ORIGINAL ARTICLE

Toxicity study of ethanolic extract of *Acorus calamus* rhizome

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Acorus calamus is widely used in traditional medicine in various ailments. However, there is no toxicological information available regarding its safety after exposure. The present study was designed to evaluate potential toxicity of an ethanolic extract of *Acorus calamus* Linn. rhizomes after acute and chronic administration in Wistar rats. In the acute toxicity study, female Wistar rats were treated with ethanolic extract by oral gavage at dose levels of 175, 550, 1750 and 5000 mg/kg body weight according to OECD 425. Animals were observed periodically during the first 24 h after administration of the extract, and daily thereafter for 14 days. In the chronic toxicity study, the ethanolic extract of *Acorus calamus* was administered orally at doses of 0, 200, 400 and 600 mg/kg body weight daily for 90 days in Wistar rats. The effects on clinical signs, body weight, food consumption, organ weight, haematology, clinical biochemistry, as well as histology, were studied. No mortality was observed, but clinical signs like abdominal breathing, piloerection and tremors were observed for 30 min in rats dosed with 1750 mg and 5000 mg/kg body weight of extract. No statistical significant data in body weight and feed consumption were observed. Haematological and biochemical analysis showed no marked differences in any of the parameters. Pathologically, neither gross abnormalities nor histopathological changes were observed. The ethanolic extract of *A. calamus* does not appear to have toxicity on acute and chronic administration in Wistar rats.

Key words: *Acorus calamus*, acute toxicity, chronic toxicity

INTRODUCTION

Acorus calamus (L.) (Araceae) is a perennial, semiaquatic and smelly plant found in the northern temperate and subtropical regions of Asia, North America, and Europe. It is six feet tall, aromatic herb with creeping rhizomes. The leaves are long, slender, sword-shaped and simple, arising alternately from the horizontal rhizomes. These are longitudinally fissured with nodes, somewhat vertically compressed and spongy internally. Flowers are small and fragrant with pale green spadix; fruits are three-celled fleshy capsule.[1-7]

All parts of the plant contain volatile oil having terpenoids, calamine, calamenol, calamenone, eugenol, camphene, pinene and asaronaldehyde. Acorafuran is a sesquiterpenoid found in calamus oil.[8-11] The rhizomes are utilized extensively by the Chinese, Indians and American Indians as well as by other cultures.[9] Its roots and rhizomes are used in treatment of various ailments including mental disorders, such as hysteria, insanity, insomnia, melancholia, neurasthenia, epilepsy, diarrhoea and asthma.[12,13] The leaves extract of *Acorus calamus* were studied for anti-inflammatory activity on keratinocyte HaCaT cells.[14] The roots and rhizomes extracts of *Acorus calamus* have been reported with various pharmacological activities such as analgesic,[12] cardiovascular,[15,16] anticonvulsant,[17] hypolipidemic,[18] antispasmodic,[19] anti-inflammatory,[20] antibacterial,[21] antiulcer and cytoprotective activity.[12] In most of studies the roots and rhizomes extracts of *A. calamus* reported for its CNS activities.[12,13,22-24]

In an earlier report, it has been shown that ethanolic extract of rhizome of this plant possesses immunosuppressive,[25] sedative, analgesic, moderately hypotensive and respiratory depressant properties.[15] *Acorus calamus* extract is also used in traditional Chinese prescription and its beneficial effects on memory disorder, learning performance,[25] lipid peroxide[26] content and its senescence effect have been reported.

Despite the wide use of *A. calamus* extract in traditional medicine, no study has been reported in the scientific literature about its toxicity. Hence, the present study was carried out to determine acute and chronic toxicity

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of the ethanolic extract of rhizomes of *A. calamus* (EAC) in Wistar rats.

MATERIALS AND METHODS

Plant Material

A. calamus rhizomes were obtained from the local market of Surat, Gujarat, India. The samples were identified and authenticated by Dr. Minoo Parabia (Professor of Botany) Veer Narmad South Gujarat University, Surat, Gujarat, India. The voucher specimen (R001) is kept in the Pharmacology Department at SPTM, SVKM's NMIMS University, Mumbai. The plant material was shade-dried, milled to powdered form and stored in airtight containers.

Preparation of EAC

Rhizome powder of *A. calamus* was extracted with 95% ethanol (Qualigens fine chemicals, Mumbai) for 12 hours in Soxhlet extractor. The obtained extract was concentrated using rotary vacuum evaporator (BUCHI, Japan) at 40-60°C. The concentrated semisolid extract was stored in refrigerator at 2-8°C till further use. The yield (w/w) of the ethanolic extract was found to be 27%.

Phytochemical Analysis

Preliminary phytochemical investigations of EAC for the presence of active phytoconstituents such as carbohydrates, alkaloids, proteins, volatile oils, triterpenes, flavonoids, saponins, phenols, resins and tannins were carried out using the methods previously described.[27]

Preparation of Dosing Solution

EAC (200,400,600 mg) was dissolved in 10 ml of 0.5% carboxy methyl cellulose (CMC) each to obtained concentration of 20, 40, 60 mg/ml, respectively, and administered orally at the dose volume of 10 ml/kg body weight to achieve the dose level of 10 mg/kg body weight.

Acute Toxicity Study

Female Wistar rats, 8-10 weeks old (150-200 g) were obtained from Breeding Facility of Jai research foundation, Vapi, India. Animals were kept under controlled environmental conditions (22±0.5°C, relative humidity 65-67%, 6 am to 6 pm alternate light–dark cycles, food and water *ad libitum*) in polypropylene cages covered with stainless steel grid and an autoclaved clean rice husk breeding. The animals were allowed to acclimatize for seven days prior to the commencement of experiment. The animal protocol was approved by the Institutional Animal Ethical Committee (IAEC) protocol number R-290 as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India.

Rats had free access to drinking water filtered through an Aquaguard water filter system tap water and nutrilab rodent feed, except overnight fasting before the treatment with a single dose of the EAC. EAC was suspended in 0.5% CMC on the day of the experiment. Dose progression slope 2 of OECD 425[28,29] was followed throughout the study with the starting dose of 175 mg/kg body weight to 5000 mg/kg body weight. The rats were observed for signs of toxicity and mortality at 0.5, 1, 2, 3, 4 and 6 hours post dosing. Subsequently, the rats were observed twice a day for morbidity and mortality for a period of 14 days following oral dosing. The clinical signs were recorded once a day. Individual animal body weight was recorded prior to dosing on day 0, 7 and 14. At the end of the 14 days observation period, all the treated rats were euthanised by carbondioxide asphyxiation. They were subjected to gross pathological examination, consisting of external examination and opening of the abdominal and thoracic cavities. Abnormalities, if any were recorded. $etal_{50}$ *Journal of Applied Toxicology*The LD_{50} values were determined according to the method of Dixon's maximum likelihood method using software (AOT 425 StatPgm).

Chronic Toxicity Study

Wistar albino rats of either sex, 5-6 weeks old (100-190 g), were housed in polypropylene cages covered with stainless steel grid top (one in a cage) under the standard conditions. The animals were divided into four groups (I, II, III and IV) of ten rats each (five females and five males). EAC was suspended in 0.5% CMC on the day of the experiment, and was given daily in morning by oral gavage for 60 days at doses of 200 mg/kg (Group II), 400 mg/kg (Group III), and 600 mg/kg (Group IV), while the control rats (Group I) received only the vehicle. Toxic manifestations and mortality were monitored for 60 days. Clinical signs were recorded immediately after dosing, if any. Body weight, feed consumption were measured weekly. At the end of study, blood samples were obtained by the retro-orbital puncture under light diethyl ether anaesthesia, with or without anticoagulant (ethylenediamine tetraacetate). Blood with the anticoagulant was used immediately for the determination of haematological parameters, while blood without the anticoagulant was centrifuged at 1789 × g for 10 min at 4°C, and the serum obtained was stored at −20°C until analyzed for biochemical parameters. After blood collection, rats were sacrificed by carbon dioxide asphyxiation. All organs were examined grossly, weighed and collected for histopathological examination.[30]

Haematology

The haematological analysis was performed using automatic haematological analyzer (Sysmex, Japan). Total red blood cells (RBC), leukocyte (WBC), haematocrit, haemoglobin, platelet count, clotting time, erythrocyte

indices: -mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and differential leucocyte count, reticulocyte count (Leishman's stain) of blood sample were recorded.

Clinical biochemistry

Glucose, total cholesterol, triglyceride, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, gamma glutamyltranspeptidase (GGT), total protein, total bilirubin, albumin, globulin, albumin:globulin ratio (A/G ratio), total creatinine, blood urea nitrogen (BUN), urea, calcium and phosphorus were determined using auto analyzer (BT-2000, Japan).

Pathological examination

Gross necropsy

All animals in the study were subjected to detailed gross necropsy, which includes careful examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents. The adrenals, testes, urinary bladder, thyroid and parathyroid, kidneys, brain, heart, spleen, liver, lungs and thymus of all animals were trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying.

Histopathology

Histopathological examination of control and high dose group was performed for all gross lesions and for brain, spinal cord, stomach, small and large intestines, liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs, gonads, accessory sex organs (e.g., uterus, prostate), urinary bladder, lymph nodes, peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, and a section of bone marrow by fixing immediately in 10% formalin for routine histopathological examination. Tissues were embedded in paraffin wax; sections of tissues were cut at 3-5 m thickness with microtome and stained with haematoxylin and eosin for microscopical examination.

Statistical Analysis

Data were expressed as mean±S.E.M and assessed statistically by appropriate statistical methods (Bartlet's, ANOVA, Dunnett's '*t*' test and Student'*t*' test) were employed to assess the significance among different groups with significance level of $P<0.05$.

RESULTS

Phytochemical Analysis

Presence of carbohydrates, alkaloids, proteins, volatile oils, triterpenes, flavonoids, saponins, resins and tannins were indicated by phytochemical investigations of EAC.

Acute Toxicity Study

The results of the acute toxicity study of EAC are presented in Table 1. No mortality or change in body weight was observed in rats al dose level of the *A. calamus* extract 550 mg/kg, and 750 mg/kg body weight. Some clinical signs such as tremors, pilo erection and abdominal breathing were observed immediately after the oral dosing of 1,750 and 5,000 mg/kg body weight of EAC but no mortality or change in body weight were observed. No significant changes were observed in gross necropsy on day 14.

The acute toxicity data indicated that the calculated LD_{50} value (Dixons likelihood method) for the oral doses of the EAC was found to be more than 5,000 mg/kg body weight.

Chronic Toxicity Study

Clinical signs

No clinical signs like tremors, convulsions, piloerection, aggression, lethargy, abdominal breathing, gait and licking were observed throughout the study period of 90 days.

Body weight and feed consumption

Changes in body weight and feed consumption of control and extract treated rats are presented in Figures 1-4, respectively. Rats in control group gained weight with time (as expected), with no significant difference in feed consumption and weight gain at the end of 90 day. Moreover, no mortality was recorded up to the dose of 600 mg/kg, during the 90-day treatment.

Haematological parameters

The values for the haematological parameters in treated and control rats are shown in Table 2. Level of total RBC, leukocyte (WBC), hematocrit, and hemoglobin, platelet count, clotting time, Erythrocyte indices: -mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and Differential Leucocyte Count, Reticulocyte count (Leishman's stain) on chronic oral administration of EAC (daily for 90 days) did not exhibit any significant changes in any of hematological parameters. All values remained

Table 1: Clinical signs of toxicity observed during acute oral toxicity study of ethanolic extract of *Acorus calamus* in Wistar rats

Dose (mg/kg body weight)	Latency	Symptoms
175	-	None
550	-	None
1750	-	Piloerection, abdominal breathing
5000	-	Tremor, Piloerection, abdominal breathing
5000	-	Tremor, Piloerection, abdominal breathing
5000	-	Tremor, Piloerection, abdominal breathing

No symptoms observed during the observation period; latency – Time of death after the dose

Table 2: Effect of ethanolic extract of *Acorus calamus* on haematological parameters in chronic toxicity study for 90 days

Parameter	Male				Female			
	Control	EAC (mg/kg body weight)			Control	EAC (mg/kg body weight)		
		200	400	600		200	400	600
RBC count (10^6/μl)	7.48±0.23	7.68±0.17	7.59±0.31	7.29±0.26	6.16±0.13	6.11±0.08	6.23±0.19	6.19±0.26
Reticulocyte (%)	1.5±0.06	1.44±0.05	1.44±0.06	1.45±0.05	1.71±0.03	1.69±0.04	1.7±0.04	1.69±0.05
Hb (g/dl)	14.6±0.17	14.62±0.14	14.58±0.47	14.6±0.38	13.18±0.36	13.14±0.08	13.24±0.24	13.16±0.18
HCT (%)	40.78±0.82	40.60±0.48	38.68±1.32	40.8±1.23	37.28±1.07	37.32±0.19	37.22±0.93	37.28±1.23
MCV (fl)	54.64±1.26	55.72±0.80	57.08±1.18	56.08±0.76	60.46±0.45	57.82±0.86	56.64±1.19	56.08±0.76
MCH (pg)	19.56±0.52	18.02±0.25	19.44±0.51	20.06±0.25	21.38±0.17	20.48±0.21	20.6±0.57	20.06±0.25
MCHC (g/dl)	35.82±0.31	36.28±0.25	35.79±0.59	35.82±0.30	29.16±6.44	35.44±0.30	36.34±0.52	35.82±0.30
WBC count (10^3/μl)	4.92±0.51	5.28±0.22	5.38±0.45	4.44±0.51	2.94±0.34	3.04±0.18	4.26±0.66	3.66±0.62
Lymphocyte (%)	71.6±1.69	75.6±0.75	73.4±1.29	72.4±1.03	76.6±2.11	72.8±1.39	72.4±2.73	70.4±1.69
Neutrophil (%)	26.6±1.03	23.2±0.86	25.6±1.44	25.8±0.58	22.4±2.50	25.2±1.16	27±2.49	28±1.87
Monocyte (%)	1.6±0.75	0.6±0.40	0.8±0.49	1±0.77	0.4±0.40	1.8±0.66	0.6±0.25	1.2±0.58
Basophil (%)	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00	0±0.00
Eosinophil (%)	0.2±0.20	0.6±0.00	0.2±0.00	0.8±0.00	0.6±0.40	0±0.00	0.2±0.00	0.4±0.00
Platelet (103/μl)	707±122.18	699±62.69	685.6±93.02	702±52.79	631.4±54.25	690.2±42.43	662±64.65	702±52.79
Clotting time (seconds)	120±13.42	120±13.42	108±7.35	120±0.00	114±11.23	144±11.23	120±9.49	108±7.35

Values are expressed as Mean±S.E.M (*n*=5). *P<0.05; EAC – *Acorus calamus*; RBC – Red blood cells; Hb – Haemoglobin; HCT – Haematocrit; MCV – Mean corpuscular volume; MCH – Mean corpuscular haemoglobin; MCHC – Mean corpuscular haemoglobin concentration; WBC – White blood cells

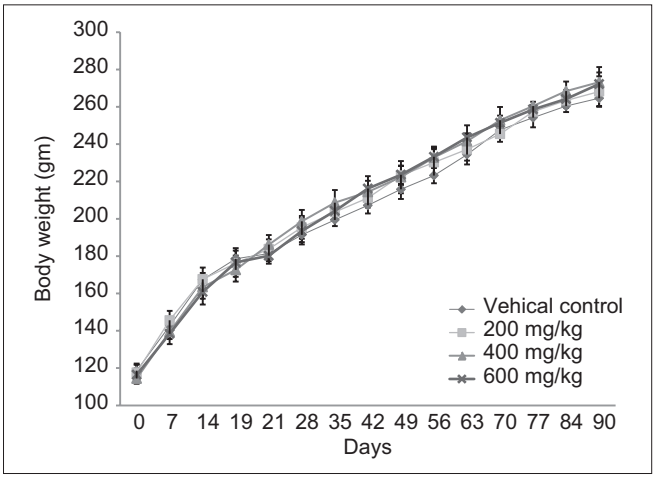

Figure 1: Effect of ethanolic extract of *Acorus calamus* (EAC) on body weight in male rat in chronic toxicity study. Each point represents Mean±S.E.M.

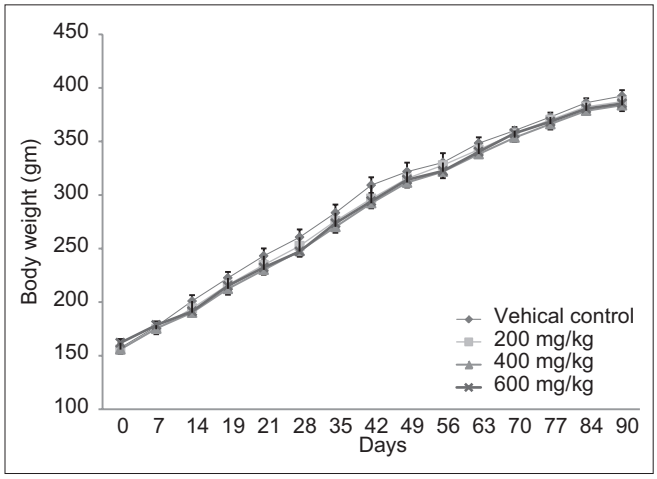

Figure 2: Effect of ethanolic extract of *Acorus calamus* (EAC) on body weight in female rats in chronic toxicity study. Each point represents Mean±S.E.M

within physiological range throughout the treatment period.

Biochemical parameters

The values for biochemical parameters in treated and control rats are presented in Table 3. Chronic oral administration of EAC (daily for 90 days) did not show any significant changes in any of the biochemical parameters. All values remained within physiological range throughout the treatment period.

Pathological examination

There was no significant difference between the control and treated groups in the organ weights of male and female rats [Table 4]. No alternations were detected in pathological examination of the tissues during the microscopic examination of the internal organs. No histopathological findings could be attributed to EAC and no differences found in the organ morphology across the treated groups.

DISCUSSION

Phytotherapeutic products are, many times, mistakenly regarded as safe because they are "natural". Nevertheless, those products contain bioactive principles with potential to cause adverse effects. A Word Health Organization (WHO) survey indicated that about 70-80% of the world's populations rely on non-conventional medicine, mainly of herbal source, in their primary healthcare. In addition,

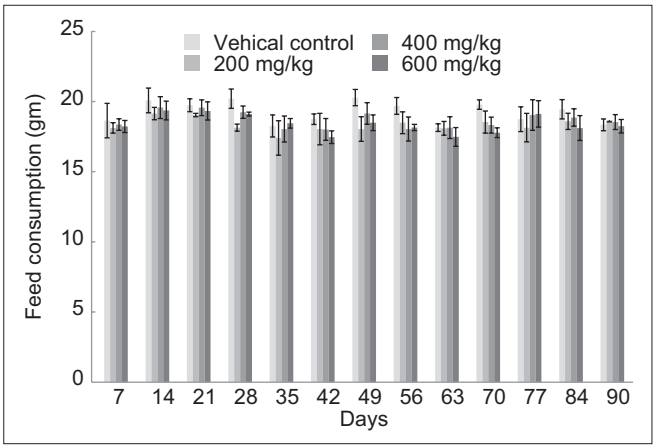

Figure 3: Effect of ethanolic extract of *Acorus calamus* (EAC) on feed consumption in male rats in chronic toxicity study. Data are expressed as Mean±S.E.M

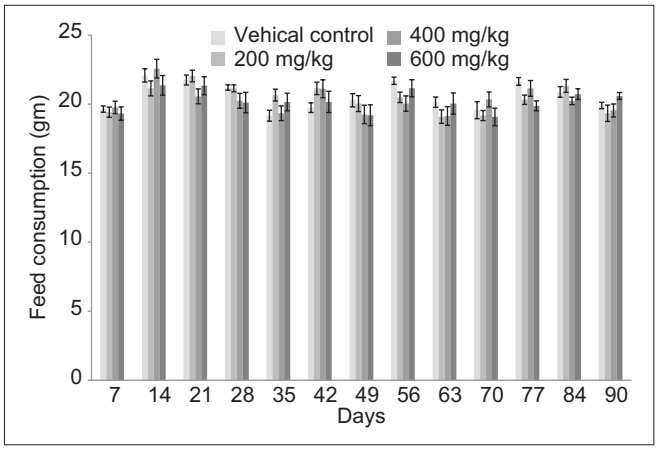

Figure 4: Effect of ethanolic extract of *Acorus calamus* (EAC) on feed consumption in female rats in chronic toxicity study. Data are expressed as Mean±S.E.M

Table 3: Effect of ethanolic extract of *Acorus calamus* on biochemical parameters in chronic toxicity study for 90 days

Parameter	Male				Female			
	Control	EAC (mg/kg body weight)			Control	EAC (mg/kg body weight)		
		200	400	600		200	400	600
GLU (mg/dL)	65±10.08	63.44±5.59	67.34±7.60	68.12±4.81	72.2±8.03	71.72±3.63	71.28±11.48	71.42±8.09
CHO (mg/dL)	79.04±4.98	83.94±3.11	83.9±2.97	83.7±3.60	85.44±9.10	89.58±11.29	87.66±11.59	90.18±2.50
TRIG (mg/dL)	109.5±16.26	103.08±13.24	109.46±11.96	105.44±17.68	83.46±5.20	83.22±8.93	89.3±24.45	75.2±6.40
ALT (IU/L)	57.44±12.41	56.1±5.04	54.42±2.61	56.74±3.60	38.96±0.72	36.16±3.90	42.22±4.06	61.94±2.67
AST (IU/L)	35.8±7.66	34.28±5.45	35.22±4.72	35.22±5.24	34.1±8.46	35.28±3.87	35.84±1.56	34±4.96
ALP (IU/L)	98.48±5.31	118.08±11.11	114.7±5.89	111.28±19.10	88.16±5.78	85.2±13.01	89.44±5.88	89.84±6.77
GGT (IU/L)	2.4±0.75	2.1±0.10	2.22±0.14	1.92±0.33	0.48±0.24	1.66±1.00	2.22±1.28	0±0.00
T.PRO (g/dL)	7.06±0.30	7.36±0.12	8.84±0.33	8.08±4.73	9.04±0.14	8.24±0.30	8±0.53	8.28±0.22
T.BIL (mg/dL)	0.06±0.04	0.04±0.00	0.06±0.00	0.08±0.00	0.28±0.07	0.26±0.04	0.4±0.07	0.24±0.05
ALB (g/dL)	5.22±0.12	5.24±0.07	5.14±0.16	5.24±0.04	6.56±0.10	6.18±0.17	5.72±0.31	6.38±0.14
GLB (g/dL)	1.84±0.19	2.12±0.07	1.82±0.18	7.84±4.74	2.5±0.13	2.32±0.16	2.28±0.26	2.48±0.12
ALB:GLB	2.92±0.26	2.59±0.07	2.89±0.11	2.66±0.38	2.66±0.16	2.45±0.17	2.56±0.21	2.36±0.10
CRE (mg/dL)	0.74±0.04	0.68±0.04	0.8±0.06	0.94±0.05	1.08±0.07	1.06±0.03	1.12±0.07	0.88±0.06
BUN (mg/dL)	22.2±0.97	22.22±2.01	22.9±2.42	22.38±1.14	28.26±2.80	30.12±1.86	30.18±1.80	28.26±0.91
UREA (mg/dL)	47.52±2.05	47.56±4.32	47±4.16	47.58±2.57	60.46±5.98	64.48±3.99	64.58±3.81	60.5±1.95
CAL (mg/dL)	9.58±0.27	9.84±0.34	9.58±0.32	9.62±0.31	11.66±0.29	10.74±0.29	10.54±0.20	10.78±0.28
PHO (mg/dL)	7.06±0.60	8.12±0.46	8.08±0.18	8.14±0.18	5.4±0.47	5.66±0.54	5.68±1.14	5.54±0.05

Values are expressed as Mean±S.E.M (n=5). *P<0.05. EAC – *Acorus calamus*; GLU – Glucose; CHO – Total cholesterol; TRIG – Triglyceride; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase; ALP – Alkaline phosphatase; GGT – Gamma glutamyltranspeptidase; T.PRO – Total protein; T.BIL – Total bilirubin; ALB – Albumin; GLB – Globulin; CRE – Creatinine; BUN – Blood urea nitrogen; CAL – Calcium; PHO – Phosphorous

Table 4: Effect of ethanolic extract of *Acorus calamus* on organ weight (g) in chronic toxicity study for 90 days

Parameter	Male				Female			
	Control	EAC (mg/kg body weight)			Control	EAC (mg/kg body weight)		
		200	400	600		200	400	600
Adrenals	0.092±0.01	0.091±0.01	0.09±0.01	0.085±0.01	0.108±0.01	0.105±0.01	0.119±0.01	0.11±0.01
Testes	3.446±0.05	3.339±0.11	3.125±0.12	3.054±0.14	-	-	-	-
Urinary bladder	0.142±0.01	0.219±0.06	0.129±0.01	0.135±0.02	0.093±0.01	0.1±0.01	0.089±0.00	0.125±0.02
Thyroid and parathyroid	0.027±0.00	0.025±0.00	0.033±0.00	0.036±0.00	0.032±0.00	0.026±0.00	0.022±0.00	0.025±0.01
Kidneys	2.41±0.07	2.296±0.09	2.364±0.15	2.304±0.06	1.471±0.05	1.681±0.07	1.516±0.06	1.527±0.06
Brain	2.132±0.05	2.052±0.06	2.048±0.03	2.095±0.04	1.921±0.05	1.933±0.02	1.89±0.03	1.97±0.08
Heart	1.192±0.09	1.008±0.03	1.102±0.04	1.13±0.03	0.779±0.01	0.746±0.03	0.713±0.03	0.738±0.02
Spleen	0.646±0.04	0.572±0.02	0.507±0.03	0.633±0.05	0.427±0.01	0.533±0.06	0.408±0.02	0.424±0.02
Liver	10.854±0.31	10.555±0.29	10.124±0.14	10.408±0.51	6.713±0.46	7.331±0.11	7.312±0.47	6.704±0.14
Lungs	1.773±0.09	1.885±0.15	3.088±0.38	3.308±0.18	1.48±0.13	1.444±0.06	1.347±0.08	1.45±0.13
Thymus	0.506±0.04	0.423±0.02	0.511±0.07	0.457±0.06	0.395±0.02	0.432±0.03	0.394±0.03	0.422±0.06

Values are expressed as Mean±S.E.M (n=5). *P<0.05; EAC – *Acorus calamus*

the poor pharmacovigilance services in this area make it difficult to determine the frequency of adverse effects caused by the use of phytotherapeutic products. Thus, all the "natural" products used in therapeutics must be submitted to efficacy and safety tests by the same methods used for new synthetic drugs.[31]

Although medicinal plants may produce several biological activities in humans, but very little is known about their toxicity and the same applies for *Acorus calamus*.

Usually acute (single dose) toxicity study is carried out on laboratory animals by using high dose (sufficient to produce death or morbidity) of the substance in question and/or based on previous report on its toxicity or toxicity of structurally related compounds. There was no previous report on toxicity of EAC. Therefore, the doses starting at 175, 550, 1,750 and limit dose 5,000 mg/kg were selected for acute toxicity study, as per OECD 425. No mortality was observed in animal at all selected dose levels.[29]

Some signs of toxicity were observed on administration of doses 1,750 and 5,000 mg/kg body weight Table 1, but they were all reversible in a maximum period of 24 h after the administration of the extract. The LD_{50} value was found to be more than 5,000 mg/kg body weight for EAC.

There is no real correlation between acute dose LD_{50} and prediction of adverse effects of chronic daily dosing. In addition, the LD_{50} in animals does not predict the human lethal dose of a drug or the symptomatology of acute poisoning after overdose. Nevertheless, the acute dose study provides a guidance for selecting doses for the chronic low-dose study, which may be more clinically relevant.[32]

Therefore, on basis of clinical observations of acute toxicity study of ethonolic extract of *Acorus calamus*, doses 200 mg/kg, 400 mg/kg and 600 mg/kg were selected for chronic toxicity study.

No mortality or clinical signs were observed with the above mentioned dose levels. However, it is very important as it indicates the capability of reaction of the organism that received the drug.

No statistical significant reduction in body weight Figures 1 & 2 and feed consumption Figures 3 & 4, respectively, were observed. Body weight changes if any are an indicator of adverse side effects, as the animals that survive cannot lose more than 10% of the initial body weight. The determination of such parameters is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients and water are essential to the physiological status of the animals and to the accomplishment of the proper response to the drug tested instead of a "false" response due to improper nutritional conditions.[33]

After 90 days of treatment, there were no significant changes in the hematological parameters between control and treated groups [Table 2].

The results indicate that the *Acorus calamus* extract was neither toxic to the circulating red cells, white blood cells and platelets nor interfered with their production. Haematopoiesis and leucopoiesis were also not affected even though the haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals.[34,35] Therefore, it plausible to assume that the extract is not haematotoxic.

All biochemical parameters were in range for 90 days indicating no sever liver or kidney damage [Table 3]. Significant changes in enzymes like ALP, AST and ALT represent liver impairment, since these are important indices of liver toxicity.[29] Serum cholesterol and proteins are mainly regulated via synthesis in the liver and increase or decrease in serum concentrations of constituents suggests liver toxicity.

Kidney toxicity has also been reported after use of phytotherapeutic products what makes essential its evaluation. In that case, creatinine and urea determinations are critical as these substances are markers of kidney function.[32] This was also confirmed through histopathological examination of the kidney.

The organ weight examinations of important organs like adrenals, testes, urinary bladder, pancrease, thyroid and parathyroid, kidneys, brain, heart, spleen, liver, lungs and thymus indicated no significant difference [Table 4].

Further, the safety of EAC in rats was supported by histopathological examinations, suggesting that there was no difference or sever damage in all important organs.

The studies carried out suggest that in 600 mg/kg dose, the extract seems to be safe. Thus, considering that *Acorus calamus* extract to be used for undetermined time, further studies in non-rodents must be performed to prove its safety.

CONCLUSION

The present investigation demonstrates that the traditional medicine, the ethanolic extract of *Acorus calamus* lack potential toxicity, as it neither cause any lethality nor

changes the general behaviour in both the acute and chronic toxicity studies.

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REFERENCES

1. Nadkarni KM. Indian Materia Medica. 3rd ed. Bombay, India: Popular Prakashan; 2007. p. 35-7.
2. Prajapati ND, Purohit SS, Sharma DD, Tarun k. A Handbook of Medicinal Plants. 1st ed. Jodhpur, India: Agrobiaos; 2003. p. 13-4.
3. Wyk VB, Wink M. Medicinal plants of the world. Times ed. Pretoria: Bria publication. 2004. p. 32.
4. The Wealth of India, Raw Material. Revised ed. New Delhi: Council of scientific and industrial research; 2005. p. 63-5.
5. Kapoor LD. Handbook of Ayurvedic Medicinal Plants. 1st ed. Boca Raton, London: CRC Press; 2005. p. 18-9.
6. Kirtikar KR, Basu BD. Indian medicinal plants. Vol. 3. Dehradun: International book distributors; 1999. p. 2034.
7. Agrawal SS, Paridhvai M. Herbal Drug technology.1st ed, Vol. 4. Hyderabad Universities press private limited, Distributed by Orient Longman private limited; 2007. p. 4:350-1,354,358-60.
8. Tkachev AV, Gurev AM, Yusubov MS. Acorafuran a new sesquiterpenoid from *Acorus calamus* essential oil. Chem. Nat. Compd.2006;42:696-8.
9. Pandy V, Jose N, Subhash H. CNS activity of methanol and acetone extracts of *Acorus calamus* leaves in mice. J Pharmacol Toxicol 2009;4:79-86.
10. Shiva MP, Lehri A, Shiva A. Aromatic and medicinal plants. 1st ed. Dehradun: International book Distributors Book Seller And Publishers; 2002. p. 677-81.
11. Bruneton J. Pharmacognosy - phytochemistry medicinal plants. 2nd ed. USA Lavoisier publishing, intercept Ltd; 2001. p. 571-2.
12. Mukherjee PK. Quality control of herbal drugs-An approach to evaluation of botanicals. 2nd ed. Business horizon, Kolkata Pharmaceutical Publishers. 2007. p. 688-94.
13. Hazra R, Ray K, Guha D. Inhibitory role of *Acorus calamus* in ferric chloride-induced epileptogenesis in rat. Hum Exp Toxicol 2007;26:947-53.
14. Kim H, Han TH, Lee SG. Anti-inflammatory activity of a water extract of *Acorus calamus* L. leaves on keratinocyte HaCaT cells. J Ethnopharmacol 2009;122:149-56.
15. Pulok KM, Venkatesan K, Mainak M, Houghton PJ. *Acorus calamus*: Scientific validation of ayurvedic tradition from natural resources. Pharm Biol 2007;45:651-66.
16. Panchal GM, Venkatakrishna-Bhatt H, Doctor RB, Vajpayee S. Pharmacology of *Acorus calamus* L. Indian J Exp Biol 1989;27:561-7.
17. Achliya GS, Wadodkar SG, Dorle AK. Evaluation of CNS activity of *Bramhi ghrita*. Indian J Pharmacol 2005;37:33-6.
18. Parab RS, Megni SA. Hypolipidemic activity of *Acorus calamus* L. in rats. Fitoterapia 2002;73:451-5.
19. Gilani AU, Shah AJ, Ahmad M, Shaheen F. Antispasmodic effect of *Acorus calamus* Linn. is mediated through calcium channel blockade. Phytother Res 2006;20:1080-4.
20. Vohora SB, Shah SA, Dandiya PC. Central nervous system studies on an ethanol extract of *Acorus calamus* rhizomes. J Ethanopharmacol 1990;28:53-62.
21. Aqil F, Ahmad I. Antibacterial properties of traditionally used Indian medicinal plants. Methods Find Exp Clin Pharmacol 2007;29:79-92.
22. Shukla PK, Khanna VK, Ali MM, Maurya R, Khan MY, Srimal RC. Neuroprotective effect of *Acorus calamus* against middle cerebral artery occlusion–induced ischaemia in rat. Hum Exp Toxicol 2006;25:187-94.
23. Shukla PK, Khanna VK, Ali MM, Maurya RR, Handa SS, Srimal RC. Protective effect of *Acorus calamus* against acrylamide induced neurotoxicity. Phytother Res 2002;16:256-60.
24. Prasad L, Khan TH, Jahangir T, Sultana S. *Acorus calamus* extracts and nickel chloride-Prevention of oxidative damage and hyperproliferation response in rat kidney. Biol Trace Elem Res 2007;113:77-92.
25. Mehrotra S, Mishra KP, Maurya R, Srimal RC, Pandey R, Singh VK. Anticellular and immunosuppressive properties of ethanolic extract of *Acorus calamus* rhizome. Int Immunopharmacol 2003;3:53-61.
26. Manikandan S, Srikumar R, Jeya Parthasarathy N, Sheela Devi R. Protective effect of *Acorus calamus* LINN on free radical scavengers and lipid peroxidation in discrete regions of brain against noise stress exposed rat. Bio Pharma Bull 2005;28: 2327-30.
27. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Pune: Nirali Prakashan; 2004. p. 593-7.
28. Hayes AW. Guidelines for acute oral toxicity testing. In: Principles and Methods of Toxicity. New York: Raven Press Ltd; 1989. p. 184.
29. OECD. Guidelines for the testing of chemicals 425: Acute oral toxicity-up-and-down procedure. Paris, France: Organisation for Economic Co-operation and Development; 2001.
30. OECD. Guidelines for the testing of chemicals 407: Repeated dose 28-day oral toxicity study in rodents. Paris, France: Organisation for Economic Co-operation and Development; 1995.
31. Feres CA, Madalosso RC, Rocha OA, Leite JP, Guimarães TM, Toledo VP, *et al*. Acute and subchronic toxicological studiesof *Dimorphandra mollis* in experimental animals. J Ethnopharmacol 2006;108:450-6.
32. Lahlou S, Israili ZH, Lyoussi B. Acute and subchronic toxicity of a lyophilised aqueous extract of *Tanacetum vulgare* leaves in rodents. J Ethnopharmacol 2008;117:221-7.
33. Ramesh T, Lee K, Lee HW, Kim SJ. Acute oral toxicity study of *Asiasari radix* extract in mice. Int J Toxicol 2007;26:247-51.
34. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, *et al*. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 2000;32:56-67.
35. Adeneye AA, Ajagbonna OP, Adeleke TI, Bello SO. Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of *Musanga cecropioides* in rats. J Ethnopharmacol 2006;105:374-9.

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