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# Effects of aqueous extract of *Sorghum bicolor* on hepatic, histological and haematological indices in rats

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Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries for primary health care. In Nigeria today, there is an upsurge in the acceptance and utilization of these herbal medicine partly because of scientific support for some of their medicinal uses. In recent times, findings from medicinal plants research indicate that extracts from some plants both hepatotoxic and hematotoxic, while some are reported to possess both hepatoprotective and hemopoietic properties. We investigated the effects of aqueous extract of *Sorghum bicolor* leaf sheaths on the biochemical hepatic functions, histological integrity and hematological indices in Sprague-Dawley albino rats. Phytochemical screening of the aqueous extract of *S. bicolor* leaf sheath was carried out. Also, male and female rats (100 – 210 g) and divided into 5 groups were employed for this study. Four groups of 6 rats each were orally administered with 1.0 ml of 200, 400, 800 and 1600 mg/kg body weight daily doses of aqueous extract of *S. bicolor* leaf sheath, respectively for 14 days. The control group consisted of 6 rats treated to a daily dose of 0.5 ml of 0.9% normal saline. At the end of the administration period, the rats were sacrificed; the blood samples were collected through orbital sinus and cardiac puncture. The liver tissues were harvested and used for the hematopoietic and liver functions investigations. Phytochemical analysis of the plant leaf sheath showed the presence of Anthracine glycosides, reducing compounds, saponins, flavonoids, glycosides and polyphenols. Liver function tests revealed that the serum alanine amino transferase (ALT) concentration in the experimental rats showed a significant ( $P \leq 0.05$ ) increase with the increases in dosage concentrations of the extract compared with the control. Aspartate amino transferase (AST) and alkaline phosphatase (ALP) as well as the concentrations of total protein and albumin in male and female experimental rats were not significantly ( $P \geq 0.05$ ) altered compared with the control by the oral administration of the extract. However, red blood cell counts, hemotocrit and haemoglobin concentrations increased significantly ( $P \leq 0.05$ ) on administration of the extract in both male and female experimental rats compared with the control. Histopathological examination did not reveal any lesion or alteration in the morphological features of the liver tissues in all the animals. Data of the present study indicate that aqueous extract of *S. bicolor* leaf sheath is both hepatoprotective and hematopoietic in male and female Sprague-Dawley rats. These findings are therefore of clinical importance given the various reported medicinal potentials of the plant.

**Key words:** *Sorghum bicolor*, liver, rats, aminotransferases, hemoglobin.

## INTRODUCTION

The use of plants for remedies has long been in existence and is among the most attractive sources for

developing drugs (Chevellier, 1996). Any part of plant can be considered as herbs including leaves roots, flower, seeds, resins, leaf sheath, bark, inner bark (cambium), berries and sometimes the pericarp or other portion. These ancient indigenous practices were discovered by series of 'trial and error' which then could not be substantiated by proven scientific theories (Holetz et al., 2002).

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However, research has shown that these practices have produced results of proven efficacies comparable to conventional modern medicine. In recent times, herbal remedies have become indispensable and forming an integral part of the primary health care system of many nations (Nwogu et al., 2007).

*Sorghum bicolor* is locally called 'oka pupa' in the Southern part of Nigeria is one of the major grain crops for human food throughout the drier areas of Africa and India and its grain is extensively used for animal feeding, some of its common names are 'Jowar' in India, 'Bachanta' in Ethiopia and here in Africa it has variety of names such as 'Karan dafi' and the Yoruba's of Southern part of Nigeria call it 'Oka pupa' (Dalziel, 1948; Ogwumike, 2002). In West Africa, dye can be extracted from the plant to color leathers, cloths, calabashes and as body pigment (Cobley and Steele, 1976).

Sorghum is used largely for forage in the U.S. It is very important in the world as part of human diet, with over 300 million people dependent on it (Bukantis, 1980). It is grown for grain, forage, syrup and sugar, and industrial uses of stems and fibers. Grain sorghum is a staple cereal in hot dry tropics, the threshed grain ground into wholesome flour. It serve as stalks used as animal feed for silage or green soiling, or for hay when grown irrigated in very dry areas. The grain can be cooked like rice or ground into flour. Sorghum, with large juicy stems containing as much as 10% sucrose, used in manufacture of syrup; sugar can be manufactured from sorghum. Broomcorn used for making brooms. The seed is used as food, in brewing "kiffir beer", the kiffir corn malt and cornmeal is fermented to make *Leting* (a sour mash), the pith is eaten and the sweet culm chewed (Watt and Brayer-Brandwijk, 1962). Arubans make porridge and muffins from sorghum meal. Parched seed are used as coffee substitutes or adulterants (Morton, 1981).

However, the use of the leaf sheath as a remedy against anaemia (reduction of red blood cells or its function) by traditional medicine healers is common in Nigeria as well as within the local people of the Yoruba and Hausa tribes (Ogwumike, 2002). Malted *S. bicolor* grain is higher in protein and lower in fat content than corn and this is partly responsible for its haemopoietic ability (Makokha, 2002). It has been reported that sorghum can be used as antiabortive, cyanogenetic, demulcent, diuretic, emollient, intoxicant and poison. Sorghum is a folk remedy for cancer, epilepsy, flux and stomach ache (Duke and Wain, 1981). The root is used for malaria in Southern Rhodesia; the seed has been employed for the treatment of breast disease and diarrhoea while the stem has been used for tubercular swellings treatment. In India, the plant is considered antihelminthic and insecticidal and in South Africa, in combination with *Erigeron canadense*, it is used for eczema (Watt and breye-Brandwijk, 1962). In China, where the seed are used to make alcohol, the seed husk is braised in brown sugar with a little water and applied to

the chest of measles patients. The stomachic seeds are considered beneficial in fluxes (Perry, 1980). According to Morton (Curacao natives drink the leaf decoction for measles, grinding the seeds with those of the calabash tree (*Crescentia*) for lung ailments. Venezuelans toast and pulverize the seeds for diarrhea. Brazilians decoct the seed for bronchitis, cough and other chest ailments, possibly using the ash for goiter. Arubans poultice hot oil packs of the seeds on the back of those suffering pulmonary congestion. According to Grieve (1931), a decoction of a 50 g seed to a litre of water is boiled down to half a litre as a folk medication for kidney and urinary complaints. However it has also been reported that dyes from *S. bicolor* may be carcinogenic (Owolagba et al., 2009 Avwioro et al., 2009). Avwioro et al. (2006) also reported that crude ethanolic extract of *S. bicolor* used as stain on red blood cells, collagen and muscle fibres indicated that the dye may be apigeninidin in nature.

Recently focus has been on the leaf sheath of *S. bicolor* being used as herbal remedy for anaemia and having a boosting effect on blood concentration hematinic potentials (Ogwumike, 2002; Friday et al., 2010).

The rising cost of material services and medication including blood tonics are becoming unaffordable for many patients thus preventing these individuals from receiving adequate healthcare and since the importance of blood cannot be over emphasized, then any alternative, easy and locally available means of improving blood concentration is of paramount importance (Little, 2001). According to Okonkwo et al. (2004), accurate laboratory determination of blood parameters remains the only sensitive and reliable foundation for ethical and rational research, diagnosis, treatment and prevention of anaemia.

This study therefore investigated the hematopoietic potential of *S. bicolor* leaf sheath extract in rat model with the aim of showing the effects of aqueous extract of *S. bicolor* leaf sheath on the biochemical indices of liver function and some haematological parameters.

## MATERIALS AND METHODS

### Identification and preparation of plant Materials

Dry leaf sheaths of *S. bicolor* plants weighing 2.8 g were purchased in May 2008 from herb sellers at Mushin market, Lagos, Nigeria. The sample of the plant specimen was identified and authenticated at the herbarium of the Department of Botany, University of Lagos, Akoka and the specimen was deposited in the herbarium of the same University. The dry leaf sheaths of sorghum plants was air-dried for 3 weeks and subsequently dried to completion in an oven for 48 h at 60°C (after which the lightly coloured part of the leaf sheath were cut off leaving the dark red part of the leaf sheath) and ground into fine powder using electric dry mill. A total of 480 g of the ground powder divided into two (240 g each) was boiled in a 4.5 L of distilled water for 10 min. This was allowed to cool then sifted to remove shafts. The liquid extract was then concentrated using rotary evaporator to produce a gel-like extract which was further evaporated to dryness at 50°C using oven, which weighed 40.2 g.

Subsequently, appropriate concentrations of the extract were made by dilution with water into 200, 400, 800 and 1600 mg/kg body weight and administered to the rats.

#### Phytochemical screening of the leaf extract of *S. bicolor*

The phytochemical screening of the aqueous extract of the leaves *S. bicolor* was carried out according to the method of Trease and Evans (1983). Qualitative analysis of alkaloids, anthracine glycosides, flavonoids, glucosides, saponins, proteins, tannins and phenolic compounds were studied. Freshly prepared ground samples of *S. bicolor* leaf sheath extract from samples soaked in solvent overnight and filtered was employed for phytochemical screening.

#### Test for anthracine glycosides

A mixture of 0.5 ml dilute sulphuric acid and 5.0 ml ferric chloride solution was added to 1 ml of the extract. The resulting mixture was boiled for 5 min, cooled and filtered into a separator funnel. The filtrate was shaken with an equal volume of carbon tetrachloride. The lower organic layer was carefully separated into a test tube and 5.0 ml of dilute ammonia solution added with gentle shaking. Pink coloration in ammonia indicated the presence of anthracine glycosides.

#### Test for alkaloids

1.0 ml of extract of the sample was added and shaken with 5.0 ml of 2% HCl on a steam bath and filtered. Five drops of Meyer's reagent (potassium mercuric iodine solution) was then added to 1.0 ml of the filtrate and observed for cream colored precipitate which is a positive test for alkaloids.

#### Test for flavonoids

1.0 ml of 10% ferric chloride was added to 1.0 ml of extract. The formation of a greenish brown or black precipitate or color was positive test for phenolic nucleus. To 1.0 ml extract, 1.0 ml of dilute NaOH was added. Addition of 1.0 ml dilute NaOH to 1.0 ml extract gave a precipitate which shows presence of flavonoids.

#### Test for protein

5.0 ml of distilled water was added to 4.0 ml extract and allowed to stand for 3 h then boiled. 2.0 ml of the boiled mixture was then added to 0.1 ml of mercuric nitrate (Million's reagent) and shaken. A pinkish precipitate indicates presence of protein.

#### Test for saponins

1.0 ml of extract was boiled with 5.0 ml of distilled water for 5 min and decanted while still hot. The filtrate was used for the following test:

#### Frothing test

1.0 ml of the filtrate was diluted with 4.0 ml of distilled water shaken vigorously and observed on standing for stable froth which confirms the presence of saponins.

#### Emulsion test

Two drops of olive oil was added to 1.0 ml of filtrate. The solution was shaken and observed for formation of emulsion which confirms the presence of saponins.

#### Test for tannins

5.0 ml of extract was added to 2.0 ml of 1% HCl. Deposition of a red precipitate was an evidence for the presence of phlobotannins.

#### Test for glycoside

5.0 ml of extract was treated with 2.0 ml of glacial acetic acid containing 1 drop of 0.1% ferric chloride, and then mixed with 1.0 ml concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

#### Care and treatment of animals

18 males and 12 females Sprague-Dawley albino rats weighing 100 – 210 g were collected from the Laboratory Animal Centre of the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. They were housed in wooden cages and allowed to acclimatize for two weeks, fed with rat chow and water *ad libitum* and under standard conditions (ambient temperature,  $29.0 \pm 2.0^\circ\text{C}$  and humidity 46%, with a 12 h light/darkness cycle). The rats were divided into five groups of six animals each. The animals were administered various agents as follows: the control group was not given the extract but was received a daily dose of 0.5 ml of 0.9% of normal saline while the second, third, fourth and fifth groups were treated to 1.0 ml 200, 400, 800 and 1600 mg/kg of the extract respectively in daily oral doses for 14 days. All the animal experiments were carried out in accordance with the guidelines of the Institutions Animal Ethical Committee.

#### Collection of blood and biochemical analysis

The rats were sacrificed after last day of extract administration and blood samples were collected from each animal through ocular bleeding into two sets of plain and EDTA-treated sample bottles, respectively.

The blood in the plain samples bottles were allowed to clot after 3 h. The clotted blood samples were spun in a bench top centrifuge to obtain sera. The serum samples were thereafter separated into another set of plain sample tubes and stored in the refrigerator pending enzyme assay. The whole blood collected into EDTA-treated sample bottles were used for the assay of haematological parameters. All assays were done within 24 h of the sample collection. The assay of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total proteins and albumins assays were carried out according to the procedures described by Roche Laboratories Ltd, USA. Packed cell volume (PCV) was measured by the microhaematocrit technique using a Hawksley microhaematocrit centrifuge and spinning for 5 min at 12,000 xg before reading with the hematocrit reader. Red blood cell counts (RBC counts) were estimated using the hemocytometer method. Haemoglobin levels (Hb) were measured colorimetrically by the oxyhemoglobin methods using Reichert's haemoglobinometer.

#### Histology

The rat liver from each group was fixed in 10% formal saline and for

72 h. The organ was dehydrated in graded alcohol, cleaned in xylene and embedded in paraffin. The resulting blocks were exhaustively sectioned. The sections were randomized, while the selected sections were stained in haemotoxylin and eosin. The slides were then examined at magnifications of  $\times 400$  under optical microscope.

### Statistical analysis

The results from the study were analyzed by one-way analysis of variance (ANOVA) and Students t-test. Statistical significance was tested at  $P \leq 0.05$ .

## RESULTS

The results of this study are presented in Tables 1 - 3, as well as Plates 1 - 4. The phytochemical screening of the leaf sheath indicated the presence of Anthracine glycosides, saponins flavonoids, glycosides and polyphenols (Table 1).

The results obtained in this present study for some serum liver enzymes, total proteins and albumin in normal male and female rats treated with aqueous extract of *S. bicolor* leaf sheath are presented in Table 2, while the results of hematological analysis are presented in Table 3. It was observed from these results that treatment of rats with aqueous extract of *S. bicolor* leaf sheath have no significant effect ( $P \geq 0.05$ ) on the activities of the serum liver enzymes as well as the concentrations of serum total proteins and albumins, compared respectively with the control (Table 2).

This observation indicated that the aqueous extract *S. bicolor* leaf sheath did not show marked hepatotoxic effect in the animal model. Plates 1 and 2 show photomicrograph of the liver tissues of the test rats, while plates 3 and 4 show the photomicrograph sections of the liver tissues of the control rats, respectively. The liver section of the animal in control groups showed a central vein with prominent small-sized nuclei, with the hepatocytes well separated by sinusoids. While the tissue section of the test rats showed a prominent central vein with a relatively large-sized nuclei; also, the sinusoids separating the hepatocytes in the test rats are observed to be relatively more prominent than that of the rats in the control groups. Generally, the liver sections of rats in the control and test groups showed that the cords of hepatocytes well preserved, cytoplasm not vacuolated and the sinusoids well demarcated. Also, no area of infiltration by inflammatory cells and fatty degenerative changes were observed in the tissue sections. These features gave an indication of normal hepatic integrity for rats in both control and test groups.

The results of this study also showed that the red blood cell counts, hematocrit and hemoglobin concentrations increased significantly ( $P < 0.05$ ) on administration of the extract to the rats. Also, no significant effect ( $P > 0.05$ ) on the white blood cell counts was observed to be associated with treatment of the rats with extract (Table 3).

The different dosage administration of the aqueous extract of *S. bicolor* suggests the hematinic potentials of the plant extract.

Furthermore, very significant increase in the haematological indices took place with the administration of the highest dosage of the extract and it is an indication of the fact that the hematopoietic effects of the extract is dose dependent.

This present study therefore suggests that aqueous extract of *S. bicolor* leaf sheath is not hepatotoxic, but rather possess hemapoietic property in rats. These findings are therefore, of clinical importance given the various reported medicinal potentials of the plant.

## DISCUSSION

The results of this study showed that the administration of the aqueous leaf extracts of *S. bicolor* has hemopoietic and antioxidant properties in the rat. The present study further confirmed the previous report on the hematinic potentials of *S. bicolor* (Ogwumike, 2002; Avwioro et al., 2009). The extract contains phenolic compounds and saponins which are powerful antioxidant. The medicinal value of this plant lies in those chemical substances that produce a definite physiological action on the human body such as alkaloids, flavonoids and tannins. This is in agreement with observations made previously (Hill, 1952; Trease and Evans, 1996). These phytochemicals are known to perform many functions in plants and may exhibit different biochemical and pharmacological actions in animal species when ingested (Duke and Wain, 1981; Owolagba et al., 2009). Saponins are known to possess hypocholesterolemic effects (Price et al., 1987) and as such its presence in the leaf sheath extract of *S. bicolor* may aid in lessening the oxidative stress on the liver. The results of this study also indicate that leaf sheath extract of *S. bicolor* may function as blood booster in anaemic condition and this possibly could be as a result of its direct effect on the hematopoietic systems (Friday et al., 2010).

Stimulations of hematopoietic growth factors and erythropoietin systems have been reported to enhance rapid synthesis of blood cells (Murray, 2000). It is also safe at the concentrations used in the experiment on the basis of the result of liver function tests and histological studies which did not indicate significant changes between the tests and the control. Avwioro et al. (2006) however, using crude ethanolic extract of *S. bicolor* to stain red blood cells, collagen and muscle fibres indicated that the dye may be apigenindin. However, result from oral administration of the same extract up to a concentration of 1500 mg/kg on albino rats did not show any adverse reaction while the rats administered 2500 mg/kg died. It is therefore necessary to conduct further works on this plant in order to obtain clearer pictures on its medicinal values since the works done so far are based on short term exposure of the experimented

**Table 1.** Phytochemical profile of aqueous extract of *Sorghum bicolor* leaf sheath.

Phytochemicals	Occurrence
Anthracine Glycosides	+ve
Alkaloids	-ve
Flavonoids	-ve
Glucosides	+ve
Protein	-ve
Saponins	
Frothing Test	-ve
Emulsion Test	+ve
Tannins	-ve
Phenolic compound	+ve

Note: +ve: present, -ve: absent

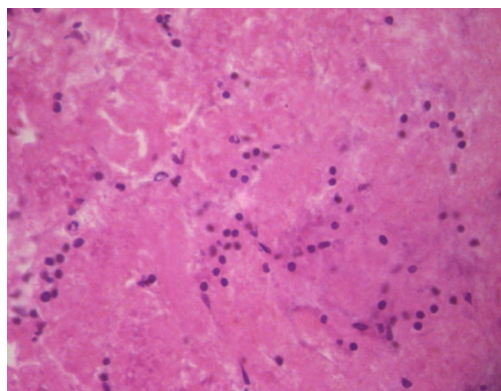
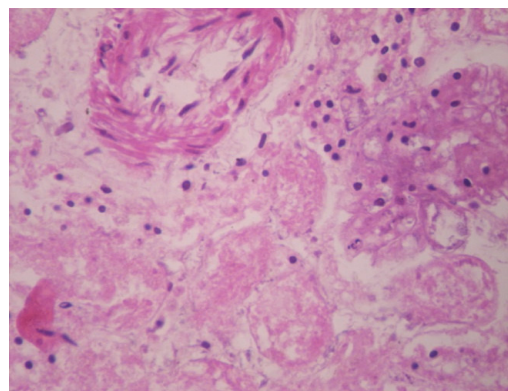
**Table 2.** Effect of *Sorghum bicolor* aqueous leaf extract on liver enzymes, total proteins and albumins levels of Sprague-Dawley albino rats.

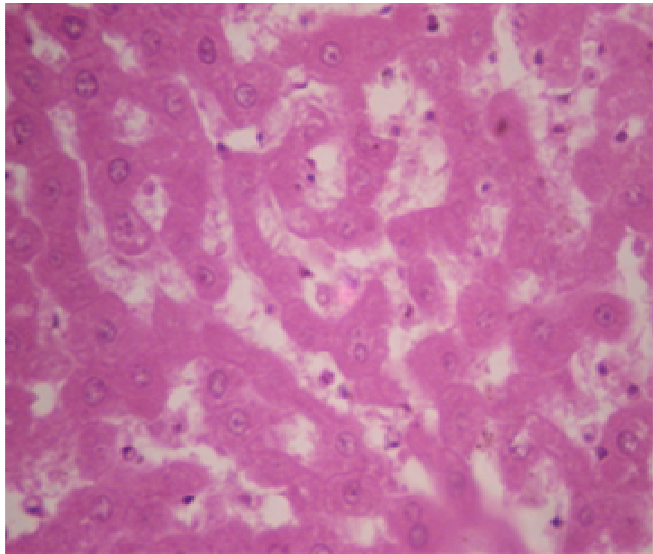
Treatment group (mg/kg)	AST	ALT	ALP	Total serum protein	Serum albumin
Control Saline	89.83 ± 17.26	84.50 ± 3.33	154.34 ± 26.12	66.67 ± 15.22	27.88 ± 9.33
Extract 200	102 ± 11.08	86.6 ± 2.7	167.38 ± 18.37	65.80 ± 17.45	26.56 ± 6.21
Extract 400	106.6 ± 19.42	92. ± 4.18	148.24 ± 29.98	68.75 ± 12.22	29.06 ± 8.43
Extract 800	93.25 ± 9.54	88.20 ± 2.95	187.34 ± 20.54	62.63 ± 18.72	28.86 ± 5.11
Extract 1,600	104.8 ± 11.52	88.75 ± 0.5	129.5 ± 15.02	70.21 ± 13.85	30.18 ± 4.25

**Table 3.** Effect of *Sorghum bicolor* aqueous leaf extract on some hematological parameters of Sprague-Dawley albino rats.

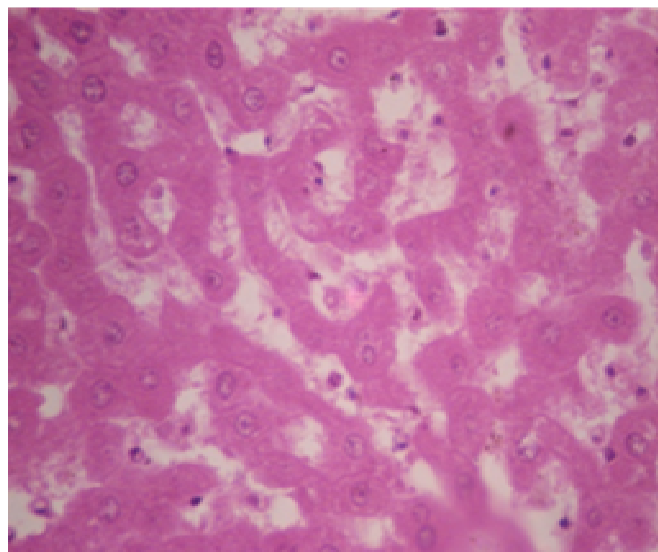
Treatment group (mg/kg)	PCV (%) ± SD	Hb (g/dl) ± SD	RBC × 10 <sup>6</sup> mm <sup>-3</sup>	MCV × 10 <sup>-6</sup>	MCH × 10 <sup>-6</sup> (Pg)	MCHC (g/dl)
Control saline	29.00 ± 4.16	9.60 ± 1.51	3.17 ± 0.49	9.16 ± 0.29	3.03 ± 0.10	31.26 ± 0.91
Extract 200	38.67 ± 4.13	12.81 ± 1.37	4.22 ± 0.48	9.18 ± 0.14	3.04 ± 0.05	32.02 ± 0.67
Extract 400	37.80 ± 1.48	12.52 ± 0.49	4.02 ± 0.18	9.41 ± 0.20	3.12 ± 0.07	32.51 ± 1.07
Extract 800	41.00 ± 2.94	13.58 ± 0.98	4.40 ± 0.29	9.32 ± 0.27	3.09 ± 0.09	33.28 ± 1.12
Extract 1,600	39.60 ± 1.95	13.11 ± 0.65	4.26 ± 0.26	9.30 ± 0.21	3.09 ± 0.09	32.02 ± 1.41

PCV = Packed cell volume or hematocrit; Hb = Hemoglobin; RBC = Red blood cell; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration.

**Plate 1.** Photomicrograph of hepatocytes of male rats treated with extract of *S. bicolor*.**Plate 2.** Photomicrograph of hepatocytes of female rats treated with extract of *S. bicolor*.



**Plate 3.** Photomicrograph of hepatocytes of male control rats.



**Plate 4.** Photomicrograph of hepatocytes of female control rats.

animals.

## Conclusion

Data of the present study do suggest that the leaf sheath extract of *S. bicolor* possess hemopoietic potential. There is no indication of the likelihood of it being hepatotoxic or hematotoxic in rats employed in this study. It could therefore be said to possess hepatoprotective and hematopoietic potentials.

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