

Full Length Research Paper

# Phytochemical screening and toxicity studies of the aqueous extract of the pods pulp of *Cassia sieberiana* DC. (*Cassia Kotchiyana Oliv.*)

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The study was conducted to screen for the phytochemical constituents of *Cassia sieberiana*. The acute toxicity value and the effect of the aqueous extract of the pods of *C. sieberiana* on serum transaminases and alkaline phosphatase (ALP) were also measured in rats after five weeks of daily oral administration of grade doses (200 - 1600 mg/kg body weight) of the extract. Phytochemical analysis of the *C. sieberiana* pods pulp (fruit) revealed the presence of tannins, alkaloids, saponins, steroids, flavonoids, phlobatannins, cardiac glycosides, cyanogenic glycosides and reducing sugars. The concentration of tannins and saponins were found to be much higher than the other phytochemical components. The result of the oral acute toxicity study showed that LD<sub>50</sub> of the extract is 1950 mg/kg, indicating that the extract is of low toxicity. In this study, the extract used had caused a significant elevation ( $P < 0.05$ ) ( $27.60 \pm 1.25^b$ ) of serum levels of ALT in the group that received the highest dose (1600mg/kg body weight) of the extract and a significant increase ( $P < 0.05$ ) ( $43.00 \pm 1.14^b$ ,  $49.40 \pm 2.71^c$ ,  $52.60 \pm 3.90^d$ ,  $60.00 \pm 3.55^e$ ) increase in the serum level of AST in all the treated group when compared to the control rats ( $22.80 \pm 0.66^a$ ). The result also show a statistically significant ( $P < 0.05$ ) ( $208.80 \pm 11.95^b$ ,  $220.20 \pm 8.47^c$ ,  $234.80 \pm 4.33^d$ ) increase in the serum level of ALP in those groups that received 400, 800 and 1600mg/kg body weight of the extract when compared to the control rats ( $146.80 \pm 7.20^a$ ). From these result, even though the pods (fruit) of *C. sieberiana* has been reported to have medicinal value and despite the fact that the calculated LD<sub>50</sub> of this fruit indicated a low toxicity, this study has shown that using the extract at a high dose such as 400 – 1600 mg/kg body weight for a long period can cause liver damage. We therefore conclude that the plant should be taken at a very low dosage  $\leq 200$  mg/kg body weight and should not be taken over a long period of time.

**Key words:** Phytochemical, toxicity, transaminases, alkaline phosphatase, *Cassia sieberiana*.

## INTRODUCTION

*Cassia sieberiana* DC. (*Cassia kotchiyana Oliv.*) belongs to the family Caesalpiniaceae and grows up to 10 – 20 m high with drooping branches. The fruit (pods) is cylindrical, smooth, 40 - 60 (80) cm long and about 1.5 cm in diameter; indehiscent, dark brown. The plant was described by Gledhill (1991) as an open savannah tree found in drier area of forest and thickets. The plant is widely distributed in the southern sahel and sudan savanna from Senegal to Cameroun, as far as Sudan and the Republic

of Congo (Michael, 2004; Von maydell, 1986). It is also found in most parts of Nigeria. In the North-West it is found in places like Zamfara, Zurumi and forest reserve near Sokoto. It is found widely distributed in Borno, Yobe, Bauchi and some part of Adamawa State in the North eastern part of Nigeria. It is mostly found in Agodi in Ibadan and Awka near Onitsha in the South-west and South-east respectively (Keay et al., 1964). Its indigenous names in Nigeria include "marga" in hausa, the fulani people called it "margaje", in kanuri it is called "kiskatigrai" and in yoruba it is called "ifo" or "aridan-tooro." The fruits are used for the treatment of fever, jaundice, stomach ache, gonorrhoea, piles and ulcers. It is

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also used as a vermifuge, laxative or for wound dressing (Aubreville, 1950; Shahina, 1989).

Phytochemical analysis on the root and fruit pulp of the plant as reported by Modusolomuo et al. (1999) shows the presence of four active principles; tannins, saponins, quinones and alkaloid, which are of varying concentrations. Saponin was found in high concentrations in the fruit pulp which suggest the use of the pulp in the treatment of dysentery and urinogenital infections. This is also due to the fact that triterpenoid saponins have been shown to have antimicrobial properties (Mahato et al., 1988). It is known that phytochemicals confer pharmacological relevance on plants generally (Ojo et al., 2008). The growing interest in herbal medicine (Atawodi, 2005) demands information on various plant preparations used in the treatment of diseases (Sofowora, 1991). Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risks associated with the use of either herbal preparations or conventional drugs of plant origin.

Scientific information on the medicinal use and side effect of the extract from *C. sieberiana* pods pulp is however lacking. It is against this background that this study was designed to provide scientific information on the safety or toxicity of the extract from pods pulp of the plant in rats.

## MATERIALS AND METHODS

### Plant materials: Sample collection and identification

The plant material (fruits or pods) to be used in this study was collected from the University of Maiduguri campus. It was identified and authenticated by Dr. S.S Sanusi a plant taxonomist in the department of Biological Sciences, University of Maiduguri. A voucher specimen was deposited at the herbarium of the Botany Department, University of Maiduguri. The pods pulp of the plant sample was dried, pulverized to powder using pestle and mortar and stored in cellophane bags at room temperature until required for the experiment.

### Extraction techniques

Aqueous extract of the pods pulp was prepared following the methods of Mittal et al. (1981), Fernando et al. (1989). Two hundred grams (200 g) of the powdered pods pulp was mixed with 1 L distilled water in a 5 L beaker and heated at 65°C for 30 min. It was allowed to cool and then filtered using Whatman No. 1 filter paper. The extract (filtrate) was stored at 4°C in a concentration of 0.2 g/ml until use.

### Phytochemical screening

Phytochemical analysis of the extract was performed according to the standard methods of Wall et al. (1952) and Sofowora (1993) to screen for tannins, alkaloids, saponins, steroids, flavonoids, phlobatannins, cardiac glycosides, cyanogenic glycosides and reducing sugars.

### Animals

Albino rats of both sexes weighing between 100 – 225 g obtained

from Anatomy Department, University of Maiduguri were used for the experiment. They were kept for period of one week so as to get acclimatized to laboratory condition in the Research Complex of the Department of Biological Sciences of the Adamawa State University, Mubi. They were fed on standard diet, grower mesh (ECWA Feeds, Jos) and water *ad libitum*. Thirty of the rats were randomly divided into six groups of five rats each for oral acute toxicity and the remaining Twenty-five rats were divided into five groups of five rats each for the sub acute toxicity study.

### Acute toxicity study (determination of LD50)

Acute toxicity of the aqueous pods (fruit) pulp extract of *C. sieberiana* DC. was determined orally in rats. Thirty albino rats were randomly divided into six groups of 5 rats each. The first groups was given 2 ml of normal saline while groups 2 - 6 were given graded doses of 1000, 1500, 2000, 2500, 3000 mg/kg body weight of the extract respectively orally using gastric tube. The animals were observed for 24 h for toxicity signs and death. The LD<sub>50</sub> of the extract was calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982).

$$LD_{50} = \text{Least dose that killed all the animals} - \frac{\text{Sum of (Dose difference} \times \text{Mean dose)}}{\text{No. of animals/group}}$$

### Sub acute (prolonged) toxicity study

Twenty five Wister albino rats weighing between 100 – 175 g were used for this study. The rats were randomly divided into five groups of five rats each. The first group served as control, while groups 2 - 4 was treated orally daily with graded doses of 200, 400, 800 and 1600 mg/kg body weight respectively of the aqueous extract of *C. sieberiana* DC. pods for five weeks. On the last day of the experiment, the animals were sacrificed by decapitation. Blood was collected in test tubes without anticoagulant, it was allowed to clot to obtain the serum for estimation of liver enzymes; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum alkaline Phosphatase (ALP). Assay for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were done by the method described by Reitman and Frankel (1957) and serum level of alkaline phosphatase was measured by the method of Klein et al. (1960) using Randox test kits.

## RESULTS

The result of the qualitative phytochemical screening of *C. sieberiana* DC. pods pulp is presented in Table 1. The result indicated that *C. sieberiana* DC. pods pulp (fruit) contains tannins, alkaloids, saponins, steroids, flavonoids, phlobatannins, cardiac glycosides, cyanogenic glycosides and reducing sugars.

The result of toxicity signs observed following administration of the extract and calculated LD<sub>50</sub> are presented in Tables 2 and 3. The results of the oral acute toxicity shows that there was no mortality in the groups that received 2 ml of normal saline and the group that received 1000 mg/kg body weight of the extract. Mortality occurred in groups that received 1500, 2000, 2500 mg/kg body weight within 24 h, with 100% death recorded in the group that received 3000 mg/kg body weight within 11 h.

The result also indicate that, the rats showed signs of

**Table 1.** Result of the phytochemical analysis.

Test	Result
Tannins	+++
Alkaloids	+
Saponins	+++
Cardiac glycosides	++
Steroids	+
Flavonoids	+
Phlobatannins	++
Anthraquinone	-
Reducing sugar	++
Cyanogenic glycosides	+

+++ = High, ++ = Moderate, + = Trace, - = Not detected.

toxicity (Table 2) such as dizziness, insensitivity to pain, lack of appetite, itching, depression, unsteady movement and death within 24 h.

The result of the oral acute toxicity study showed that LD<sub>50</sub> of the extract is 1950 mg/kg, indicating that the extract is of low toxicity.

The result of the effect of prolonged oral administration of various doses of aqueous pods (fruit) pulp extract of *C. sieberiana* DC. on the serum liver enzymes (ALT, AST and ALP) are shown in Table 4. The result shows that the extract administered to the rats at 200, 400 and 800 mg/kg body weight though has raised the serum level of ALT, but it was not statistically significant ( $P > 0.05$ ) ( $25.60 \pm 1.17^a$ ,  $26.20 \pm 2.67^a$ ,  $27.20^a$ ) when compared to the control ( $22.80 \pm 0.66^a$ ). However, there was a statistically significant ( $P < 0.05$ ) ( $27.60 \pm 1.25^b$ ) increase in serum level of ALT observed in the group of rats that received 1600 mg/kg body weight of the extract when compared to the control rats ( $22.80 \pm 0.66^a$ ).

It was also observed that all the doses of the extract (200, 400, 800 and 1600 mg/kg body weight) administered to the rats caused a statistically significant ( $P < 0.05$ ) ( $43.00 \pm 1.14^b$ ,  $49.40 \pm 2.71^c$ ,  $52.60 \pm 3.90^d$ ,  $60.00 \pm 3.55^e$ ) increase in the serum level of AST when compared to the control rats ( $22.80 \pm 0.66^a$ ).

Result on the serum level of ALP indicate that there is no statistically significant difference ( $P > 0.05$ ) ( $155.60 \pm 9.41^a$ ) between the group that received 200mg/kg body weight of the extract and the control rats ( $146.80 \pm 7.20^a$ ), but there was a significant ( $P < 0.05$ ) ( $208.80 \pm 11.95^b$ ,  $220.20 \pm 8.47^c$ ,  $234.80 \pm 4.33^d$ ) increase in those group that received 400, 800 and 1600 mg/kg body weight of the extract when compared to the control rats ( $146.80 \pm 7.20^a$ ).

## DISCUSSION

In this study, the result of the phytochemical screening of *C. sieberiana* DC. pods pulp indicated the presence of tannins, alkaloids, saponins, steroids, flavonoids, phloba-

tannins, cardiac glycosides, cyanogenic glycosides and reducing sugars. Tannins and Saponins were found to be much higher than the other phytochemical components (Table 1). The result of this study is supported by the report of Modusolomuo et al. (1999) who also reported that the roots and pods (fruit) pulp shows the presence of four active principles; tannins, saponins, quinones and alkaloid, which are of varying concentrations. However, in contrast to the study of Modusolomou et al. (1999), this study showed that the pods did not show the presence of quinones. This difference may be explained by the fact that variation may sometimes occur in bioactive compounds of the different part of the same plant and even in the same plant parts found in different environment. Elujoba (1989) reported that variation may occur in bioactive compounds of the same plant due to different environment where they are found. The presence of these phytochemical components in this plant pods (fruit) provide an empirical basis for its traditional medicinal uses. Phytochemicals have been reported to have medicinal uses (Tella and Ojo, 2005).

This study shows that the calculated LD<sub>50</sub> of the aqueous pods (fruit) pulp extract of *C. sieberiana* DC. is 1950 mg/kg indicating that it is of low toxicity. According to the toxicity scale of Hodge and Sterner (1943), any compound with an oral LD<sub>50</sub> of between 500 – 2000 mg/kg should be considered practically non toxic. This also agrees with the findings of Allain (2000) who ranks all plants whose LD<sub>50</sub> figures are less than 25 mg/kg in "very toxic" group, between 25 and 200 mg/kg in "toxic" group, and from 200 to 2000 mg/kg in "noxious" group. This could be attributed to the fact that orally administered drugs and compounds do undergo some events that potentially decrease the amount reaching systemic circulation for pharmacological effects (Brander et al., 1991).

The result also indicate that the rats showed signs of toxicity (Table 2) such as dizziness, insensitivity to pain, lack of appetite, itching, depression, unsteady movement and death. The manifestation of depression and lack of appetite observed in the rats may be linked to some chemical constituents present in the extract such as tannins (Hotellier and Delaveau, 1975; Nwafor et al., 1995). Alldredge (1993) attributed reduce feed intake in animals fed tannin containing diets to strong astringent property of tannins and induction of internal malaise in mammals, which may contribute to reduce feed intake. In the assessment of liver damage by drugs or any other hepatotoxin, the determination of enzyme levels such as ALT and AST is largely used (Dobbs et al., 2003; Xu et al., 2002). Necrosis or membrane damage releases the enzyme into circulation; therefore, it can be measured in serum. High levels of AST indicate liver damage, such as that due to viral hepatitis, cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a

**Table 2.** Toxicity signs observed in rats that received single dose by intubation of the aqueous Pods (fruits) pulp extract of *C. sieberiana*.

Group	Dose (mg/kg body weight)	Signs of toxicity n = 5						
		Dizziness	Insensitivity to pain	Lack of appetite	Itching	Depression	Unsteady movement	Death
1	2 ml normal saline	-	-	-	-	-	-	0
2	1000	+	-	-	-	+	-	0
3	1500	+	-	+	-	+	-	2
4	2000	+	+	+	+	+	+	3
5	2500	+	+	+	+	+	+	3
6	3000	+	+	+	+	+	+	5

+ = Present, - = Absent

**Table 3.** LD<sub>50</sub> of the aqueous Pods (fruits) pulp extract of *C. sieberiana* calculated by arithmetic method of Karber.

Group	Dose (mg/kg body weight)	n = 5			
		Dose difference	Dead	Mean death	Dose difference × mean death
1	2 ml normal saline	-	0	0	0
2	1000	500	0	0	0
3	1500	500	2	1.0	500
4	2000	500	3	2.5	1250
5	2500	500	3	3.0	1500
6	3000	500	5	4.0	2000

$$LD_{50} = \frac{\text{Least dose that killed all the animals} - \text{Sum of (Dose difference} \times \text{Mean dose)}}{\text{No. of animals/group}}$$

$$LD_{50} = 3000 - 5250/5, LD_{50} = 3000 - 1050, LD_{50} = 1950 \text{ mg/kg.}$$
**Table 4.** Effect of aqueous fruit pulp extract of *C. sieberiana* on serum level of ALT, AST and ALP of treated rats.

	Group I control	Group II (200 mgkg <sup>-1</sup> )	Group III (400 mgkg <sup>-1</sup> )	Group IV (800 mgkg <sup>-1</sup> )	Group V (1600 mgkg <sup>-1</sup> )
ALT (iu/l)	22.80 ± 0.66 <sup>a</sup>	25.60 ± 1.17 <sup>a</sup>	26.20 ± 2.67 <sup>a</sup>	27.20 ± 2.41 <sup>a</sup>	27.60 ± 1.25 <sup>b</sup>
AST (iu/l)	31.20 ± 0.49 <sup>a</sup>	43.00 ± 1.14 <sup>b</sup>	49.40 ± 2.71 <sup>c</sup>	52.60 ± 3.90 <sup>d</sup>	60.00 ± 3.55 <sup>e</sup>
ALP (iu/l)	146.80 ± 7.20 <sup>a</sup>	155.06 ± 9.41 <sup>a</sup>	208.80 ± 11.95 <sup>b</sup>	220.20 ± 8.47 <sup>c</sup>	234.80 ± 4.33 <sup>d</sup>

All values represent mean ± standard error of mean (SEM); n = 5. Comparison was done between the groups and values with different superscript are statistically different (p < 0.05).

better parameter for detecting liver injury (Williamson et al., 1996). Serum ALP level on the other hand, is related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis of the enzyme, in presence of increasing biliary pressure (Moss and Butterworth, 1974).

In this study, as shown in Table 4, the extract used had caused a significant elevation (P<0.05) of serum levels of ALT in only the group that received the highest dose; 1600 mg/kg body weight and a significant increase P<0.05) of serum level of AST in groups II to V (200 – 1600 mg/kg body weight) and ALP in groups III to V (400

– 1600 mg/kg body weight), the increase in the serum level of ALP at these doses could be due to increase biliary pressure probably caused by the extract there by increasing the synthesis of the enzyme above normal. Increase in the level of aminotransterases especially ALT is an indication of liver damage. From the results, this study shows that using the extract at a high dose such as 1600 mg/kg body weight for a long period can cause liver damage. Even though the pods (fruit) of *C. sieberiana* has been reported to have medicinal value (Von maydell, 1986), Modusolomuo et al. (1999), and despite the fact that the calculated LD<sub>50</sub> of this fruit indicated a low toxic

city, this study has also shown that using the extract at a high dose such as 400 – 1600 mg/kg body weight for a long period can cause liver damage. We therefore conclude that the plant should be taken at a very low dosage  $\leq$  200 mg/kg body weight and should not be taken over a long period of time.

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