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Acute toxicity studies of Andrographolide

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ABSTRACT

Andrographolide is a diterpenoid lactone present in *Andrographis paniculata.* It is a major active constituent of *A.paniculata.* Extraction was carried out by cold maceration. Andrographolide was isolated from the extract. The present study has been designed with the objective to examine the andrographolide (isolated form *A.paniculata*) in order to evaluate its acute toxicities in experimental animal swiss albino mice. In acute toxicity studies the andrographolide 2000 mg/kg body weight was administered orally, observed after dosing and also observed for 14 days. Andrographolide effects on body weight, gross necropsy, hematological parameters, and biochemical parameters were studied. No significant variation in the body weight and organ weight between the control and the treated group was observed after single administration of andrographolide. Hematological and biochemical parameters of the control and the treated group revealed no toxic effect of the Andrographolide. No mortality was observed during 14 days study. From this study it may nontoxic through the oral route upto 2000mg/kg body weight dose level.

Keywords: *Andrographis paniculata*, Andrographolide, acute toxicity, cold maceration.

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INTRODUCTION

The Indian pharmacopoeia mentioned that it is a predominant constituent of at least 26 Ayurvedic formulations. Extensive research has revealed that *A. paniculata* has a broad range of pharmacological effect [1]. *A. paniculata* is a herbaceous plant belongs to family Acanthaceae, native to India and Srilanka mostly leaves and roots were used for medicinal purpose[2]. Andrographolide, Neo Andrographolide and Kalmeghnin present in the plant have been reported to be a active principles [3]. Andrographolide is a bicyclic terpenoid lactone having bitter taste with various activities [4]. Herbal drug therapy is the most trusted system of medicine in countries like India, where people strongly believe in Ayurveda as herbs are the part of rural Indian life style. Most of the diseases which have no medicine in allopathic system can be cured successfully using traditional medicines [5]. The present studies were undertaken to evaluate acute toxicity of Andrographolide in experimental animals.

MATERIALS AND METHODS

Collection and identification of plant materials

The aerial parts of *Andrographis paniculata*, were collected from Thirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The aerial part of *A. paniculata,* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Animals

Healthy swiss albino mice (20-25g) were divided into two groups (one treated groups and one control group) each group consists of three animals. Experiment animals were obtained from the central animal house, Raja Muthiah Medical College, Annamalai University. They were acclimatized to the standard laboratory conditions for two weeks prior to initiation of the study. All experiments were carried out according to the guideline for care and use of experimental animals and approved by Institution Animal Ethical Committee Affiliated (750/2010) to CPCSEA India.

Extraction isolation & Identification

Leaf powder *A. paniculata* was extracted exhaustively with a 1:1 mixtures of Dichloromethane and Methanol by cold maceration and solvent were removed by vacuum evaporation. The obtained extract was washed with toluene several times for removal of colored matters then the toluene was removed from the residue. The crystalline material left behind was dissolved in hot methanol and cooled in a refrigerator for crystallization. The isolated Andrographolide identified by compared with standard Andrographolide by IR & NMR [3].

Acute oral toxicity

For evaluation of acute toxicity a dose of 2000mg/kg was used in OECD guideline 423. The animals were examined at 30, 60,120min and at 4 and 24hrs for gross behavioral changes and mortality. Once daily cage side observations included changes in skin, fur, eyes, autonomic (salivation, lacrimation, urination and defecation) and central nervous system (ataxic, tremors and coma) changes. Mortality if any was determined over a period of two weeks [6].

Body weight analysis

Individual weight of animals was recorded before the administration of drug on 1st day of the study and 14th day of the experiment before withdrawal of the blood from the individual animals. Changes in the weight compared to that of the control animals [7].

Gross necropsy

All animals in the study were subjected to a full detailed gross necropsy like careful examination of the external surface of the body and abdominal cavities and their contents were examined. Organ weight (heart, kidney and liver) were also recorded [8].

Hematological parameters

Blood samples were collected by cardiac puncture of all the test mice and the control mice. Samples were analyzed for routine hematological parameters. Blood cell counts were done with blood smears [9].

Biochemical parameters

For the determination of total cholesterol, triglycerides, sugar, SGOT, SGPT, protein and Creatinine, Blood samples were collected separately for each of the group (Control and Treated) by using standard procedures [5].

Statistical Analysis

The experimental results were expressed as the mean ± SEM. Student'*t*' test for the sample treated group and control group were performed. The data were considered significant with probability less than 0.05.

RESULTS AND DISCUSSION

It is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Screening active compounds from plants has lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against

various diseases [10]. The isolated Andrographolide form *Andrographis paniculata* extract was characterized by its spectral analysis like (IR & NMR spectral analysis) and found that isolated Andrographolide matched well with that of reference standard Andrographolide.(result not disclosed). The study period of 14 days, there was no mortality or morbidity observed in the experimental animals followed by single administration of Andrographolide at dose of 2000mg.kg^{-1} body weight. The results of current study revealed no adverse change in cage side observation. All animals in the control and the treated group were found healthy as well as active. Similarly, no significant differences were observed in the body weight between the groups and data was presented in Fig.1.

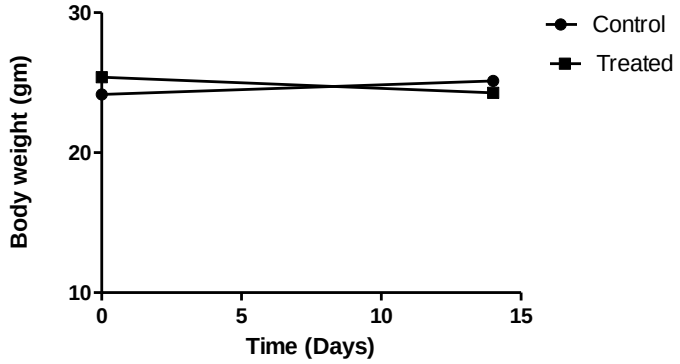

Fig 1: Effect of Andrographolide on the body weight

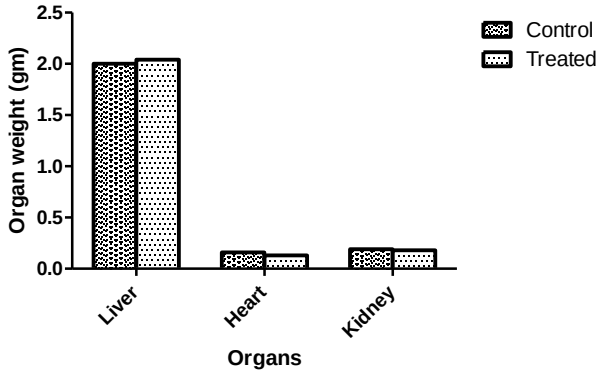

Fig 2: Effect of Andrographolide on the Organ weight

Morphological observation of abdominal content and organs like heart, liver and kidney were studied and found that there were no sign of inflammation or toxicity in both groups. Organ weight of the control and the treated groups were not significantly changed. The results were summarized in Fig 2.

All hematological parameters, which was useful in the detection of infection in the animal that may be due to adverse effect of the drugs administered. All hematological parameters of Andrographolide treated group was compared to that control group. There were no significant changes in hematological parameters like Hb, WBC, RBC, PCV and platelet counts. The results were shown in Table 1.

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Table 1: Effect of Andrographolide on hematological parameters

Parameter	Control Group(n=3)	Treated Group(n=3)
Hb (gm %)	15.05±0.44	14.1±0.37
WBC x(10^3/mm)	8.99±0.11	8.79±0.18**
RBC x(10^6/mm)	9.69±0.81	9.52±0.10
PCV (%)	42±2.3	41±1.15**
Platelets x(10^3/mm)	179±3.76	175±4.06

Mean ± SEM p<0.01,**p<0.001 value compared with control

Table 2: Effect of Andrographolide on biochemical parameters

Parameter	Control Group(n=3)	Treated Group(n=3)
Total cholesterol (mg /dl)	59 ±1.73	57.33 ±0.81
Triglycerides (mg /dl)	60 ± 0.58	57.33 ± 0.87
Sugar (mg /dl)	68.15±0.70	66.31±1.36
SGOT (IU/L)	28.22 ±1.14	27.89 ±0.71**
SGPT (IU/L)	71.74 ± 1.41	70.19 ±1.5**
Protein (mg/dl)	3.83 ±0.06	3.63 ±0.11
Creatinine	0.36±0.03	0.3± 0.05**

Mean ± SEM p<0.01,**p<0.001 value compared with control

Evaluation of hepatic and renal function is of prime important to assess the inherent toxic properties of drugs. However the results of assay of these enzymes in plasma revealed no difference between control and treated group. There were no significant changes in the total cholesterol, triglyceride, sugar level, SGOP, SGPT, Protein and Creatinine treated group when compared with control group. The biochemical parameters of the treated and control group were presented in Table 2.

CONCLUSION

In view of the increasing popular consumption of medicinal plants as after native therapy, it is necessary to conduct research to support the therapeutic claims and also ensure that the plants are indeed safe for the human consumption. The results of acute toxicity studies of Andrographolide clearly demonstrated that Andrographolide treated animals were devoid of any toxic sign and indicates that it is safe up to the dose of 2000 mg.kg^{-1} body weight.

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