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Acute oral toxicity assessment of ethanolic extract of *Cassia auriculata linn* whole plant in Albino mice

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ABSTRACT: The current study was conducted to assess the safety of an ethanol extract of the Cassia auriculata linn entire plant by detecting its potential toxicity following acute administration in Swiss albino mice. For the acute investigation, an ethanol extract of Cassia auriculata linn entire plant was supplied to albino mice in a single dose of 0-2000 mg/kg via feeding. After feeding, behavioral changes, adverse effects, and death were assessed for up to 14 days. Histopathological examinations were performed 14 days following oral dosing. At the end of the observation period, animals were sacrificed. In an acute investigation with ethanol extract of Cassia auriculata linn, the whole plant showed no behavioral alterations indicating detrimental effects or deaths. There were no significant differences in organ weights or gross necropsy between the treatment and control groups. The ethanol extract of Cassia auriculata linn entire plant can be deemed relatively harmless at the oral dose tested because it did not induce any mortality or unfavorable behavioral changes in albino mice, according to a necropsy investigation in acute toxicity.

KEY WORDS: Acute oral toxicity, *Cassia auriculata linn,* behavioral pattern study, LD_{50} value, Histopathological study, Albino mice

INTRODUCTION

Folklore use of herbals was quite popular in rural areas, and the use of herbal preparations for the treatment of various ailments remains very common [1]. Because natural herbal treatments are widely used, researchers are now focusing their efforts on studying the efficacy and safety of medicinal plants [2]. Plants with therapeutic activity should have low toxicity due to their long-term use in humans. However, numerous medicinal plants employed in folkloric medicine have been shown to have harmful consequences [3-4]. A huge proportion of current medicines are made from natural ingredients. Many of these formulations rely on substances found in traditional medicine [5]. The Organization for Economic Cooperation and Development (OECD) defines acute toxicity as an impact that occurs shortly after oral ingestion of a single dosage of a chemical or repeated doses administered within a 24-hour period [6]. Acute, subacute, and chronic toxicological assessments are classified based on the duration of animal exposure to chemicals. Acute toxicity studies are conducted to investigate the short-term toxicity effect of a toxicant (1 to 2 weeks). An estimate of safe acute dosages for humans. The possibility of acute poisoning in humansTimeline of drug-induced clinical observations, The probable target organs of toxicity include The recommended dosage for multiple-dose toxicity studies, Toxicity varies by species, The LD50, or median fatal dose, and gross behavior are calculated using the above information from the acute toxicity research.

Cassia auriculata Linn (Family: Caesalpiniaceae), also known as Tanner's Senna and Avartaki. It is a densely branching shrub with smooth cinnamon colored bark and tightly pubescent branchlets. It is abundantly dispersed in India's hot deciduous woods. Cassia auriculata linn's leaves include O-methyl-d-glucose, resorcinol, alpha-tocopherolbeta-mannosidase, and carboxylic acid (7), while its fruits contain The seeds of Cassia auriculata Linn contain alkaloids, glycosides, saponins, phenols, terpenoids, flavonoids, tannins, and steroids (8,9). Palmitic acid, linoleic acid, benzoic acid 2-hydroxyl methyl ester, 1-methyl butyl ester, and resorcinol(10) and roots of Cassia auriculata Linn contain anthraquinone glycosides and flavone glycosides (11). Cassia auriculata linn shrub parts contain a variety of chemical constituents that are used to treat a variety of diseases, including diabetes, hyperlipidemia (12-14), hepatoprotective (15-17), cancer (18), inflammation, analgesic (19-21), anthelmintic (22), immunomodulatory (23), ulcer (24), nephroprotective (25), arthritis (26) and anthelmintic activity (27). The purpose of this study was to utilize an acute toxicity test to assess the LD50, or median fatal dose, of an ethanol extract of Cassia auriculata Linn entire plant in Swiss albino mice.

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MATERIALS AND METHODS

Collection of plants;

The whole Plant of *Cassia auriculata* linn were collected from Vadakkankulam, Perungudi areas of Tirunelveli dist, Tamilnadu, India. The identification of the plant was confirmed by Dr M.U. Sharief, Scientist 'E' & Head of Office, Botanical survey of India, Southern Regional Centre, Coimbatore (BSI/SRC/5/23/2020/Tech/708)

Preparation of Crude extract:

Freshly collected whole plant *Cassia auriculata linn* were dried in shade place and 25 g was weighed. Dried plant was grounded into powder and mixed with 80% ethanol in round bottom flasks. Flasks were separately attached with soxhlet apparatus and extracted at the temperature 60°C for 4 hours. After extraction collect the supernatant and allow evaporating to obtain concentrated extract.

Approval from Animals ethics committee

The study was performed after getting approval from Animals Ethics Committee of Arulmigu Kalasalingam College of Pharmacy, Ref. No 509/PO/Re/01/CPCSEA

Acute toxicity Assay

An acute oral toxicity test was conducted to determine the LD50 value of EECA4 (Cassia Auriculuta). The experiment was carried out on Swiss albino mice (20-25 g in body weight). Each group of three animals was used independently. The animals were given free access to a conventional pellet meal (Sai Enterprises Pvt. Ltd, Chennai, India) and unlimited water. The samples were kept in a controlled laboratory environment with a 12-hour dark/light cycle, temperatures of 22 ± 2°C, and humidity of 50 ± 5% per CPCSEA recommendations. The EECA4 were given separately, orally to mice. The animals were closely monitored for three days and then observed for up to 14 days to determine delayed mortality. Any hazardous symptoms, including mortality and morbidity in the animals, were noted. Animals were fasted prior to treatment. The meal was removed overnight, and the water was removed three hours before the medicine was administered. It was a step-by-step approach in which the animals were given 5 mg/kg and then escalated to 50, 300, and 2000 mg/kg p.o. body weight. The mortality of the animals dosed in one phase determined the subsequent step. Animals were monitored for behavioural changes, toxic symptoms, and mortality for up to three days. Observations were carried out over a 14-day period to determine any delayed mortality. If the animal survived, the second group of animals received a higher dose. If the first animal died or appeared to be moribund, the second animal received a reduced dose (OECD guidelines-423). The identical approach was used for the control group animals. The control group received only the vehicle (1% of Tween 80). After killing mice by cervical dislocation, vital organs and livers were removed; organ weights were recorded and kept in 10% formalin for histological examination. Finally, behavioral patterns were observed and documented.

Behavioral pattern Study:

In the acute toxicity trial, EECA was delivered to mice orally at a dose of 2000mg/kg. Gross behavior activities such as respiration, tremor, convulsions, hind limb paralysis, sense of touch and sound, salivation, urination, diarrhea, and death were seen.

Histopathological study:

At the end of the investigation, mice were sacrificed via cervical decapitation with Xylazine and Ketamine (16 + 100 mg/kg i.m.), and the liver was excised for histological analysis (Sini Sadasivan et al., 2006) (28). The separated liver was sliced into 5 mm pieces, preserved in a neutral formalin (10%) solution for 3 days, then rinsed under running water for around 12 hours. This was followed by 12 hours of dehydration with progressively stronger alcohols (70%, 80%, and 90%). Absolute alcohol was used for final dehydration, with approximately three changes at 12-minute intervals. Cleaning was done by using xylene with changes at 15 to 20 minute intervals. After cleaning, the pieces were infiltrated with paraffin in an automatic tissue processing device. The pieces were thoroughly rinsed with running water to remove all formalin. The tissue was then immersed in fixative, which promptly killed it and blocked enzymatic digestion, retaining the usual structure. Another goal of repairing the tissues was to harden them. For the fixative, formalin was utilized. 10% formalin was the most commonly used solution for wet tissue storage. Following the application of the primary fixative (formalin), the surplus fixative was removed by gently rinsing the tissue in water before immersing it in 70% alcohol.

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Tissues were processed using the paraffin and wax procedure. Water was removed from fixed tissues using the dehydration agent ethyl alcohol. Xylene was utilized as the cleansing agent. It was miscible with alcohol and paraffin wax. As a result, it aids in the replacement or clearing of alcohol to make room for paraffin during infiltration and impregnation processes.

After cleaning, tissues were transferred to an embedding oven to be infiltrated and impregnated with molten paraffin wax. During this combination process, the clearing agent (xylene) was removed from the tissues by diffusing into the surrounding melted wax (infiltration), and the wax then diffused into the tissue to replace the clearing agent (impregnation). This was carried out in the paraffin oven (50–56°C) for 2-3 h.

The infiltrated - impregnated tissue was placed in warm liquid paraffin (embedding media), which solidified into a hard block when cooled to room temperature. It was also known as casting or blocking. The method of embedding allowed the tissue to be sliced with a microtome. Paraffin wax with a higher melting point (56°C-58°C) was employed for routine embedding. The implanted tissues were prepared for section cutting. The microtome was used to cut thin sections of organs with uniform thickness for microscopic investigation of their interior structure. Tissues immersed in paraffin were sliced with a rotary microtome.

The chopped portions were floated on warm water in a shallow water bath maintained at a temperature of around 460°C (100°C below wax melting point). Microscope slides were prepared and coated with an adhesive to keep the slice securely attached to the slide. A well-known adhesive, Mayer's glycerol-albumin combination, was used. I drained the surplus water from the slide. The slide was thoroughly dried in the oven. Warm slides were allowed to cool to room temperature before being deparaffinized.The slides were immersed in xylene for 5 minutes, followed by an additional 5 minutes in another xylene bath. Decreasing grades of alcohol (absolute, 90%, 80%, and 70%) were fed through them for 30 to 60 seconds each for the hydration process before being rinsed in distilled water. The slides were stained with Harris haematoxylin for 5 minutes before being rinsed under running tap water. Then it was swiftly dipped in 0.5% HCl and briefly rinsed in water (40-60 seconds). The slides were repeatedly dipped in weak ammonia water, cleaned with water, and rinsed with 95% alcohol. The slides were agitated with eosin staining solution for 10-60 seconds. The stain was drained before proceeding to the next procedure. It was cleaned with 70% alcohol (30 to 60 seconds), 95% alcohol (30 to 60 seconds), absolute alcohol 23 times (30 to 60 seconds each), and xylene twice (30 to 60 seconds each). The surplus xylene was allowed to drain and then mounted on DPX with a cover slip. The piece was then placed in diphenyl xylene. The prepared slides were examined under a light microscope for histological features, and images were taken. The slides were examined under a light microscope to obtain enlarged images of tissue structure for future investigation (Uzma Saleema et al. 2017) 29.

RESULTS AND DISCUSSION

Behavioral pattern Study:

When EECA was administered individually at a dose of 2000mg/kg, there was no morbidity or mortality as compared to the control. Table 1 shows the findings of an acute toxicity investigation in mice treated with the control group. The LD50 values of EECA were determined to be 2000mg/kg/oral based on gross behavioral experiments in mice. Even 72 hours after administration, there was no change in breathing or writhing reaction. This reaction demonstrated that EECA had no effect on neurohumoral transmission. This amount does not cause tremors even after 72 hours, and the EECA does not alter the dopaminergic level, basal ganglia, or cholinergic receptors, demonstrating the extracts' safety in mice. Animal poisons and their release may change GABA inhibitory and glutamate excitatory actions at the postsynaptic level, resulting in convulsion. This acute toxicity dosage level does not cause convulsions. The dose did not result in any paralysis. It was found that EECA does not affect CNS stimulation or the NMDA receptor. Some extracts and anesthetic medicines affect sound and touch by acting on the ascending reticular activating system. The acute toxicity dose of EECA did not change sound, touch, or sense, demonstrating the extracts' safety. The does not cause diarrhoea, showing that there was no significant change in gastrointestinal motility, and the animals remained alive until the end of the trial, just like the control group mice. The work was supported by the earlier author work (Qin et al.,2009 30; Fraschini et al.,2002 31). Table 2 displays the findings from the acute toxicity investigation. There was no moribundity or mortality up to the treatment level of 2000mg/kg p.o., and the animals survived until the end of the trial.

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Table 1: Results of acute toxicity in mice treated with control group

Observation	Effect					
Gross behavior activity	1h	4 h	24 h	48 h	7 days	14days
Respiration	N	N	N	N	N	N
Writhing	-	-	-	-	-	-
Tremor	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-
Sense of touch and sound	N	N	N	N	N	N
Salivation	N	N	N	N	N	N
Urination	N	N	N	N	N	N
Diarrhoea	-	-	-	-	-	-
Mortality	-	-	-	-	-	-

(-) No Effect (N) Normal effect

Table 2: Results of acute toxicity in mice treated with EECA

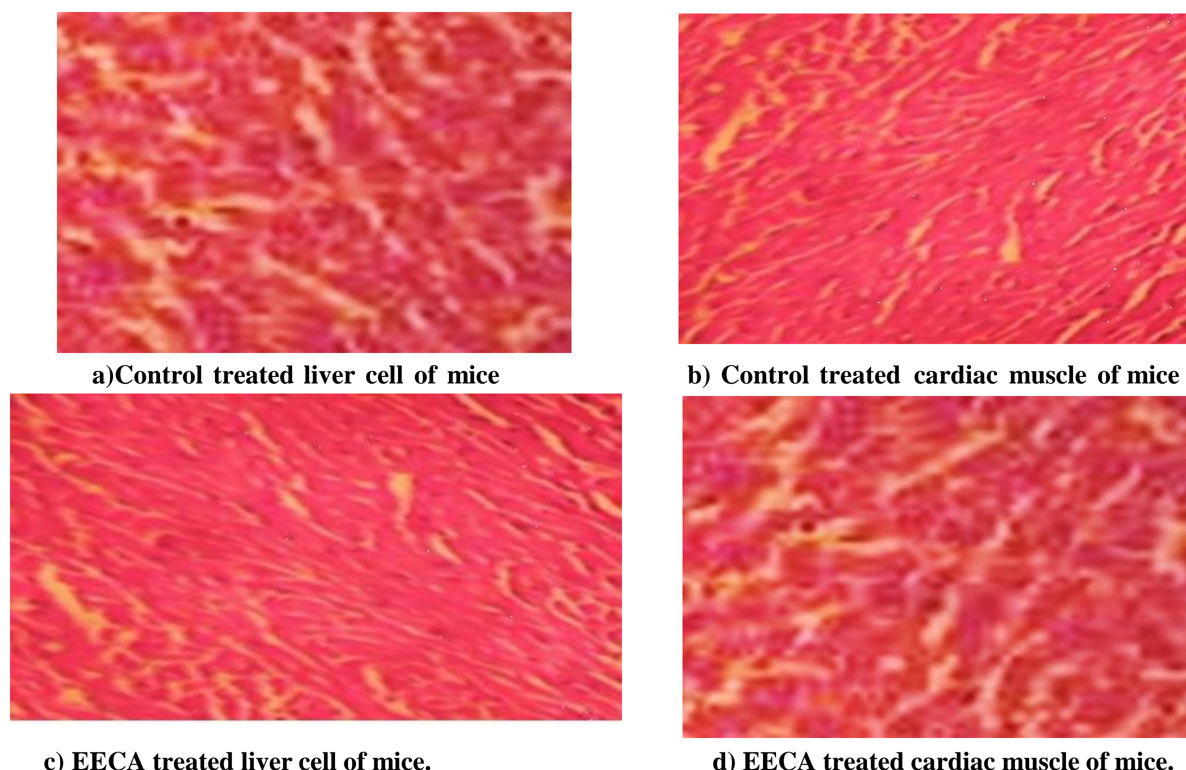
Observation	Effect					
Gross behavior activity	1h	4 h	24 h	48 h	7 days	14days
Respiration	N	N	N	N	N	N
Writhing	-	-	-	-	-	-
Tremor	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-
Sense of touch and sound	N	N	N	N	N	N
Salivation	N	N	N	N	N	N
Urination	N	N	N	N	N	N
Diarrhoea	-	-	-	-	-	-
Mortality	-	-	-	-	-	-

(-)No Effect (N)Normal effect

Histopathological study

The histological slides of liver slices of the mice treated with 2000 mg/kg/oral of EECA- Fig: 1(a) demonstrated hepatic cells with normal sinusoidal space and central vein with normal architecture when compared to the control Fig: 1(c) The investigation also found no abnormalities, liver cell degeneration, necrosis, sinusoidal haemorrhages, or dilatations. This study confirms the safety of EECA alone, on the normal cellular architecture of the liver. The histological slides of the cardiac muscles of mice treated with 2000 mg/kg/oral of EECA-Fig: 1(b) revealed normal architecture, the lack of nuclear fatty infiltration, oedema, inflammatory cells, and normality. Muscle fiber fragmentation as compared to the control animals (Fig. 1(d). The study supports the safety of an acute toxicity dose (2000mg/kg/oral) of EECA on normal cellular architecture of cardiac cells.

The LD50 value for EECA was determined to be 2000/mg/kg/body weight. When compared to control group animals, there was no visual difference in gross behaviour impacts. Histopathological examination of the slides of cardiac muscles and liver from animals given with 2000 mg/kg/oral of EECA, respectively, revealed normal architecture, confirming the safety of LD50 dosages.



a)Control treated liver cell of mice **b) Control treated cardiac muscle of mice**

c) EECA treated liver cell of mice. **d) EECA treated cardiac muscle of mice.**

Figure 1: Histopathological results of EECA and Control in mice liver, Cardiac cell (2000 mg/kg/oral)

CONCLUSION

Based on the results of acute toxicity testing, it was determined that the ethanolic extract of Cassia auriculata linn entire plant does have toxic effects since it stimulates behavioral pattern research and histopathological characteristics. Nonetheless, preliminary findings indicated that it should be further estimated for long-term use and repeated dose effects to ensure the herb's safety.

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