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Toxicological evaluation of ethanolic root extract of *Cassia sieberiana* in rats

A. WEREMFO[1], M. DUWEIJUA[2] and S. ABASSAH-OPPONG[3]

[1]Biochemistry Department, School of Biological Sciences, University of Cape Coast, (Ghana).
[2]Pharmacology Department, Faculty of Pharmacy, KNUST, Kumasi (Ghana).
[3]Biochemistry Department, School of Biological Sciences, University of Cape Coast (Ghana).

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ABSTRACT

The root of *Cassia sieberiana* (Caesalpinaceae) has been used extensively in Ghanaian traditional medicines as a remedy for the treatment of abnormal pain, dysmenorrhoea, ulcer and general body pains. The analgesic activity of the root extract has previously been established. Even though the plant has been used extensively, little is known about its possible side effects associated with its use. This work evaluated the ethanolic root extract of *Cassia sieberiana* for their behavioural and pharmaco-toxicological effects. No acute toxic effect was observed after a single oral dose up to 3.2 g/Kg of the extract. After administering the extract (40, 160 and 640 mg/Kg, p.o.) to the rats for 30 days, no deaths were found and the histopathological analysis of the vital organs did not show alterations. The extract also did not cause significant changes in the biochemical parameters examined except bilirubin level. For the haematological parameters studied, the red blood cells and haemoglobin concentrations increased which may be due to haemolysis.

Key words: Toxicological evaluation, root extract, *Cassia sieberiana.*

INTRODUCTION

Medicinal plants play a key role in the human health care especially in developing countries where western pharmaceuticals are very expensive and traditional medicines are generally more acceptable. About 80% of the world population relies on the use of traditional medicine which is predominantly based on plant materials (WHO, 2002).

A large part of the commonly utilised medicines in Ghana are thus still derived from plants and large volumes of plants or their extracts are sold in both informal and commercial sectors of the economy. This presents a valuable resource for research into the development of new pharmaceuticals as there is growing interest in natural and traditional medicines as a source of new commercial products (Gilani and Rahman, 2005 and Patwardhan, 2005).

Although plant extracts have been used in the treatment of diseases according to knowledge accumulated over centuries, scientific research has shown some substances present in these medicinal plants to be potentially toxic and carcinogenic (Pak *et al.*, 2004). Investigation of traditionally used medicinal plants is thus important as a source of potentially chemotherapeutic drugs and also as a measure of safety for continued use of medicinal plants.

Cassia sieberiana a member of the Caesalpinaceae family is a tree widely distributed in Africa. It is commonly referred to as Africa laburnum. *Cassia sieberiana* has been used extensively in traditional medicine. In Ghana the root back extract is used to treat abdominal pains, dysmenorrhoea, ulcers, general body pains, strangulated hernia and also as aphrodisiac (Irvine, 1961). In spite of its potential therapeutic usefulness, no toxicological investigations have been carried out.

This work aimed to evaluate the safety of its use by investigating the acute and subacute toxicity of the ethanolic root extract of *Cassia sieberiana.*

EXPERIMENTAL

Experimental animals

Wister rats of either sex (180 - 220g) were obtained from the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology. The animals were housed, fed and treated in accordance with the in-house guidelines for animal protection. Animals were kept for 10 days to be acclimatised prior to the investigation. They were housed in groups of 5 in stainless steel cages (34 x 47 x 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet and water *ad libitum*. All animals were euthanised after each experiment and each animal was therefore used only once.

Preparation of plant extract

Air-dried powdered root of *Cassia sieberiana* was collected from the production unit of the Centre for Research into Plant Medicine, Ghana. The powdered root was extracted with 70% ethanol in water at room temperature for 5 days. The resulting filtrate was concentrated at 50°C in rotary evaporated. The concentrated solution was dried over anhydrous $Ca(OH)_2$ in a dessicator. From a 550g sample of root, 73.9 g of solid material was obtained, giving a percentage yield of 13.43%. This was kept in refrigerator. Suitable quantities were suspended in saline when required.

Acute toxicity

Rats (90 – 110 g) of either sex were used. They were randomly divided into five groups (n=5). Doses of extract (50, 200, 800, and 3200 mg/kg) were given orally, a dose to each group, and the effects observed. The control group was given normal saline.

The overall behaviour exhibited by the animals was recorded using a check list, which include; urinary frequency, defecation, changes in locomotor activity, convulsion, lacrimation, and salivation, within the first 6 hours after extract administration. The animals were further kept and observed for 48 hours during which time the number of death were recorded.

Subacute toxicity

Animals were randomised into four groups each containing 20 rats. Doses of extract (40, 160, and 640 mg/kg) were respectively administered orally to three of the groups and the remaining group put on normal saline. The various doses of extract and saline were given daily and the animals observed for 30 days. Five rats in each group were sacrificed every 10 days and blood collected by cardiac puncture for biochemical and haematological analyses. The lung, heart, liver, kidney, and spleen were removed and preserved in 10% formalin for histological analysis.

Plasma biochemistry

The blood samples taken every 10 days were centrifuged at 2000 g for 10 minutes to separate the serum and kept at 4°C to assay the activities of serum enzymes. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the method of Reitman and Frankel (1957). Alkline phosphatase (ALP) was estimated according to Kind and King (1954). Serum bilirubin, urea and creatinine levels were estimated according to (Malloy and Evelyn (1937), Varley (1967), and Bartels and Bohmer (1972), respectively.

Haematological studies

Blood collected by cardiac puncture during subacute treatment was put into tubes containing 0.l ml of a 4% aqueous solution of the di-potassium salt of ethylenediamine tetra-acetic acid (EDTA). The number of red blood cells and white blood cells were obtained by counting directly in a haemocytometer after suitable dilutions of the blood using a Thoma pipette. Haemoglobin content was determined using haemoglobinometer after diluting the blood with Drabkins Solution.

Histopathological studies

Liver, kidney, heart, lung, and spleen removed from rats in subacute toxicity studies were fixed in 10% formalin. These were processed for paraffin embedding following standard microtechnique. Sections of organs stained with

haematoxylin and eosin were examined for histopathological changes under a light microscope. Photomicrographs of sections were made.

Effect of *C. sieberiana* on barbiturate-induced sleeping time

Male rats, weighing between 90-110 g and fed a standard laboratory diet were used in 4 groups of six per treatment. Three groups were given orally a daily dose of 40 mg/kg, 160 mg/kg, and 640 mg/kg of *C. sieberiana* for 14 days. An equivalent volume of saline was given orally to the fourth group serving as vehicle control. On the fourteenth day, each rat was given an intraperitoneal injection of pentobarbitone at a dose of 30 mg/kg. The rats were separately caged and appropriately marked to monitor individual sleeping times. The righting reflex was monitored by gently placing the rat on its back. If the righting reflex is still present, the animal will 'right' itself. The sleeping time was measured as the time the rat lost its reflex to the time it regained its reflex.

Statistical method

All the data collected during this study were expressed as mean ± SEM. One way analysis of variance (ANOVA) with subsequent Bonferroni's test was used to detect further differences between groups. Unpaired Students t-test was used for comparison of two means. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Natural products have proved to be of great importance in the development of new pharmaceuticals. Consequently, numerous works have been published signalling the potential toxicological effects that these products could also posses (Marcus and Snodgrass, 2005 and Pak et al., 2004). Based on experimentally demonstrated properties, *C. sieberiana* is an interesting candidate for developing new phytotherapeutics, and it is therefore necessary to establish its toxicological effects.

The data gathered from the toxicity study indicate that the different concentrations of ethanolic extract of *C. sieberiana* given acutely did not induce any observable effects in the rats. From Table 1 daily oral administration of doses of *C. sieberiana* to rats did not have significant effect on pentobarbitone-induced sleeping time. Because pentobarbitone is metabolised by the hepatic microsomal cytochrome P-450, it means therefore that the extract neither induced nor inhibited the action of the metabolising enzymes, thus ruling out the possibility of any liver damage as changes in the structure or function of the liver alter the action of the metabolising enzyme. This was supported by the extract having no significant effect on the serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Table 2). Damage to the structural integrity of liver is reflected by an increase in the level of serum aminotransferases (ALT and AST) as these are cytoplamic in location and are released into circulation after cellular damage (Sallie *et al*., 1991). Although serum ALP cannot be used to assess acute liver damage or even cirrhosis, it is an excellent indicator of space-occupying lesion in liver primarily because of destruction of biliary canaliculi within the liver.

Kidney damage increases the levels of urea and creatinine in the blood plasma. However, the extract did not significantly change the levels of plasma urea and creatinine when compared to the untreated rats (Table 2), thereby ruling out the possibility of any kidney damage during subacute study.

Table 1: The effect of ethanolic root extract of *C. sieberiana* on pentobarbitone-induced sleeping time in rats.

Treatment (mg/kg)	Mean sleeping time (min.) S.E.M.
control	125.6 ± 7.0
40	134 ± 6.0 ^{ns}
160	120 ± 3.8 ^{ns}
640	141 ± 9.1 ^{ns}

The values represent mean ± s.e.m. n = 6 rats.
^{ns} = 6 rats. $^{ns}P > 0.05$, compared to control. n=6.

The significant difference was verified using one-way analysis of variance (ANOVA).

Table 2: The effect of ethanolic root extract of *C. sieberiana* on certain biochemical parameters in rats after 10, 20, and 30 days of oral administration.

Treatment (mg/kg)	Alkaline phosphatase conc. (U/L) 10 days	20 days	30 days
Control	44.55 ± 0.55	39.60 ± 1.10	37.60 ± 2.00
40.0	41.80 ± 1.10 ^{ns}	39.60 ± 3.30 ^{ns}	43.45 ± 11.45 ^{ns}
160.0	42.90 ± 3.30 ^{ns}	39.05 ± 0.55 ^{ns}	37.95 + 0.55 ^{ns}
640.0	48.40 ± 2.20 ^{ns}	41.80 ± 2.20 ^{ns}	36.85 ± 0.55 ^{ns}
	Alanine aminotransferase conc. (U/L)		
Treatment (mg/kg)	10 days	20 days	30 days
Control	27.65 ± 0.64	28.28 ± 4.23	26.75 ± 1.36
40.0	25.85 ± 4.42 ^{ns}	30.62 ± 1.44 ^{ns}	28.82 ± 0.18 ^{ns}
160.0	32.42 ± 5.95 ^{ns}	30.89 ± 0.99 ^{ns}	29.72 ± 2.16 ^{ns}
640.0	28.82 ± 2.89 ^{ns}	24.67 ± 1.26 ^{ns}	28.19 ± 1.72 ^{ns}
	Aspartate aminotransferase conc. (U/L)		
Treatment (mg/kg)	10 days	20 days	30 days
Control	101.05 ± 1.54	85.71 ± 14.72	102.59 ± 7.36
40.0	104.10 ± 10.61^{ns}	89.82 ± 5.41^{ns}	103.46 ± 2.17 ^{ns}
160.0	100.64 ± 1.52 ^{ns}	91.12 ± 1.09 ^{ns}	109.73 ± 03.25 ^{ns}
640.0	102.59 ± 7.36 ^{ns}	78.78 ± 5.19 ^{ns}	108.00 ± 2.38 ^{ns}
	Bilirubin conc. (mg/dL)		
Treatment (mg/kg)	10 days	20 days	30 days
Control	0.41 ± 0.03	0.42 ± 0.01	0.37 ± 0.02
40.0	0.60 ± 0.05 ^{ns}	0.54 ± 0.02 ^{ns}	0.54 ± 0.09 ^{ns}
160.0	0.48 ± 0.03 ^{ns}	0.43 ± 0.01 ^{ns}	0.66 ± 0.01 *
640.0	0.50 ± 0.01 ^{ns}	0.69 ± 0.08 *	0.70 ± 0.02**
	Creatinine Concentration (U/L)		
Treatment (mg/kg)	10 days	20 days	30 days
Control	0.54 ± 0.14	0.45 ± 0.09	0.48 ± 0.03
40.0	0.54 ± 0.07 ^{ns}	0.55 ± 0.10 ^{ns}	0.60 ± 0.05 ^{ns}
80.0	0.56 ± 0.04 ^{ns}	0.41 ± 0.05 ^{ns}	0.55 ± 0.15 ^{ns}
160.0	0.60 ± 0.13 ^{ns}	0.50 ± 0.05 ^{ns}	0.55 ± 0.02 ^{ns}
	Urea concentration (mmol/L)		
Treatment (mg/kg)	10 days	20 days	30 days
Control	1.01 ± 0.29	1.45 ± 0.26	1.62 ± 0.63
40.0	1.08 ± 0.27 ^{ns}	1.36 ± 0.68 ^{ns}	1.58 ± 0.55 ^{ns}
80.0	0.96 ± 0.19 ^{ns}	1.31 ± 0.02 ^{ns}	1.57 ± 0.36 ^{ns}
160.0	1.01 ± 0.14 ^{ns}	1.73 ± 0.37 ^{ns}	1.37 ± 0.09 ^{ns}

Values represent mean ± s.e.m. n = 5 rats. ^{ns}P>0.05, compared to control group.
The significant difference was verified using one-way analysis of variance (ANOVA).

The haematological investigations revealed a significant decrease in haemoglobin and RBC levels in the treated groups (Table 3). It could be speculated that the components of the extract are metabolised into intermediates which require conjugation with endogenous glutathione (GSH). GSH acts both as nucleophilic scavengers of reactive metabolites and as a substrate in GSH peroxidase-mediated detoxification of hydroperoxidase. Accumulation of the normal oxidative metabolites in the cell or the action of toxic chemicals, leads to depletion of GSH, resulting in oxidative stress (Reed, 1990). RBC's under oxidative stress are liable to undergo haemolysis. This could account for the decreased levels of RBC and haemoglobin after treatment.

Excessive haemolysis leads to an increase in the formation of bilirubin, resulting in increased serum bilirubin levels. When the liver is damaged, it is unable to conjugate and excrete bilirubin. The increase in bilirubin could also be due to renal damage which prevents excretion of urea, creatinine, and bilirubin. Since any possible liver and kidney damage has been ruled out from earlier results, the increase in bilirubin (Table 2) is probably due mainly to haemolysis.

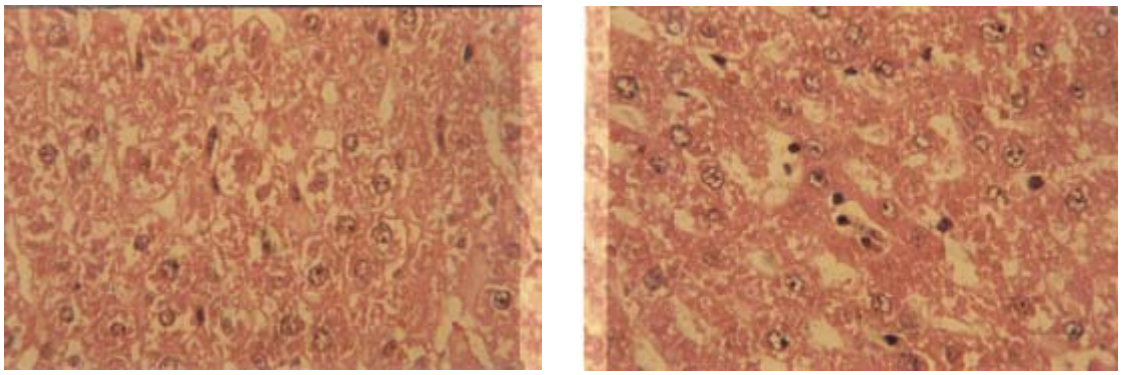
The above inferences were confirmed in the histopathological studies where the microscopic examination of organs of rats treated with *C. sieberiana* did not show necrosis, fatty depositions in cells, or fluid accumulation in the liver, kidney, lung, heart, and spleen after 30 days of oral treatment (Plates 1–5). Also there were no significant changes in the relative weights of the organs when compared with the control (results not shown). This implies that the extract of *C. sieberiana* when taken daily cannot cause any degeneration of cells in the major organs examined.

Table 3: The effect of ethanolic root extract of *C. sieberiana* on haematological indices of rats after 10, 20, and 30 days of oral administration.

	RBC(x 10^6/mm^3)		
Treatment (mg/kg)	**10 days**	**20 days**	**30 days**
control	5.98 ± 0.45	6.42 ± 0.19	6.19 ± 0.35
40.0	5.77 ± 0.43 ^{ns}	5.71 ± 0.48 ^{ns}	5.71 ± 0.09 ^{ns}
160.0	6.53 ± 1.49 ^{ns}	6.24 ± 0.21 ^{ns}	5.76 ± 0.03 ^{ns}
640.0	6.74 ± 0.96 ^{ns}	5.01 ± 0.36 ^{ns}	4.94 ± 0.04 *
	WBC (x 10^3/mm^3)		
Treatment (mg/kg)	10 days	20 days	30 days
control	9.00 ± 1.35	9.24 ± 0.09	8.36 ± 0.31
40.0	9.65 ± 0.10 ^{ns}	7.81 ± 0.04 ^{ns}	8.13± 0.03 ^{ns}
160.0	9.51 ± 0.56 ^{ns}	7.96 ± 0.44 ^{ns}	7.94 ± 0.09 ^{ns}
640.0	9.95 ± 0.98 ^{ns}	7.48 ± 0.65 ^{ns}	7.55 ± 0.60 ^{ns}
	Haemoglobin Concentration (g/dl)		
Treatment (mg/kg)	10 days	20 days	30 days
Control	12.20 ± 0.20	12.20 ± 0.20	12.10 ± 0.10
40.0	12.60 ± 0.20 ^{ns}	11.90 ± 0.10 ^{ns}	10.90 ± 0.10*
160.0	12.30 ± 0.10 ^{ns}	11.50 ± 0.10 ^{ns}	10.90 ± 0.09*
640.0	11.30 ± 0.30 ^{ns}	11.30 ± 0.10	10.40 ± 0.05 *

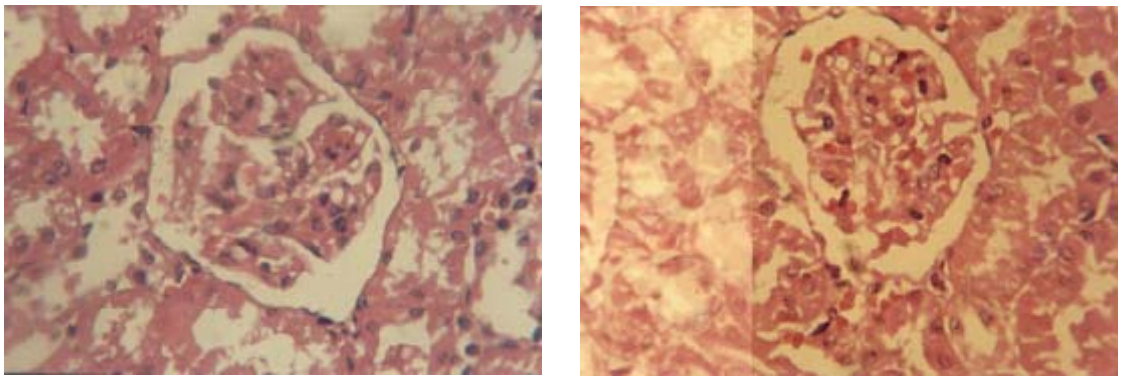
Values represent mean ± s.e.m. n = 5 rats. ^{ns}P>0.05, *P<0.05, compared to control group.

The significant difference was verified using one-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparison tests.



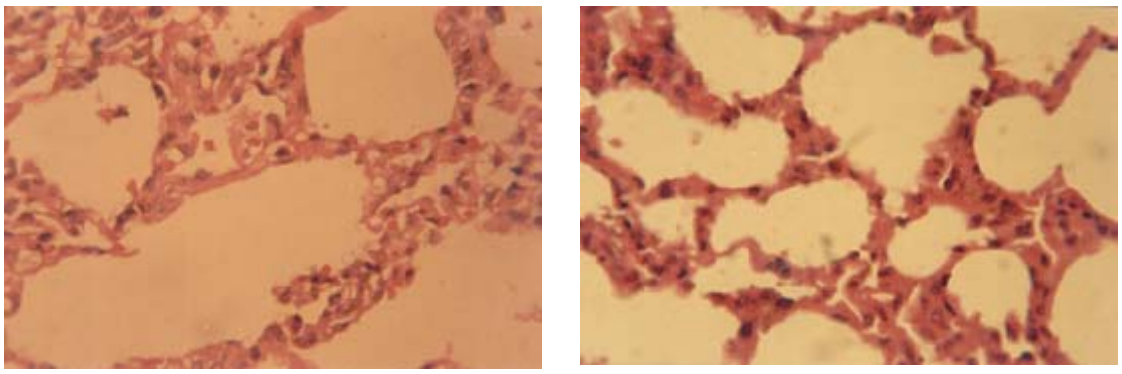
A B

Plate 1: Photomicrograph of a transverse section of the liver of rat treated with (A) vehicle (B) 640 mg/kg of *Cassia* extract, for 30 days (H & E; Magnification x400)



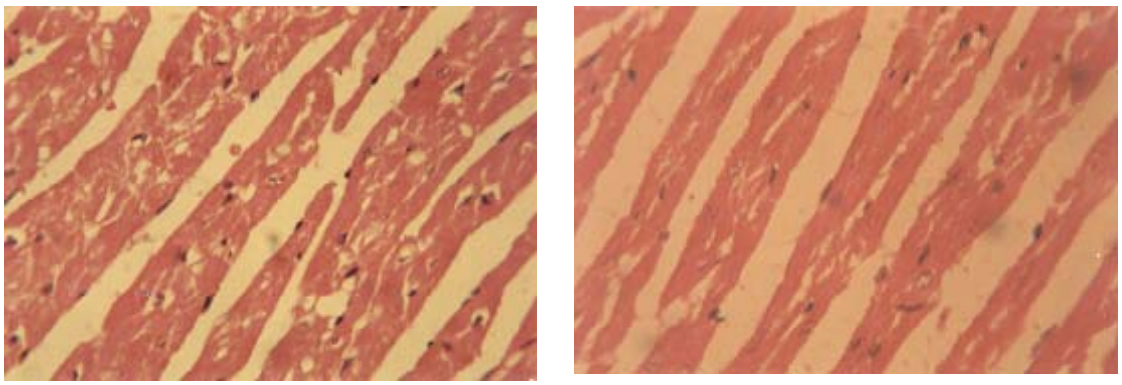
A B

Plate 2: Photomicrograph of a transverse section of the kidney of rat treated with (A) vehicle (B) 640 mg/kg of *Cassia* extract, for 30 days (H & E; Magnification x400)



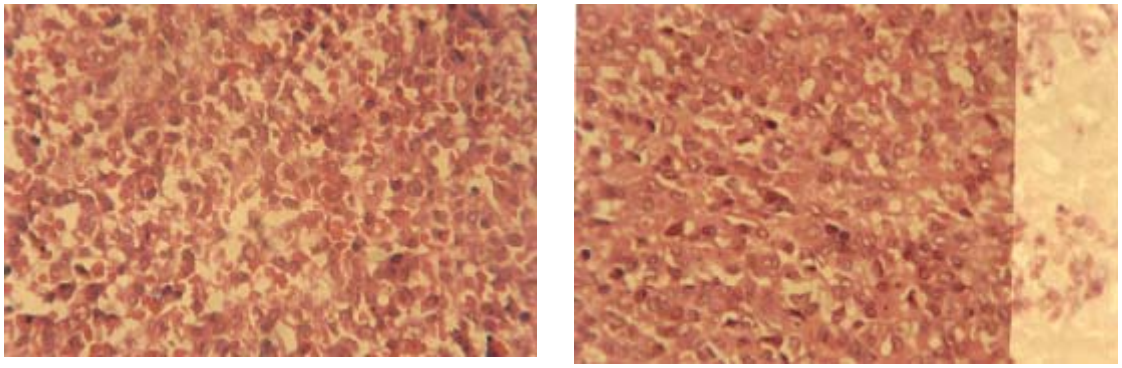
A B

Plate 3: Photomicrograph of a transverse section of the lung of rat treated with (A) vehicle (B) 640 mg/kg of *Cassia* extract, for 30 days (H & E; Magnification x400)



A B

Plate 4: Photomicrograph of a transverse section of the heart of rat treated with (A) vehicle (B) 640 mg/kg of *Cassia* extract, for 30 days (H & E; Magnification x400)



A B

Plate 5: Photomicrograph of a transverse section of the spleen of rat treated with (A) vehicle (B) 640 mg/kg of *Cassia* extract, for 30 days (H & E; Magnification x400)

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