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Research Article

Evaluation of Acute and Sub-acute Oral Toxicity Study of Aqueous and Ethanolic extracts of *Ipomoea carnea* on Experimental Rats

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Abstract

Aim of the study: The present research was aimed to evaluate the ethanol and aqueous leaf extracts of *Ipomoea carnea* for acute and sub-acute toxicity study and to identify the range of dose that could be used for further studies.

Materials and Methods: Acute toxicity study was conducted in rats by using OECD 423 guidelines whereas sub-acute toxicity study was carried out in rats by using OECD 407 guidelines. In the acute toxicity study, rats were administered a single dose of 2000 mg/kg and 5000 mg/kg orally and then observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days. In the Sub-acute toxicity studies, extracts was given orally at doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight daily for 28 days to male and female rats respectively. General behavior, adverse effects and mortality were observed throughout the experimental period. Food intake, water intake, body weight, organ weight, hematological and biochemical parameters, were evaluated.

Results: The limit doses of 2000 mg/kg and 5000 mg/kg did not cause any mortality or signs of acute toxicity in the rats tested during the observation period. In sub-acute toxicity tests, the results did not show any treatment related abnormalities in terms of hematological and biochemical parameters.

Keywords: *Ipomoea carnea*, OECD guidelines, Hematological and biochemical parameters, Acute toxicity study, Sub-acute toxicity studies

Introduction : The toxicity study is done for data profiling and safety of the herbal drugs, the toxicity study of various plant and herbal formulation are reported. **[1]** Toxicity associated with herbal products has alerted many national and international regulatory authorities to develop and implement various set of guidelines for assessing, monitoring and preventing the toxicity associated with the herbal products.

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For example, Uppsala monitoring committee (UMC) of the world health organization (WHO) collates and communicates information regarding herbal adverse drug reactions whereas Organization for Economic Cooperation and Development (OECD) sets guidelines for conducting various toxicity studies. Toxicity tests are most widely used to examine specific adverse events or specific endpoints such as cancer, cardio-toxicity and skin/eye irritation. Toxicity testing also helpful in determining the No Observed Adverse Effect Level (NOAEL) dose and is helpful for further clinical trials. **[2]** A number of plants and their constituents traditionally used as medicines are suspected of being carcinogens to rodents and/or humans. Some of the traditionally used medicinal plants such *as Larrea tridentata (DC.), Piper methysticum G. Forst., Atractylis gummifera L., Callilepsis laureola DC.* etc., are known to cause human liver injuries. **[3]** Acute, sub-acute and chronic toxicity tests are routine toxicity tests carried out by the pharmaceutical companies in the development of new medicines. **[2]**

- **Toxicity studies are divided into:**

1. Acute toxicity studies **[5]**
2. Sub-acute toxicity studies **[4]**

- **Acute toxicity studies:** To determine the therapeutic index, i.e. ratio between the lethal dose and the pharmacologically effective dose in the same strain and species (LD_{50}/ED_{50}). The greater the index, safer is the compound. LD_{50} with confidence limits is to be established on one common laboratory species such as mouse/rat using the standard method. The LD_{50} dose thus found was administered to guinea pigs, rabbits, cats or dogs on weight basis (on basis of relative surface area gives better results). **[5]**

- **Sub-acute toxicity studies:** This study is conducted to determine organs affected by different dose levels. This study access the nature of toxic dose under more realistic situation than the acute toxicity studies. Three dose levels are normally used. Doses are generally selected on the basis of information obtained in acute toxicity studies

using both LD50 and the slope of the dose response curve. The duration of sub-acute toxicity studies depend on intended duration of the test substance. **[4]** Changes in physiologic functions and electrophysiology Biomarkers are measurements of test model (animal) parameters that can provide important quantitative data about the biological state of the test model, predictive of effects in humans. These biomarkers in toxicology are preferably shared by both test animals and humans and in a manner that the relationship of findings in one species as applying to another is known. **[6]**

- *Ipomoea carnea*, the pink morning glory, is a species of morning glory. This flowering plant has heart-shaped leaves that are a rich green and 6–9 inches (15–23 cm) long. The plant is also of medicinal value. The literature survey reveals that the plant possess various bioactive compounds such as glycosides, alkaloids, reducing sugars, flavonoids, fatty acid, esters, alcohol and tannins. The leaves of this plant showed the presence of thirteen compounds which include hexadecanoic acid, stearic acid, 1, 2 diethyl phthalate, n-octadecanol, octacosane, hexatriacontane, tetracontane, 3-diethylamino-1-propanol. Ipomea carnea Also possess various pharmacological activities like Anti-Inflammatory Activity, Antioxidant Activity:, Antidiabetic Activity, Antibacterial Activity, Wound Healing Activity, Immunomodulatory Activity, Cardiovascular Activity, Hepatoprotective Activity, Anxiolytic Activity, Anti HIV activity, Embryotoxic effect. **[7]**

Materials and Methods

- **Material and reagents:** Ethanol obtained from Himedia Laboratories Mumbai, India. Chloroform obtained from LOBA Chemie (P) Ltd, Mumbai India. CMC obtained from central drug house (P) LTD, New Delhi India. All other chemicals used in the study were purchased from Rankem Gurgaon, Haryana, India. All reagents were of analytical grade.
- **Collection of plant:** ***Ipomoea carnea*** was collected from (M.P.) during the month of May 2019. The plant has been identified and authentication by Dr. Saba Khan, Head of the Department Botany at the Safia College of Science, Bhopal (M.P.). The plant part specimen was submitted as herbarium with a voucher specimen no 463/Boi/Safia/18.

Sample preparation: The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

➢ **Sample extraction**

● **Aqueous extract:** 100 grams of I. carnea fine powdered sample was extracted with 800 ml distilled water by continuous refluxing for 2 h at 100˚C. The greenish liquid obtained was filtered with muslin cloth and the filtrate was further evaporated to dryness in a water bath at 100˚C.

● **Ethanolic extract:** 100 grams of I. carnea fine powdered sample was extracted with 250 ml ethanol for 48 h by using Soxhlet apparatus at 65˚C. The extract was evaporated under reduced pressure by using a rotary evaporator and further concentrated in a water bath at 65˚C**.**

● **Experimental animals:** Wistar albino rats (weighing between 130 gms-200 gms) of both sexes were selected for Acute and sub-acute toxicity studies. They had free access to food and water and were maintained under standard laboratory conditions which included 12-hour light-dark cycle and temperature of 28-30 degrees centigrade. Animals are allowed for a one week of acclimatization period prior to the study. The experimental protocol was approved by the IAEC (institutional animal ethical committee) and care of the experimental animals was taken according to the CPCSEA guidelines.

➢**Phytochemical Analysis:** The crude extracts of plant were subjected to various qualitative tests to detect the presence of common chemical constituents. **[9]**

➢**Acute oral toxicity studies (OECD 423):** Before experimentation Wistar albino rats were fasted overnight with water ad libitum. Extracts of ***Ipomoea carnea*** *Linn* was dissolved in suitable solvent (1% aqueous CMC), to prepare dose of 2000 and 5000 mg/kg. Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, Administered dose was found tolerable (as no death found). **[2, 8]**

Table 1: Animals were grouped as follows for acute oral toxicity studies

Group	Name of Group	Treatment
1	Control	Normal saline (5 ml/kg p.o. body weight)
2	Test-1	***AEIC*** **(**2000 mg/kg p.o. body weight)
3	Test-2	***EEIC*** (2000mg/kg p.o. body weight)
4	Test-3	***AEIC*** (5000 mg/kg p.o. body weight)
5	Test-3	***EEIC*** (5000 mg/kg p.o. body weight)

AEIC: Aqueous extract of *Ipomoea carnea*
EEIC: Ethanolic extract of *Ipomoea carnea*

➢ **Sub acute oral toxicity studies:** About 42 albino rats were randomly grouped into 7 (control, Test-1, Test-2, Test-3, Test-4, Test-5 and Test-6) of six rats each. Group A served as control and was administered with 0.5 ml of normal saline once daily for 28 days. Rats in groups Test-1, Test-2, Test-3, Test-4, Test-5, and Test-6 were orally gavaged with 250, 500 and 1000 mg/kg body weight of the aqueous and ethanolic extracts, respectively once daily for 28 days. The rats were observed daily for any signs of toxicity, and their body weights were also recorded weekly throughout the experimental period. **[2, 8]**

➢ **Termination of the experiment**

On the 29th day of the protocol, following an overnight fast of 8 h, all animals in various groups were anesthetized under chloroform and blood samples were collected into non-heparinised and heparinised bottles for haematological and biochemical investigations respectively. Blood samples collected into clean non-heparinised bottles were allowed to clot and centrifuged according to groups; and serum was separated from the clot into clean bottles for the biochemical analyses.

➢ **Calculation ratio of organ-to-body weight**

Organ-to-body weight ratio was calculated by dividing the weight (gm) of each organ by the weight (gm) of rats before sacrifice.

➢ **Hematological parameters:** The heparinised blood was used for the analysis of hematological parameters such as hemoglobin, red blood cell count, white blood cell count, platelet count were measured using fully automated hematology analyser (PE 6000).

➢ **Biochemical Parameters:** The serum was separated from non-heparinized blood and the serum biochemical parameters including total cholesterol, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), triglycerides, total cholesterol, albumin, bilirubin and total protein were analysed by using semi-automatic biochemical analyser.

Table 2: Animals were grouped as follows for Sub acute oral toxicity studies

Group	Name of Group	Treatment
1	Control	Normal saline (5 ml/kg p.o. body weight)
2	Test-1	***AEIC*** (250 mg/kg p.o. body weight)
3	Test-2	***EEIC*** (250 mg/kg p.o. body weight)
4	Test-3	***AEIC*** (500 mg/kg p.o. body weight)
5	Test-4	***EEIC*** (500 mg/kg p.o. body weight)
6	Test-5	***AEIC*** (1000 mg/kg p.o. body weight)
7	Test-6	***EEIC*** (1000 mg/kg p.o. body weight)

➢ **Histopathological examination**

All rats were sacrificed after the blood collection. The experimental rats were fixed in 10% buffered formalin in labeled bottles, and processed for histological examination. Tissues embedded in paraffin wax were sectioned 5 mm thick, stained with haematoxylin and eosin, mounted on glass slides and examined under a standard light microscope.

➢**Statistical analysis:** Results are expressed as mean ±standard error mean (SEM). Data obtained was analyzed by using one way ANOVA followed by Dunnett's test and $p<0.05$ was considered as statistically significant.

Results and discussion

Phytochemical screening: There is a presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins and saponins in extracts of ***Ipomoea cornea*** **leaves**

The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. However, there is a lack of proven scientific studies on the toxicity and adverse effect of these treatments.

Therefore, the present research was aimed to evaluate the ethanol and aqueous leaf extracts of *Ipomoea carnea* for acute and sub-acute toxicity study and to identify the range of dose that could be used for further studies. The oral acute toxicity study of the tested plant extracts was carried out on wistar rats at single dose of 2000 and 5000 mg/kg body weight and was continuously monitored for first 4 h, followed for a period of 72 h for any toxic effect after the treatment period. No major changes in behavior and mortality were observed in all groups. In the acute toxicity study, **AEIC** and **EEIC** extracts, regardless of dose used, neither appeared to affect the body weight of the rats nor did it cause significant changes in their food intake or utilization of food. However, there were significant decreases in body weight gains of rats (Kidney) when treated sub acutely with repeated oral doses of 500 mg/kg and 1000 mg/kg. The weights of organs (liver, kidney, lung, heart, and spleen) in the treated male did not differ significantly from those of the control group, in sub acute studies. The extract seems to be safe at a dose level of 5000 mg/kg, and the LD50 is considered be >5000 mg/kg. Any pharmaceutical drug or compound with the oral LD50 higher than 1000 mg/kg could be considered safe and low toxic. This suggests that the ethanol and aqueous leaf extract is practically non-toxic in single dose of level 5000 mg/kg body weight. However in case of multiple dose uses in the treatment of the chronic disorder like cancer, diabetes or hyperlipidemia, whether it will be safe and have no effect on relative organ weight, hematological and biochemical parameters that can be confirmed from its sub acute toxicity study.

A sub-acute toxicity study was therefore carried out with doses of 250, 500, 1000 mg/kg of extract as per OECD guideline. Decreases or increases in the body weights are associated with toxic effects of chemicals and drugs. There were slight but significant elevations in the levels of ALT and AST at sub acute doses of 500 mg/kg and 1000 mg/kg in rats. These findings suggest that sub acute ingestion of ethanolic extract at relatively high doses is hepatotoxic in rats. The transaminases (AST and ALT) are biomarker enzymes used to predict possible toxicity. Generally, damage to the parenchymal liver cells results in elevation of both transaminases. Interestingly, the elevation of AST level was about twice the increase in ALT. The isolated elevation of AST levels, in the presence of normal levels of other cholestatic markers, could be justified apart from liver injury. AST is present in different tissues (including kidney, liver) while ALT is localized primarily in the liver. Moreover, AST can exist as a complex with an immunoglobulin, and this macromolecule can cause an elevation in serum AST activity, which may be detected in blood chemistry analysis and mistakenly be considered to highlight the occurrence of liver dysfunction. Thus, extract might influence previous mechanisms that cause more elevations of the AST than ALT levels, rather than acting on the liver. However, scientific evidence confirmed that increases or decreases in the body weights are accompanied with accumulation of fats and physiological adaptation responses to the plant extracts rather than to the toxic effects of chemicals or drugs that lead to decrease appetite and, hence, lower caloric intake by the animal. The average organ body weight of the vital organs like liver, kidney, stomach, pancreas and small intestine were found normal indicating no toxic effect in both control and treated group and was statistically non-significant differences ($P > 0.05$). The absence of any significant differences in the liver, kidney, heart and small intestine weight provides support for the safety of

Ipomoea carnea. After 28 days of treatment with tested plant extract, the hematological parameters showed no significance P > 0.05 when compared to control group.. Hence, the tested plant extract may not have harmful effects on bone marrow function and justify the fact that at all doses of *Ipomoea carnea* does not induce anemia, making it safe. Similarly, estimation of serum biochemical parameters in treated animals showed non-significance (P > 0.05) compared to control group. However, the transaminases enzyme SGOT (AST) and SGPT (ALT) were observed positive and showed a remarkable significant elevation (P < 0.001) in plant treated animal for 500 and 1000 mg/kg extract as compared to respective control group. Many studies have confirmed that elevated serum levels of hepatic enzymes, transaminases (SGPT and SGOT) are not a directly linked for liver injury but increase levels are responsible to cause inflammation, cellular leakage and damage of cell membrane to cells in the liver. The main target organ for drug or bioactive active compound is liver where exposed to the foreign substances being absorbed in intestines and metabolized to other compounds which may or may not be hepatotoxic to the rat. Therefore, the increase in liver hepatic enzyme (SGPT and SGOT) after administration of the ethanolic plant extract might be because of certain phytochemical compound that might have toxic potential on liver with increasing dose and result liver injury. However, these changes may not be toxicologically significant, as they were not corroborated by the biochemical findings (ALT, AST). Further specific assays of toxicity and more histological study could provide more information regarding to the toxic effect of the extract on liver. This study provides very important data on the acute and sub acute toxicity profile of the ethanolic & aqueous extract of *Ipomoea carnea* that should be very useful for any future in vivo and clinical study of this plant medicine. *Ipomoea carnea* both extract was found to be less toxic when oral sub-acute toxicities in mice were performed. These results showed that the use of extract of ethanolic and aqueous *Ipomoea carnea* is safe.

Conclusion

This study validated the toxic effects of *I. carnea* leaves extracts at the doses of 250, 500 and 1000 mg/kg with prolonged use. The toxic effects comprised changes in the hematological compositions with end-organ damage to the liver, leading to alterations in the normal physiological functions and weakening of the immune system of the animals. In summary, the *I. carnea* extracts were found to be fairly nontoxic when oral acute and sub-acute toxicities in rats were performed. Chronic toxicity study is needed for further support the safe use of this plant.

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Figure 1: *Ipomoea carnea*

Table 3: Percentage yield of *Ipomoea cornea* leaves

Extracts	Yield (gm)	Extracts	Yield (gm)
Aqueous	11.25	Ethanolic	8.25

Observation	Control group	2000 mg/kg AEIC	5000 mg/kg AEIC	2000 mg/kg EEIC	5000 mg/kg EEIC
General Observation					
Body weight	No change	No change	No change	No change	No change
Temperature	Normal	No change	No change	No change	No change
Food Intake	Normal	No change	No change	No change	No change
Water Intake	Normal	No change	No change	No change	No change
Mood					
Grooming	Normal	No change	No change	No change	No change
Vocalization	Normal	No change	No change	No change	No change
Restlessness	Normal	No change	No change	No change	No change
Irritability	Normal	No change	No change	No change	No change

Fearfulness	Normal	No change	No change	No change	No change
CNS Excitation					
Straub Tail	Normal	No change	No change	No change	No change
Tremors	Normal	No change	No change	No change	No change
Convulsions	Normal	No change	No change	No change	No change
Reflexes					
Pinna	Normal	No change	No change	No change	No change
Corneal	Normal	No change	No change	No change	No change
Autonomic					
Writhing	Normal	No change	No change	No change	No change
Pupil Size	Normal	No change	No change	No change	No change
Urination	Normal	No change	No change	No change	No change
Salivation	Normal	No change	No change	No change	No change
Respiratory Rate	Normal	No change	No change	No change	No change
Hypothermia	Normal	No change	No change	No change	No change
Skin Color	Normal	No change	No change	No change	No change
Death					
Acute	Alive	Alive	Alive	Alive	Alive
Delayed	Alive	Alive	Alive	Alive	Alive

Table 5: Effect of AEIC extracts on Average Organ weigh (gm)

Organ weight (g/100 g body weight)	Control group	250 mg/kg AEIC	500 mg/kg AEIC	1000 mg/kg AEIC
Liver	3.21 ± 0.13	3.38 ± 0.01	3.41 ± 0.01	3.56 ± 0.20
Kidney	0.46 ± 0.07	0.43 ± 0.02	0.40 ± 0.01	0.45 ± 0.01
Pancreas	0.134 ± 0.011	0.139 ± 0.007	0.141 ± 0.013	0.142 ± 0.011
Stomach	0.663 ± 0.019	0.642 ± 0.020	0.692 ± 0.069	0.702 ± 0.011
Spleen	0.26 ± 0.04	0.28 ± 0.01	0.24 ± 0.02	0.27 ± 0.01

Values are expressed as mean ± SEM. P > 0.05 when compared to normal control group.

Table 6: Effect of EEIC extracts on Average Organ weigh (gm)

Organ weight (g/100 g body weight)	Control group	250 mg/kg EEIC	500 mg/kg EEIC	1000 mg/kg EEIC
Liver	3.21 ± 0.13	3.24 ± 0.19	3.37 ± 0.08	3.45 ± 0.23
Kidney	0.46 ± 0.07	0.42 ± 0.02	0.44 ± 0.02	0.43 ± 0.03
Pancreas	0.134 ± 0.011	0.129 ± 0.007	0.139 ± 0.117	0.141 ± 0.011
Stomach	0.663 ± 0.019	0.677 ± 0.021	0.691 ± 0.034	0.700 ± 0.091
Spleen	0.26 ± 0.04	0.25 ± 0.05	0.21 ± 0.04	0.28 ± 0.03

Values are expressed as mean ± SEM. P > 0.05 when compared to normal control group.

Table 7: Effect of AEIC extract on biochemical parameters

Biochemical & Haematological Parameters	Control group	250 mg/kg AEIC	500 mg/kg AEIC	1000 mg/kg AEIC
Glucose (mg/dL)	114 ± 23.1	136 ± 4.70	130 ± 16.2	126 ± 22.1*
Creatinine (mg/dL)	0.38 ± 0.02	0.43 ± 0.07	0.60 ± 0.03*	0.66 ± 0.12*
Total cholesterol (mg/dL)	115.90 ± 14.01	107.00 ± 0.50	104.50 ± 0.30	112.00 ± 0.50
SGOT (AST) (IU/L)	102 ± 10.2	118 ± 14.9	162* ± 10.1	166* ± 27.3
SGPT (ALT) (IU/L)	43.1 ± 4.50	52.7 ± 6.10	67.3* ± 6.90	74.5* ± 11.4
Haemoglobin (g/L)	11.500 ± 0.610	10.220 ± 1.520	12.270 ± 1.300	11.560 ± 1.432

Values are expressed as mean ± SEM. P > 0.05 when compared to normal control group.

Table 8: Effect of EEIC extract on biochemical parameters

Biochemical & Haematological Parameters	Control group	250 mg/kg AEIC	500 mg/kg AEIC	1000 mg/kg AEIC
Glucose (mg/dL)	114 ± 23.1	139 ± 10.1	142 ± 10.6	122* ± 10.8
Creatinine (mg/dL)	0.38 ± 0.02	0.44 ± 0.16	0.71* ± 0.19	0.77* ± 0.04
Total cholesterol (mg/dL)	115.90 ± 14.01	127.00 ± 0.50	120.50 ± 0.30	132.00 ± 0.50
SGOT (AST) (IU/L)	102 ± 10.2	119 ± 16.9	128 ± 10.1*	151 ± 12.3*
SGPT (ALT) (IU/L)	43.1 ± 4.50	59.7 ± 11.1	63.3 ± 14.1*	77.5 ± 12.1*
Haemoglobin (g/L)	11.500 ± 0.610	11.11 ± 1.210	11.270 ± 0.690	11.560 ± 1.112

Values are expressed as mean ± SEM. P > 0.05 when compared to normal control group.