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Phytochemical Components and Acute Toxicity Study of *Cassia Tora* Seed Extract in 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 work is to determine the phytochemical constituents and acute toxicity of ethanolic seed extract of *Cassia tora* in rats for potential pharmaceutical development. Twelve (12) male rats of 100 g – 120 g body weight after 7 days acclimatization, were fasted overnight for the toxicity study. The study was done in two phases. In the first phase, 3 groups of three rats each were orally administrated with 10, 100, 1000 mg/kg body weight of the extract respectively. The rats were observed for a sign of toxicity and mortality for 0, 6, 12, 24 h for 24 hrs at 6 h interval. In the second phase, another 3 groups each containing 1 rat were further orally administered with 1600, 2900, and 5000 mg/kg body weight of the extract. The rats were observed for sign of toxicity and mortality at regular intervals for 0, 6, 12, 24 h, for 3 days. The result of determination of phytochemical constituents of ethanolic seed extract of *Cassia tora* showed the presence of flavonoids, anthraquinones, steroids, saponins, tannins and terpenoids. The acute toxicity study of the extract of *Cassia tora* showed that the plant extract was toxic to the rats even at a lower dose (100 mg/Kg) (phase I) and caused death at a dose of 500 mg/Kg body weight of the live rats (phase II), and the LD_{50} was found to be 2,236 mg/kg body weight. The results obtained from the present study demonstrated that the extract had toxic effect in rats, however, the ethanolic seed extract of *Cassia tora* could be potential for pharmaceutical development as a result of the phytochemicals present.

Keywords: phytochemical, toxicity, *Cassia tora*, extract, Wister rats, metabolite

INTRODUCTION

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. This can be attributed to increased poverty, ignorance as well as unavailability of modern health facilities (Omonike 2010; Christiana *et al*., 2012). 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 (Krippner, 2003). According to Tom *et al*., (2008) comprehensive knowledge system that encompasses the utilization of substances, dosages and practices based on cultural norms and religious beliefs as well as witnessed experiences and observations of a specific group. Plants that have some medicinal properties or pharmacological effect in human body that plant are denoted as medicinal plants. These plants could be used directly or for in the form of extract because of the presence of some natural medicinal properties (Ashis, 2003). Medicinal plant naturally synthesize secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, glycosides, volatile oil, etc. (Palanivelu *et al*., 2015). 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 (Palanivelu *et al*., 2015). Herbs have been used by all cultures throughout history and thus, 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 compound continue to play a dominant role in maintenance of human health since antiquities (Palanivelu, 2015). Herbal medicine is gaining popularity once again and there is an increased interest in green medicine simply because it is considered as safe. Traditionally also plants and plant extracts are used to cure many diseases and disorders. However, before usage it is of utmost importance 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 (SenthamilSelvan *et al*., 2015). Hence, toxicity assessment of plants with proven therapeutic use is imperative. Toxicity reports are needed to foretell the safety associated before their usage (Baris *et al*., 2006).

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 the livestock, avian and fish. *Cassia species* possess several medicinal value like hepatoprotective activities (Upadhyay *et al*., 2000), prevent skin disorder (Sridhar and Bhagya, 2007), anti-inflammatory and anti-pyretic activity (Gobianand *et al*., 2010). In Ayurveda, it helps in the treatment of skin diseases like ring worm, leucoderma, eczema etc. It is an anthroquinone containing plant which also has a certain bioactive compounds such as emodin, rhein, palmatic, isostearic, etc (Albasarah *et al*., 2010). *C. tora* has been reported to contain many active substances, including Anthraquinone, Quarcetine, chrysophenol, emodin, rhein, etc. *Cassia tora* has been reported to exhibit significant antimutagenic activity (Obdoni and Ochuko, 2001).

Cassia tora is well known for its medicinal value in Asian and African countries including Nigeria, owing to the different scientific investigations conducted especially on their leaves and seeds. However, several studies need to be conducted well in different regions to ascertain its phytochemical components and safety in different methods. For this reason, an ethanolic seed extract of *Cassia tora* was selected for the determination of phytochemical components and acute toxicity study. Therefore, the present study aims at evaluating phytochemical components and acute toxicity study of ethanolic seed extract of *Cassia tora* in Wistar rats.

METHODLOGY

Experimental Animals

Twelve (12) male Wistar rats of 120 g – 150 g body weight were obtained from the Animal House, Department of Biochemistry. The animals were maintained in a well-ventilated room in appropriate cages bedded with dry clean wood shavings. The animals were fed with standard growers mash/feeds (pallet contain produced by Grand Cereals Ltd. Nigeria and water *ad libitum.* The experimental room was cleaned and disinfected regularly. The water containers and animal cages were washed regularly.

Plant Materials

The plant material (*C. tora*) used in this study, as described by Adamu *et al*., (2025) was collected from the natural environment growing behind the Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria, in December 2024. It was then identified and authenticated by a Taxonomist from the Department of Biological Sciences, University of Maiduguri, Nigeria.

Methods

Plant Sample Preparation

The seed of *Cassia tora* was collected, weighed, washed with tap water and rewashed to remove particles and dust, and weighed again after it was air-dried under shade, to check if it was dried. The dried seed was then pulverized into powder using wooden pestle and mortar.

Ethanolic Extraction of *Cassia tora* Seed.

Three hundred gram (300 g) of the *Cassia tora* seed powder was packed in a thimble which was placed into the extractor. In the first stage, 3000 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 (Azwanida, 2015). The continuous cycles were carried out for 5 hours a day for 3 days until the clear white solutions from the siphon were obtained. After the Soxhlet extraction, a thick dark solvent containing the extracted material was found in the round bottom flask. Extract was collected and dried in a porcelain dish and placed into desiccators. The percentage yield obtained was 12.53% (w/w). This extract was used in all the subsequent experiments.

Preparation of Stock Concentration of the Extract

The solution was prepared by dissolving 20 grams of the crude extract in 100 ml of distilled water to give a concentration of 0.2 g/ml and was stored at 4 ^{0}C until required.

Phytochemical Analysis

The phytochemical analysis (Screening) of senna tora *(C. tora)* seeds was carried out according to standard method of Evan (2002).

Test for Alkaloid

About 0.5g of the powdered material was warmed with 10ml of 2% H_2SO_4 (Sulfuric acid) for two minutes and filtered. Three-one portions were treated with a few drop of Drangendroff's reagent, Wagner's reagent and Mayer's reagent.

Determination of Steroids

This was carried out using the method described by Akinyeye *et al*., (2014).Two milliliters of acetic anhydride was added to 5 mg of the extract, thereafter, 2 ml of conc. H_2SO_4 was added. The colour changed from violet to blue or green in some samples which indicates the presence of steroids.

Test for Tannins

This as determined by the method of Trease and Evans, (2002). Two milligrams of the extract was added to 5 ml distilled water, followed by addition of 2 ml $FeCl_3$. Tannin was indicated by a brownish green or blue-black precipitate.

Determination of Terpenoids

This is carried out using the method described by Adamu *et al.,* (2025). Five milligrams of the ethanolic seed extract of *C. tora* were mixed with 2 ml of chloroform and conc. H_2SO_4. 3 ml was carefully added to form a layer. The reddish brown colour in inner face indicated the presence of terpenoids.

Determination of Anthraquinones

Bontrager's test was used to test the presence of free anthraquinone. The plant extract (0.5 g) was shaken with 10 ml of benzene and then filtered. Five milliliters (5 ml) of ammonium solution was added to the filtrates. The mixture was then shaken and appearance of a pink, red or violet colour in the ammonical (lower) phase was taken as an indicator for the presence of free anthraquinones (Trease and Evans, 2002).

Test for Saponin

About 0.5g of the powdered material *(Cassia tora)* seeds extract was shaken with 5ml of distilled water and heated to boiling point. Frothing shows the presence of saponin. The filtrate from the extract was added to 3ml of paraffin oil and thoroughly shaken to form a stablseedse emulsion. This was left to stand for about 5minutes, the presence of stable emulsion indicates the presence of saponins (Trease and Evans, 2002).

Test for Phlobatanins

0.5ml of the filtrate was boiled with 5ml of 1% HCl (Hydrochloric acid), a red precipitate shows the presence of phlobatanins.

Test for Flavonoid

2ml of dilute sodium hydroxide (NaOH) was added to 2ml of the extract. The appearance of a yellow color indicates the presence of flavonoids.

Test for Carbohydrates

a. Fehling's Test:

5ml of an equal mixture of Fehling's solution of A and B was added to a small portion of the *C. tora* seedss extract in a testtube and was boiled on a water bath, brick red precipitate indicates the presence of reducing sugar (Evans, 2002).

b. Molisch Test:

To a small portion of the extract *(Cassia tora)* seedss, a few drops of Molisch reagent was added to the test tube containing the extract and the concentrated sulfuric acid (H_2SO_4) was added down the sides of the test tube to form a lower, reddish coloring at the interphase indicates the presence of carbohydrates (Evans, 2002).

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

The lethal dose (LD_{50}) of the ethanolic seed extract of *C. tora* was determined in Wistar rats as described by Adebayo *et al*., (2015).

Twelve (12) male rats of 120 g – 150 g body weight after 7 days acclimatization, were fasted overnight for the toxicity study. The study was done in two phases. In the first phase, 3 groups of three rats each were orally administrated with 10, 100, 1000 mg/kg body weight of the extract respectively. The rats were observed for a sign of toxicity and mortality for 0, 6, 12, 24 h for 24 hrs at 6 h interval. In the second phase, another 3 groups each containing 1 rat were further orally administered with 1600, 2900, and 5000 mg/kg body weight of the extract. The rats were observed for sign of toxicity and mortality at regular intervals for 0, 6, 12, 24 h, for 3 days.

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

By definition,

Oral Median Lethal Dose (LD_{50}) = √(min toxic dose x max toxic dose)

RESULTS AND DISCUSSION

The result of phase I and phase II Acute Toxicity Study is presented in Table 1 and 2 respectively below:

Table 1. Phase I Acute Toxicity Studies of Ethanolic Seed Extract of *Cassia tora* in Wistar Rats

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

Table 2. Phase II Acute Toxicity Studies of Ethanolic Seed Extract of *Cassia tora* in Wistar Rats

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

In the present study phytochemical analysis of *Cassia tora* seeds extracts revealed the presence of flavonoids, anthraquinones, phenols, saponins, tannins, steroids, terpenoids, which is partly in line with the one reported by Surana *et al*., (2012), which showed the presence of carbohydrates, glycosides, flavanoids and saponins in methanolic and aqueous extract *Cassia tora* seeds. Suradkar *et al*., (2017) also reported that ethanolic extract of *C. tora* possess alkaloids, flavanoids, terpenoids, saponins, tannins, and glycosides. The findings in this study is also at par with Noha *et al*., (2015), who reported that alcoholic seed extract of *C. tora* possesses saponins, flavonoids carbohydrates, steroids and glycosides.

Mortality is the main criteria used in assessing the acute toxicity (LD_{50}) of any compound. Ethanolic extract of *C. tora* was orally administered to each group of rats sequentially at doses of 10 mgand, 100 mg/kg and 1000 mg/kg as shown in Table 1. Each concentration was given to group of three rats. Mortality was not observed in the first phase of the test animals.

During the period of the experiment, acute toxicity studies, the rats that were given 100 mg/kg showed some physical changes such as inactivity, lethargy, fur erection, loss of appetite, sedation and pilo erection. But the rats in the control group did not show any of these 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 result of phase II experiment showed that there was mortality in the highest dose group (5000 mg/kg). Based on this, LD_{50} was calculated using the formula (presented above), which was found to be 2236 mg/kg as calculated below.

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

The acute oral toxicity of ethanolic seed extract of *Cassia tora* was determined in the present study. The screening of the toxicity of the plant seed extract was crucial to assure the safety and effectiveness of the plant extract. Physical changes in the tested animals were detected in the assays. In this study of acute oral toxicity, twelve (12) male Wister rats were employed to observe the toxicity effects of ethanolic seed extract of *Cassia tora*. From the result, mortalities were reported as well as adverse toxicity signs were observed on the tested rats. The physical appearance as signs of toxicity such as inactivity, lethargy, fur erection, sedation, pilo erection and loss of appetite, were observed which indicated that the extract did affect the animals right from the lower dose 100 mg/kg. Hence, ethanolic seed extract of *Cassia tora* has caused acute toxicity effects with an LD_{50} value of 2,236.07 mg/kg which suggest the possibility of the use of the extract as a potential source for the development of pharmacological agent to treat various types of ailment, unlike a research carried out by Adebayo *et al*., (2015) that the leaves extract was found to be higher than 5000mg/kg body weight. The result of this study was agreement with a similar to the acute oral toxicity study described on ***Cassia tora*** by Sanjaya Kumar et al., (2022) that the ethanol extract of *Cassia tora* seeds was found to be safe up to dose of 2000 mg/kg body weight upon 13 weeks consecutive oral administration in Sprague Dawley rats. Also, methanol extract of *Cassia tora* leaves were found to be safe up to 2000 mg per kg in rats during acute oral toxicity study. Moreover, acute oral toxicity study of ethanol extract of leaves 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 reasonably demonstrated by Sanjaya Kumar *et al*., (2022).

CONCLUSION

The aim of this research work is to determine the phytochemical constituents and acute toxicity of ethanolic seed extract of *Cassia tora* in rats for potential pharmaceutical development. In conclusion, the results obtained from the present study demonstrated that the extract had toxic effect in rats, however, the ethanolic seed extract of *Cassia tora* could be potential for pharmaceutical development as a result of the phytochemicals present.

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