Research Article

ACUTE ORAL TOXICITY, DERMAL IRRITATION AND EYE IRRITATION STUDY OF *ECLIPTA ALBA* AQUEOUS EXTRACT IN SPRAGUE DAWLEY RATS AND NEWZEALAND WHITE RABBITS

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ABSTRACT

Eclipta alba is used as a medicinal herb in many herbal preparations including most of hair oils produced in India. The *E. alba* extracts are part of many Ayurvedic/herbal medicines with hepatoprotective, anti-diabetic, anti-inflammatory and anti-microbial properties. Aqueous extract, hydroalcoholic extracts and methanolic extract are reported to possess hepatoprotective, anti-diabetic, anti-inflammatory and anti-microbial properties. Studies in Burkina Faso have demonstrated that soaking seeds with 2.5% *E. alba* aqueous extract for 6 h improved seed quality parameters followed by increased yield in sorghum. Accordingly, it has been proposed to use *E. alba* for seed treatment on a larger scale. Hence it was relevant to test the toxicity of *E. alba*. The purpose of this study was to test the acute oral toxicity, dermal irritation and eye irritation of aqueous extract of *E. alba* dried leaves in Sprague Dawley rats and New Zealand white rabbits. The acute toxicity studies were carried out based on OECD guidelines 423. The highest dose administered at 2000 mg/kg body weight did not produce mortality or changes in general behaviour of the test animals indicating safety of the oral administration of aqueous *E. alba* extract in Sprague Dawley rats. The acute dermal irritation study in New Zealand white rabbits was investigated according to OECD test guideline No. 404. The *E. alba* fine powder applied to the intact left flank of female rabbit did not elicit any skin reactions at the application site of animal at any of the observation time points and hence 'Non Irritant' to the rabbit skin. The acute eye irritation study on New Zealand white rabbits did not cause corneal opacity, iris and conjunctivae in any of the treated animals and did not cause staining of the treated eye and is termed as 'not irritating' to the rabbit eyes / eye mucosa. The toxicological studies prove that the *E. alba* aqueous extract are safe to be used as seed treatment and can be handled safely by humans under field conditions.

KEYWORDS: *Eclipta alba*, OECD, toxicity, seed treatment, acute oral toxicity, derma irritation, eye irritation

INTRODUCTION

Eclipta alba Hassk. (Family: *Asteraceae*) is an erect or prostrate, much branched, strigosely hirsute, annual herb, often rooting at nodes. Leaves are opposite, sessile, oblong-lanceolate, 2.5-7.5 cm. long with white appressed hairs. Floral heads are 6-8 mm in diameter, solitary and white. Fruit is an achene, compressed and narrowly winged. This plant is widely distributed in the warm humid tropics with plenty of rainfall. It grows commonly in moist places as a weed all over plains of India, ascending up to 6,000 ft on the hills. *E. alba* is commonly known as 'safed bhangra' (Hindi) when in flower and as 'kala bhangra' when in fruit. 'Pila bhangra' is the name given to the closely related plant, *Wedelia chinensis* Merrill (syn. *W. calendulacea* Less.), which is used to some extent, vicariously for *E. alba*.

The plant contains broad array of active principles which includes coumestans, alkaloids, flavonoids, glycosides, polyacetylenes, and triterpenoids. The leaves contain stigmastanol, β -terthienylmethanol, wedelolactone, demethylwedelolactone and demethylwedelolactone-7-glucoside¹, furanocoumarins, oleanane and taraxastane glycosides². The aerial parts of the plant contain a phytosterol, β -amyirin in the n-hexane extract and luteolin-7-glucoside, β -glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone in polar solvent extract, roots contain

polyacetylene substituted thiophenes³. The major coumestan isolated from *E. alba* has been wedelolactone⁴.

The antimicrobial and beneficial pharmacological effects of aqueous and solvent extracts of *E. alba* as a herbal medicine have been reviewed recently^{5,6,7,8}. Studies in Burkina Faso by Zida *et al.*⁹ reported seed treatment of sorghum with *E. alba* aqueous extract clearly reduced seed infection by *Phoma* spp. followed by positive impact on sorghum grain yield. Seed health testing and field trials conducted in Burkina Faso to evaluate the effects of seed treatment with aqueous extracts of *Eclipta alba* and a binary pesticide Calthio C 50 WS on seed-borne fungi, seedling emergence, and yield of sorghum revealed a stimulatory effect on the seedling emergence and yield increase¹⁰. Even a shortened soaking time of seeds (6 h) and a reduced concentration of *E. alba* was effective in improving growth parameters¹¹. A detailed analysis of 118 field experiments conducted in Burkina Faso in different location resulted in increased yield in comparison to control¹².

In continuation of studies on beneficial effects of *E. alba* seed treatments, one of the major parameters to be considered for seed treatment is whether the aqueous extract used can cause any toxic effects on humans as most farmers in Burkina Faso and Tanzania use bare hands to treat seeds and to sow in farms. Scientific publications reveal that fine dry *E. alba* powder

consumed in the form of capsules 3 g/day) is diuretic, hypotensive and hypocholesterolemic and helps in alleviating oxidative stress-induced complications in hypertensives. Clinical studies in humans have established *E. alba* as hepatoprotective with no side effects¹³. However pre-clinical animal studies related to acute oral toxicity, dermal irritation and eye irritation study of *Eclipta alba* aqueous extract is not reported in detail so far. Therefore to corroborate the promising results obtained in enhancing seed quality parameters in fields of Burkina Faso, Tanzania and other African nations, the present study was undertaken to evaluate the acute oral toxicity, dermal irritation and eye irritation study of *Eclipta alba* aqueous extract in Sprague Dawley rats and New Zealand white rabbits

MATERIALS AND METHODS

Plant material and source

Eclipta alba is an easily accessible plant and was collected from paddy fields in Mysore, Karnataka, during April 2014. The plant was identified by Dr. K. K. Sampath Kumara, taxonomist. The voucher specimen of the plant is kept in the Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore for future reference with the number DAB/Ea/01/04/2014-15. The root portion was cut off and the plant was washed thoroughly to free from debris. The leaves and shoot portion were shade dried, ground to fine powder in waring blender and stored at room temperature for further use.

Preparation of plant material

The test material was prepared by soaking *E. alba* powder in sterile water at room temperature for 24 h. One hour before administration, mechanical stirring was done. The required quantity of test item was weighed as per the dose. The test item formulations were prepared using sterile containers.

Acute oral toxicity study of *E. alba* aqueous extract in Sprague Dawley rats

Experimental animals

The study was conducted on Sprague-Dawley rats as per the OECD guideline for testing of chemicals, "Acute Oral Toxicity – Acute Toxic Class Method", Test No. 423¹⁴, was inspected and audited by the Quality Assurance Unit at test facility centre. This study was performed at Vipragen Biosciences Private Limited, a CPCSEA approved laboratory under Registration number 1683/RO/c/13/CPCSEA following all ethical practices as laid down in the guidelines for animal care. This study has been approved by the Institutional Animals Ethics Committee (VIP/IAEC/ 27/2015) of the test facility. Experiments were performed using healthy non-pregnant adult female Sprague Dawley rats weighing 180-200 g.

Animals were housed under standard laboratory conditions in air-conditioned environment with adequate fresh air supply (Air changes 12-15 per hour), room temperature $22 \pm 3^\circ\text{C}$, relative humidity 30-70 %, with 12 h light and 12 h dark cycles. The animal husbandry conditions such as temperature and relative humidity were recorded once daily. Maximum of three animals of same group were housed per cage in standard polysulfone (Size: L 370 x B 200 x H 185 mm) with stainless steel mesh top grill having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube. The animals were acclimatized for 5 days to laboratory conditions and were observed for clinical signs daily. Veterinary examination of all the animals was performed on the day of receipt and on day 5 of acclimatization period. The pellet feed was provided *ad libitum* throughout the acclimatization and experimental period. Nutrient rodent feed manufactured by

M/s Rayans Biotechnologies Private Limited Hyderabad, India was provided. Reverse osmosis filtered water was provided *ad libitum* throughout the acclimatization and experimental period. Water was provided in water bottles with stainless steel sipper tubes.

Grouping of animals

Grouping of animals was done based on body weight stratification and randomization. The animals stratified based on body weight were distributed to study groups in equal numbers such that body weight variation of animals used doesn't exceed $\pm 20\%$ of the mean body weight. The grouping was done one day prior to the initiation of test item administration. Body weight of the animals was recorded and statistically analyzed to rule out the statistical significant difference in mean body weight. For sequential step wise testing, three female animals were used for the study.

Dose selection

Based on the toxicity testing OECD 423 guideline specifications, sequential testing procedure was followed to allow selection of the appropriate starting dose for the next level. In this method of testing, the effect of various doses was investigated in sequential manner. A starting dose of 300 mg/kg body weight was selected.

Formulation of test doses

For 300 mg/kg body weight dose, the test item was soaked in 10 ml and for 2000 mg/kg body weight dose, the test item was soaked in 20ml of sterile drinking water. The required quantity of test item was weighed as per the dose. The test item formulations were prepared using sterile containers. Formulations of the test item were prepared freshly, shortly before dosing.

Route of administration

The test item *E. alba* was administered through oral route by gavage. The dose of test item is expressed as mg/kg body weight/day. The test item was administered as a single dose.

Test item administration

The test item was administered to animals by oral gavage as a single dose using oral gavaging tube. The dosage volume administered to individual rat was adjusted according to its body weight recorded on the day of dosing. A dose volume of 2 ml /100 g body weight of animal was maintained.

The animals were administered with 300 mg/kg body weight. Based on the outcome of clinical signs viz., moribund or mortality, further next step-wise sequential testing was conducted at higher 2000 mg/kg bodyweight fixed dose as per OECD 423 guideline for dosing of other set of animals. The animals were observed for a period of 24 h before administering the next sequential dose level. The time interval between dosing at each level was determined by the onset, duration and severity of toxic signs.

Clinical signs, mortality and viability

All the animals were observed for clinical signs of toxicity and mortality at 30 min, 1h, 2 h, 3 h and 4 h on day 1 following administration of test item and thereafter once daily during the 14 day observation period. The 14 days duration of observations was determined by the toxic reactions, rate of onset and length of recovery period. The appearance, change and disappearance of these signs were recorded. Clinical observations were also made on skin, fur, eyes, mucus membranes, autonomic activity, circulatory and central nervous system, somatomotor activity, behavior pattern, tremors, convulsions, salivations, stool

consistency, lethargy, sleep, changes in gait, posture and response to handling.

Body weights

Individual animal body weights was taken and recorded on Day 1 of the study before the test item administration. There after weekly body weights were determined on day 8 and 15, during the study period.

Gross pathology and Necropsy

After the completion of 14 days of observation, all surviving animals were sacrificed humanely by Carbon-di-oxide asphyxiation method in a euthanasia chamber. The external and internal gross pathological changes were recorded.

Statistical analysis

The raw data (body weights) was subjected to One-way ANOVA Test using Graph Pad Prism Version 5.0 computer statistical processing.

Acute dermal irritation/corrosion study of *E. alba* aqueous extract in New Zealand white rabbits

Experimental animals

This study was performed at Vipragen Biosciences Private Limited, a CPCSEA approved laboratory under Registration number 1683/RO/c/13/CPCSEA following all ethical practices as laid down in the guidelines for animal care. This study has been approved by the Institutional Animals Ethics Committee (VIP/IAEC/ 28/2015) of the test facility. Three animals of same group were housed per cage in standard polysulfone individually ventilated cages (Size: L 430 x B 270 x H 150 mm) with stainless steel mesh top grill having facilities for holding pelleted food and drinking water in water bottle fitted with stainless steel sipper tube in autoclaved corn cob bedding.

The pellet feed was provided *ad libitum* throughout the acclimatization and experimental period. Nutrient Rabbit feed manufactured by M/s Rayans Biotechnologies Private Limited, Hyderabad, India was provided. Autoclaved reverse osmosis water was provided *ad libitum* throughout the acclimatization and experimental period. Water was provided in water bottles with stainless steel sipper tubes.

Test item preparation

0.5 g (per animal) test item was moistened with 0.5 ml water and applied to the intact skin. The pH of the test item was measured before the study initiation date. The pH was found to be 7.4

Preparation of test system

Approximately, one day before treatment, both the flanks were clipped using a clipper, exposing an area of approximately 100 cm² (10 cm x 10 cm). Since, all the animals were normal and no skin reactions were observed, all the animals were used in the test.

Treatment

A single female rabbit was treated first. As no severe skin reaction was observed after 4 h exposure, the test was completed using the remaining two rabbits for an exposure period of 4 hours. On the day of treatment, 0.5 g of test item was moistened with 0.5 mL of water and applied to the skin of the animal and covered with a surgical gauze patch (approximately, 4 cm x 4 cm), which was held in place with non-irritating tape. The test item was applied to the treatment site of the clipped area (left flank), while the control site (right flank) was treated with 0.5 mL of water. The patches were covered with a semi-occlusive

dressing. The dressing was wrapped around the abdomen and anchored with tape. The duration of treatment was 4 hours. Then the dressing was removed and the skin was gently cleaned with water.

Clinical signs, mortality and viability

Clinical signs were checked once daily from acclimatization of the animals to the termination of test, mortality and viability were checked twice daily.

Body weights and irritation scores

On the day of acclimatization start, test item application and at termination of observation, the skin reaction was assessed according to the numerical scoring system listed in OECD guidelines No. 404, "Grading of Skin Reactions" (24th April 2002)¹⁵ approximately at 1, 24, 48 and 72 h after the removal of the dressing, gauze patch and test item.

Pathology and necropsy

After completion of the experiment period, all the surviving animals were subjected to following pathological examinations. There was no mortality during the course of the experiment. All animals were humanely sacrificed by carbon dioxide asphyxiation at termination and discarded after the gross/macrosopic pathological changes are observed and recorded. No gross pathological changes was observed, thus no histopathological examination was carried out.

Acute eye irritation/ corrosion study of *E. alba* aqueous extract in New Zealand white rabbits

This study was performed at Vipragen Biosciences Private Limited, a CPCSEA approved laboratory under Registration number 1683/RO/c/13/CPCSEA following all ethical practices as laid down in the guidelines for animal care. This study has been approved by the Institutional Animals Ethics Committee (VIP/IAEC/ 29/2015) of the test facility.

Test item preparation

0.1 g (per animal) test material was administered as such in to the conjunctival sac of the animals eye. The pH of the test item was measured before the study initiation date. The pH was found to be 7.4

Preparation of test system

The eyes of the animals were examined one day prior to the test item administration. Since, all the animals were normal and no eye abnormalities were observed, all the animals were used in the test.

Treatment

A single female rabbit was treated first. As no severe eye reactions were observed after the 48-hour exposure, the test was completed using the remaining two female rabbits for an exposure period of 24 hours. On the day of treatment, 0.1 g of the test item was applied in the conjunctival sac of the left eye of each animal after gently pulling the lower lid away from the eyeball. The lids were then gently held together for about one second to prevent loss of test item. The right eye remained untreated and served as the control. The treated eyes were washed with water at 24 h after instillation.

Clinical signs, mortality and viability

Clinical signs were checked once daily from acclimatization of the animals to the termination of test, mortality and viability were checked twice daily.

Body weights and irritation scores

On the starting day of acclimatization, test item application and

at termination of observation. The skin reaction was assessed according to the numerical scoring system listed in OECD guidelines No. 405, "Grading of Ocular Lesions"¹⁶ approximately at 1, 24, 48 and 72 hours, after the administration of test item.

Pathology and necropsy

After completion of the experiment period, all the surviving animals were subjected to pathological examinations. There was no mortality during the course of the experiment. All animals were humanely sacrificed by carbon dioxide asphyxiation at termination and discarded after the gross/macrosopic pathological changes are observed and recorded. No gross pathological changes were observed, thus no histopathological examination was carried out.

RESULTS

Acute oral toxicity study of *E. alba* aqueous extract in Sprague Dawley rats

The test item *E. alba* extract administration at 300 and 2000 mg/kg body weight did not have any effect on the body weight of the animals during the study period. The individual animal bodyweight and the summary are presented in table 1. The test item *E. alba* extract administration at 300 and 2000 mg/kg body weight did not produce any of the clinical signs of toxicity or mortality of the animals during the study period.

The summarized clinical signs of toxicity and mortality are presented in table 2 and the individual animal clinical signs of toxicity and mortality are presented in table 3. The test item *E. alba* extract administration at 300 and 2000 mg/kg body weight did not produce any of the external or internal gross pathological lesions in the animals. The summarized gross pathological observations are presented in table 2 and the individual animal gross pathological observations are presented in table 3.

The Acute Oral Toxicity – Acute Toxic Class Method (OECD 423) described by OECD test guidelines is not intended to allow the calculation of a precise LD₅₀ value. The test item was classified to one of a series of toxicity classes defined by fixed LD₅₀ cut-off values according to the Globally Harmonised System (GHS) for classification of chemicals which cause acute toxicity (OECD Series on Testing and Assessment, Number 33; Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures; ENV/JM/MONO (2001)6). Based on the trials, it can be concluded from the present study, that *E. alba* extract is classified to GHS Category 5 or Unclassified and the cut-off LD₅₀ value was found to be 5000 mg/kg body weight or ∞ (infinity).

Acute dermal irritation/corrosion study of *E. alba* aqueous extract in New Zealand white rabbits

All the animals appeared normal throughout the acclimatization period. No clinical signs were observed in the animals during the course of experiment and no mortality occurred. The mean score was calculated across 3 scoring times (24, 48 and 72 h, after patch removal) for each animal for erythema/eschar grades and for oedema grades, separately. The individual mean score for erythema or edema for each of female were 0.0. The test item did not reveal any abnormal reactions after 1, 24, 48 and 72 h after exposure (removal of the dressing, gauze patch and test item). No abnormal findings were noticed on the control skin of any animal at 1, 24, 48 and 72 h after treatment and at the end of the observation period. The test item did not produce any staining on the skin of treated rabbits. No corrosive effects were observed on the skin.

The body weights of all the animals were considered to be within the normal range of variability. Since, the outcome of the result did not qualify for any of the classification criteria, *E. alba* aqueous extract is classified as per the Harmonised Integrated Classification System (14th Aug, 2001) as "Non Irritant" to the rabbit skin.

Acute eye irritation/ corrosion study of *E. alba* aqueous extract in Newzealand white rabbits

All the animals appeared normal throughout the acclimatization period. No clinical signs were observed in the animals during the course of experiment and no mortality occurred. The mean score was calculated across 3 scoring time points (24, 48 and 72 h after administration) for each animal for corneal opacity, iris, conjunctivae and chemosis. The individual mean score of opacity, iris and conjunctivae for all test animals were 0.0. The individual mean score of chemosis for animal no. 1, 2 and 3 were 0.00, 0.33 and 0.33 respectively.

The administration of *E. alba* into the eye of the rabbits did not produce any severe irritation or corrosion till 72 h after exposure. Animal #1 showed some swelling of nictating membrane above normal at 1 hour observation and appeared normal at 24, 48 and 72 h observation, whereas animal # 2 and 3 shown some swelling of nictating membrane above normal and obvious swelling respectively and continue to show some swelling of nictating membrane at 24 h observation; animals appeared normal at 48 and 72 h observation. Test item did not cause corneal opacity, iris and conjunctivae in any of the treated animals. The observation was terminated at 72 h since, there were no serious reactions observed in any of the treated animals. The test item did not produce any staining on the eye / eye lid of treated rabbits. No corrosive effects were observed on the skin. The body weight of all the animals were considered to be within the normal range of variability.

Table 1: Summary of body weights (g)

Sequential steps	Dose (mg/kg bwt)	Animal No.	Body weights (g)		
			Days		
			1	7	14
Step-1	300	01-03	190.2 ± 10.0	213.0 ± 7.9	228.9 ± 8.8
Step-2	300	04-06	199.2 ± 10.3	229.6 ± 15.4	227.8 ± 6.2
Step-1	2000	07-09	196.9 ± 9.3	216.6 ± 4.8	226.8 ± 7.4
Step-2	2000	10-12	215.0 ± 9.1	241.9 ± 13.5	244.4 ± 13.1

n=3; Values are in Mean ± Standard deviation

Table 2: Summary of clinical signs and mortality data

Sequential steps	Dose (mg/kg bwt)	Animal No.	Clinical signs	Mortality
			Days (1-15)	Days (1-15)
Step-1	300	01-03	1	0
Step-2	300	04-06	1	0
Step-1	2000	07-09	1	0
Step-2	2000	10-12	1	0

1= Normal

Table 3: Summary of gross pathology observations

Sequential steps	Dose (mg/kg bwt)	Animal No.	Necroscopic findings	
			External	Internal
Step-1	300	01-03	NAD	NAD
Step-2	300	04-06	NAD	NAD
Step-1	2000	07-09	NAD	NAD
Step-2	2000	10-12	NAD	NAD

NAD – No abnormalities detected

Table 4: Individual animal clinical signs and mortality observation data

Sequential steps	Dose (mg/kg bwt)	Animal No.	Clinical signs														
			Days														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Step-1	300	01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		02	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		03	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Step-2	300	04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		05	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		06	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Step-1	2000	07	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		08	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		09	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Step-2	2000	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

1=Normal

Sequential steps	Dose (mg/kg bwt)	Animal No.	Mortality														
			Days														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Step-1	300	01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Step-2	300	04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Step-1	2000	07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Step-2	2000	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

DISCUSSION

The whole world is dependent on food through agriculture. The use of fungicide and insecticide is inevitable to produce large quantity of food crops worldwide. Fungicide and insecticide are not only expensive but the damage caused on environment is irreversible. Resource poor farmers with small holdings of land find it expensive and suffer due to side effects on improper handling of fungicide and insecticide worldwide. Alternatives such as bio-control agents, bio-fertilizers and plant products are successful, if handled properly and at right time of cropping. Botanicals are the best source, as they are eco-friendly and mainly they can be directly handled by farmers if they can identify the plants which are grown locally.

Eclipta alba is a well known plant of medicinal importance and reported for hepato-protective activity², Anti-tumour activity¹⁷, Anti-depressant¹⁸ and antimicrobial activity¹⁹. The non-toxic nature of aqueous *E. alba* extract is evident by the absence of mortality of test animals at oral treatment of 2000 mg/kg body weight of rats, no effect on skin and eye of treated rabbits.

The acute toxicity studies were carried out based on OECD guidelines 423 and fixed dosage studies was adopted where the limit dose is 2000 mg/kg body weight of test animal. This dose did not produce mortality or changes in general behaviour of the test animals. These results indicate the safety of the oral administration of aqueous extract *E. alba*. The same low level or absence of acute toxicity was reported for ethanolic extracts of freshly harvested leaves of *E. alba* in mice²⁰.

In similar studies Jayashree *et al.*²¹ classified methanolic leaf extract of *Ficus virens* (White fig, fruits are edible) in Wistar albino rats as category 5 as the study did not reveal any clinical signs of toxicity and mortality in acute and repeated dose toxicity study at given dose and duration indicating non-toxic nature of the methanolic leaf extract. The non-toxic nature of *Eichhornia crassipes* (water hyacinth, leaves are edible) aqueous, ethyl acetate extract and methanol fractionate was evident from the acute oral toxicity from normal behavior of the test animals during a period of 14 days and could be safe up to the dose of 2000 mg/kg body weight of the animal²².

The safe dosage limit depends on clinical signs of toxicity in the studies. The ethanolic extract of *Momordica charantia* (bitter melon, medicinal plant) was investigated to be safe consumed below 2000 mg/kg. However highest dosage could provoke the toxic effects to the blood, tissue and vital organ especially liver²³. Methanolic extract of *Tridax procumbens* (tridal daisy, medicinal plant) exhibited toxic effects when given orally at concentration of 2000 mg/kg body weight. However the normalcy and insignificant changes in wellness parameters and body weights revealed the safety of methanolic extract at a dose of 300 mg/kg body weight in Sprague Dawley's Rats as per OECD guidelines 423²⁴.

CONCLUSION

The results of acute oral toxicity, dermal irritation and eye irritation study of aqueous extract of *E. alba* dried leaves in Sprague Dawley rats and New Zealand white rabbits clearly indicate the safety of the extract for handling by humans during seed treatment and during field applications.

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