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Original Research Article

Acute Dermal Toxicity Study of *Acacia concinna* Pods Extract in Wistar Rats

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ABSTRACT

Acacia concinna is abundantly available in southern Asian rainforests. The fruits of this plant were employed as cleansing agents in various herbal shampoos and used as an expectorant in traditional treatment. The pods of this plant contain acacic acid based saponins. The plant holds the surfactant property due to the presence of saponins it is ideal to use in skin and transdermal preparations. However, details of the dermal toxicity of *Acacia concinna* pods are still not reported. The aim of this study was to investigate the *in vivo* acute dermal toxicity of *Acacia concinna* pods extract at a dose of 2,000 mg/kg body weight in Wistar rats. According to OECD Guidelines 402 for acute toxicity protocols, the acute dermal toxicity of *Acacia concinna* pods extract was examined in rats. To determine the median lethal dose (LD_{50}) of the extract, the body weight, likelihood of death, general indications, and behavior activity measures were monitored over the course of 14 days. All the animals in the treatment group were euthanized at the end of the study. The LD_{50} was found to be >2,000 mg/kg body weight. There was a significant weight increase ($p<0.05$). No mortality was recorded in 14 days study period. A single dose of 2000 mg/kg of body weight didn't produce any toxic signs in the study animals. A single dermal dose of *Acacia concinna* pods extract had no toxic effects like mortality, clinical signs, body weight changes, and gross findings in female rats at a dose of 2000 mg/kg of body weight. Subsequently, the preparation can be used as a natural surfactant or excipient in pharmaceutical dosage forms.

Keywords: *Acacia concinna*, Single dose, Extract, Biochemical, OECD, Acute toxicity .

Introduction

It is known that drug and excipients from the plant source are safe. However, sometimes it might not be true because few plants are highly toxic and poisonous. Any chemical substance at a higher dose can be toxic. Hence all excipients and drugs must be evaluated from natural and synthetic sources. The toxicity studies not only reveal the possible toxic effects but, also safe concentration. *Acacia Concinna* is abundantly available in southern Asian rainforests. The fruits of this plant were employed as cleansing agent in various herbal shampoos and used as an expectorant in traditional treatment. The pods of this plant contain acacic acid based saponins. As this plant possess the surfactant property due to the presence of saponins, it is ideal to use in skin and transdermal preparations. Sometimes few natural agents can be toxic and allergic when applied on to the skin. Therefore, the present study aimed to carry out acute dermal toxicity of administered single dose of 2000 mg/kg on rats according to OECD 402 guidelines.[1]

Material and Methods

Plant material
The pods of *Acacia Concinna* were collected from the forest area of Warangal, Telangana during the month of December to January and authenticated by Dr. K. Madhava Chetty, Associate Professor, Department of Botany, Sri Venkateshwara University Tirupathi (Voucher Number 0339, respectively).

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The pods of the plant were dark brown in colour with longitudinal structures[9], and the fruits were washed with distilled water to make that free from dirt and dried using a tray drier (SISCO scientific instruments, Mumbai India) at 60^0C for 2 days. After making pericarp seedless it was ground and sieved to make a solution. The dried fruit powder was added to six folds of water to amount of *Acacia Concinna* powder and stirred for 8 hours at room temperature and underwent ultrasonic bath sonication for 5mins for solubilization of material, solvent was evaporated by Rota evaporation technique. The obtained crude extract was lyophilized and stored in airtight container. [2-4]

Test Animals (AKRGCP/Pheuc/2018-2)
The Experiment was carried out in A.K.R.G College of Pharmacy, Nallajerla 534-112, and East Godavari district. A.P, and approved by IAEC viz., Ref. No. AKRGCP/Pheuc/2018-2. The studies were conducted on test animals (female Wistar rats) according to CPCSEA guidelines. All the animals were kept under standard conditions like 12h light and dark at a temperature of 25± 5ºC, and humidity during the entire study period. Water and standard diet pellets are used as food for the animals.

Acute dermal toxicity
The animals were randomly allocated and kept in standard environmental conditions for three days prior to the study, as per OECD 402 guidelines. To carry out the study the rats were chosen on random basis and divided into two groups according to study protocol. Each group has six rats (n = 6). One of the groups was administered 2000 mg/kg extract (applied topically) and the other group served as the control. The prepared extract (natural surfactant) was thoroughly blended with paraffin and applied at the appropriate place gently without friction .[1, 5]

Skin preparation
According to the protocol general anesthesia was given to study the skin toxicity. After administration of anesthesia with ketamine 50 mg/Kg and xylazine injection of 5 mg/kg. The fur was removed on the dorso

thoracic part with a razor blade. The shaved portion was covered with NS and paraffin gauze.[1, 5-7]

Treatment
The dorsal surface of the rats was shaved, then clipped closely to apply *Acacia concinna* extract (natural surfactant) prior day to start the trial, and *Acacia concinna* was applied as evenly as possible and wrapped to keep in contact for a 24 h exposure period using nonporous gauze bandage along with non-irritating tape to retain the gauze on the test site and wrapped properly. Then animals were immediately observed after dosing for the first 30 minutes, frequently for the first 24 h. Care was taken especially for the first 2-6 h after exposure every day for 14 days. At the end of the experiment, all the rats were euthanized by using the CO_2 inhalation method. Complete gross examination was conducted to find any indication on skin and blood samples were collected from the posterior vena cava of each necropsied rat and preserved in EDTA tubes for hematology studies. By using Hettichzent –EBA20 Germany centrifuge at 5000 rpm for 5min to separate serum components then allowed to clot for a minimum of 30 minutes and samples were stored at -3^{0}C in the freezer. Further analysis followed by excision of kidneys, liver, and weight of both organs were noted both organs and shaved skin was preserved in 10%buffered formalin solution until further analysis.[9-11]

Hematological and serum biochemical analysis
Using a Cell-Dyn®, 3700, Abbot, USA, automatic hematology analyzer, a hematology analysis was performed. The analyzer analyzed the red blood cells (RBC), white blood cells (WBC), platelet, and hemoglobin concentrations. While the values for plasma protein and packed cell volume (PCV) were manually determined using standard methods. Wright's stain was used manually to determine the proportion of each WBC type in the blood smear, and the percentage value was multiplied by the total number of WBCs to calculate the absolute number of each WBC type. A completely automated biochemistry analyzer was used for the biochemical analysis (TRX 7070, Biorex, Germany). Urea, creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), creatine kinase (CK), total protein, and albumin are a few of the parameters that were chosen. By subtracting the concentration albumin concentration from the total protein, the globulin concentration was estimated .[10, 11]

Statistical analysis
The results was expressed as the mean ± S.E.M and analyzed by one-way ANOVA followed by Dunnetts's test. Values at $p<0.05$ were considered statistically significant. Data was computed for statistical analysis by using graph pad prism 5 Software

Results and Discussion

All rats were inspected after 24 hours upon dermal application of NS and showed no erythema or edema. Moreover, rats looked healthy, didn't feel pain to touch, were normal in motion, and ate and drank normally. There was death in neither groups nor significant mean weight difference between the treated animals. After 48 hours the rats still looked good and were not struggling when handled. There were no signs of erythema, or edema at the site of application. In addition, the absolute organ weight was found within the range. No remarkable alterations were noticed in the biochemical analysis.

Behavior of study animals
Changes in typical behavior are the main indicators of toxicity. The way a poisonous drug affects salivation, sleep, and other such factors is a sign of toxicity. Observation of such kind of parameters is presented in Table1.
There were no signs of change in their behavior from 0th day to14th day of acute toxicity study.

Organ and body weight
Any toxic compound or adverse reaction of a drug can cause alterations in body weight. The body weight of the rats was noted on 1st, 2^{nd,} and 14th day of the experiment. All of the animals had normal body weights, and no minor changes were seen throughout the study period. The control and the test group's body weights were within the normal range, and no significant variations were found in Figures 1A &1B.
After 14 days of observation, the animals were sacrificed and their vital organs weighed. The liver, kidney, lungs, and heart weights were recorded. When organs' weights fluctuate as a result of toxicity, the organs' functions may change. There was no significant change noted (Figure 2).

Biochemical Parameters
Experimental animal group serum samples were tested for various parameters like serum creatinine, serum urea, potassium, and sodium. The findings indicated that there were no alterations reported from the treated extract group at 2000 mg/kg (Figure 3).
It is desired for a substance to have therapeutic effects at low doses and few undesirable side effects and harmful effects on people. The findings of this study stated that *Acacia concinna* extract had been studied for unknown toxic effects in rats at a dosage of 2000mg/kg body weight. The extract has various applications in the pharmaceutical and cosmetics field. Therefore, this study may contribute to the significance of safe application of *Acacia concinna* onto the skin. Additionally, organ weight is a crucial indicator of physiological and obsessive state. The relative organ weight is crucial when determining whether an organ has been damaged. The body weight of rats in the treated group revealed a significant increase in body weight during the study period.

Table 1: Behaviour of study animals

Parameter	Control group		Treatment group	
	Group 1 6 hrs.	Group 2 14 hrs.	Group2 6 hrs.	Group 2 14 hrs.
Skin and Fur	N	N	N	N
Eyes	N	N	N	N
Mucous Membrane	N	N	N	N
Behavioral pattern	NC	NC	NC	NC
Salivation	NC	NC	NC	NC
Lethargy	NC	NC	NC	NC
Sleep	NC	NC	NC	NC
Diarrhea	NF	NF	NF	NF
Coma	NF	NF	NF	NF
Tremors	Nil	Nil	Nil	Nil

N= Normal; NC= No change; NF= Not Found

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Skin serves as a barrier, keeping harmful substances and bacteria outside and bodily fluids inside. The cornerstone of dermatology treatment, topical medications, are given with the goal and expectation that minimal systemic side effects will result from any percutaneous absorption. Nearly the majority of the skin's barrier characteristics are provided by the stratum corneum, the epidermis' outermost layers. Most medication absorption happens transcellular; significant absorption through sweat pores or hair follicles is uncommon. It is a passive diffusion process, and the strength will rely on the strength and effectiveness of epidermal barrier, as well as how the medication affects it.

Conclusion

The findings revealed that the aqueous extract of *Acacia concinna* pods has no toxic effects in Wistar rats at 2000 mg/kg body weight. Hence this can be employed as a surfactant in any topical formulation. Acacia *concinna* pods might be used as a surfactant in oral suspensions after conducting an oral toxicity study.

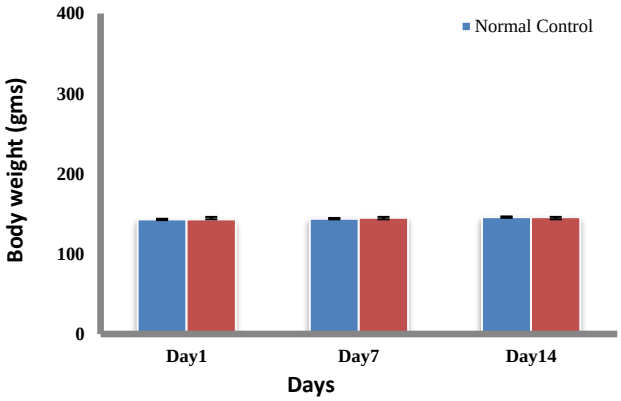

Figure 1A: Effect of body weight in days

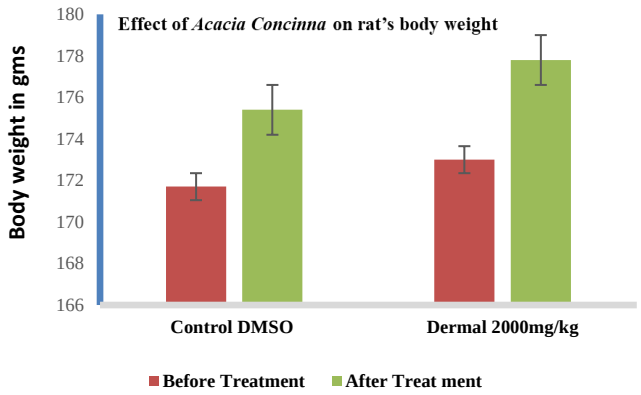

Figure 1B: Effect of extracts on animal's body weight

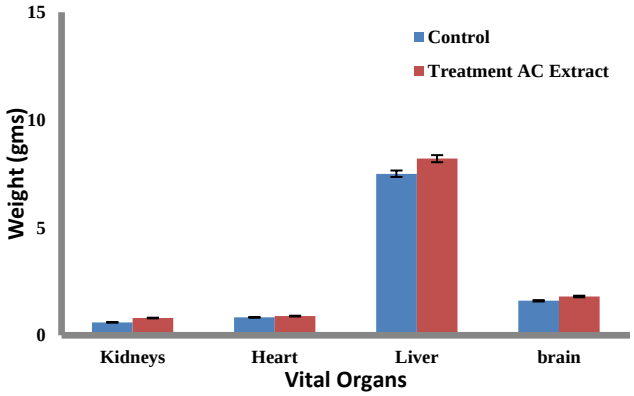

Figure 2: Weight of organs in grams

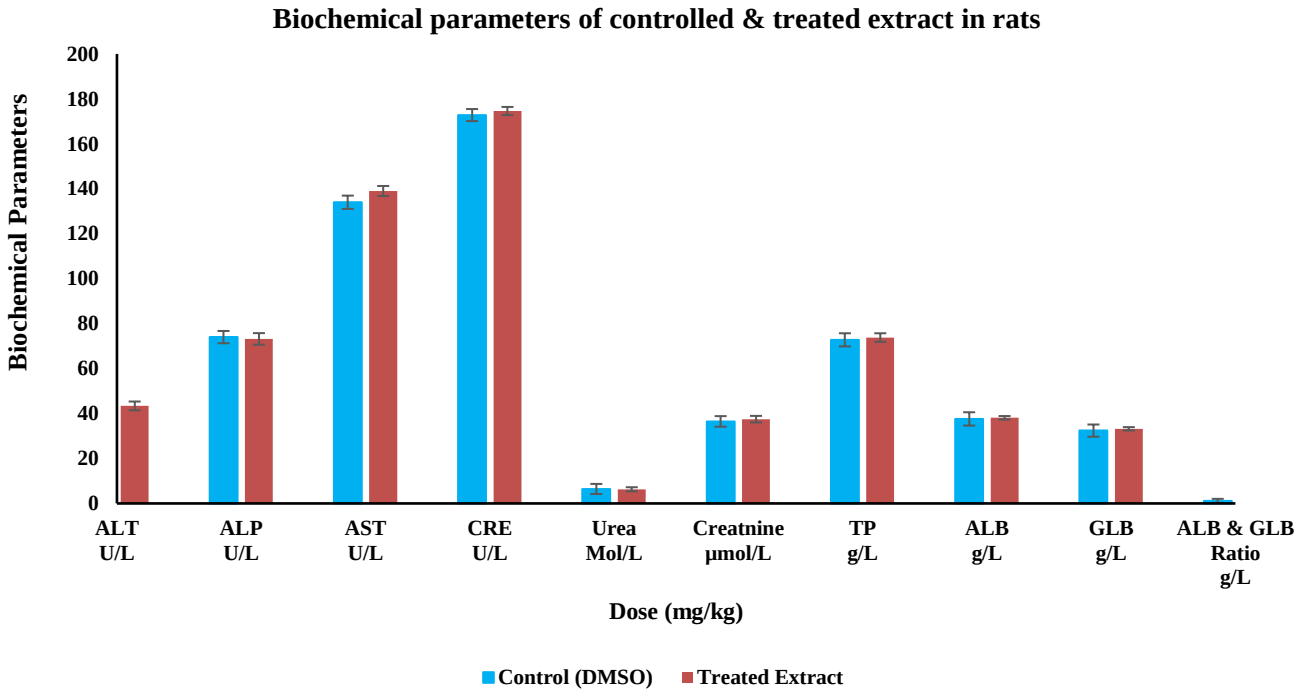

Figure 3: Biochemical Parameters

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. OECD. Acute Demal Toxicity 402. Paris: Organisation for Economic Co-operation and Development; 1987. p. 1-7.
2. Khanpara K, Renuka V, Harisha C. A detailed investigation on shikakai (*Acacia concinna* Linn.) fruit. J. Curr. Pharm. Res. 2012; 9:6-10.
3. Muhammad MT, Khan MN. Eco-friendly, biodegradable natural surfactant (*Acacia Concinna*): An alternative to the synthetic surfactants. J. Clean. Prod. 2018 Jul 1; 188:678-85.
4. Purwoningsih E, Arozal W, Lee HJ, Munim A. Neuroprotective and antioxidant activities of aqueous extract *moringa oleifera* leaves. Int. J. Appl. Pharm. 2022 Dec; 14(Special Issue 5):127-31.
5. Sari LM, Suyatna FD, Subita GP, Auerkari EI. Acute dermal toxicity study of *areca catechu* linn. Extract in sprague-dawley rats. Asian J. Pharm. Clin. Res. 2016 Dec 1; 9:209.
6. Sari DK, Sari LM, Heryanto R. Oral and Acute Dermal Toxicity with *Passiflora edulis Sims* Aqueous Extract in Sprague-Dawley Rats: doi. org/10.26538/tjnpr/v4i10. 6. Trop. J. Nat. Prod. Res. 2020 Oct 1; 4(10):691-4.
7. Reduan FH, Shaari RM, Sayuti NS, Mustapha NM, Abu Bakar MZ, Sithambaram S, Hamzah H. Acute and subacute dermal toxicity of ethanolic extract of Melastomamalabathricum leaves in Sprague-Dawley rats. Toxicological Research. 2020 Jul; 36:203-10.
8. Ahmed R, Nuhu HD, Ibrahim H, Nuhu A, Maje IM. Toxicological Assessment of Aqueous and Methanol Leaves Extracts of *Scoparia dulcis* Linn (Plantaginaceae) in Wistar Ra: doi. org/10.26538/tjnpr/v3i3. 1. Trop. J. Nat. Prod. Res. (3):64-70.
9. Shaikh IA, Muddapur UM, Bagewadi ZK, Chiniwal S, Ghoneim MM, Mahnashi MH, Alsaikhan F, Yaraguppi D, Niyonzima FN, More SS, Mannasaheb BA. Characterization of bioactive compounds from *Acacia concinna* and Citrus limon, silver nanoparticles' production by *A. concinna* extract, and their biological properties. Molecules. 2022 Apr 22; 27(9):2715.
10. Adeneye AA, Olagunju JA. Preliminary hypoglycemic and hypolipidemic activities of the aqueous seed extract of *Carica papaya* Linn in Wistar rats. Biol Med. 2009;1(1):1-0. Fazliana MS, Muhajir H, Hazilawati H, Shafii K, Mazleha M. Effects of *Ficus deltoidea* aqueous extract on hematological and biochemical parameters in rats. Med. J. Malaysia. 2008 Jul 1; 63:103-4.