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Acute Toxicity Assessment of *Cassia tora* Seed and Leaf Extracts in Wistar Rats

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ABSTRACT

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders as a result of the secondary metabolites synthesized by plants. The aim of this research is to evaluate the acute toxicity of ethanolic seed and leaf extracts of *Cassia tora*, in Wistar rats for potential medicinal use. Male rats (24) of 100 g – 120 g body weight were prepared for toxicity test and orally treated with different doses of ethanolic seed and leaf extracts of *Cassia tora*. The rats were observed for a sign of toxicity and mortality. The result of the study showed that both plant extracts were toxic to the rats even at a lower concentration (100 mg/kg). There was a case of mortality in the rats treated with ethanolic seed extract at the highest dose of 5000 mg/kg. The LD_{50} was found to be 2,236 mg/kg of the extract. However, there was no case of mortality in the rats treated with ethanolic leaf extract, and the LD_{50} was found to be high at 5000 mg/kg. Though the results proved that both extracts had toxic effect in rats, both seed and leaf extracts of *Cassia tora* could be potential candidates for medicinal use.

Keywords: Toxicity, *Cassia tora*, extract, Wister rats, metabolite

INTRODUCTION

Traditional medicine is a comprehensive knowledge system that encompasses the utilization of substances, dosages and practices based on socio-cultural norms and religious beliefs as well as witnessed experiences and observations of a specific group [1]. The practice is used in various therapies by the indigenous population all over the world. It has been documented that about 80% of the people in developing countries rely on traditional medicines for their primary health care need [2]. This can be attributed to increased poverty, ignorance as well as unavailability of modern

health facilities [2-3]. Ethnomedicine refers to the study of traditional medical practices which is concerned with the cultural interpretation of health, diseases and illness and also addresses the health care need and healing practices. It also denotes plants, animal products and minerals used by tribal communities of a particular region or country for medicinal purposes [4]. Plants that have some medicinal properties or pharmacological effect in human body are denoted as medicinal plants. These plants could be used directly or in the form of extracts because of the presence of some natural medicinal properties [5].

Medicinal plants naturally synthesize secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, glycosides and volatile oil [6]. Herbal plants produce and contain a variety of chemical substances with varied physiological effects. They are huge reservoir of various chemical substances with potential therapeutic properties. Herbal plants are being increasingly utilized to treat a wide variety of clinical diseases [6]. Herbs have been used by all cultures throughout history. Herbal medicine is the oldest form of health care known to mankind. It was an integral part of the development of modern civilization. Many drugs commonly used today are of herbal origin. Higher plants, as source of medicinal compounds, continue to play a dominant role in maintenance of human health since antiquities [7] Herbal medicine is gaining popularity once again and there is an increased interest in green medicine because it is considered as safe [8].

Traditionally, plants and plant extracts are used to cure many diseases and disorders. However, before usage it is important to ensure its safety. The extract may be therapeutically very efficient but if its toxicity assessment is not worked out, then it will not be accepted [8]. Therefore, toxicity assessment of plants with proven therapeutic use is imperative. Toxicity reports are needed to predict the safety associated before their usage [9].

C. tora is a weed abundantly grown in the forest, road sides and fallow lands during monsoon. The seed is rich in protein which can be fed to livestock, avian and fish. Cassia species possess several medicinal value such as hepatoprotective activities [10], prevent skin disorder [11], anti-inflammatory and anti-pyretic activity [12]. *Cassia tora* is a popular plant for medicinal value in Asian and African countries including Nigeria, owing to the different scientific research conducted especially on their leaves and seeds. However, several studies need to be conducted in different regions to ascertain its safety profile for their medicinal use. Therefore, the present study aims at assessing acute toxicity of ethanolic seed and leaf extracts of *Cassia tora* in Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Male Wistar rats (24) of 100-120 g body weight were obtained from the Animal House, Department of Biochemistry, University of Maiduguri, Nigeria. The animals were maintained in a well-ventilated room in appropriate cages bedded with dry clean wood shavings. The animals were fed with water *ad libitum*, and standard growers mash/feeds (Pallet contain, produced by Grand Cereals Ltd. Nigeria), and acclimatized for seven days. The experimental room was cleaned and disinfected regularly. The water containers and animal cages were washed regularly.

Plant Materials

The plant materials (*C. tora)* used in this assessment were collected from the natural environment growing behind the Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria, in December 2024. It was identified and authenticated by a Taxonomist at the Department of Biological Sciences, University of Maiduguri, Nigeria, and assessed, as described by Adamu et al., [13].

Plant Sample Preparation

The seeds and leaves of *Cassia tora* were collected, weighed, washed with tap water and rewashed to remove particles and dust, and weighed again after air-drying under shade. The dried seeds and leaves were pulverized into powder using wooden pestle and mortar.

Ethanolic Extraction of *Cassia tora* Seed and Leaf

The *Cassia tora* seed and leaf powder (200 g) were respectively packed in a thimble and placed into the extractor. In the first stage, 2000 ml of 70% ethanol and 30% distilled water (ethanolic extraction), was added into a round bottom flask, in 1:10 w/v sample to solvent ratio [14]. The continuous cycles were carried out for 5 hours a day for 3 days until clear white solutions from the siphon were obtained. After the Soxhlet extraction, a thick dark solvent containing the extract in the round bottom flask was dried in a porcelain dish and placed into desiccators. The percentage yield obtained was 12.53% (w/w). This extract was used in the subsequent experiments.

Preparation of Stock Concentration of the Extraction

The solution was prepared by dissolving 20 g of the seed and leaf extracts of *C. tora* in 100 ml of distilled water to give a concentration of 0.2 g/ml and was stored at 40^0C until required.

Acute Toxicity (LD_{50}) Evaluation (Lorkes method)

The lethal dose (LD_{50}) of the ethanolic seed and leaf extracts of *C. tora* was determined in Wistar rats as described by Adebayo et al. [15]. Mortality is the main criterion used in assessing acute toxicity (LD_{50}) of any compound. Twenty-four (24) male rats of 100 g-120 g body weight were divided into two categories of twelve (12) rats each:

Category I: Rats treated with ethanolic seed extracts of *C. tora*

Category II: Rats treated with ethanolic leaf extracts of *C. tora*

After 7-day acclimatization, the rats in Category I were fasted overnight for the toxicity test. The assessment was carried out in two phases. In phase I, 3 groups of three rats each were orally treated with 10, 100, 1000 mg/kg of the extract respectively. The rats were observed for a sign of toxicity and mortality for 0, 6, 12, 24 h for the first 24 h at 6 h interval. In phase II, another 3 groups each containing 1 rat were further orally treated with 1600, 2900, and 5000 mg/kg of the extract respectfully. The rats were observed for sign of toxicity and mortality at regular intervals for 0, 6, 12, 24 h, for 3 days. The volume of the seed and leaf extracts of *C. tora* were administered as stated in equation (1).

Volume of extract solution = (Weight of rat x Dose)/Concentration (1)

By definition,

Oral Median Lethal Dose (LD_{50}) = $\sqrt{}$(min toxic dose x max toxic dose) (2)

The same procedure was applied to the rats in Category II (rats treated with leaf extracts of *C. tora*)

RESULTS AND DISCUSSION

Acute Toxicity Studies (LD_{50})

Mortality is the main criteria used in assessing the acute toxicity (LD_{50}) of any compound. Ethanolic seed and leaf extracts of *C. tora* were orally administered to each group of rats sequentially at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg as shown in Table 1.

During the period of the experiment for acute toxicity studies, the rats that were given 100 mg/kg showed some physical changes. In the second phase, three treatment groups of 1 rat each were administered the following higher doses of the extract i.e. 1600 mg/kg, 2900 mg/kg and 5000

mg/kg as shown in Table 2. The results of phase II experiment of category I showed that there was mortality in the highest dose group (5000 mg/kg). Based on this, the LD_{50} was calculated using the formular presented in equation (2), which was found to be 2236 mg/kg as calculated from equation (3).

$\sqrt{1000 \times 5000} = 2{,}236.07$ mg/kg (3)

The results of acute toxicity assessment of ethanolic seed and leaf extracts in phase I and phase II are presented in Tables 1 and 2 respectively

Table 1: Phase I Acute Toxicity Studies of Ethanolic Seed and Leaf Extracts of *Cassia tora* in Wistar Rats

DOSE		SIGN OF TOXICITY	SURVIVAL	
			Seed Extract	Leaf Extract
10 mg/kg	Rat 1	None	1	1
	Rat 2	None	1	1
	Rat 3	None	1	1
100 mg/kg	Rat 1	Lethargy, erect fur, sedation, and pilo	1	1
	Rat 2	erection	1	1
	Rat 3	Lethargy, erect fur, sedation, and pilo erection	1	1
		Lethargy, erect fur, sedation, and pilo erection		
1000 mg/kg	Rat 1	Lethargy, erect fur, sedation, and pilo	1	1
	Rat 2	erection	1	1
	Rat 3	Lethargy, erect fur, sedation, and pilo erection	1	1
		Lethargy, erect fur, sedation, and pilo erection		

Table 2: Phase II Acute Toxicity Studies of Ethanolic Seed and Leaf Extracts of *Cassia tora* in Wistar Rats

DOSE		SIGN OF TOXICITY	SURVIVAL	
			Seed Extract	Leaf Extract
1600 mg/kg	Rat 1	Erect fur, sedation, lethargy	1	1
2900 mg/kg	Rat 2	Erect fur, sedation, lethargy	1	1
5000 mg/kg	Rat 3	Death	0	1

The acute oral toxicity of ethanolic seed and leaf extracts of *Cassia tora* was determined in the present study. The screening of the toxicity of the plant was crucial to assure the safety and effectiveness of the plant extracts. Signs of toxicity such as inactivity, lethargy, fur erection, loss of appetite, sedation and pilo erection, physical changes in the tested animals were detected in the assays.

In this study of acute oral toxicity, twelve Wister rats were employed to observe the toxicity effects of ethanolic seed and leaf extracts of *Cassia tora*. From the result, adverse signs of toxicity were observed on rats administered with both ethanolic seed and leaf extracts of *Cassia tora*, from the lower dose (100 mg/kg). The physical appearance such as, lethargy, erect fur, sedation, and pilo erection were observed which indicated that the extract did affect the animals. Ethanolic seed extract of *Cassia tora* caused acute toxicity effects with an LD_{50} value of 2,236.07 mg/kg. However, there was no case of mortality in the rats treated with ethanolic leaf extract, hence the LD_{50} was higher in 5000 mg/kg concentration which suggests the possibility of the use of the extract as a potential source for the development of pharmacological agent to treat various types of ailments.

The result of this study is in agreement with the acute oral toxicity study described on *Cassia tora* by Sanjaya Kumar et al., [15] in which the ethanol extract of *Cassia tora* seeds was safe up to dose of 2000 mg/kg, upon 13-week consecutive oral administration in Sprague Dawley rats. Also, methanolic extract of *Cassia tora* leaves were found to be safe up to 2000 mg per kg in rats during acute oral toxicity study. Similarly, acute oral toxicity study of ethanol extract of leaf of *Cassia tora* in Swiss albino mice showed that the extract was safe up to 2000 mg/kg upon single

exposure as has also been demonstrated by Sanjaya Kumar et al. [15]. The LD_{50} was higher in 5000 mg/kg seed extract. This agrees with the research carried out by Adebayo et al. [16] in which the LD_{50} was found to be higher in 5000 mg/kg dose.

CONCLUSION

The aim of this study was to determine the acute toxicity assessment of ethanolic seed and leaf extracts of *Cassia tora* in Wistar rats. Though the results proved that both extracts had toxic effect in rats, both seed and leaf extracts of *Cassia tora* have potentials for medicinal use.

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