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EVALUATE ACUTE AND SUBACUTE TOXICITY OF AQUEOUS LEAF EXTRACT OF *CALOTROPIS GIGANTEAN. LINN*

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ABSTRACT

Present study results it was concluded that aqueous leaf extract in acute toxicity study at doses of 50 mg,300m,2000mg does not exhibit any mortality and does not show any signs of toxicity excdpt the animals exhibit drowsiness, further the sub acute toxicity study carried out for 28 days at low doses 100mg 400mg and high dose 750 mg the animals does not represent any signs of toxicity and mortality hence it is found from the study results *Calotropis gigantean* leaft extract possesses LD50 at doses more than 2500mg.drowsiness represented in this study indicate the plant extract can be used for anitiepileptic and antianxiety activities.
Keywords: *Calotropis gigantean* leaft extract, acute toxicity study, sub acute toxicity study,

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INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine. About 80% of the people in developing countries use traditional medicines for their health care. The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for Pharmaceuticals. With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-infective compounds. As there are approximately 500-1000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds. Herbs have remained useful not only as remedy for different diseases that affect humans and animals, but also as good starting points for the discovery of bioactive molecules for drug development. The scientific exploitation of herbs used ethno medicinally for pain relief, wound healing and abolishing fevers has resulted in the identification of a wide range of compounds that have been developed as new therapies for cancer, hypertension, diabetes and as anti-infectives The earliest report of the toxicity of

herbs originated from Galen, a Greek pharmacist and physician who showed that herbs do not contain only medicinally beneficial constituents, but may also be constituted with harmful substances. In many countries including the U.S, herbal medicines are not subjected to the same regulatory standards as orthodox drugs in terms of efficacy and safety. This raises concern on their safety and implications for their use as medicines. Toxicity testing can reveal some of the risks that may be associated with use of herbs, therefore avoiding potential harmful effects when used as medicine (1-3).

The aim of the present study is to evaluate acute and subacute toxicity of aqueous leaf extract of *calotropis gigantean. Linn*

MATERIALS AND METHODS

Plant Collection

Calotropis gigantean Linn traditional medicinal herb used in ayurveda ,sidda, unani system of medicine,native of India is selected for the proposed study and suitable plant part leaf aqueous extract is prepared.

Animals

The acute and subacute toxicity studies were carried out on female albino rats selected randomly. The animal species are selected as per the guide lines of OECD 423 guide lines.All the animals are procured from licensed breeder Hyderabad after approval of protocol by IAEC of AKRG college of Pharmacy Nallajerla West Godavari dist, AP.

Administration of Doses

Acute oral toxicity studies (4-6)

Toxicity studies conducted as per internationally accepted protocol drawn under OECD No 420 guidelines. The overnight fasted rats were divided into 3 groups, each group consisting of 3 animals. The aqueous extract of *Calotropis gigantean* was given separately in various doses (50, 300 2000 mg/kg) by oral route. After administration of the extract, the animals were observed continuously for the first two hours and 24 hrs to detect changes in the behavioural responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma and monitored for any mortality.

Subacute toxicity studies

Three groups of rats were used in subacute toxicity study of extract of *Calotropis gigantean* and each group consists of 3 rats. The groups and treatment were designed as follows: Group 1 - Control treated with saline (2 ml/kg, p.o.); Group 2 - *C. pendantra* (100 mg/kg, p.o.); Group 3 - *C. pendantra* (400 mg/kg, p.o.); Group 4 - *C. pendantra* (750 mg/kg, p.o.

Experimental design

Acute oraltoxicity study

OECD 423 (Acute toxic class method) guide lines are used to carry out acute toxicity studies for selected plant extract, this procedure is reproducible .It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; − no further testing is needed, − dosing of three additional animals, with the same dose − dosing of three additional animals at the next higher or the next lower dose level.

Sub Acute toxicity study

The study is carried as per the method of B. Gandhare, et al with minute modifications. Four groups of rats received Normal saline,100, 400 and 750 mg/kg plant extract , (low dose, intermediate dose and high dose) orally for 28 days. The group which served as control received equivalent quantity of normal saline orally. Animals were observed for signs and symptoms, behaviour alteration, food and water intake and body weight changes. All animals were observed twice daily for mortality during the 28 day period of study. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. The groups mean body weights were calculated.

Histopathological studies

Liver tissue of all experimental animals are collected at the end of the study on 14 th day of acute toxicity study and 28 day of subacute toxicity study. Liver tissue is collected through sacrificing the animal by employing the principles of euthanasia. The tissues collected are stored in 10% formalin solution which acts as a fixative. Preparation of Fixative: 10 ml of formaldehyde is mixed with 90ml of distilled water and was mixed with sodium biphosphate.

RESULTS AND DISCUSSION

After safe evaluation of dose at 50mg/kg the study was continued further with increasing dose as per OECD 420 guide lines with 300mg/kg .It is from the study results the animals administered with 300mg/kg also does not exhibit any drowsiness except some signs of drowsiness. The study is continued by treating the animals with dose levels of 2000mg/kg body weight animals at this dose range also found to be safe exhibit no mortality. The symptoms of drowsiness are also reported in this group of animals and there was no significant change in body weight of animals (Table-1).

Table-1 Effect of Calotropis gigantean leaf extract on body weight of animals in acute toxicity studies

S No	Group treated with conc mg/kg	Body weight	
		Before Administration of drug	After Administration of Drug
Group I	50 mg /kg	130gms	130gms
Group II	300 mg/kg	140 gms	144gms
Group III	2000mg/kg	180gms	178gms

Sub acute toxicity studies are carried out in three group of animals receiving low dose (100mg/kg and 400mg/kg) and high dose 750 mg/kg) of *Calotropis gigantean* leaf extract once daily for 28days.Sub acute toxicity studies are carried out after prediction of LD50 of plant extract in acute toxicity studies. The study results of sub acute toxicity clearly revealed the long term safety level of plant extract. The study results indicate no mortality of animals in both low dose and high dose of plant extract with no signs of toxicity shown in table-2. Drowsiness of animals is reported in both all doses of plant extract.

Table-2 Effect of Calotropis gigantean leaf extract on body weight of animals in subacute toxicity studies

S No	Group treated with conc mg/kg	Body weight	
		Before Administration of drug	After Administration of Drug
Group I	100 mg /kg	133gms	130gms
Group II	400 mg/kg	190 gms	194gms
Group III	750mg/kg	165gms	163 gms

Histopathology studies are carried out for all the study animals at the last day of study by sacrificing the animals. Liver tissue was isolated in all groups of study animals they were kept in 10%formalin solution and permanent tissue slides are prepared and observed. Histopathological observations indicate that no damage was happened to tissues of animals in all doses of acute toxicity studies as well as in low and high doses of sub acute toxicity studies. All the cells of livers are found to be normal they clearly indicate hexagonal arrangement no fat tissue accumulated central vein is clearly present no rupture or damage happened to hepatocytes (Fig-1 and 2).

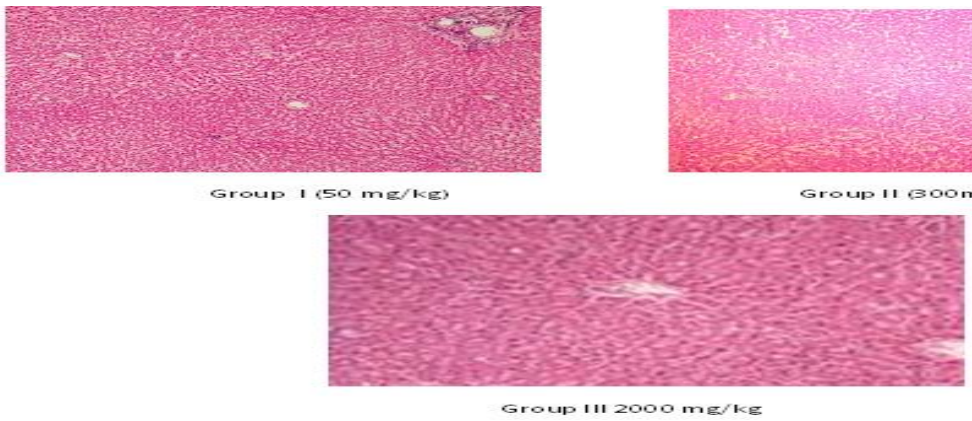

Fig-1 Effect of *Calotropisgigantean* aqueous leaf extract on liver of albino rats in acute toxicity study

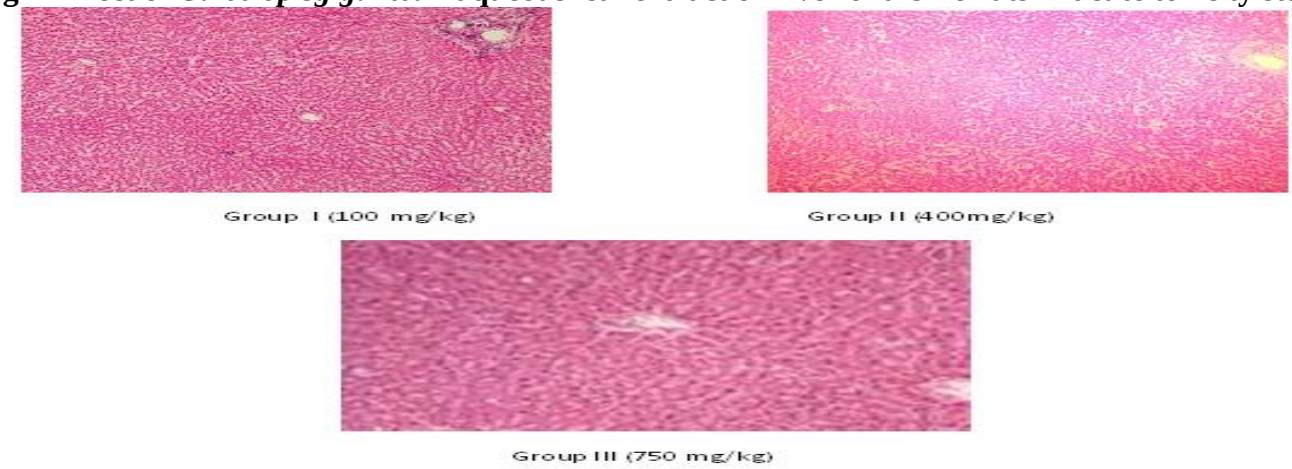

Fig-2 Effect of *Calotropisgigantean* aqueous leaf extract on liver of albino rats in subacute toxicity study

Oral Acute toxicity Study LD 50 Calculation

Animals did not show any signs of toxicity andmortality at doses treated with *Calotropis gigantean* leaf extract 50mg,300mg,2000mg/kg body weight hence it was found that LD 50 of leaf extract may be at dose more than 2500mg/kg of body weight.

CONCLUSION

The aqueous leaf extract was prepared and its acute toxicity and sub acute toxicity was studied in different doses in rodent species albino rats. Acute toxicity study involved in administration of drug for very short period of single day .It involves Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg /kg of body weight The animals were regularly and individually Observed for behavioral changes and general toxicity signs after dosing for the first 24 h, with special attention being given during the first 4 h. Thereafter, observation was continued daily for a total of 14 days followed by observation of animals for next 14 days the approximate acute lethal dose (LD50) can be calculated by these study which gives the information about the minimum safe dose of aqueous leaf extract of plant. The oral acute toxicity study results clearly showed that animals exhibit no mortality and signs of toxicity. The animals treated with doses of 50mg/kg after administration of drug are found to be normal does not show any abnormal toxic indications their body weight is found to be same before and after administration of drug. After safe evaluation of dose at 50mg/kg the study was continued further with increasing dose as per OECD 420 guide lines with 300mg/kg .It is from the study results the

animals administered with 300mg/kg also does not exhibit any drowsiness except some signs of drowsiness. The study is continued by treating the animals with dose levels of 2000mg/kg body weight animals at this dose range also found to be safe exhibit no mortality. The symptoms of drowsiness are also reported in this group of animals and there was no significant change in body weight of animals. Sub acute toxicity studies are carried out in three group of animals receiving low dose (100mg/kg and 400mg/kg) and high dose 750 mg/kg) of *Calotropis gigantean* leaf extract once daily for 28days.Sub acute toxicity studies are carried out after prediction of LD50 of plant extract in acute toxicity studies. The study results of sub acute toxicity clearly revealed the long term safety level of plant extract. Histopathology studies are carried out for all the study animals at the last day of study by sacrificing the animals. Liver tissue was isolated in all groups of study animals they were kept in 10%formalin solution and permanent tissue slides are prepared and observed. Histopathological observations indicate that no damage was happened to tissues of animals in all doses of acute toxicity studies as well as in low and high doses of sub acute toxicity studies. All the cells of livers are found to be normal they clearly indicate hexagonal arrangement no fat tissue accumulated central vein is clearly present no rupture or damage happened to hepatocytes. Both acute and sub acute toxicity studies clearly indicate that leaf extract of *Calotropis gigantean* is safe with least toxic effects and the plant extract can be carried forward for future toxicological studies followed by therapeutic exploratory studies. Drowsiness noticed in the study results indicates that plant extract having some effect on CNS. Hence plant can be further studied for antiepileptic and antipsychotic effects.

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