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Acute And Chroniic Toxicity Study Of Ethyl Acetate Extract Of *Azima Tetracantha* Lam.

B Edwin Jose[1]*, and P Panneerselvam[2].

[1]Department of Pharmaceutical Chemistry, SSM College of Pharmacy, Erode, Tamil Nadu, India.
[2]Department of Pharmaceutical Chemistry, Bharath Institute of Higher education and research, Chennai, Tamil Nadu, India.

ABSTRACT

To evaluate acute and chronic oral toxicity of ethyl acetate extract of Azima tetracantha LAM. Extract was prepared by maceration in ethyl acetate. Acute oral toxicity test was conducted in wistar rats using the conventional test. Sixty mice were divided into 6 groups, namely control group and five test groups, each given the extract at 0.19 g/kg body weight (b.w), 0.56 g/kg b.w, 1.67 g/kg b.w, 5 g/kg b.w and 15 g/kg b.w, respectively. Chronic toxicity test was carried out in Wistar rat after daily administration of ethyl acetate extract of Azima tetracantha LAM for 90 days at 100 mg/kg b.w, 400 mg/kg b.w, and 1000 mg/kg b.w. The rats in all groups were observed for behaviour, body weight development, haematological, clinical biochemistry, organ to body weight ratio, and organ histology. No mortality was observed both in acute and chronic toxicity test in male and female animals. Observed behaviour, body weight profile and organ histology among experimental groups were comparable. Haematological, clinical biochemistry parameters, organ to body weight ratio were not significantly different ($p>0.05$). There were no toxic effects after the use of single dose and repeated dose of ethyl acetate extract of Azima tetracantha LAM in animal tested. Results of the present study suggest that ethyl acetate extract of Azima tetracantha LAM is safe after single administration at high dose and repeated administration during 90 days.

Keywords: Acute, chronic, Oral toxicity, Ethyl acetate extract, Azima tetracantha LAM

Corresponding author

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INTRODUCTION

Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. However, there is little information or evidence available concerning the possible toxicity that medicinal plants may cause to the consumers [1]. In relation to drug discovery and development, there are different weights of concern of all relevant groups such as health authorities, pharmaceutical industry, and patients which need to be taken into consideration [2].

Azima tetracantha (Salvadoraceae) is a well known medicinal herb, termed 'Mulsangu' in Tamil and 'Kundali' in Sanskrit. Root, root bark and leaves of Azima tetracantha (lam) are used with food as a remedy for rheumatism, diuretic and as stimulant. Traditionally Indian medical practitioners use Azima tetracantha (lam) in inflammatory conditions, cough, asthma, small pox and diarrhoea [3,4]. The major phyto-constituents reported in Azima tetracantha (lam) are azimine, azecarpin, carpine, isorhamnitine-3-O-rutinoside, friedelin, lupeol, glutinol and β-sitosterol [5,6]. Azima tetracantha (lam) is reported to have antifungal [7], antitumour [8], antidiabetic [9], antidiarrhoeal [10] and hepatoprotective [11] activities.

Azima tetracantha (lam) is a low, spinouts, highly branched bush, woody below but with pale green, herbaceous, almost quadrangular young branches. The leaves are in opposite to sub-opposite, decussate pairs. They are shortly petiolate, about 2x4cm long, entire, elliptic, acute, sharply mucronate, rigid, pale green with an acute base. Usually, there are two laterally placed spines in the axil of a leaf. The spines which morphologically represent the first pair of leaves of the auxiliary shoot are about three cm long, more or less, triangular in cross section, very sharp and with an indurate apex. The plant is dioeciously. The flowers are borne in the axils of leaves. Generally, there is cymes of three flowers in the axil of a leaf which is the upper branches, especially of the male plants become greatly reduced or even completely suppressed.

Besides efficacy, ethyl acetate extract of Azima tetracantha LAM should meet the safety requirement for its development as an alternative medicine. In this study acute and chronic toxicity test was carried out on the extract to explore its safety feature.

Uses

The plant is used in indigenous medicines for rheumatism, microbial infections, diahorrea, inflammatory conditions, reduce lipid and as hepato-protective.

MATERIALS AND METHODS

Collection of plants

The aerial part (leaves) of Azima tetracantha (lam) was collected from the Panayur area of Madurai, Tamilnadu as raw material, during the second week of February 2015 and a voucher specimen is stored in C.L. Baid Mehta College of Pharmacy (001/ATL/CLBP) and the plant material was authenticated by a renowned botanist. About 500 g of coarse powdered leaf in 2.5 L water is boiled, cooled and filtered. The filtrate is evaporated to dryness in desiccator and stored in refrigerator (Yield- 26.5% w/w). The all extracts of Azima tetracantha (lam) was subjected to preliminary phytochemical analysis [12,13]

Various extraction methods for isolation of constituents

The whole plant will be subjected to shade drying and extraction with petroleum ether (60-80^{o}C) chloroform, Ethyl acetate and 80% ethanol in soxhlet apparatus by simultaneous extraction each for 72 hours. Concentrate the solvents in vacuum. The crude solid obtained on evaporation are to be studied for preliminary qualitative phytochemical evaluation.

Phytochemical Screening

The extract was subjected to phytochemical analysis to test the presence of carbohydrates, lycosides, alkaloids, flavonoids, tannins, sterols, and saponins in leaf extracts.

Animals

Wistar rats weighing 180 – 200 gm of male sex , fed on standard diet and access to tap water ad libitum were used . They were housed in identical wire-mesh bottomed stainless steel cages and maintained in an air – conditioned room at 25± 2°c , 50-60% relative humidity and artificial illumination between 06:00 and 18:00 h. The animals were kept under controlled condition in accordance with the national institute of health (NIH) guidelines. All procedures concerning animal treatments and experimentations in the study were renewed and approved

Acute toxicity

The toxicity study was carried out using thirty-five (35) male wistar rats weighing 180-200 g each. The animals were randomly distributed into one control group and six treated groups, containing five animals per group. They were maintained on animal cubes , provided with water ad libitum and were allowed to acclimatize to the laboratory conditions for seven days before the experiment after overnight fasting , the control group received normal saline and treated group received ethyl acetate extract of AT at the doses of 0.5, 1, 1.5,2, 2.5 and 3 g/kg body weight. The calculated doses were prepared with 0.5% carboxy methyl cellulose (CMC) in distilled water. These doses were given by intraperitoneal route. The animals were observed continuosly for the first 4h and then each hour for the next 24 h and 6 hourly intervals for the following 48 h after administering the extract, to observe any death or changes in general behaviour and other physiological activities. Acute toxicity and gross behavioural screening were studied [14].

Chronic toxicity

Animals were divided into two groups were , one was control and other was test group . Dose of 100 mg/kg body weight /day of EA AT was administered in drinking water to each animal in the test group. This dose was selected on the basis of anti-diabetic effect of the EA AT in the mice in another experiment. The treatment was continued for a period of three months during which changes in body weight was observed mice were analyzed for body and organ weight changes , haematological studies, and serum biochemical parameters [15,16].

Body weight and organ weights

The body weights of mice of each group and after the chronic treatment were measured. After this period the animals were killed by cervical dislocation and weight of vital organs (heart, lungs , liver , kidney , spleen, testis ,seminal, caudae epididymis) were measured.

Haematological studies

The blood was analyzed for WBC, RBC and Hb level by using contravesdigicell 3100H (zurich)

Serum biochemical parameters

Collected blood from chronically treated animals at fasting , serum was separated and analyzed for aspartate amino transferase (AST) alanine amino transferase (ALT) , creatinine kinase iso-enzyme MB (CK-MB) ,Glucose, urea and creatinine. These parameters were analysed on a spectrophotometer (Ultrascope , LKB).

Statistical analysis

The results were expressed as mean± standard error of mean (SEM), and statistical analysis were carried out by student t-test. Differences were considered significant at $p<0.05$.

RESULTS

In acute toxicity and gross behavioural screening of EAAT, the tested animals had no sign of toxicity and mortality up to the dose of 3g/kg body weight. However, during gross behavioural screening some sign of

passivity and decrease spontaneous activity were observed in group 3 animals who received 3g/kg body weight of EAAT at 1-4 hour.

In chronic toxicity, no symptoms of toxicity were observed upon chronic treatment with EA AT during 0-30 day; however two male mouse died in control group. While in treated group two mice died (one male mouse during 0-30 day and other male mouse died on day 70). After chronic treatment with EA AT, the total body weight gain in post treated group of male mice was significant ($p < 0.05$) as compared to pre-treated male mice group. While in post treatment, weight gain in male mice was highly significant ($p < 0.005$) as compared to pre-treatment male mice group. Organ weight (per 100 g body weight) in mice after chronic treatment with EA AT (100mg/kg body weight/day) was normal and comparable to the control.

While in biochemical parameters, the results showed a significant decrease ($p < 0.05$) in blood glucose level of treated animals as compared to control. There was no significant difference in other parameters studied as compared to the control group except mild reduction in CK-MB and slight rise in serum AST, which was statistically non-significant. Hematological studies revealed no significant difference in level of WBC, RBC and Hb levels in treated animals as compared to control.

DISCUSSION

Medicines of herbal origin are nowadays used as alternative to synthetic medicine. Therefore it is essential to carry out the safety and efficacy of these herbal products by experimental studies. In acute toxicity and gross behavioural study, the dose was safe up to 3g/kg body weight of wistar rats . No sign of toxicity and mortality were observed. During chronic toxicity study there were no toxic symptom during 0-30 days, except two deaths one between 0-30 and other between 61-90 day interval in the treatment group. All the treated male and female mice throughout the study were normal and comparable to the control.(Table 1)

Gaining body weight in both group rat indicates that the extract does not interfere with growth processes and may have promoted growth by stimulating the synthesis of body proteins. This increase in weight may be due to increase of appetite by the extract. Biochemical studies revealed the significant decrease in blood glucose level after chronic oral treatment. The hypoglycemic action of this extraction might be due to its insulin mimetic action or by some other mechanism. Antioxidant property of the extract in preventing these changes may also be considered to play a vital role. (Table 2)

There were no significant differences in other parameters studied as compared to control except mild reduction in CK-MB and slight rise in serum AST, which was non-significant. Similarly haematological studies revealed no significant difference in the level of WBC, RBC, and Hb levels in treated animals as compared to the control group. . (Table 3&4)

The present acute, gross behavioural and chronic toxicity results of EAAT tend to sup-port the safe folklore use of this plant as a drug. In the light of the results and above discussion, it is concluded that EAAT is safe in Swiss albino mice. It did not affect the biochemical and haematological parameters and also has no effect on the growth of vital body organs. However, further studies are necessary to be carried out about its safety in other species of rodents and humans.

Table 1: Mortality in chronic toxicity study with ethyl acetate extract of Azimatetracantha in rat

TREATMENT	DEATH OF MICE IN NUMBER(MALE)				LETHALITY %
	0-30 days	31-60 days	61-90 days	Total	
Control	1,1	0,0	0,0	2	10
EA AT	1,0	0,1	1,0	2	10

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Table 2: Change in body weight after 90 days treatment of ethyl acetate extraction of Azimatetracantha in rat

Treatment N=20	Body weight in gram mean±(SEM)	
	PRE TREATMENT	POST TREATMENT
Control	24.4± 1.3	30.7± 1.2
EA AT	23.8± 1.1	27.3± 1.5

Table 3: Change in organ weight with 90 days treatment of ethyl acetate extraction of Azimatetracantha in rats

Organs (n=5)	Organ weight in gram/100g body weight (Mean ± SEM)	
	control	Test
Heart	0.49 ± 0.01	0.50 ± 0.03
Lungs	0.79 ± 0.06	0.82 ± 0.09
Liver	6.00 ± 0.14	6.57 ± 0.24
Kidney	1.61 ± 0.05	1.72 ± 0.04
Spleen	0.52 ± 0.08	0.62 ± 0.04
Testis	0.66 ± 0.02	0.65 ± 0.04
Seminiferous tubules	0.58 ± 0.03	0.56 ± 0.03
Caudaeepidedymis	0.23 ± 0.02	0.25 ± 0.02

Table 4: Effect of 90 days treatment by ethyl acetate extraction of Azimatetracantha on blood parameters in mice

Parameters	Control (n=5)	EA CT (n=5)
ALT (U/L)	24.63 ± 4.71	27.35 ± 7.61
AST (U/L)	56.88 ± 6.32	57.73 ± 9.50
CK-MB (%)	127.55 ± 16.26	125.71 ± 17.83
Creatinine (µmol/L)	66.14 ± 4.76	65.95 ± 3.39
Urea (µmol/L)	6.71 ± 0.74	6.56 ± 1.52
Glucose (µmol/L)	11.42 ± 0.85	9.14 ± 0.64*
WBC(x 103) /mm3	5.6 ± 0.7	5.7 ± 0.9
RBC (x 106) /mm3	7.9 ± 0.2	6.8 ± 0.3
Hemoglobin(g/dl)	12.4 ± 0.3	13.0 ± 0.4

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