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Acute and sub-acute oral toxicity profile of *Acorus calamus* (Sweet flag) in rodents

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ABSTRACT

Objective: To determine the acute and sub-acute oral toxicity profile of the hydroalcoholic extract of *Acorus calamus* (HAE-AC) in mice and rats respectively. **Methods:** In acute toxicity study, mice were assessed to any alteration of general behavior and mortality rate within 24 h. Further, in sub-acute toxicity study, rats were used for assessment of mortality, body weight, hematological, biochemical and histopathological changes. **Results:** Single oral administrations of the HAE-AC 2500–10000 mg/kg induced increase in general behavioral abnormalities in mice. The mortality rate also increased with increasing dosage (median lethal dose; LD₅₀ = 5070.59 mg/kg). Daily single oral doses of HAE-AC 200, 500 and 1000 mg/kg were observed to be well tolerated behaviorally after 28 days of dosing and induced no significant changes in body and organs weights of rats. Further, a mild rise in the levels of alanine transaminase (ALT), aspartate transaminase (AST) and histopathological changes in liver tissue was noted at 1000 mg/kg dose of HAE-AC. **Conclusions:** Overall, the findings of this study indicate that, HAE-AC is non-toxic and has at high dose, a mild but acceptable toxicity potential.

1. Introduction

Acorus calamus (Family: *Araceae*) is an indigenous plant commonly known as sweet flag. The plant is a perennial herb growing throughout India, China, Europe, and America. Traditionally, it has been used in remedies for numerous ailments such as insomnia, melancholia, neurosis, remittent fevers, delirium, hysteria, headache, migraine, muscle pain, joint pain, vascular and nerve injury associated severe inflammatory and neuropathic pain[1–3]. Leaves of *Acorus calamus* (AC) have been reported to possess anti-fungal, anti-inflammatory action as well as anti-oxidative actions[4–6]. The rhizome part of the AC plant has also been shown to possess several medicinal properties, which is used in the treatment of insomnia, melancholia, neurosis, remittent fevers, delirium and hysteria[2–4]. The aqueous and

hydroalcoholic extracts of rhizome are reported to express hypolipidemic, neuropharmacological and neuroprotective actions[7,8]. Hydroalcoholic extract of AC (HAE-AC) in our recent studies have also shown to possess ameliorative potential in painful peripheral neuropathy[9,10].

The preliminary phytochemical studies have revealed that AC possess various secondary metabolites such as glycosides, flavonoids, saponins, tannins, polyphenolic compounds, mucilage, volatile oil and bitter principles[11]. Chemical compositions of AC in different parts and essential oil have also been thoroughly studied by Mittal *et al*[2]. Major chemical constituents identified in AC are α - and β -asarones along with other constituents, such as caryophyllene, isoasarone, methyl isoeugenol, and safrol[2,11]. Most of the biological actions of AC have been attributed to presence of α - and β -asarones[12,13]. In a recent finding beta-asarone was shown to possess ameliorative potential in cognitive impairment thereby suppressing the neuronal apoptosis[14]. Moreover, alpha-asarone is also noted reduce the excitatory action by stimulation of glutamate uptake and inhibition of excitatory

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neurotransmitter transporter mediated current^[15]. Some chemical constituents of AC beta-asarone in particular have been demonstrated to possess toxic effects like prolonged vomiting, hallucinogen, carcinogenic, and genotoxic action in dose dependent manner^[16–18]. Thus low level of beta-asarone could only be acceptable for therapeutic use, and the level of beta-asarone can be minimized by decoction process^[17]. Although a significant data advocate therapeutic potential of AC in various ailments but there is no conclusive evidence regarding its acute and sub-acute toxicity. Therefore, the purpose of this study was to investigate the acute and sub-acute oral toxicity profiles of AC in mice and rats respectively.

2. Materials and methods

2.1. Chemicals

Ethylenediaminetetraacetic acid (EDTA), ethanol and carboxy methyl cellulose (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), and sodium fluoride (S.D. Fine, Mumbai India), were procured for the present study. All the chemicals were used as analytical grade in the present study.

2.2. Plant material

The fresh rhizome part of AC were collected at Kodaikanal of Tamilnadu, India and authenticated through department of botany, American college, Madurai district, Tamilnadu. Plant sample has been kept in voucher specimen (PUP–218/2009–2010) at Punjabi University, Patiala for future reference. After authentication, fresh rhizome of AC were collected, cleaned thoroughly with distilled water and dried under shade. The shade dried rhizome was pulverized in a mechanical grinder to obtain coarse powder (sieve no.10/40).

2.3. Extraction

The coarsely powdered plant material (500 g) was subjected to extraction with mixture of ethanol: water (1:1, 50%) at room temperature for 24 h. The solvent was then completely removed by vacuum drying at low temperature (<50). The yield of hydroalcoholic extract was found to be 26.4 % (w/w). This HAE–AC was subjected to phytochemical analysis and acute and sub-acute oral toxicity studies in rodents.

2.4. Phytochemical analysis

Phytochemical analysis was carried out in HAE–AC for identification of flavonoids, saponins, tannins, steroids, alkaloid, and phenolic compounds as described by Evans^[19]. Briefly, Mg–HCl and Zn–HCl were used for flavonoids, foam test was used for saponins, ferric chloride and gelatin

were used for tannins, acetic anhydride and sulphuric acid were used for steroids, Wagner's and Heger's reagents were used for alkaloid, and ferric chloride was used for phenolic compounds. The experiments were carried out three times for each phytochemical constituent.

2.5. Experimental animals

Mice (either sex, 20–30 g) and Wistar rats (either sex, 200–230 g), procured from disease free small animal house, CCS–Haryana Agricultural University, Hisar were employed in the present study. They were housed in the animal cages with free access to water and standard laboratory pellet chow diet (Kisan Feeds Ltd., Mumbai, India). The rats were exposed under a controlled environment in the institute's animal house at (22±1) °C, 55%±10% humidity and a 12 h light–dark cycle. The experimental protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) and the care of the animals was carried out as per guidelines of the Committee for the Purpose of Control and supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No.–107/1999/CPCSEA). The experimental procedure for toxicity studies was followed according to the Organization for Economic Co–operation and development (OECD) guide line no. 401 and 425^[20].

2.6. Acute oral toxicity test in mice

Acute toxicity tests were performed in both male and female mice in order to evaluate the toxic effect of hydro–alcoholic extract of AC (2500, 5000, 7500, and 10000 mg/kg, *p.o.*) in different gender groups. All deviations in general behavior *i.e.*, convulsions, disorientation, hypoactivity, hyperventilation, pilo–erection and mortality rate in mice were monitored continuously for 24 h after dosing completed. The LD₅₀ was calculated according to the method of Miller and Tainter^[21].

2.6.1. Experimental protocol for acute oral toxicity studies

Six groups, each comprising of aged matched ten mice (five males and five females), were employed in the present study.

Group I (Normal control): Mice were not subjected to any drugs and vehicle administration. The behavioral changes were observed for 24 h.

Group II (Vehicle control): Mice were subjected to single dose of vehicle [Carboxy methyl cellulose (CMC) (0.5 % w/v, *p.o.* 10 mL/kg)] administration. The behavioral changes were observed as mentioned in group I.

Group III (HAE–AC, 2500): Mice were subjected to single dose of HAE–AC (2500 mg/kg; *p.o.*) administration. The behavioral changes were observed as mentioned in group I.

Group IV (HAE–AC, 5000): Mice were subjected to single dose of HAE–AC (5000 mg/kg; *p.o.*) administration. The

behavioral changes were observed as mentioned in group I.

Group V (HAE–AC, 7500): Mice were subjected to single dose of HAE–AC (7500 mg/kg; *p.o.*) administration. The behavioral changes were observed as mentioned in group I.

Group VI (HAE–AC, 10000): Mice were subjected to single dose of HAE–AC (10000 mg/kg; *p.o.*) administration. The behavioral changes were observed as mentioned in group I.

2.7. Sub-acute oral toxicity test in rat

Sub-acute toxicity tests were also performed in rats of both sexes, in order to evaluate the toxic effect of the HAE–AC in different gender groups. Based on LD₅₀ values (5070.59 mg/kg) obtained from acute toxicity studies, the selection of doses for sub-acute toxicity study was carried out. The dose selected for sub-acute toxicity studies ranges from 200 mg/kg, *p.o.* to 1000 mg/kg, *p.o.*. The dosing was done daily and the rats were observed for 28 days for signs of sub-acute toxicity *i.e.* mortality, and the body weight changes. On the 28th day, the animals were anaesthetized with chloroform and sacrificed. Whole blood samples were collected by cardiac puncture and transferred into ethylenediaminetetraacetic acid (EDTA), sodium fluoride and gel containing tubes for hematological and biochemical analysis. Tissue sample *i.e.*, liver, heart, and kidney were collected from the rats. Further, tissue samples were weighed, observed colour changes of the vital organs and then subjected to histopathological examinations.

2.7.1. Experimental protocol for sub-acute oral toxicity studies

Five groups, each comprising of aged matched ten Wistar rats (five males and five females), were employed in the present study.

Group I (Normal control): Rats were not subjected to any drugs and vehicle administration. The changes of mortality and the body weight were observed at different time intervals, *i.e.*, day 0, 1, 7, 14, 21 and 28. Thereafter, all the animals were sacrificed and subjected to evaluation of biochemical and histopathological changes in blood and tissue respectively.

Group II (Vehicle control): Rats were subjected to administration vehicle *i.e.*, carboxy methyl cellulose (CMC, 0.5% w/v; *p.o.*) for 28 consecutive days. The body weight, biochemical and histopathological changes were evaluated as mentioned in group I.

Group III (HAE–AC, 200): Rats were subjected to administration of HAE–AC (200 mg/kg; *p.o.*) for 28 consecutive days. The body weight, biochemical and histopathological changes were evaluated as mentioned in group I.

Group IV (HAE–AC, 500): Rats were subjected to administration of HAE–AC (500 mg/kg; *p.o.*) for 28 consecutive days. The body weight, biochemical and histopathological changes were evaluated as mentioned in

group I.

Group V (HAE–AC, 1000): Rats were subjected to administration of HAE–AC (1000 mg/kg; *p.o.*) for 28 consecutive days. The body weight, biochemical and histopathological changes were evaluated as mentioned in group I.

2.8. Blood analyses

Hematological and biochemical analyses were performed at a commercial laboratory. Complete blood cell count *i.e.* red blood cell count (RBC), white blood cell count (WBC), platelet count, lymphocytes, hemoglobin, hematocrit (packed cell volume, PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were determined on a fully automated analyzer. Blood chemistry tests were performed on an auto analyzer (Beckman Coulter CX7, Fullerton, CA, USA) using a kinetic rate method for the enzymes *i.e.* alanine transaminase (ALT), aspartate transaminase (AST); modified rate Jaffe method for creatinine; the timed endpoint method for urea, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and glucose oxidase peroxidase method for glucose^[22].

2.9. Histopathological evaluation

Tissue samples liver, heart and kidney were stored in the fixative solution (1% formalin) and about 4 µm thick sections (forward mode wax ribbon cutting method) were cut by using a microtome device (Radical Scientific Equipments Pvt. Ltd., Ambala, India). Staining was done by using hematoxylin and eosin (H&E) staining method. Microscopic slides were analyzed qualitatively under light microscope (450×).

2.10. Statistical analysis

Results are presented as mean±SD. The Student's *t*-test was used for statistical comparison of data between groups. The *P*≤0.05 was considered to be statistically significant. The LD₅₀ was calculated according to method of Miller and Tainter^[21].

3. Results

3.1. Phytochemical analysis

The phytochemical screening of HAE–AC has indicated very high levels of saponins, flavonoids, and phenolic compounds; medium level of tannins and alkaloids and very low levels of steroids (Table 1).

3.2. Effect of HAE–AC on acute oral toxicity in mice

Table 1.

Phytochemical analysis for the HAE-AC.

| Extracts / Phytoconstituents | Flavonoids | Saponins | Tannins | Steroids | Alkaloids | Phenolic compounds |
|------------------------------|------------|----------|---------|----------|-----------|--------------------|
| HAE-AC | +++ | +++ | ++ | + | ++ | +++ |

+++; High level; ++; Medium level; +; low level. The experiments were carried out three times for each phytochemical constituent. Classification was performed based on observation of colour intensity and the precipitation with suitable reagent.

Table 2.

Effect of HAE-AC on acute oral toxicity test in mice.

| Groups | % Mortality | Symptoms of toxicity |
|----------------|-------------|--|
| Normal | None | None |
| Vehicle | None | None |
| HAE-AC (2500) | None | None |
| HAE-AC (5000) | 40 | Pilo-erection and hypo activity |
| HAE-AC (7500) | 80 | Convulsions, disorientation, hypoactivity, hyperventilation, pilo-erection |
| HAE-AC (10000) | 100 | Convulsions, mortality |

Digits in parentheses of HAE-AC indicate dose in mg/kg. Symptoms of toxicity were observed at least in six out of ten treated mice.

Table 3.

Effect of HAE-AC on haematological and biochemical parameters of the rats.

| Parameter (units) | Normal | Vehicle | HAE-AC (200) | HAE-AC (500) | HAE-AC (1000) |
|------------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------------------|
| RBC ($\times 10^{12}/L$) | 7.100 \pm 0.200 | 7.200 \pm 0.100 | 6.900 \pm 0.300 | 7.000 \pm 0.200 | 6.900 \pm 0.200 |
| WBC ($\times 10^9/L$) | 2.200 \pm 1.200 | 2.300 \pm 1.200 | 2.100 \pm 1.100 | 1.900 \pm 1.200 | 1.900 \pm 1.100 |
| Platelet count ($\times 10^9/L$) | 796.500 \pm 23.500 | 787.500 \pm 24.200 | 789.000 \pm 19.600 | 793.800 \pm 22.100 | 794.200 \pm 20.600 |
| Lymphocytes (%) | 39.100 \pm 0.300 | 39.300 \pm 0.100 | 38.600 \pm 0.400 | 38.900 \pm 0.300 | 38.800 \pm 0.200 |
| Haemoglobin (g/dL) | 14.200 \pm 0.300 | 14.100 \pm 0.200 | 14.300 \pm 0.200 | 14.100 \pm 0.100 | 13.900 \pm 0.300 |
| Hematocrit (PCV) (L/L) | 0.430 \pm 0.003 | 0.410 \pm 0.001 | 0.420 \pm 0.002 | 0.400 \pm 0.001 | 0.390 \pm 0.004 |
| MCV (fL) | 59.500 \pm 0.800 | 58.300 \pm 1.200 | 59.700 \pm 1.100 | 57.800 \pm 0.600 | 58.400 \pm 0.900 |
| MCH (pg) | 20.200 \pm 0.200 | 19.600 \pm 0.100 | 20.000 \pm 0.100 | 20.100 \pm 0.300 | 19.900 \pm 0.200 |
| MCHC (g/dL) | 34.300 \pm 0.100 | 34.600 \pm 0.300 | 33.900 \pm 0.200 | 34.200 \pm 0.100 | 34.100 \pm 0.200 |
| HDL (mg/dL) | 53.500 \pm 0.100 | 52.900 \pm 0.200 | 53.200 \pm 0.100 | 53.400 \pm 0.300 | 52.800 \pm 0.400 |
| LDL (mg/dL) | 169.200 \pm 0.600 | 171.400 \pm 1.100 | 170.100 \pm 0.400 | 171.500 \pm 0.300 | 172.100 \pm 0.500 |
| Glucose (mg/dL) | 122.400 \pm 1.900 | 121.650 \pm 1.600 | 119.210 \pm 1.200 | 120.810 \pm 2.200 | 126.980 \pm 2.300 |
| Urea (mg/dL) | 23.900 \pm 0.600 | 24.100 \pm 0.200 | 24.800 \pm 0.400 | 23.900 \pm 0.100 | 24.900 \pm 0.300 |
| Creatinine (μ M/L) | 23.600 \pm 1.900 | 24.100 \pm 2.200 | 23.900 \pm 1.600 | 25.800 \pm 2.400 | 21.500 \pm 1.600 |
| ALT (U/L) | 74.100 \pm 0.400 | 74.300 \pm 0.100 | 75.600 \pm 0.200 | 77.800 \pm 0.400 | 83.200 \pm 0.200 ^a |
| AST (U/L) | 116.100 \pm 2.900 | 117.500 \pm 3.100 | 118.400 \pm 2.700 | 120.100 \pm 2.900 | 135.600 \pm 2.400 ^a |

Digits in parentheses of HAE-AC indicate dose in mg/kg. Values are expressed as mean \pm SD, $n=10$ rats per group. a: $P < 0.05$ as compared with control. HAE-AC, hydro-alcoholic extract of *Acorus calamus*; RBC, red blood cell count; WBC, white blood cell count; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine transaminase; AST, aspartate transaminase; g/dL, gram per liter; L/L, liter per liter; fL, femtolitre; pg, pictogram.

The effects of HAE-AC in mice after oral administration are summarized in Table 2. HAE-AC at a dose of 2500 mg/kg, p.o. did not show any mortality and behavioral changes. The 40 percentage of mice died at a dose of 5000 mg/kg, 80 percentage of mice died at a dose of 7500 mg/kg and 100 percentage of mice died at a dose of 10000 mg/kg of HAE-AC. Moreover, behavioral changes i.e., convulsions, disorientation, hypo-activity, hyperventilation and pilo-erection were also observed at doses of 5000 and 7500 mg/kg. The SE of LD₅₀ is found to be 6.97. The calculated LD₅₀ is found to be 5070.59 mg/kg of body weight (95% confidence intervals: 5063.62 – 5077.56 mg/kg).

3.3. Effect of HAE-AC on body weight and mortality in sub-acute oral toxicity in rat

No significant changes were recorded in body weight in the HAE-AC treated rats as compared to the normal control. All the group of animals (normal, vehicle control and HAE treated groups) appeared consistently healthy throughout the 28 day period of the study. No mortality was recorded in HAE-AC treated rats (data not shown).

3.4. Effect of HAE-AC on hematological and biochemical parameters in sub-acute oral toxicity in rat

The effects of HAE-AC in rat after oral administration for 28 days are summarized in Table 3. The hematological parameters (i.e. RBC, WBC, platelet count, lymphocytes, haemoglobin, PCV, MCV, MCH, and MCHC) and the blood biochemical parameters (i.e. creatinine, urea, HDL, LDL and glucose) showed no significant changes ($P \leq 0.05$) in HAE-AC

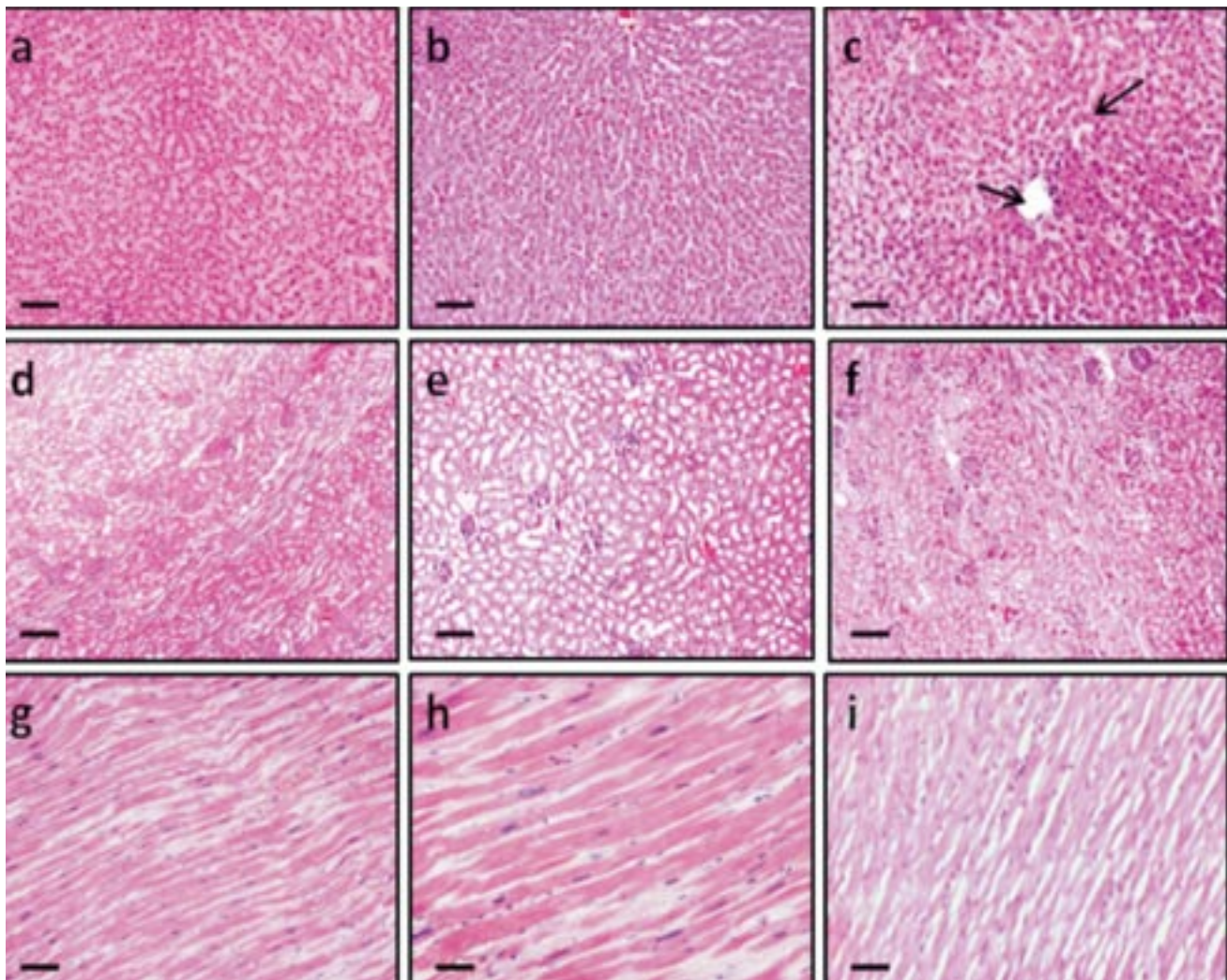


Figure 1. Effect of HAE-AC on histopathological changes.

Figure 1 show cross section of liver (1a–1c), kidney (1d–1f), and heart (1g–1i) tissues of normal, HAE-AC (500, and 1000 mg/kg) treated groups respectively. Tissues were stained with hematoxylin and eosin (H & E) staining. Figure 1c, shows mild histopathological changes (disturbance of the normal architecture of liver tissue with slight dilatation of central vein) in liver section. Figures 1e, 1f, 1h, and 1i did not show any significant changes in the histology of kidney, and heart sections. Microscopic examinations were performed under 450 X under light microscopy, scale bar 35 μm .

(200, 500 and 1000 mg/kg, *p.o.*) treated rats as compared to the normal control rats. However, rats treated with 1000 mg/kg of HAE-AC have shown a slight insignificant rise in the levels of ALT and AST when compared to normal control group. No such effect was noted at 200 mg/kg and 500 mg/kg of HAE-AC.

3.5. Effect of HAE-AC on organ weight changes in sub-acute oral toxicity in rat

The effects of HAE-AC on wet weight changes of rat organs *i.e.*, liver, heart, and kidney are summarized in Table 4. The chronic oral ingestion of HAE-AC (200 and 500 mg/kg, *p.o.* for 28 days) caused no significant changes in the weights of the vital organs as compared to normal control group. Although a slight difference was observed between the normal control and 1000 mg/kg of HAE-AC treated group but results were not statistically significant ($P \leq 0.05$).

Table 4.

Effect of HAE-AC on organ weight changes in the rats.

| Groups | Organ weight changes (g) | | |
|---------------|--------------------------|-----------------|-------------------|
| | Liver | Kidney | Heart |
| Normal | 9.39 \pm 1.65 | 2.47 \pm 0.64 | 1.092 \pm 0.067 |
| Vehicle | 8.54 \pm 1.57 | 2.46 \pm 0.52 | 0.995 \pm 0.043 |
| HAE-AC (200) | 8.82 \pm 1.61 | 2.48 \pm 0.61 | 0.981 \pm 0.071 |
| HAE-AC (500) | 7.69 \pm 1.48 | 2.51 \pm 0.57 | 0.997 \pm 0.054 |
| HAE-AC (1000) | 7.43 \pm 1.57 | 2.58 \pm 0.46 | 0.941 \pm 0.051 |

Digits in parentheses of HAE-AC indicate dose in mg/kg. Values are expressed as mean \pm SD, $n=10$ rats per group. HAE-AC: hydro-alcoholic extract of *Acorus calamus*.

3.6. Effect of HAE-AC on histopathological changes in sub-acute oral toxicity in rat

Chronic oral administration of HAE-AC (200 and 500 mg/kg, *p.o.*) over 28 days produced no significant changes in the histology of the vital organs as compared to normal control

group. However, mild histopathological changes were observed in liver tissue at a dose of 1000 mg/kg of HAE-AC without any effect on kidney and cardiac tissues (Figure 1).

4. Discussion

Phytomedicine is indeed worldwide accepted therapeutic approach for chronic diseases and practically it cannot be avoided in the health care systems. In developing (low and middle income) countries, it often plays a heart of medicine in traditional therapy, because it is believed that phytomedicines are harmless^[23]. There has been enormous rise in the number of uses of traditional medicine and new scientific evidences are coming up regarding the safety of the medicinal plants. Therefore, this creates a warning regarding the toxicity and therapeutic effect of drugs from plant origin including AC^[16,17]. The phyto-constituents of AC have been documented to possess a number of beneficial effects on certain disease^[2]. The scientific toxicological reports of AC will be of great help in optimizing its safety in long term management of chronic illness.

The results of the present study indicate that HAE-AC is well tolerated in mice up to oral dose of 2500 mg/kg. Mortality and moderate behavioral symptoms were observed at doses of 5000 mg/kg of HAE-AC, 100 % mortality was noted at a single oral dose of 10000 mg/kg of HAE-AC. According to classification of Loomis and Hayes, LD₅₀ value within the range of 5000 to 15000 mg/kg is considered as practically non-toxic^[21]. The LD₅₀ value of AC in our study suggested that the AC plant could be regarded as practically non-toxic in acute ingestion. Body weight changes are marker of adverse effects of drugs and chemicals, sub-acute administration for 28 consecutive days of HAE-AC (200, 500 and 1000 mg/kg, *p.o.*) have shown no significant changes in the body weight, general behavior, as well as mortality of rats. This implicate that long oral administration of HAE-AC could be safely used for the chronic ailments. However, there was no gender based difference observed in this study.

The hematopoietic system is one of the most sensitive targets for toxic substances and it also an important marker of physiological and pathological status in human and animal studies^[24,25]. The results of hematological and biochemical analyses have shown that sub-acute oral administration of HAE-AC at doses of 200 and 500 mg/kg did not show any significant effect on hematological and biochemical parameters. However, a mild rise in levels of ALT and AST in HAE-AC 1000 mg/kg treated group was noted. This indicates that HAE-AC does not have any toxic effects on hematological (circulating blood cells), renal and cardiac functions *etc.* However, rise in the levels of ALT and AST at high dose indicate hepatotoxicity. Indeed, the ALT and AST are well-known enzymes markers of liver function and hepatotoxicity^[26]. Elevation of transaminases in blood is due to the damage of cell organelle *i.e.*, parenchyma, smooth

endoplasmic reticulum, and mitochondria of hepatocytes^[27]. Thus, the mild changes in ALT and AST activities suggest that the sub-acute administration of HAE-AC at high dose (1000 mg/kg) may cause alteration of the hepatic metabolism in rats and probably hepatotoxicity. This is further supported by histopathological studies that high dose has induced mild histopathological changes in liver tissue while sparing renal and cardiac tissues. It is important to note that no significant difference in the weights of the vital organs, body weight and color of the organs was observed therefore, further advocating good safety profile of HAE-AC in rats. Hence, it may be concluded that oral administration of HAE-AC extract has shown good safety profile in acute and sub-acute toxicity studies. Nevertheless, further studies are needed to establish full toxicity profile of HAE-AC.

Conflict of interest statement

We declare that there is no conflict of interest in the present study.

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