Sahel Journal of Life Sciences FUDMA (SAJOLS)
March 2025 Vol. 3(1): 276-281
ISSN: 3027-0456 (Print)
ISSN: 1595-5915(Online)
DOI: *https://doi.org/10.33003/sajols-2025-0301-33*

Research Article

Assessment of Oral Administration of Ethanol Extract of *Cassia sieberiana* Root in Male Wistar Rats: Haematology, Lipid Profile and Histopathology of the Heart

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ABSTRACT

Cassia sieberiana root is commonly used in the management of erectile dysfunction or as a sexual enhancer in traditional medicine. The present study evaluated the safety potential of ethanolic extract of *C. sieberiana* roots on haematology, lipid profile, and heart histology of male rats. Male Wistar rats were randomly distributed into a control group, and three treatment groups orally administered 100, 200, and 400 mg/kg body weight respectively of the ethanolic crude extract for 21 days. The control group was administered 10 mL/kg distilled water only. At the end of the experiment, blood samples were collected and haematological parameters and lipid profile were evaluated. The hearts of experimental animals were excised and processed histologically. The results showed that there were no significant alterations ($p > 0.05$) in haematological indices except for a significant decrease in lymphocytes (58.54 ± 2.25) in the group administered 200 mg/kg body weight of the extract when compared to the control. The lipid profile and organ weight of treated groups were not significantly different ($p > 0.05$) from the control. Photomicrographs of heart sections of both the control and treated groups show normal features. The results suggest that *C. sieberiana* had no deleterious effect on blood, lipid profile, and heart.

Keywords: *Cassia siberiana*; Haematology; Lipid profile; Heart; Wistar rats

Citation: Omaji, G.O., Lawal, N., Ganiyu, A.I., Salihu, A.K., Yakubu, A., Sani, B.U. & Tasiu, M. (2025). Assessment of Oral Administration of Ethanol Extract of *Cassia sieberiana* Root in Male Wistar Rats: Haematology, Lipid Profile and Histopathology of the Heart. *Sahel Journal of Life Sciences FUDMA*, 3(1): 276-281. DOI: *https://doi.org/10.33003/sajols-2025-0301-33*

INTRODUCTION

The use of medicinal herbs in the treatment of reproductive diseases is as old as mankind. However, undetermined quantities of herbal remedies are often taken without consideration for potentially fatal and other adverse effects (Ojatula and Afolabi, 2022). Phytochemicals in plant extracts target biochemical pathways, making herbal medicines a safer alternative to synthetic pharmaceuticals.

Studies on the aphrodisiac enhancing potential of herbal plants have shown that they contain phytochemicals that support their activities. Reports have suggested that some of these aphrodisiac herbal components may be harmful or may cause harm when used extensively and over an extended period (Abudayyak *et al*., 2015). In contrast, some of these substances are anti-nutrients that adversely affect the internal organs and overall health of all animals, including humans. The latent damage or impairment of internal organs produced by the indiscriminate use of herbal supplements or treatments has made their usage cautious. Thus, a thorough investigation into their impact on the visceral organs and biochemical indices is also necessary since detoxification is the exclusive duty of these essential organs.

Cassia sieberiana is a plant in the family Fabeceace. The plant is found in Sahel savannah, Sudan savannah and West African countries (Mshelia *et al*., 2017). Hausa call it 'Malga' while Yoruba call it 'Aidan toro' in Nigeria (Yakubu *et al*., 2019). *C. sieberiana* has a significant history of traditional uses in many cultures. The roots of *C. sieberiana* are

commonly used as aphrodisiac in the treatment of erectile dysfunction and have fertility enhancing potentials (Yusuf *et al*., 2019). It is also widely used to treat ulcer, arthritis, burns and general body pain (Duwiejua *et al*., 2007). Due to the many applications of *C. sieberiana* in traditional medicines, there is the need to investigate the toxic potential of the plant. Therefore, this study aims to investigate the effect of the root extract on haematology, lipid profile and histology of the heart in male Wistar rats.

MATERIALS AND METHODS

Collection of Plant Material

Roots of *Cassia sieberiana* were obtained from its natural habitat in Dutsin-ma town, Katsina State. Nigeria. The plant was identified at the Department of Biological Sciences, Federal University Dutsin-ma. They were further identified at the Department of Plant Biology, Bayero University Kano and assigned voucher number 0065.

Preparation of Crude Extract

Fresh *C. sieberiana* roots were washed, chopped, dried under shade, grinded to powder using mechanical grinder and stored in air tight container until further analysis. The weighed amount of 750 g of powdered sample was macerated with ethanol for 48 hours with shaking at regular intervals. The extract was filtered using a muslin cloth and then with Whatman filter paper number 1. The filtrate was concentrated using a rotatory evaporator. The semi-solid residue obtained was dried in a desiccator to obtain the ethanol crude extract. The percentage yield calculated was 17.2 % (w/w).

Experimental Animals

The experiment was performed using adult male rats purchased from a reputable supplier and housed in the animal house of the Department of Biochemistry and Molecular Biology, Federal University Dutsin-ma, Nigeria. The male rats were kept in standard cages, acclimatized and maintained for two weeks under standard conditions (12 h light/12 h dark cycle). The animals were fed with normal commercial pellets and water *ad libitum*. All animal experiments were carried out according to the guide for the care and use of laboratory animals published by the National Research Council (2011).

Experimental Design

A total of twenty (20) male wistar rats weighing between 119 g – 225 g were used in this study. The rats were randomly divided into four groups of five (5) rats each. Normal control rats (Group 1) received distilled water at 10 mL/kg and treatment rats (Group 2-4) received different extracts (100, 200, 400 mg/kg/day) for 21 days respectively.

Haematological Analysis

At the end of 21 days treatment, blood samples were collected from abdominal aorta. Blood sample for haematological evaluations were collected in bottles containing EDTA. The sample bottle containing sample and EDTA was gently mixed to prevent clothing. The haematological indices of the blood samples were determined using an automated analyser. Parameter that were determined include total haemoglobin concentration (HGB), haematocrit (HCT), red blood cell count (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell count (WBC), Lymphocyte count (LYM), MID – sized cells (MID), Granulocyte (GRAN) and Mean plate volume (MPV).

Biochemical Analysis

Second portion of the blood was collected into plain sample bottles for analysis of serum lipid profile. Total Cholesterol (TCHOL) was estimated based on the method of Roeschlau *et al*. (1974), Triglycerides (TG) (Tietz, 1986), High-density lipoprotein cholesterol (HDL-c) (Albers *et al*., 1978), low-density lipoprotein cholesterol (LDL-c) were estimated according to the method described by Frieldewald *et al*. (1972).

Histological analysis

The heart of the experimental animals were excised, weighed and preserved in 10% formal saline. Tissues were stained using the Haematoxylin-Eosin staining technique before histopathological evaluation.

Data Analysis

All the values are expressed as mean ± SEM (n = 5). Data was analysed by One-way analysis of variance (ANOVA) using a statistical package for social science (SPSS version 16). The value of $p < 0.05$ was considered to be statistically significant

RESULTS

Weight of organ

Table 1 shows the effect of the crude ethanolic extract of *C. sieberiana* root on organ weight. There was no significant increase ($p > 0.05$) in the weight of the heart in the group administered *C. sieberiana* crude extract as compared to the control.

Haematological parameters

The effect of oral administration of ethanol crude extract of *C. sieberiana* root is shown in Table 2. The data indicate that there was no significant difference in haematological indices except for the value of lymphocyte that was significantly reduced ($p < 0.05$) when compared to the control.

Lipid Profile

Table 3 shows the effect of ethanol crude extract of *C. sieberiana* on lipid profile of male rats. There were no significant alteration ($p > 0.05$) in TCHOL

and TG when compared to the control. Similarly, the serum level of HDL-c and LDL-c in test rats administered the various doses of the extract were not significant different ($p > 0.05$) from those of the control.

Table 1. Effect of ethanol crude extract of *C. sieberiana* on organ weight

Group	Weight of Heart (g)
Control	0.80 ± 0.60[a]
100 mg/kg	0.70 ± 0.60[a]
200 mg/kg	0.78 ± 0.10[a]
400 mg/kg	0.80 ± 0.70[a]

Data are represented as mean ± SEM, n = 5 determinations. Values with different alphabets in the same column indicate a significant difference ($p< 0.05$).

Table 2. Effect of ethanol crude extract on haematological parameters

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
WBC ($10^{\wedge 3}$/µl)	4.50 ± 0.54[a]	4.78 ± 0.32[a]	5.18 ± 0.24[a]	4.68 ± 0.31[a]
LYM (%)	65.18 ± 1.72[b]	62.42 ± 2.25[ab]	58.54 ± 2.25[a]	61.60 ± 1.32[ab]
MID (%)	4.34 ± 0.27[a]	5.52 ± 0.42[a]	4.72 ± 0.38[a]	5.8 4± 0.96[a]
GRAN (%)	30.42 ± 1.66[a]	32.10 ± 2.22[a]	36.10 ± 2.39[a]	33.34 ± 1.53[a]
RBC (x$10^{\wedge 6}$/µl)	6.16±0.47[a]	6.08±0.07[a]	6.04±0.07[a]	5.96±0.12[a]
HGB (g/dl)	13.18±0.56[a]	12.74±0.47[a]	13.02±0.04[a]	15.80±2.09[a]
HCT (%)	39.20±1.56[a]	38.60±2.01[a]	39.60±1.47[a]	44.80±3.69[a]
MCV (fl)	88.06±2.97[a]	85.22±1.65[a]	88.48±0.52[a]	88.86±0.82[a]
MCH (pg)	32.74±1.89[a]	28.68±0.84[a]	31.88±1.81[a]	30.46±0.84[a]
MCHC (g/dl)	32.30±0.59[a]	33.60±0.26[a]	33.38±1.56[a]	33.42±0.62[a]
MPV(fL)	7.62±0.31[a]	9.02±0.72[a]	7.86 ± 0.28[a]	8.18±0.23

Data are represented as mean ± SEM, n = 5 determinations. Values with different alphabet in the same row indicate a significant difference ($p < 0.05$). WBC = white blood cell, LYM = lymphocytes, MID = mixed-sized cells, GRAN = granulocyte, RBC= Red blood cell, HGB= haemoglobin, HCT= Haematocrit, MCV= Means corpuscular volume, MCH= Means corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration, MPV= mean platelet volume

Table 3. Effect of ethanol crude extract of *C. sieberiana* root on lipid profile

Groups	TCHOL (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Control	33.88±8.01[a]	20.54±3.87[a]	7.40±1.54[a]	22.36±6.41[a]
100 mg/kg	55.20±8.10[a]	53.70±23.39[a]	6.60±1.03[a]	37.86±6.80[a]
200 mg/kg	38.36±11.88[a]	13.84±3.97[a]	7.80±2.08[a]	27.66±12.55[a]
400 mg/kg	35.54±2.59[a]	25.62±8.77[a]	9.80±0.49[a]	6.80±3.93[a]

Data are represented as mean ± SEM, n = 5 determinations. Values with different alphabet in the same column indicate a significant difference ($p< 0.05$). TCHOL = total cholesterol, TG = triglyceride, HDL-c = high-density lipoprotein, LDL-c = low-density lipoprotein.

Histological Evaluation

Figure 1a shows the photomicrograph of heart section from the control. There were no abnormal features or lesions around the myocardium after the 21 Days experimental period. Photomicrograph of heart sections from groups administered 100 mg/kg, 200 mg/kg and 400 mg/kg body weight of the extract (Figure 1b, 1c, and 1d) also show normal histological feature of the heart.

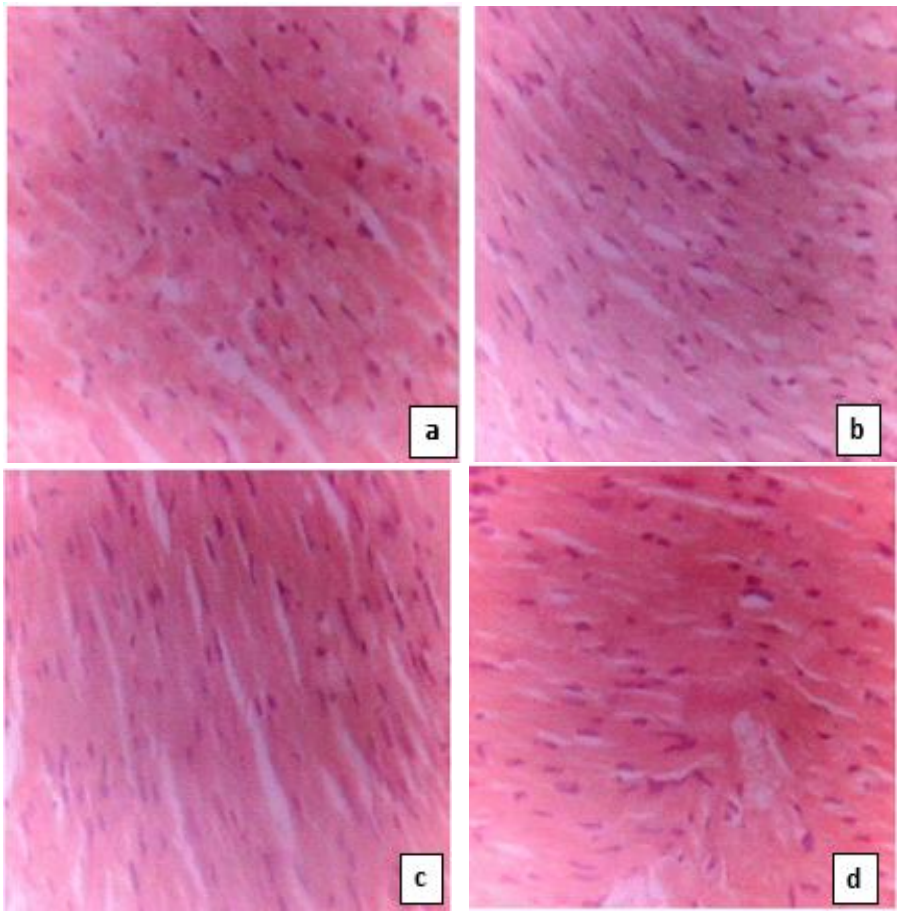

Figure 1: Photomicrographs of sectioned hearts of male rats. (a) Control (b) 100 mg/kg body weight (c) 200 mg/kg body weight (d) 400 mg/kg body weight. Both control and *Cassia sieberiana* treated groups show normal features of the myocardium (H & E x400)

DISCUSSION

Herbal products from medicinal herbs are generally considered safe due to the natural bioactive compounds available in them. Thus, they are often indiscriminately consumed without considering their safety limits and possible adverse effects. *C. sieberiana* has been shown to contain various phytochemicals which may justify its traditional use in the management of erectile dysfunction/enhancement of sexual function, among others. The toxicity of medicinal plants is often associated with the presence of phytochemicals. It has therefore become necessary to assess the safety of *C. sieberiana* root extract.

Findings of this study revealed no significant alteration in haematological indices except for a significant decrease in lymphocyte level in the group treated with 200 mg/kg body weight of the extract as compared to the control. A decrease in lymphocytes may indicate an immune response to stressful situations following antigen entry. As lymphocytes migrate to sites of inflammation, their number may also decrease from circulation (Nigatu *et al*., 2017). It is known that certain haematological parameters such as haemoglobin, neutrophils and platelets are associated with the development of cardiovascular disease (Liao *et al*., 2021). The non-significant changes in haematological parameters suggest that the ethanolic extract of *C. sieberiana* root has no haematotoxic effect on the normal physiology of the blood. Thus may not increase the risk of coronary heart disease. The results obtained are similar to those of Donkor *et al*. (2014).

Lipid profile studies provide vital information on predisposition of the heart to atherosclerosis, lipid metabolism, and other associated coronary heart diseases. In this study, administration of the extract did not cause a significant change in the level of TCHOL and TG when compared to the control. Cholesterol is a precursor for steroid hormones, bile acid and is also an essential structural element of biological membranes. However, high concentrations of cholesterol have been linked with the development of coronary heart disease (Abdulmumin *et al*., 2020). The non-significant changes in TCHOL and TG suggest that *C. sieberiana* root extract may not increase the risks of developing coronary heart disease and erectile dysfunction due to the close association between ED and cardiovascular disease. Studies have shown

that elevation of serum LDL-c levels are among the risks factor for coronary heart disease, and there is a direct correlation between LDL-c and atherosclerosis (Bahramikia and Yazdanparast, 2008). Results from this study showed that the level of LDL-c was not significantly altered following administration of the extract. One major function of HDL-c is in reverse cholesterol transport. HDL-c remove excess cholesterol from the bloodstream and transport it back to the liver for elimination. Therefore, a decrease in HDL level can contribute to higher risk of atherosclerosis. Administration of *C. sieberana* extract had no significant effect on the level of HDL-c, suggesting that the extract has low tendency to induce atherosclerotic plaque.

The effect of *C sieberiana* on heart was assessed by measuring organ weight and evaluating the histoarchitecture of the heart. There were no significant differences in heart weight when the treated groups were compared to the control. Although, organ weight provide useful insight about the effect of drugs on organ of interest, organ weight data are best interpreted with gross pathology and histopathological findings among others (Ugwah-Oguejiofor *et al*., 2019). Histological evaluation of sectioned hearts in control and treated groups showed normal histoarchitexture, as there were no pathological lesions in myocardial fibres, hypertrophy, congestion, necrosis and degeneration. The heart is a vital organ responsible for pumping blood throughout the body. Thus enabling the transport of oxygen, hormones and other vital substances to various tissues, and removal of metabolic wastes (Ojatula and Afolabi, 2022). The result indicate that the extract at the graded doses had no toxic effect on the heart and therefore, may not increase the risk of heart disease which is a predictive factor for cardiovascular diseases, erectile dysfunction, hypertension, stroke and diabetes among others.

CONCLUSION

Findings from this study revealed that administration of the ethanolic extract of *C. sieberiana* root at the graded doses had no deleterious effect on haematological indices, lipid profile and histoarchitexture of the heart. The result suggest that *C. siberiana* may be safe, however, it effect should also be assessed on liver, kidney, male sexual and accessory organs.

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