotometry (Olympus AU400, Olympus Inc.) following the manufacturer's instructions.

Histopathological examination: Immediately after sacrifice and collection of blood samples, some major organs (heart, liver, spleen, kidneys and lungs) were harvested, cleared of fats and connective tissues and examined macroscopically. All tissues were preserved in 10% neutral buffered formalin solution. For histopathological examination, tissues were cleaned in physiological saline, fixed in Bouin's fluid, dehydrated in increasing concentrations of ethanol and embedded in paraffin wax. Thereafter, sections of tissues were cut at 5 mm with a rotary microtome and mounted on clean glass slides. Sections were stained with haematoxylin and eosin (H and E). The stained tissues were observed through an Olympus CX41 microscope and photographed at total magnifications 100x.

Data analysis: Data obtained were presented as Mean±SEM. Significant differences were determined using a one-way ANOVA with Newman Keul's *post hoc* test and differences were considered significant if $p<0.05$, with the exception of the histopathology which was shown by photomicrographs.

RESULTS

Effect of *Sorghum bicolor* and saltpetre on haematological parameters: Results obtained show that sub-acute administration of SB and Sp either separately or in combination did not produce significant changes in haematological parameters in the experimental rats when compared to control groups (Table 1).

Effect of *Sorghum bicolor* and saltpetre on serum biochemical parameters: Data obtained from biochemical analysis is presented in Table 2. The effect of the treatments on serum biochemical parameters after 14 days showed no significant differences at all treatment levels except ALP, AST and glucose concentration which were significantly ($p<0.05$) different from control group.

Effect of *Sorghum bicolor* and saltpetre on organ histology: Histological assessment of the various organs showed effects such as necrosis, congestion, dilatation of tubules and sinusoidal and central vein, haemosiderosis, infiltration of inflammatory cells, oedema and fatty changes.

Figure 1 presented kidney micrographs of control rats and rats that received either *Sorghum bicolor* or saltpetre or a combination of both. No morphological changes were observed in control rats (Fig. 1a). *Sorghum bicolor* (100 mg kg^{-1}) induced necrosis, congestion and dilatation of renal tubules (Fig. 1b). Tubular necrosis was observed in rats that received *Sorghum bicolor* (300 mg kg^{-1}) while those that received *Sorghum bicolor* (1000 mg kg^{-1}) exhibited necrosis and dilatation of the tubules together with hypertrophy of glomerulus (Fig. 1c, d). On the other hand, saltpetre (100 mg kg^{-1}) induced congestion with infiltration of inflammatory cells, glomerular atrophy with increased capsular space (Fig. 1e). Rats that received saltpetre (300 and 1000 mg kg^{-1}) developed tubular necrosis and dilatation in addition to congestion at 1000 mg kg^{-1} (Fig. 1f, g). Administration of *Sorghum bicolor* and saltpetre in combination induced tubular necrosis at 100 mg kg^{-1} (Fig. 1h) and tubular necrosis and dilatation at 300 and 1000 mg kg^{-1}, respectively (Fig. 1i, j).

Figure 2 presents liver micrographs of the various treatment groups. Control rats exhibited normal histology (Fig. 2a). *Sorghum bicolor* (100 mg kg^{-1}) caused sinusoidal

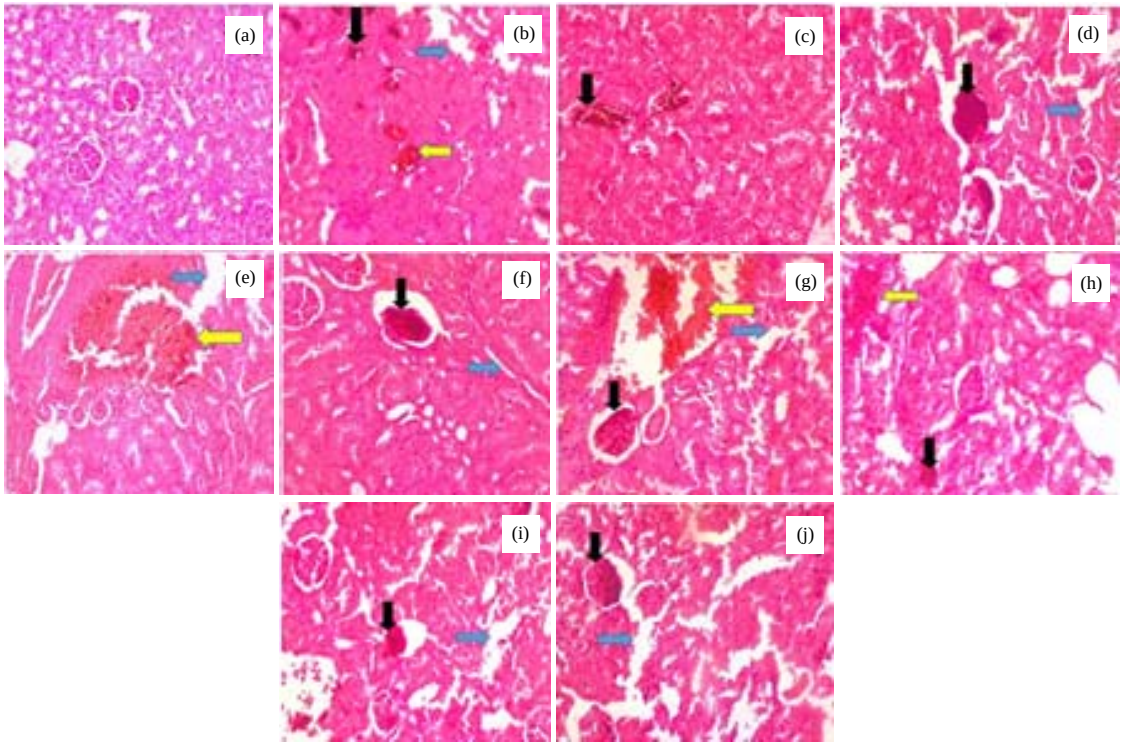

Fig. 1(a-j): Kidney micrographs for (a) Control, (b) SB (100 mg kg^{-1}), (c) SB (300 mg kg^{-1}), (d) SB (1000 mg kg^{-1}), (e) Sp (100 mg kg^{-1}), (f) Sp (300 mg kg^{-1}), (g) Sp (1000 mg kg^{-1}), (h) SB+Sp (100 mg kg^{-1}), (i) SB+Sp (300 mg kg^{-1}) and (J) SB+Sp (1000 mg kg^{-1})
SB: *Sorghum bicolor*, Sp: Saltpetre

Table 1: Haematological parameters in rats treated with *Sorghum bicolor* and saltpetre

Parameters	*Sorghum bicolor* only			Saltpetre only			*Sorghum bicolor* and Saltpetre			
Dose (mg kg^{-1})	100	300	1000	100	300	1000	100	300	1000	Control
WBC ($\times 10^3$ µL^{-1})	7.9±2.0	5.1±2.0	3.70±2.0	4.1±0.1	7.6±0.01	6.3±5.2	3.4±2.2	5.85±0.9	6.1±4.1	4.9±0.8
RBC ($\times 10$ µL^{-1})	6.1±0.3	6.2±0.5	6.30±0.4	7.2±0.1	6.8±0.1	6.7±0.4	4.4±2.7	6.20±1.0	5.7±0.5	6.7±0.2
HGB (g dL^{-1})	12.0±0.6	12.0±0.4	12.00±0.4	13.5±1.2	12.7±0.6	12.2±0.4	6.5±6.7	11.50±1.9	11.1±1.2	12.3±0.5
HCT(%)	37.7±1.8	37.8±2.5	36.70±0.2	42.1±1.2	39.3±1.5	38.5±1.3	26.3±17.4	36.30±7.2	32.9±4.3	35.8±1.3
MCHC (g dL^{-1})	32.0±0.8	31.8±1.1	32.80±1.1	31.8±2.1	32.2±0.2	31.6±0.2	14.9±14.9	31.90±1.0	33.8±0.9	34.3±0.1
PLT ($\times 10^3$ µL^{-1})	853.0±10.5	1010.0±41	908.00±237.5	343.0±3.0	1261.5±20.5	814.5±276.5	524.0±349	1098.50±108.2	1089.0±221	949.6±175
LYM (%)	9.2±1.4	3.2±2.0	3.55±0.6	6.5±1.4	4.9±4.1	3.1±1.9	0.7±0.7	5.30±3.3	1.5±0.6	2.6±0.4

Values are Mean±SEM, (n = 5). PCV: Pack cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular, MCHC: Mean corpuscular haemoglobin concentration, LYM: Lymphocyte, MPV: Mean platelet volume, PLT: Platelet

Table 2: Serum biochemistry parameters in rats orally treated with saltpetre and *Sorghum bicolor*

Parameters	*Sorghum bicolor* only			Saltpetre only			*Sorghum bicolor* and Saltpetre			
Dose (mg kg^{-1})	100	300	1000	100	300	1000	100	300	1000	Control
TP (g L^{-1})	82.50±4.4	81.90±1.2	78.7±3.2	75.70±9.6	82.10±19.7	81.70±0.2	39.2±23.3	81.90±19.5	88.80±17.8	68.60±86.7
AST (U L^{-1})	190.20± 8.6*	229.60±10.5**	231.8±15.4**	154.40±3.4*	204.50±7.6**	229.30±20.8**	160.3±20.3*	203.90±30.4**	248.80±29.5**	86.70±3.59
ALP (U L^{-1})	335.70±16.9	494.00±20.6*	416.6±63.0	404.50±155	529.80±66.4*	656.70±43.4**	469.5±160.2	447.20±86.5	658.20±41.8**	158.30±20.0
ALT (U L^{-1})	66.80±3.7	67.30±3.6	75.6±17.7	46.50±14.1	63.60±1.8	84.30±8.9	54.5±37.2	62.00±28.6	67.10±17.7	86.80±1.8
CHOL(mmol L^{-1})	1.80±0.3	1.65±0.3	1.8±0.1	1.40±0.1	2.10±0.5	1.90±0.0	1.9±0.7	1.67±0.8	2.10±0.0	2.65±0.8
TG (mmol L^{-1})	0.97±0.1	0.84±0.1	0.7±0.2	0.83±0.8	0.95±0.2	0.98±0.1	1.5±0.8	0.81±0.1	1.09±0.1	1.55±0.2
GLU (mmol L^{-1})	6.40±0.6	8.90±0.6	8.8±1.3	6.80.4	8.10±0.4	8.90±1.9	7.6±0.5	9.70±0.1*	10.60±1.2*	5.70±2.3

Values are Mean±SEM (n = 5). TP: Total protein, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, CHOL: Cholesterol, TG: Triglyceride, GLU: Glucose. *p<0.05, **p<0.01 (one-way ANOVA followed by Newman Keul's *post hoc* test compared to control)

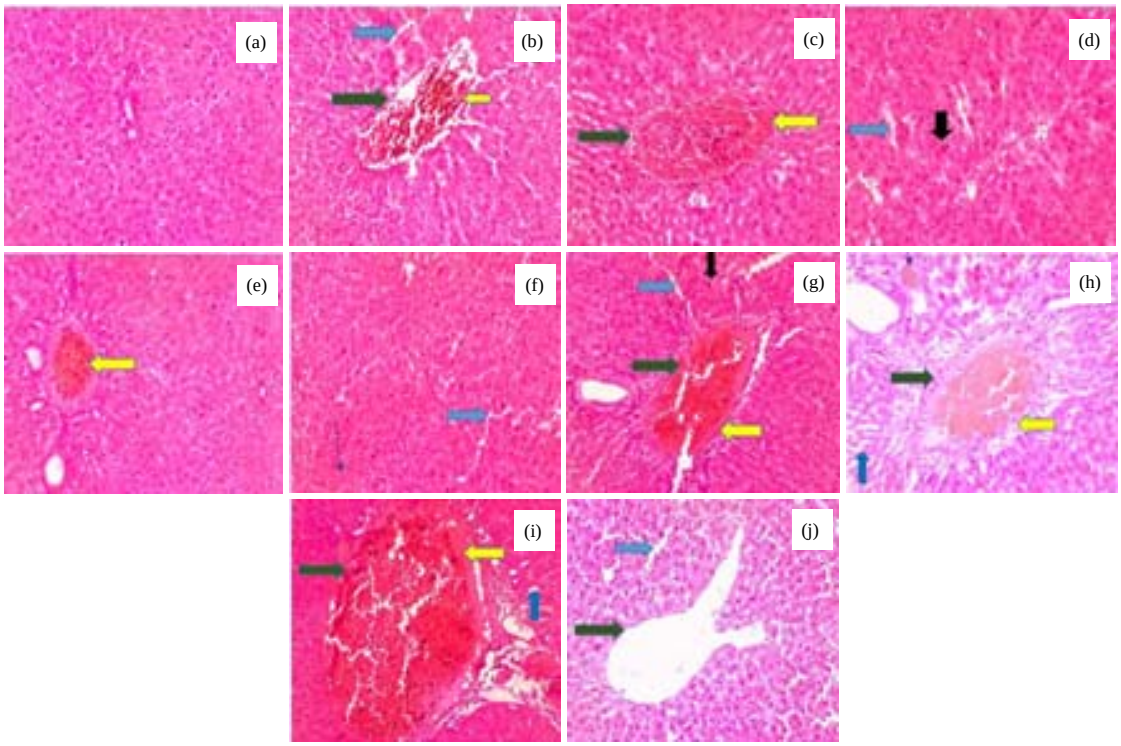

Fig. 2(a-j): Liver micrographs for (a) Control, (b) SB (100 mg kg^{-1}), (c) SB (300 mg kg^{-1}), (d) SB (1000 mg kg^{-1}), (e) Sp (100 mg kg^{-1}), (f) Sp (300 mg kg^{-1}), (g) Sp (1000 mg kg^{-1}), (h) SB+Sp (100 mg kg^{-1}), (i) SB+Sp (300 mg kg^{-1}) and (j) SB+Sp (1000 mg kg^{-1})
SB: *Sorghum bicolor*, Sp: Saltpetre

dilatation, congestion and dilatation of central vein (Fig. 2b) while it induced congestion and dilatation of central vein at 300 mg kg^{-1} (Fig. 2c). At 1000 mg kg^{-1}, *Sorghum bicolor* caused hepatic necrosis and dilatation of sinusoids (Fig. 2d). Saltpetre, on the other hand, caused congestion at 100 mg kg^{-1}, haemasiderosis and dilatation of sinusoids at 300 mg kg^{-1}, congestion, focal necrosis and dilatation of central vein in liver at 1000 mg kg^{-1} (Fig. 2e-g). The combination of the two additives (*Sorghum bicolor* and saltpetre) induced congestion, sinusoidal dilatation, haemasiderosis and fatty changes in the liver at 100 mg kg^{-1} (Fig. 2h), congestion and dilatation of central vein at 300 mg kg^{-1} (Fig. 2i) and dilatation of central vein, fatty change and sinusoidal dilatation at 1000 mg kg^{-1} (Fig. 2j).

Photomicrographs of heart sections of the various treatment groups are presented in Fig. 3(a-j). Control rats (Fig. 3a) showed normal heart histology. There was also no major histological alteration or damage to the cardiac tissues of rats in other treatment groups, except mild congestion observed in rats given *Sorghum bicolor* (300 mg kg^{-1}), saltpetre (300 mg kg^{-1}) and a combination of *Sorghum bicolor* and saltpetre (300 mg kg^{-1}) (Fig. 3c, g, j).

Similarly, spleen micrographs of rats in the various treatment groups as presented in Fig. 4 revealed normal histology for rats in the control group (Fig. 4a), necrosis and haemosiderosis in the group treated with 100 mg kg^{-1} and 300 mg kg^{-1} doses of *Sorghum bicolor* (Fig. 4b, c) and necrosis, sinusoidal dilatation and haemosiderosis in rats that received 1000 mg kg^{-1} *Sorghum bicolor* (Fig. 4d). Administration of saltpetre induced necrosis and haemosiderosis, necrosis and sinusoidal dilatation and haemasiderosis, dilatation of sinusoids and necrosis at 100, 300 and 1000 mg kg^{-1} doses, respectively (Fig. 4e-g). Necrosis, sinusoidal dilatation and haemosiderosis were observed in the spleen of rats given a combination of *Sorghum bicolor* and saltpetre at 100, 300 or 1000 mg kg^{-1} (Fig. 4h-j).

Figure 5 presents lung micrographs of various treatment groups with the control rats showing normal histology (Fig. 5a). Infiltration of inflammatory cells was observed in the lungs of animals given *Sorghum bicolor* (100, 300 and 1000 mg kg^{-1}). The infiltration of inflammatory cells was accompanied by oedema except in the 100 mg kg^{-1} group (Fig. 5b-d). Similarly, infiltration of inflammatory cells

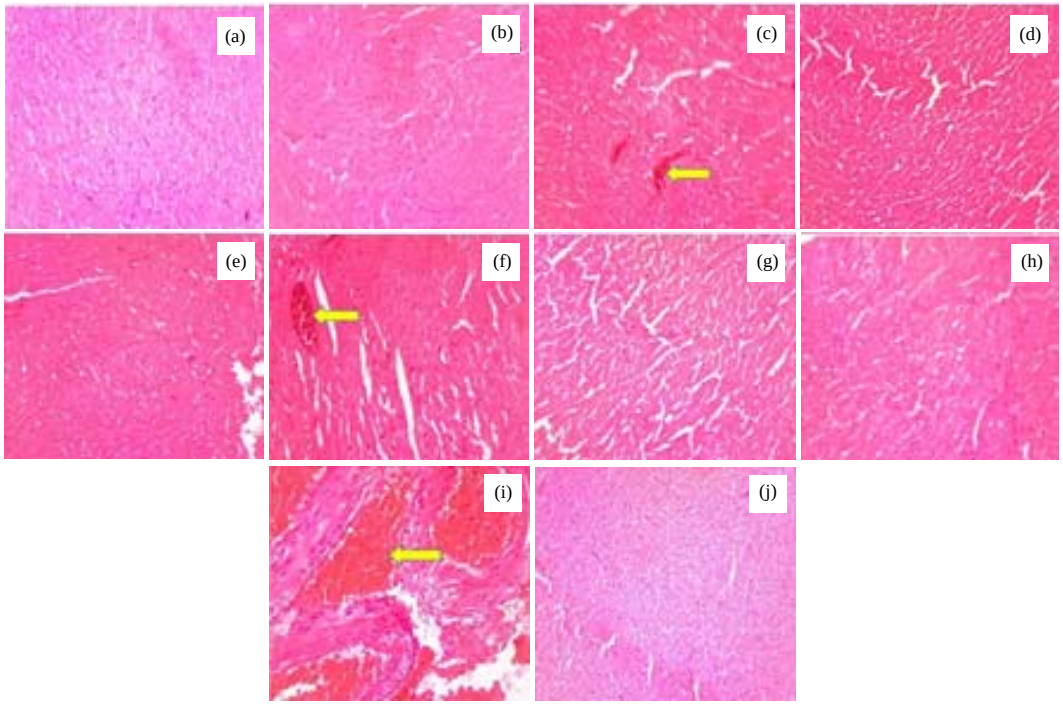

Fig. 3(a-j): Heart micrographs for (a) Control, (b) SB (100 mg kg^{-1}), (c) SB (300 mg kg^{-1}), (d) SB (1000 mg kg^{-1}), (e) Sp (100 mg kg^{-1}), (f) Sp (300 mg kg^{-1}), (g) Sp (1000 mg kg^{-1}), (h) SB+Sp (100 mg kg^{-1}), (i) SB+Sp (300 mg kg^{-1}) and (j) SB+Sp (1000 mg kg^{-1})
SB: *Sorghum bicolor*, Sp: Saltpetre

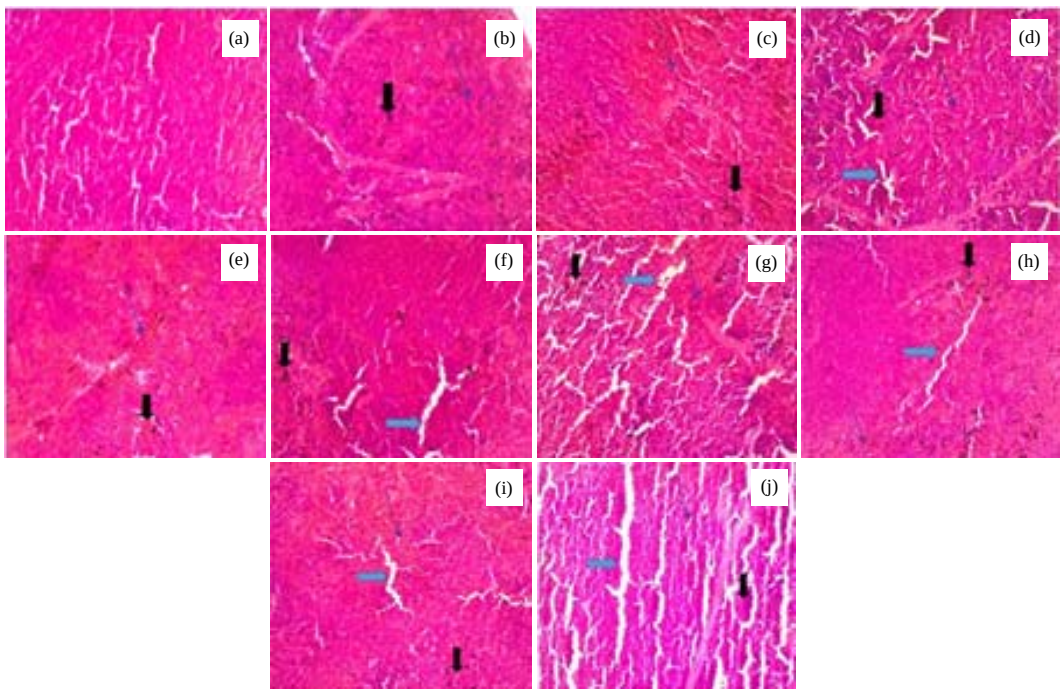

Fig. 4(a-j): Spleen micrographs for (a) Control, (b) SB (100 mg kg^{-1}), (c) SB (300 mg kg^{-1}), (d) SB (1000 mg kg^{-1}), (e) Sp (100 mg kg^{-1}), (f) Sp (300 mg kg^{-1}), (g) Sp (1000 mg kg^{-1}), (h) SB+Sp (100 mg kg^{-1}), (i) SB+Sp (300 mg kg^{-1}) and (J) SB+Sp (1000 mg kg^{-1})
SB: *Sorghum bicolor*, Sp: Saltpetre

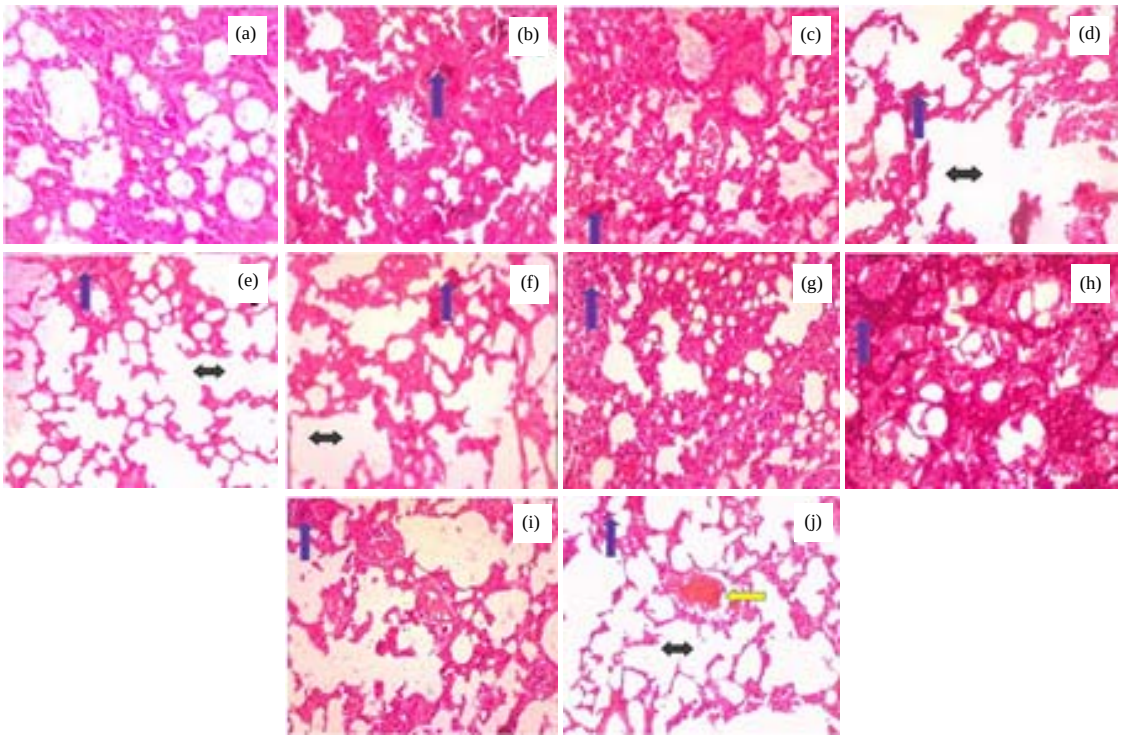

Fig. 5(a-j): Micrographs of lungs (a) Control, (b) SB (100 mg kg^{-1}), (c) SB (300 mg kg^{-1}), (d) SB (1000 mg kg^{-1}), (e) Sp (100 mg kg^{-1}), (f) Sp (300 mg kg^{-1}), (g) Sp (1000 mg kg^{-1}), (h) SB+Sp (100 mg kg^{-1}), (i) SB+Sp (300 mg kg^{-1}) and (J) SB+Sp (1000 mg kg^{-1})
SB: *Sorghum bicolor*, Sp: Saltpetre

were also observed in the lungs of animals that received saltpetre (100, 300 and 1000 mg kg^{-1}) with oedema accompanying this infiltration only at 100 and 300 mg kg^{-1} (Fig. 5e-g). Severe infiltration of inflammatory cells and congestion of vessels were observed in the lungs of rats given a combination of *Sorghum bicolor* and saltpetre at 100 mg kg^{-1} dose (Fig. 5h). Similarly, infiltration of lungs with inflammatory cells and oedema were also seen in rats that received combination of *Sorghum bicolor* and saltpetre at 300 and 1000 mg kg^{-1} (Fig. 5i and j).

DISCUSSION

In this study, the food additive-treated animals did not exhibit any overt adverse haematological effects. Changes in cellular components of the blood usually help to evaluate pathological, nutritional and physiological status of the consumer. Haematological parameters such as red blood cell, white blood cell, packed cell volume, mean corpuscular volume, mean corpuscular, mean corpuscular haemoglobin concentration, lymphocyte, mean platelet volume and platelets are valuable in monitoring the toxicity of food constituents which goes a long way to affect the health status of consumers[23]. Data obtained show that neither the individual additives nor their combination had any significant adverse effect on the blood. This agrees with earlier reports which suggest that the leaf sheath of *S. bicolor* does not have any toxic effect on haematological parameters but rather improves the haemopoietic activity of consumers[13,15].

Serum biochemical data reveal elevated levels of aspartate aminotransferase, alkaline phosphatase and glucose. Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase are usually indicative of cellular injuries. Although, alanine aminotransferase activity was not significantly affected, the activity of aspartate aminotransferase was elevated at different doses when animals were treated with only the individual food additives, as well as when administered in combination. Alkaline phosphatase activity, on the other hand, was elevated when food additives were given separately (100 mg kg^{-1} body weight of *Sorghum bicolor* and 300 mg kg^{-1} body weight of Saltpetre), pointing to possible cellular injuries in the liver, kidney or heart. Worth noting is the sharp increase in plasma glucose level in animals treated with the combination of the

food additives in a dose dependent manner. The observed hyperglycaemia could have been as a result of damage to liver cells and this might have led to reduced sensitivity to glucose or reduced secretion of insulin by the pancreas. Similar haematological and biochemical effects have been observed with administration of food additives like amaranth dye and vanillin in experimental models[24]. These food additives (amaranth and vanillin) significantly elevated parameters of renal and hepatic functions following sub-chronic exposure suggesting possible adverse consequences or damage to these organs[24].

Histopathological examination revealed no alteration or changes in organ morphology of rats in the control group as expected. For rats in the groups treated with only *Sorghum bicolor*, there were little or no tissue alterations as well as pathological changes at the various doses except for the highest dose (1000 mg kg^{-1} body weight) where, there was infiltration of inflammatory cells in the lungs and sinusoidal dilatation in the spleen. The same can be said for the treatment groups that received saltpetre only where there was also congestion in the kidney at the highest dose (1000 mg kg^{-1} body weight). The combination of the two additives, however, caused significant organ injury or morphological damage. There was severe infiltration of inflammatory cells in the lungs of the 100 mg kg^{-1} body weight treatment group. Administering the additives in combination at 300 mg kg^{-1} body weight induced severe infiltration of inflammatory cells in the lungs, haemosiderosis of the spleen, congestion in the heart and dilatation of tubules in the kidneys. At 1000 mg kg^{-1} body weight, there was necrosis in the kidney and spleen, severe oedema and infiltration of inflammatory cells in the lungs, sinusoidal dilatation in the liver and spleen and tubular dilatation in the kidney. Congestion occurs as a result of obstruction to the outflow of blood from the capillaries and this could lead to. In the spleen, obstruction could lead to sinusoidal dilatation. Also, obstruction in the spleen may resulted in hypoxia and eventually haemosiderosis which is characterized by accumulation of haemosiderin as a result of iron overload. The massive oedema in the lungs, referring to abnormal accumulation of fluid in the interstitium, can be attributed to increased pressure in the blood vessels or reduced proteins in the bloodstream to hold on to the fluid in the plasma. This could lead to respiratory depression, reduced oxygen circulation, lactic acidosis and subsequently organ failure[25].

Infiltration of inflammatory cells is the body's natural response to the presence of toxic substances in the body or when cell/tissue damage occurs. The severe inflammatory cell infiltration observed especially in the lungs indicates severe damage in the lungs, augmenting the observed massive oedema in this organ. The compromise in pulmonary function may result in reduced circulating oxygen and subsequently in shrinkage of the Bowman's capsules in the kidney. This may affect renal function and possibly lead to devastating consequences. These observations agree with earlier studies which suggest that ethanol/potash extract of *Sorghum bicolor* causes severe intrahepatic cell damage as evident by marked elevation in levels of liver enzymes in rats. Histopathological examination revealed lesions in the morphological features of the liver and kidney[17]. In another study also, a dye of *Sorghum bicolor* leaf sheath was capable of causing varying degrees of congestion in the heart, kidney and lungs[16]. Also, serum alanine aminotransferase has been reported to be elevated upon oral administration of the aqueous extract of the plant[13]. It is therefore, necessary that consumers of these food additives use them with care in order not to experience any toxic effects.

CONCLUSION

It is concluded that no mortality was recorded in the acute toxicity study, the sub-acute toxicity assessment revealed increase in biomarkers for liver and kidney function tests, pointing to possible damage to these organs. This was confirmed by histopathological examination which revealed various degrees of damage in some of these organs, with the lungs and spleen being the most hardly hit. It is also important to point out the dose-dependent hyperglycaemia produced by these food additives when administered in combination. It is not impossible, therefore, that the heavy utilization or consumption of these plants which have become household ingredients or food additives in sub-Saharan Africa may be a contributory factor to the high prevalence of diabetes in the region. However, it will be necessary to establish if there is truly a causal relationship between consumption of these food additives and the development of diabetes.

SIGNIFICANCE STATEMENTS

This study reveals the potential harmful effect that may be associated with long-term consumption of *Sorghum bicolor* and saltpetre when used as food additives or for medicinal purposes. The potential of these popular food additives to cause harm, when used separately or in combination, to some important vital organs such as the

liver, kidneys, lungs, spleen and to a lesser extent, the heart, was clearly demonstrated in this study. Significant dose-dependent elevation in blood glucose level was observed following combined administration of these food additives. This should be of important interest considering the high prevalence of diabetes in sub-Saharan Africa where these food additives have become household ingredients.

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