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Comparative Acute Toxicity Studies of Selected Indigenous Herbal Plants in Swiss Albino Mice

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Abstract:
Objective: *Toxicology may be defined as the study of harmful / poisonous effects of drugs and other chemicals with emphasis on detection, prevention and treatment of poisonings. The present study was aimed to determine LD50 and to establish the safety margin ofdifferent solvent extracts of selected herbal plants sources namelySaussurea lappa (Root), Ficus bengallensis (Root), Flacourtria romantchi (stem bark and Root), andOroxyllum indicum (Root) by acute toxicity study in Swiss albino mice as per OECD guideline 425.*
Methods: *Swiss albino mice were sequentially administered all the extracts in single dosages of 250, 500, 750, 1000, 1500, 2000 mg/kg of body weight. All the animals were individually studied for mortality, wellness parameters and body weight for 24 hours.*
Results: *No mortality and no significant changes were observed in body weight and wellness parameters at 250, 500 and 750 mg/kg body wt. doses, which reveal the safety of these extracts in the doses up to 1000 mg/kg body weight.*
Conclusion: *Conclusively, LD50 value of all extracts of Saussurea lappa (Root), Ficus bengallensis (Root), Flacourtria romantchi (stem bark and Root), and Oroxyllum indicum was found to be more than 1000 mg/kg body weight.*
Keywords: *Acute toxicity(LD50), Root extract of Saussurea lappa**(RESL)**, Root extract of Ficus bengallensis (**REFB)**, Stem bark and root extract of Flacourtria romantchi(**SBAREFR**), root extract of Oroxyllum indicum (REOI), OECD guideline 425.*

I. Introduction

'Toxicology' as the 'science of poisons' began with early cave dwellers who recognized poisonous plants and animals for the use as hunting or warfare. With advancing time, it included the practice of determining the safety margin of a particular compound. Comprehensively, in its present form, toxicology may be defined as the study of harmful / poisonous effects of drugs and other chemicals with emphasis on detection, prevention and treatment of poisonings. After gaining relevant information on the harmful effects of a compound, the levels for its safe usage or the degree of its safety is established, this is known as its Biosafety level [1-2].

Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the past 20 years as this type of medicine is easily accessible in some regions [3]. Plant-derived foods, particularly vegetables and fruits, are generally considered to be highly beneficial components of the human diet. They contribute great importance in daily life by providing wide range of nutrients, vitamins and other compounds which widen the therapeutic arsenal. In general, natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases [4-8].

*Saussurea lappa (Root), Ficus bengallensis (Root), Flacourtria romantchi (stem bark and Root), and Oroxyllum indicum (Root)*are medicinal plant. They are perennial, procumbent herb, widely distributed in North Africa, elsewhere in the Mediterranean region, in the Himalayas, in North India, and in Arabian countries.

The modern researches suggest that root of *Saussurea lappa* is a blood purifier, antiseptic and increases the cetaceous circulation. It is a good insect repellant.Hence, might be used to keep off the insects from the clothes. Internally it is a good expectorant, anti-spasmodic and neurotoxin, hence, might be used for cough, bronchitis, bronchial asthma, paralysis, facial palsy and neurasthenia. (9-13)F. *benghalensis*has showed antitumorand antibacterial activities (Mousa, et a1., 1994) while the ethanol extract of the hanging roots showed anti-diarrhoeal activity (Mukherjee et at., 1998) in various experimental models.(14)

Toxicological studies have undergone a significant evolution during the past decade, with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity. The mutations in somatic cells are not only involved in the carcinogenesis process but also play a role in the pathogenesis of other chronic degenerative diseases, such as atherosclerosis and heart diseases, which are the leading causes of death in the human population (De Flora and Izzotti, 2007) (15).

*Flacourtiaramontchi*L. Herit (Flacourtiaceae) is a small deciduous thorny shrub, found in scrub forests and rocky hills upto 900 meters throughout India. This plant is used in treatment of migraine, rheumatic pain and liver disorders. (16-17)

Oroxylum indicum is a species of flowering plant belonging to the family Bignoniaceae,It is a tree which can reach a height of 12 metres (39 ft).This plant is used in treatment of inflammation, microbial infections and liver disorders. (18-20)

The present study was aimed to determine LD50 and to establish the safety margin ofselected different extracts of plant sources namely *Saussurea lappa (Root), Ficus bengallensis (Root), Flacourtria romantchi (stem bark and Root), and Oroxyllum indicum* by acute intraperitoneal toxicity study in Swiss albino mice as per Organization for Economic Cooperation and Development (OECD) guideline 425. The test procedure described in this guideline uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonized System for the classification of chemicals that cause acute toxicity. Also, this method is of value in minimizing the number of animals required to estimate the acute intraperitoneal toxicity of a chemical. In addition to the estimation of LD50 and confidence intervals, the test allows the observation of signs of toxicity.

II. Methodology

Plant Materials

1) **Collection and authentication of Plants:**Four plants have been selected for the comparative study of acute toxicity in Swiss albino mice & different parts of these plants were collected from the following places-

- The root of ***Saussurea lappa*** was collected from General Herbal Market, Jaipur Rajasthan, India.
- The root of ***Ficus bengallensis*** was collected from the campus of Nims University Jaipur Rajasthan, India.
- *The stem bark and root of* ***Flacourtria romantchi*** were collected from road side location of Agra, District-Agra, Uttar Pradesh, India.
- The root of ***Oroxyllum indicum*** was collected from the campus of B.I.T., Deemed University, Mesra, Ranchi, Jharkhand, India.

The plants were identified and Authenticated by the botanist, from the department botany, University of Rajasthan, Jaipur, Rajasthan, India.

Processing of plant samples

The roots of *Saussurea lappa (Root), Ficus bengallensis(Root),Flacourtria romantchi (stem bark and root), and Oroxyllum indicum (Root)* are properly washed in tap water and then rinsed in distilled water. The rinsed roots are dried in an oven at a temperature of 35-40°C for 3 days. The dried roots of plant are pulverized, using a sterile electric blender, to obtain a powdered form. The powdered form of these plants is stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation of plant extracts

Extraction of plant materials were carried out in mixture of four different solvents e.g. Water, Ethanol, Chloroform and petroleum ether in the ratio of 7 : 85 : 4 : 4 respectively. Extraction is followed by evaporation of solvents under low pressure in the evaporator. The basis for selection of extraction in a mixture of water + Ethanol + petroleum ether + Chloroform is- (a) For the maximum yield according to polarity I have made a combination of a mixture of solvents for extraction of selected medicinal plants. (b) If I select separate solvent system then the numbers of extracts/samples of different four selected plants were too many and evaluation of these all extracts were very difficult and complex. (c) Due to so many extracts/samples I require much high number of animals for pharmacological evaluation that was not possible for receiving clearance certificate for animal experiments by Institutional animal ethics committee (IAEC).

Acute intraperitoneal toxicity study

Acute toxicity of a drug can be determined by the calculation of LD50, i.e., the dose that will kill minimum 50% of animals of a particular species.This study is needful before pharmacological screening on animals.The acute toxicity study was carried out as per Organization for Economic Cooperation and Development (OECD) guideline 425 which are based on a stepwise procedure with the use of a minimum number of animals per step.

The acute toxicity for the plant extract was determined by the Miller and Tainter method administering the compounds intraperitoneally. LD_{50} of the test compounds calculated by Miller and Tainter (1944) method, initially least tolerated dose (100% mortality) and most tolerateddose (0% mortality) were determined by hit and trial method. If mortality observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality not observed, the procedure was repeated for further higher doses such as100, 500 and 2000 mg / kg body weight. After determination of two doses we have selected five doses in between the least tolerated and most tolerated doses were given intraperitoneally to 6 groups of mice, 06 animals in each group. The animals were observed for first 2 hours and then at 6^{th} and 24^{th} hour for any toxic symptoms. After 24 hours, the number of deceased animals was counted in each group and percentage of mortality calculated. The percentage of animals that had died at each dose level is then transformed to probit. From the obtained data determined the LD_{50} of the test compounds by using probit value transformations.

Ethical clearance: Ethical Clearance Has Been Obtained from Institutional Animal Ethics Committee (IACE).Protocol used in this study for use of mice as animal model for cancer research was approved by Institutional Animal Ethical committee, NIMS University, Jaipur-Rajasthan (NIMSUR/IAEC/CERT/2014/07/04). The copy of the ethical clearance certificate obtained from Institutional Animal Ethical Committee (IAEC) is attached.

Animals for experiment:

Healthy Mature Swiss albino mice of either sex weighing 20–25g were taken for the study. All the animals were procured from the Central Animal House of the NIMS University.The animals were housed in polypropylene (32x24x16 cm) cages containing husk as bedding material and maintained under controlled conditions of temperature (25±2^{o}c), humidity (55±5%) and 12h light and 12h dark cycles.

The animals were fed with standard pellet diet and mixture*ad libitum*. The animals are randomized into experimental and control groups. The animals were provided with Standard pellets diet (Hindustan Lever, Kolkata, India) and tap mixture*ad libitum* and maintained at natural day night cycle. The animals were acclimatized to laboratory condition for one week before commencement of experiment.

Housing Conditions:-

All pharmacological activities were conducted in 2-3 months old either sex of Swiss albino mice. The animals were housed under specific conditions in polypropylene cages for group rearing. The cages simply contained husk bedding material. The animals were randomly selected and kept in their cages for 5 days prior to dosing to allow for acclimatization to the laboratory conditions. The animals were housed individually in clean polypropylene cages. Room temperature and humidity were maintained at 25°C (± 30°C) and 45-55% respectively with a light-dark cycle of 12 h (light from 06:00 AM to 06:00 PM). Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard pellet chow (Hindustan Lever, Kolkata, India) and mixture*ad libitum*. All experiments were approved by Institutional Animal Ethics Committee.

Determination of Median Lethal Dose (LD_{50})[3, 4]

The crude extracts (200-2000mg/kg) produced physical signs such as gasping for air, palpitation, depression, decreased respiratory rate, loss of appetite, feeling sleepy, and death depending on the dose. The LD50 values of the crude extracts in mice were calculated as following-

Procedure

Healthy, young, adult Swiss albino mice of either sex (30-35 gm.) were used for this study. Animals were fasted prior to dosing. On next day, the fasted body weight of each animal was determined and the dose was calculated according to the body weight.

36 animals were divided into six groups (n=6) for giving each plant extracts dose 250, 500, 750, 1000, 1500 and 2000 mg/kg respectively. The test extract was dissolved in distilled mixture and injected intraperitoneally to six groups of mice (each containing 5 mice) at different doses (250, 500, 750, 1000, 1500 and 2000 mg/kg). LD50 was evaluated by recording mortality after 24 hours.

Group1: 250 mg/kg, the dose of extract given to animals. At this dose animals showed normal behavior.

Group2: 500 mg/kg, the dose of extract given to animals. At this dose initially animals were uncomfortable but after sometimes they became normal.

Group3: 750 mg/kg, the dose of extract given to animals. At this dose initially animals were uncomfortable but after sometimes they became normal.

Group4: 1000 mg/kg, the dose of extract given to animals. At this dose 30 to 50% animals died.

Group5: 1500 mg/kg, the dose of extract given to animals. At this dose 60 to 80% animals died.

Group6: 2000 mg/kg, the dose of extract given to animals. At this dose 90 to 100% animals died.
Animals were observed individually 30 minutes after dosing, periodically during the first 24 hours. Following changes were examined in the treated animals

1) **Behavior intraperitoneal profile :**
   Awareness**:** Alertness, Visual placing, Stereotypy, passivity.
   Mood**:** Grooming, restlessness, irritability, fearfulness.
2) **Neurological profile**
   Motor activity: Spontaneous Activity, reactivity touches response, pain response startle response tremor gait grip strength pinna reflex, and corneal reflex.
3) **Autonomic profile:** Writhing, Defecation, Urination, Pile erection, heart rate, respiratory rate.

The percentage dead for 0 and 100 are corrected before the determination of probits as under:

Corrected % Formula for 0 and 100% mortality:
For 0% dead: 100(0.25/n)
For 100% dead: 100(n-0.25/n)
The probit values are plotted against log-doses and then the dose corresponding to probit 5, i.e., 50%, is found out.

No mortality was noticed up to 250 mg/kg body weight (i.p.), whereas, 100% mortality was noticed at the dose of 1500-2000 mg/kg (i.p.). The LD50 of the plant extracts was found to be 1000 mg/kg for *Saussurea lappa (Root),* 1500mg/kg for *Ficus bengallensis (Root),* 850mg/kg for *Flacourtria romantchi (stem bark and Root)* and 1000 mg/kg for *Oroxyllum indicum (Root)* body weight (i.p.). One-tenth of this dose was selected as the therapeutic dose for the evaluation of antitumor activity.
Selection of dose: 1/10 and 1/5 of the maximum tolerated dose was selected as treatment dose for further any other pharmacological activity. (Ghosh MN, 1984)

Statistical analysis:

The data are expressed as mean ± SD. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by Dunnet and Tukey's test. *P*-value <0.05 was regarded as statistically significant.

The test animals did not display any significant changes in behavioral pattern such as trembling, diarrhea, salivation, breathing, impairment in food intake, mixture consumption, postural abnormalities, hair loss, sleep, lethargy, restlessness, or in physical appearance such as eye colour, mucous membrane, salivation, skin/fur effects, body weight, injury, at the doses up to 250 mg/kg dose of plant extracts when compared to the control at the end of 24 hours of general observation.

The LD50 of selected four plant extracts like root extract of *Saussurea lappa* (RESL), root extract of *Ficus bengallensis* (REFB), stem bark and root extract of *Flacourtria romantchi* (SBAREFR) and root extract of *Oroxyllum indicum* (REOI) in mice was found to be 1000 mg/kg, 1500 mg/kg, 850 mg/kg and 1000 mg/kg after intraperitoneal injection, respectively. Results of intraperitoneal administration of plant extracts in mice are given in Tables-1 to 4 respectively. In the animals receiving intraperitoneal injection, the abdominal muscle contractions and ataxia was observed, which persisted for few hours. At the 6^{th} hour they were drowsy and less responsive. The severity of these effects was related to the level of dose. However, at 24th hour most of the survivors had recovered from these symptoms. The LD50 values presented here after intraperitoneal injection are 10–15 times greater than doses of plant extracts reported for its pharmacological effects.

Table-1: Results of the lethal doses of *RESL* for the determination of the LD50 after intraperitoneal injection in mice (n=06):

Group No.	Dose(mg/kg) of *RESL*	Log Dose of *RESL*	No. of Deaths	% Deaths	*Corrected %	Probits
1	50	1.7	0/6	0	4.16	3.04
2	250	2.4	0/6	0	4.16	3.04
3	500	2.7	2/6	33.33	33.33	4.56
4	1000	3	3/6	66.66	66.66	5
5	1500	3.18	5/6	83.33	83.33	5.95
6	2000	3.3	6/6	100	95.83	6.69

*Corrected % Formula: For 0 and 100 % deaths,
For 0% dead: 100(0.25/n), For 100% dead: 100(n-0.25/n)

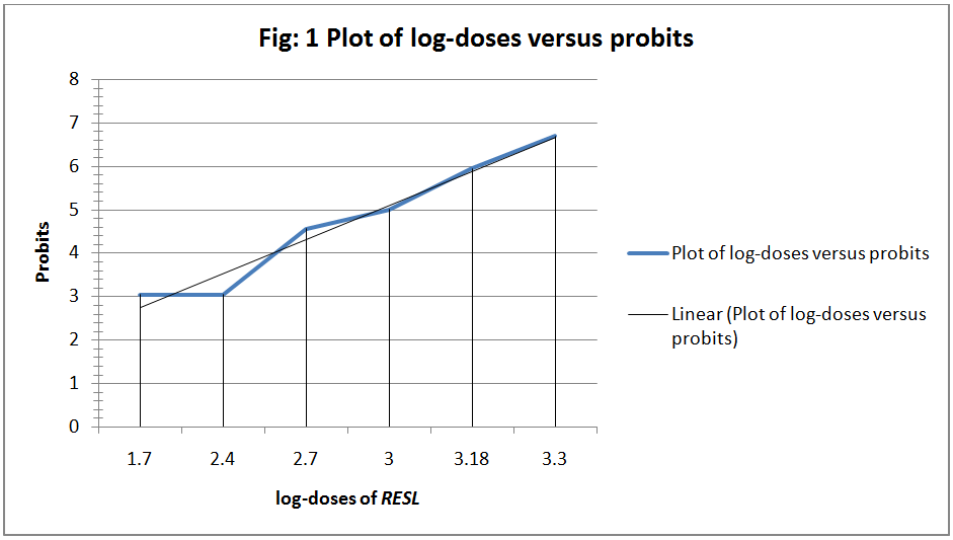


Table-2: Results of the lethal doses of *REFB* for the determination of the LD50 after intraperitoneal injection in mice (n=06):

Group No.	Dose(mg/kg) of *RESL*	Log Dose of *RESL*	No. of Deaths	% Deaths	*Corrected %	Probits
1	50	1.7	0/6	0	4.16	3.04
2	250	2.4	0/6	0	4.16	3.04
3	500	2.7	1/6	16.66	16.66	4.01
4	1000	3	2/6	33.33	33.33	4.56
5	1500	3.18	3/6	50	50	5
6	2000	3.3	6/6	100	95.83	6.69

*Corrected % Formula: For 0 and 100 % deaths,
For 0% dead: 100(0.25/n), For 100% dead: 100(n-0.25/n)

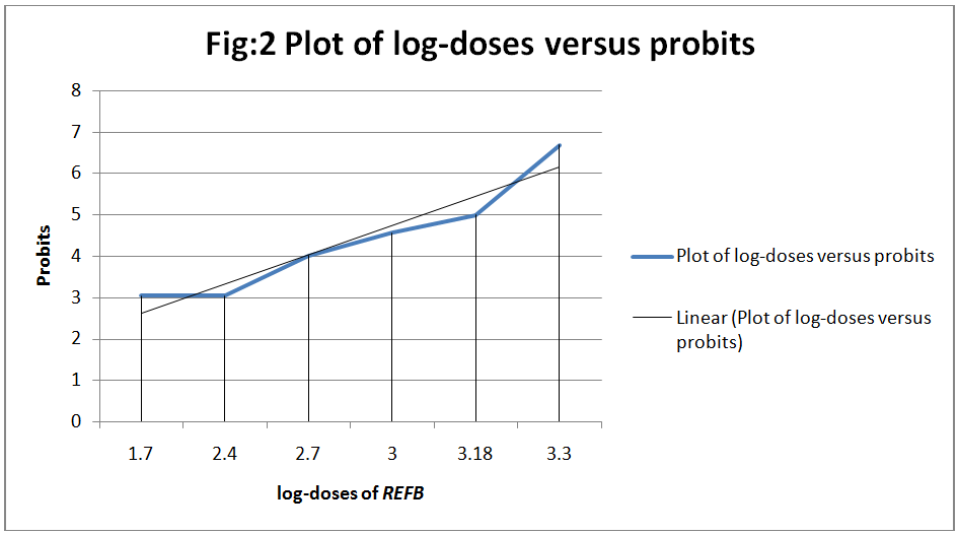


Table-3: Results of the lethal doses of *SBAREFR* for the determination of the LD50 after intraperitoneal injection in mice (n=06):

Group No.	Dose(mg/kg) of SBAREFR	Log Dose of SBAREFR	No. of Deaths	% Deaths	*Corrected %	Probits
1	50	1.7	0/6	0	4.16	3.04
2	250	2.4	0/6	0	4.16	3.04
3	500	2.7	2/6	33.33	33.33	4.56
4	1000	3	4/6	66.66	66.66	5.44
5	1500	3.18	5/6	83.33	83.33	5.95
6	2000	3.3	6/6	100	95.83	6.69

*Corrected % Formula: For 0 and 100 % deaths,
For 0% dead: 100(0.25/n), For 100% dead: 100(n-0.25/n)

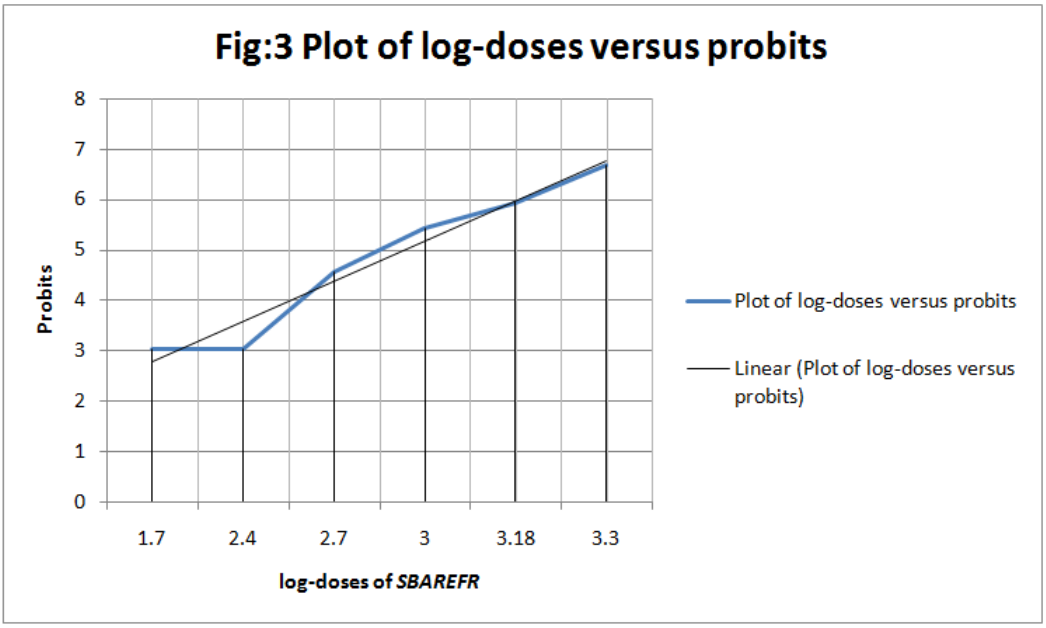
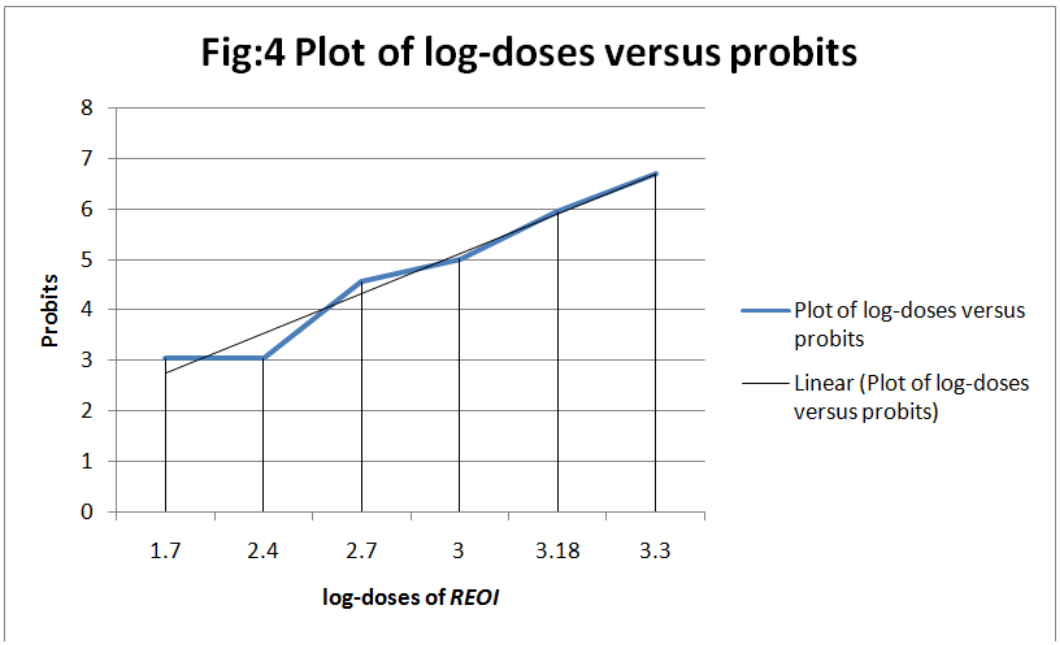
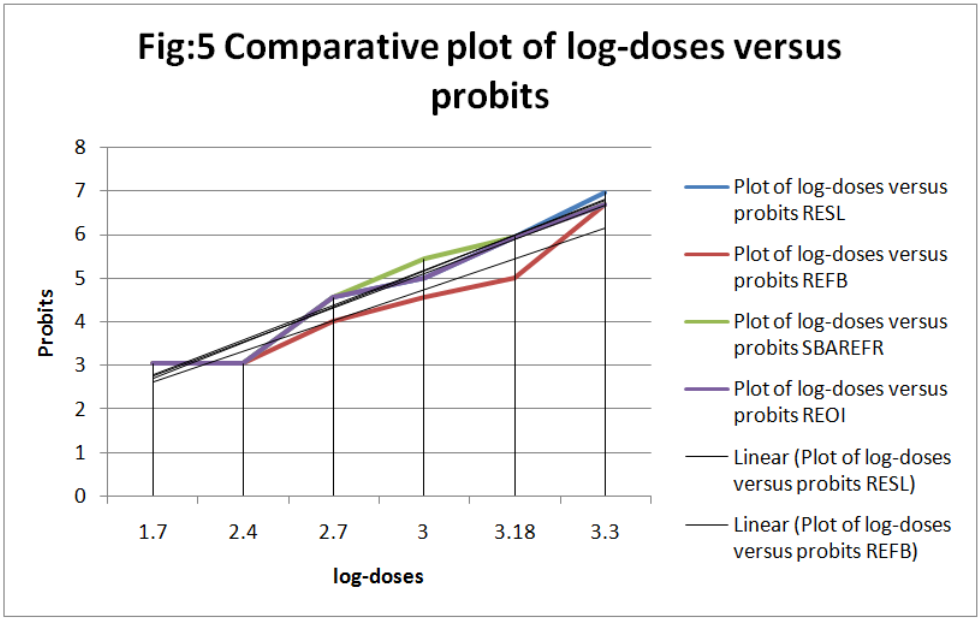


Table-4: Results of the lethal doses of *REOI* for the determination of the LD50 after intraperitoneal injection in mice (n=06):

Group No.	Dose(mg/kg) of REOI	Log Dose of REOI	No. of Deaths	% Deaths	*Corrected %	Probits
1	50	1.7	0/6	0	4.16	3.04
2	250	2.4	0/6	0	4.16	3.04
3	500	2.7	2/6	33.33	33.33	4.56
4	1000	3	3/6	50	50	5
5	1500	3.18	5/6	83.33	83.33	5.95
6	2000	3.3	6/6	100	95.83	6.69

*Corrected % Formula: For 0 and 100 % deaths,
For 0% dead: 100(0.25/n), For 100% dead: 100(n-0.25/n)





III. Discussion

The aim of the present study was to determine the LD50 of selected herbal medicinal plants given intraperitoneally in Swiss albino mice. LD50 estimated by the method of Miller and Tainter inour study, Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in thedeveloping countries for primary health care. Herbal medicines have received greaterattention as an alternative to clinical therapy and the demand for these remedies has currentlyincreased.The medicinal plants commonly contain various bioactive principles which have thepotential to cause beneficial and/or detrimental effects. Experimental screening method isimportant in order to ascertain the safety and efficacy of traditional and herbal products and alsoto establish the active component of the herbal products.The results of the acute toxicityreveals that there was no mortality observed up to the maximum dose level of 2000mg/kg b.wt ofthe each extract administered intraperitoneally, which is the single high dose recommended by OECDguidelines425 for testing acute toxicity. No changes attributable to treatment were found in bodyweight, respiration rate, heart rate. Treatment related changes observed in behavioral signs viz. In general *in vivo* toxicity study is the toxicological analysis of many medicinal plants and itspotency to evaluate qualitatively and quantitatively by acute toxicity studies. The acute toxicity testing in mice could be used to evaluate natural remedies for differentpharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose. A toxic substance might elicit interesting pharmacological effects at alower non-toxic dose.

The result of the current study showed that the LD50 of the selected herbal plants was found to be greater than 1000mg/kg, body weight which may be accepted as safe (OECD 425). The test animals did not display any significant changes in behavioral pattern such as trembling, diarrhea, salivation, breathing, impairment in food intake, water consumption, postural abnormalities, hair loss, sleep, lethargy, restlessness, or in physical appearance such as eye colour, mucous membrane, salivation, skin/fur effects, body weight, injury, when compared to the control at the end of 24 hours of general observation.

In this study, the mice in the control and treated groups were administrated with vehicles and crude extracts, respectively. The mice were monitored for 24 hours for any toxic signs and mortality. The clinical symptom is one of the major important observations to indicate the toxicity effects on organs in the treated groups. During the 24 hours of period acute toxicity evaluation, mice which are i.p. administrated with different plant extracts at single dose 1000 mg/kg showed no overt signs of distress, and there were no observable symptoms of neither toxicity nor deaths. All of the mice gained weight and displayed no significant changes in behavior. Apart from that, the physical appearance features such as skin, fur and eyes were found to be normal while the body weight of the mice showed as increase, this indicates that the administration of the crude extracts has negligible level of toxicity on the growth of the animals. Furthermore, determination of food intake and water consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drugs tested.

IV. Conclusion

The present results show that different plant extracts does not cause any apparent in toxicity of an animal model. No death or signs of toxicity were observed in rate treated with extracts at dose 2000 mg/kg thus establishing its safety in use. The extracts did not produce any toxic symptoms of mortality up to the dose level of 250 mg/kg body weight in mice, and hence the extracts were considered safe for further pharmacological screening, the 1/10 or 1/5 of the LD50 were taken as dose for the evaluation of anticancer activity.

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