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Short Communication

Acute toxicity study on aqueous extract of the leaf of *Cassia sieberiana* D.C. (*Caesalpiniaceae*) in albino rats

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ABSTRACT: In order to evaluate the toxicity of *Cassia sieberiana* leaf extract in albino rats with the aim of establishing its safe application in experimental and field trials, the acute toxicity of the aqueous extract of the leaves was studied in albino rats. Four experimental groups of albino rats (A, B, C, D) were respectively given intraperitoneal doses of 400, 800, 1600 and 3200 mg/kg body weight of the extract, while the fifth group (E) was control. The LD_{50} was calculated, clinical signs and gross and microscopic lesions of the liver and kidney were recorded. Immediate clinical signs observed were weakness, depression, starry hair coat, anorexia, while abnormal gait, lordosis, opthalmia, coma and death appeared after 2 hours. Hepatomegaly and focal necrosis were observed on gross examination, while sinusoidal congestion with periportal necrosis and Kupffer cell proliferation were the microscopic lesions observed. Gross enlargement of the kidneys, vascular degeneration, and interstitial mononuclear cell infiltration were observed. The calculated median lethal dose (LD_{50}) was 960 mg/kg, and the severity of clinical signs and hepatic and kidney lesions was dose dependent. This study has shown that *Cassia sieberiana* leaf aqueous extract could be toxic in a dose-dependent way, and should be used with caution in veterinary practice.

KEYWORDS: *Cassia sieberiana*, Leaf aqueous extract, acute toxicity, albino rats.

INTRODUCTION

Medicinal plants usually contain substances that can be used for therapeutic purposes or contain precursors used for the synthesis of useful drugs (Sofowara, 1993). The scientific evaluation of plants or their by-products is relevant in discovering novel drugs and helps to assess toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin (Sofowara, 1993).

The plant *Cassia sieberiana* is widely distributed in semi arid northeastern Nigeria (Buratai *et al*., 2011), with several parts used in the treatment of diseases (Obidah *et al*., 2009).

Aqueous extracts of *Cassia sieberiana* are used locally in Northeastern Nigeria for the treatment of inflammatory conditions (Madusolumuo et al., 1999). Other reports indicate that the extracts have been used to treat, malaria, diarrhoea, bilhazia, and as a dewormer (se Obidah *et al*., 2009).

In view of the wide medicinal usage and distribution of the plant *Cassia sieberiana* in Nigeria, this study was conducted to assess its toxicity, so as to provide a reference for its safe application in veterinary practice.

MATERIALS AND METHODS

Plant collection and identification: Fresh and matured leaves of *Cassia sieberiana* were obtained in 2010 from the University of Maiduguri campus, Nigeria, and identified by a botanist from the Department of Biological Sciences, University of Maiduguri, Nigeria. A set of voucher herbarium (species No. LCMC 228) has been deposited in the department for reference.

Extract preparation: The fresh matured leaves of *Cassia sieberiana* were air dried in a shaded area of the Veterinary Parasitology Laboratory for one week and hand crushed to obtain a 470 gram powder. This was exhaustively extracted in 700 ml distilled water at 60 ^{0}C for 8 hours using a Soxhlet extractor (Quickfit, England). The extract was concentrated on an aluminium tray, placed in an oven and maintained at 60 ^{0}C to dry. The dried sample was weighed, and a yield of 77.9% $^w/_w$ was obtained. The samples were stored at room temperature until used.

Table 1: LD_{50} of the aqueous extract of the leaves of *Cassia sieberiana* in rats

Groups (n=5)	Dose administered (mg/Kg body weight)	Dose Difference (DD)	Dead Rats (MD)	Mean Dead	DD x MD
A	400		0		
B	800	400	2	1	400
C	1600	800	5	3.5	2800
D	3200	1600	5	5	8000
Total					11200

$LD_{50} = LD_{100} - ((DD \times MD)/n$

= 960 mg/kg body weight

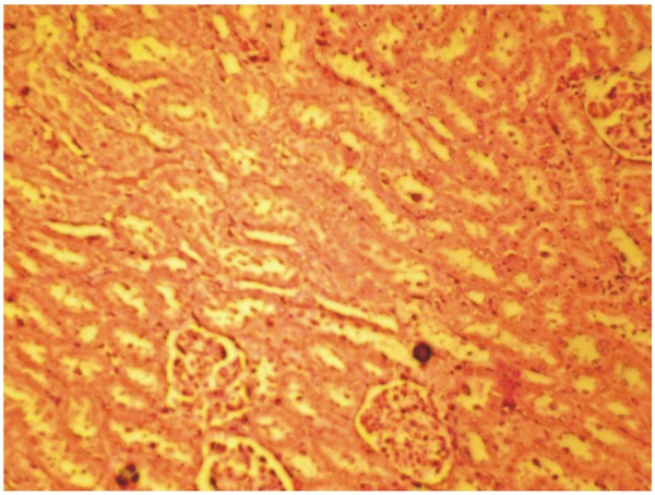

Figure 1: Photomicrograph of the liver of rats given 400 mg/kg of *Cassia sieberiana* leaf aqueous extract at day 14 showing tubular sinusoidal congestion and haemorrhage and periportal hepatic necrosis (H&E × 750)

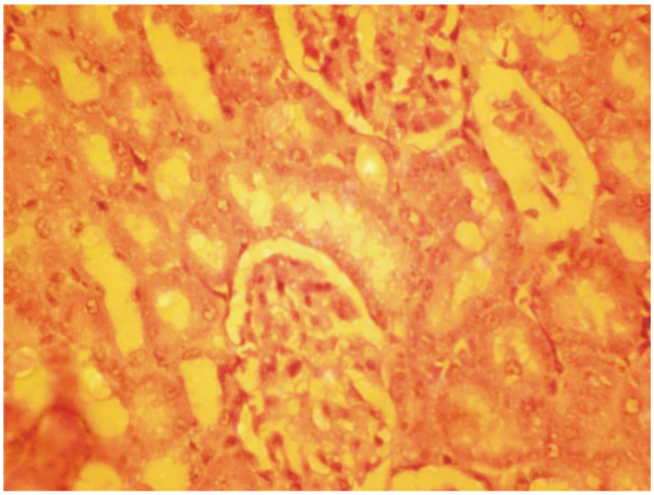

Figure 2: Photomicrograph of the liver of rats given 3200mg/kg of *Cassia sieberiana* leaf aqueous extract showing severe wide spread vacuolar degeneration and kupffer cell proliferation. (H & E × 750)

Experimental design: A total of 25 albino rats weighing between 106.5 and 166.5 grams were randomly divided into 5 groups (A, B, C, D and E) of 5 rats each. The albino rats were obtained from the Laboratory Animal Unit of the Department of Animal Science, University of Maiduguri, Nigeria; and housed in cages. Pelletized commercial feeds (ECWA, Nigeria plc) and water were given *ad libitum* to the albino rats and were acclimatized for 2 weeks to the laboratory environment before experimental treatments were administered. The acute toxicity test was conducted to determine the LD_{50} of the *Cassia sieberiana* leaf aqueous extract using groups A, B, C, and D, while group E (control) received only drinking water. The albino rats were treated intraperitoneally with graded doses of 400, 800, 1600 and 3200 mg/kg of the extract. The albino rats were observed over a 24-hour period for clinical signs and death. The LD_{50} was calculated using the modified arithmetic method of Karber (Aliu and Nwude, 1982). Post mortem examination of dead rats was performed and the liver and kidney collected in 10% formalin, embedded in paraffin wax. The tissue samples were cut to 5 μm thickness, stained with haematoxylin and eosin (Drury and Wallington, 1976), and the tissue slides examined under light microscope at x750 magnification.

RESULTS AND DISCUSSION

Clinical signs and mortality scores (LD_{50}): The animals displayed immediate clinical signs of weakness, depression, starry hair coat, and anorexia; while abnormal gait, lordosis, opthalmia, coma, and death appeared after 2 hours. The doses of 1600 and 3200 mg/kg produced 100% mortality, while 800 mg/kg produced 50% and no mortality was

observed in anim als that received 400mg/kg (Table 1). The calculated LD_{50} was 960 mg/kg (Table 1).

Gross histopathology: Hepatomegaly and focal necrosis were observed on gross examination, while sinusoidal congestion with periportal necrosis and Kupffer cell proliferation were the microscopic lesions observed (Figures 1 and 2). The kidneys were grossly enlarged and showed vascular degeneration, and interstitial mononuclear cell infiltration microscopically (Figure 3). The degenerative changes observed in the liver may probably arose from biotransformation of the active/ toxic principles of the extract, while those observed in the kidneys may be associated with toxicity-related-excretory processes in albino rats (Rabo *et al*., 2002; Mbaya, *et al*., 2007).

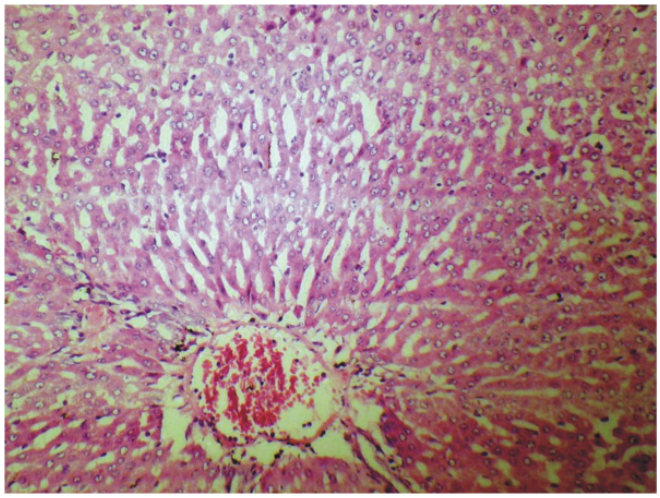

Figure 3: Photomicrograph of the kidney of rats given 3200mg/kg of *Cassia sieberiana* leaf aqueous extract showing tubular vascular degeneration and severe interstitial mononuclear cell infiltration (H & E × 750)

The findings on the acute toxicity study of *Cassia sieberiana* leaf aqueous extract revealed an intraperitoneal lethal median dose (LD_{50}) of 960 mg/kg, and clinical signs, gross and microscopic lesions that were dose-related. This suggests that the extract is moderately toxic to albino rats. Clarke and Clarke, (1975) and Biu *et al*. (2010) suggested that substances with LD_{50} of between 500 and 5000 mg/kg are moderately toxic, and should be used in veterinary practice with some degree of caution. The toxicity of plant extracts are generally related to their phytocomponents such as glycosides, saponins, terpenes, flavonoids, tannins and alkaloids. These substances have been shown to be present in high levels in *Cassia sieberiana* (Mavar-Manga, 2006; Asase *et al*., 2008).

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