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*Original Article*

# Acute and sub-acute Toxicity study of Aqueous extracts of *Canscora heteroclita* (L) Gilg in Rodents

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## ABSTRACT

**Background:** *Canscora heteroclita* (*C. heteroclita*) being used in the Ayurvedic system of medicine in India for the treatment of various diseases. No systematic toxicity study for this plant was described. **Objective:** The present study was undertaken to assess the safe use of this plant in traditional practice. **Materials and Methods:** The acute oral toxicity study of aqueous extract of *Canscora heteroclita* (AECH) was carried out as per the OECD guidelines 423 in mice and the sub-acute toxicity was carried out at a dose of 200 mg/kg and 400 mg/kg as per OECD 407 guidelines in male and female rats. **Results:** Mice administered upto 2000 mg/kg as a single dose orally not caused any signs of toxicity or mortality in mice. In sub-acute toxicity study in rats, AECH at two different daily doses of 200 and 400 mg/kg for 28 days did not cause any significant change including the hematological and biochemical parameters. Histopathological examinations showed normal architecture suggesting no morphological disturbances. **Conclusion:** No deaths or any signs of toxicity was observed after oral administration in acute toxicity study upto a dose of 2000 mg/kg of AECH in mice and upto a dose of 400 mg/kg of AECH in sub acute toxicity study in rats.

**Key words:** *Canscora heteroclita,* Acute toxicity, Sub-acute toxicity, Biochemical, Histology.

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## INTRODUCTION

*Canscora heteroclita* (L.) Gilg (Figure 1) (Gentianaceae) synonymically called as *Canscora sessiliflora and Gentiana heteroclita* is a gregarious moderately to profusely branched herb. *C. heteroclita* are common and abundantly available in south India and it is distributed widely in upper Gangetic plains, Gujarat, Madura hills, Rajasthan and Srilanka,[1,2] where ethno botanical studies and traditional practice revealed their use for treating fever, inflammation, tumor and for liver disorders.[3,4] In India, traditional medical practioners widely using this *Canscora* species for various ailments and as a substitute for shankhpushpi particularly, for the treatment of liver disorders. Decoction of the entire plant was given for cough and fever. Entire plant powder along with thadupusti legyam was used by traditional practitioners as nerve tonic powder. There is no scientific evidence or reports available in the literature for the oral acute and sub-acute toxicity study for *C. heteroclita*. Hence to ascertain and establish the safety for its use in traditional practice, acute and sub-acute toxicity studies of the aqueous extract of *C. heteroclita* was carried out as per OCED guidelines.

## MATERIALS AND METHODS

### *Plant materials*

*C. heteroclita* (L) Gilg was collected during January 2014 and the plants were authenticated (BSI/SRC/5/23/2014-15/Tech/872) by Dr. M. Palanisamy, Scientist C, Botanical survey of India, Coimbatore, India. Voucher specimen of the plant was deposited at the Department of Pharmacognosy, KMCH College of Pharmacy, Coimbatore for future reference.

### *Preparation of extracts*

Entire plant of *C. heteroclita* was cleaned with distilled water to remove earthy matter and residual materials. It was then shade dried at room temperature (32 ± 2°C) for 10 days, pulverized to coarse powder, passed through a #40 mesh sieve. Then decoction was prepared by boiling 100 g of the ground entire *plant* in 500 mL of distilled *water* for about 30 min. Filtered and concentrated separately under reduced pressure (IKA Rotary evaporator; Model No RN 10 digital V, ILMAC Germany) at 40°C, yielded 19.6 % w/w of aqueous extract of *Canscora heteroclita* (AECH).

### *Animals*

Female Albino mice (7-8 weeks old, 20-30 g) and Wistar strains of either sex rats (10-12 weeks old, 125-175 g) were obtained from the animal house of Kovai medical center research and educational trust, Coimbatore, Tamilnadu. The animals were kept in polypropylene cages at a temperature of 25 ± 2°C, RH (50 ± 5%), and 12 h light and dark cycles. They were fed with standard laboratory animal diet and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. Experiments were designed and conducted in accordance with ethical norms approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and Institutional Animal Ethical Committee (IAEC) (KMCRET/AICTE/01/2014-15, dt.31/01/2015).

### *Acute toxicity study*

Acute toxicity test was performed as per OECD guideline 423[5] for testing of chemicals (2001). Healthy young adult albino nulliparous, non-pregnant female mice weighing about 20-30 g were administered with 5, 50, 300 and 2000 mg/kg of AECH in distilled water as a single dose (1 ml) orally using oral feeding needle. Animals were observed individually for first 30 min, then for the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days to observe toxicity signs like changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and for behavioural pattern. On 15$^{th}$ day animals were anaesthetized, blood was collected from the mice for haematological and biochemical analysis. Mice were then sacrificed, dissected and the organs lungs, liver, spleen and kidney were carefully collected, weighed, processed and observed under photomicroscope for histopathological examination.

### *Sub-acute toxicity study*

The sub-acute toxicity assessment of AECH was performed as per OECD Guideline 407.[6] Wistar strain of rats (125-175 g) was divided into six groups, each consisting of six male and six female rats. Male and female Control groups received only distilled water. Two other groups of each male and female rats were administered with AECH in distilled water orally using rat oral feeding needles, daily for 28 days at a dose of 200 mg/kg and 400 mg/kg respectively. During the treatment period the body weight of animals were monitored on 0, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day. Food consumption and water intake for all the groups were observed from day 1 to 28 days.

On 29$^{th}$ day, the overnight fasted rats were anaesthetized with diethyl ether inhalation in a jar containing cotton soaked with diethyl ether. Then blood samples were withdrawn from retro-orbital sinus and the collected blood samples were evaluated for hematological parameters viz. red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), platelet count, packed cell volume (PCV), differential count, Mean Platelet Volume (MPV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red Blood Cell Distribution Width (RDW).

A portion of the blood samples were centrifuged at 10000 rpm for 10 min and the separated serum was analyzed for biochemical parameters viz. Cholesterol, triglycerides, VLDL levels SGOT, SGPT, ALP, total bilirubin, total protein, albumin, globulin, urea, uric acid and creatinine. Biochemical investigations were carried out in an auto analyzer (Photometer 5010 V5+, Robert Riely, Berlin) using Piramal healthcare limited reagent kit. The animals were sacrificed by cervical dislocation and the organs, heart, liver, spleen, kidneys, stomach, testes and ovary were isolated, weighed, processed and observed under photomicroscope for histopathological examination.

### *Statistical analysis*

The results are expressed as the mean ± SEM. The significance of the difference was evaluated by one-way ANOVA followed by Dunnett's test. Data were considered statistically significant if $p<0.05$.

## RESULTS

### *Acute Toxicity Study*

Single oral administration of AECH at a dose of 5, 50, 300 and 2000 mg/kg as per OECD guideline 423 for 14 days did not produce any mortality in tested animals (Table 1). No observable sign of toxicity was detected during the experimental period.

### *Sub-acute toxicity study*

Daily oral administration of AECH at a dose of 200 mg/kg and 400 mg/kg elicited no change in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems and behaviour pattern of the rodents. Absence of tremors, convulsions, salivation, diarrhea, and sleep also noted (Table 2). Thus in sub-acute toxicity study, no sign of toxicity viz. jerk, convulsion and mortality was observed for both the male and female rats treated with 200 and 400 mg/kg dose of AECH.

### *Parameters observed during acute and sub-acute toxicity studies*

### *Body weight*

Gain in body weight of mice and rats was observed in acute and sub-acute toxicity study respectively for control group and AECH administered animals and the recorded weights are displayed in Table 3 and 4.

### *Food and distilled water consumption*

Food and distilled water consumption of mice (acute toxicity) and rats (sub-acute toxicity) were continuously monitored, where there is no change in consumption was observed for both the control group and treated groups (Figure 2 to Figure 5).

### *Hematological analysis*

The results of hematological investigations (Table 5 and 6) conducted on day 15$^{th}$ day for acute toxicity study and on 29$^{th}$ day for sub-acute toxicity study revealed no significant changes in the values of RBC, WBC, Hb, platelet count, PCV, differential count, MPV, MCV, MCH, MCHC and RDW of treated groups when compared with the respective control mice and rats respectively.

### *Biochemical Investigations*

Biochemical investigations were performed in order to review any toxic effects produced after administration of AECH on liver and kidney. There was no significant alteration in cholesterol, triglycerides and VLDL levels in treated groups of sub-acute toxicity study when compared with control group of rats (Table 7). No significant change observed in (serum glutamic oxaloacetic transaminase) SGOT, (Serum glutamic pyruvic transaminase) SGPT, (alkaline phosphatase) ALP and total bilirubin content of treated group animals when compared with control group animals (Table 8 and 9). There was no significant alteration observed in creatinine, urea and uric acid levels of treated group animals when compared with control group animals (Table 10 and 11).

### *Organ weight*

No abnormal change in the relative weight of internal organs of mice and rats was observed when compared to control group as shown in Table 12 and 13.

### *Histopathological investigation*

### *Acute toxicity study in mice*

### *Lungs*

Normal alveoli with normal bronchioles and normal alveoli with congested blood vessels were observed in histopathological investigation of lungs of control group of mice in 10 x and 40 x magnifications respectively. Mice treated with 2000 mg/kg of AECH showed normal alveoli with bronchioles in both the magnifications (Figure 6).

### *Liver*

Normal lobular architecture and normal portal tract was observed in histopathological section of liver in control group mice in 10 x and 40 x magnifications respectively. AECH treated mice at a dose of 2000 mg/kg showed normal lobular architecture, central veins and sinusoids. No toxic signs like inflammation, fatty change or fibrosis were observed (Figure 7).

### *Spleen*

Histological spleen section showed red pulp congestion in control group mice and in AECH treated mice (2000 mg/kg). Variation in white pulp, pencillar artery and the red pulp was not observed (Figure 8).

### *Kidney*

Normal cortex, medulla and normal glomeruli were observed from the histological sections of kidney from control and AECH treated mice (2000 mg/kg). Section also showed normal interstitium and inflammation or necrosis was not observed (Figure 9).

### *Sub-acute toxicity study in rats*

### *Heart*

Histological sections of the heart of control group of rats and AECH treated male and female rats at a dose of 200 and 400 mg/kg showed normal myocytes (Figure 10 and 11).

**Figure 1:** Entire plant of *Canscora heteroclita* ( L.) Gilg.

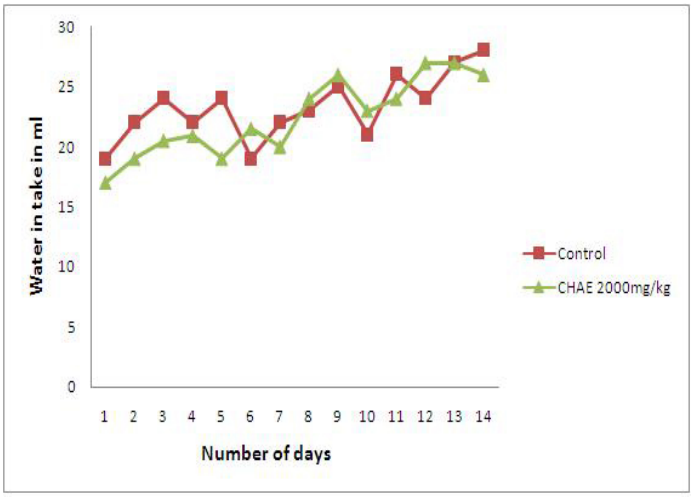


**Figure 2:** Food intake of mice in g in acute toxicity study.

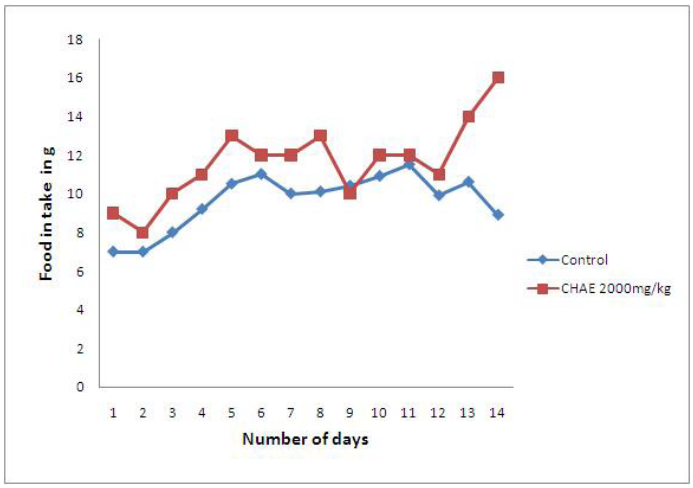


**Figure 3:** Water intake of mice in g in acute toxicity study.

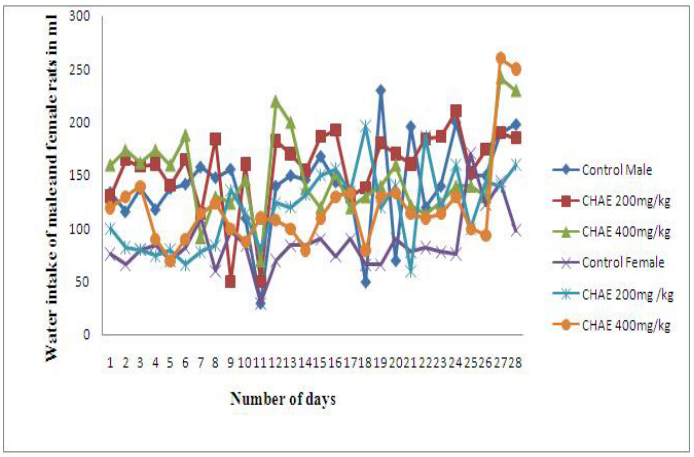


**Figure 4:** Food intake of male and female rats in g in sub-acute toxicity study.

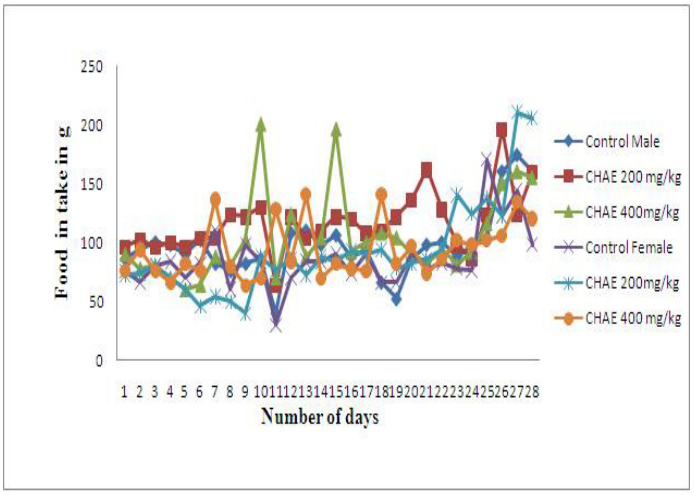


**Figure 5:** Water intake of male and female rats in g in sub-acute toxicity study.

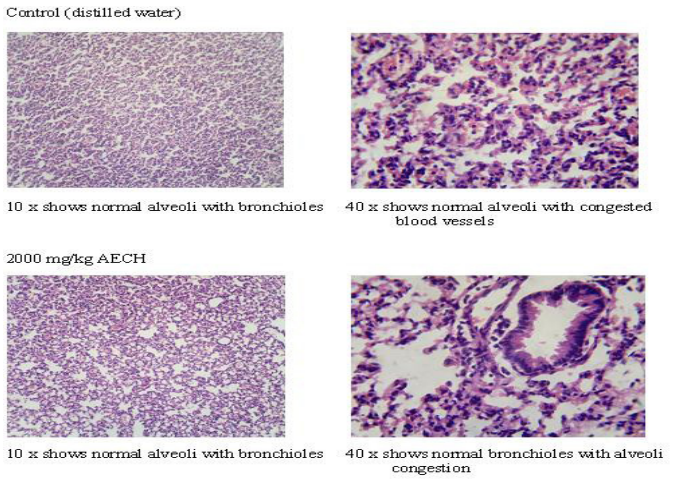


**Figure 6:** Histology of lungs for control and treated mice under 10 x and 40 x magnifications.

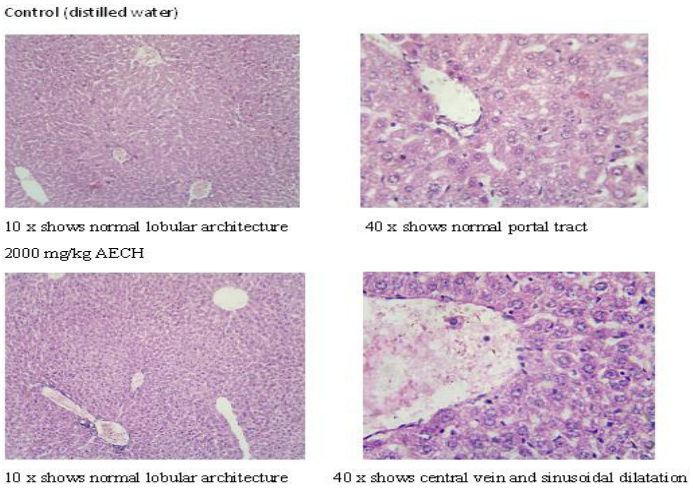


**Figure 7:** Histology of liver for control and treated mice under 10 x and 40 x magnifications.

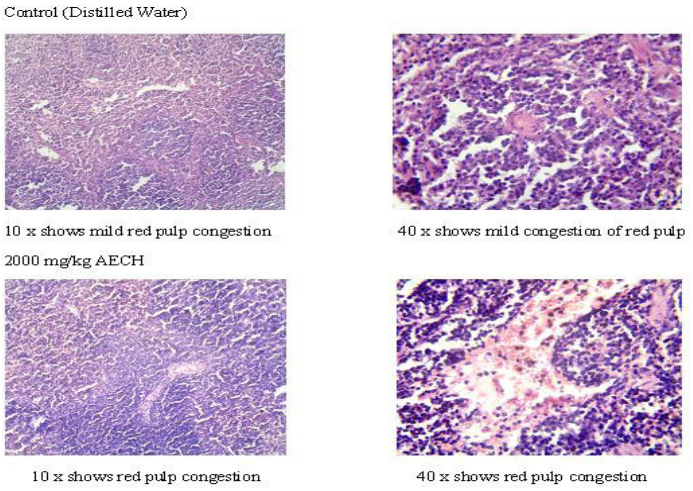


**Figure 8:** Histology of spleen for control and treated mice under 10 x and 40 x magnifications.

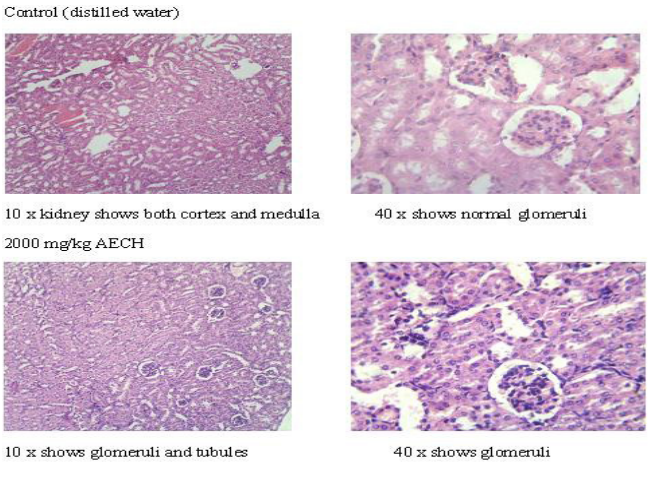


**Figure 9:** Histology of kidney for control and treated mice under 10 x and 40 x magnifications.

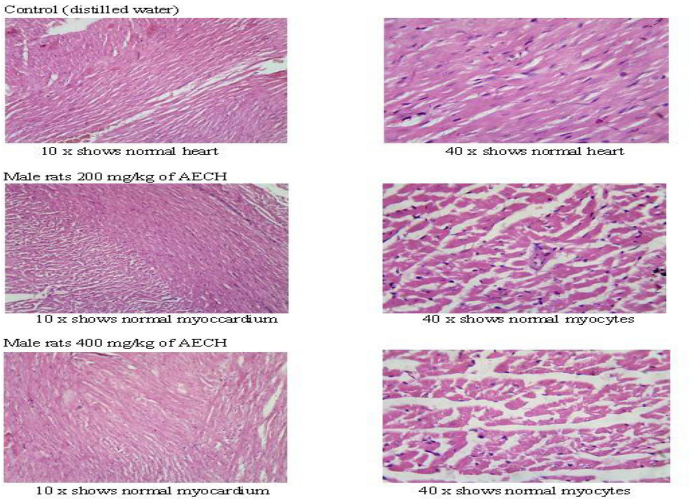


**Figure 10:** Histology of heart for control & treated male rats under 10 x and 40 x magnifications.

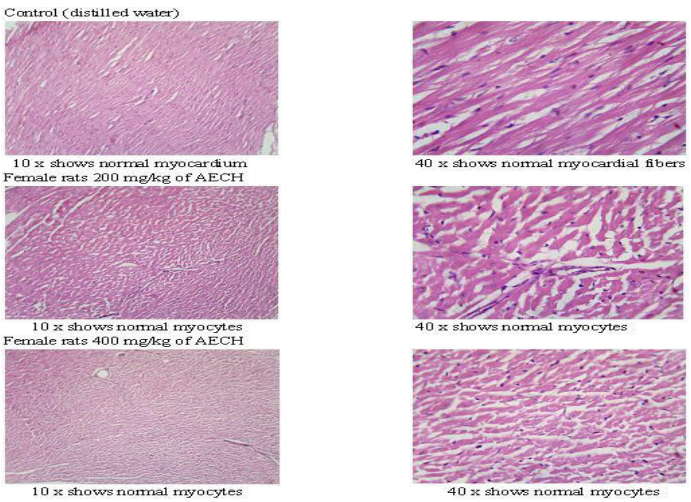


**Figure 11:** Histology of heart for control & treated female rats under 10 x & 40 x magnifications.

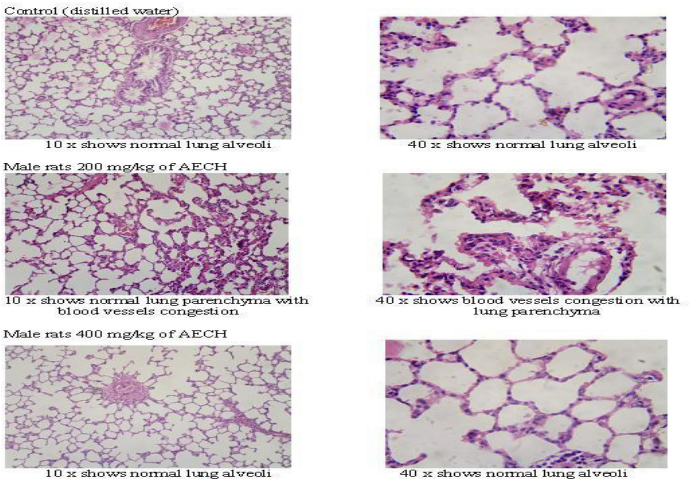


**Figure 12:** Histology of lungs for control & treated male rats under 10 x and 40 x magnifications.

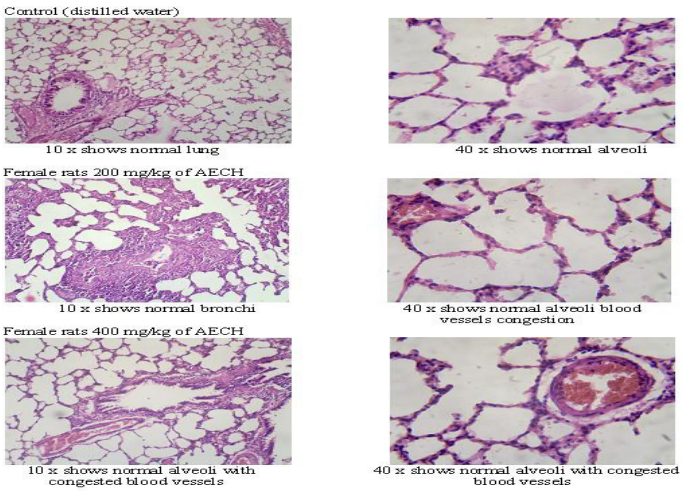


**Figure 13:** Histology of lungs for control & treated female rats under 10 x & 40 x magnifications.

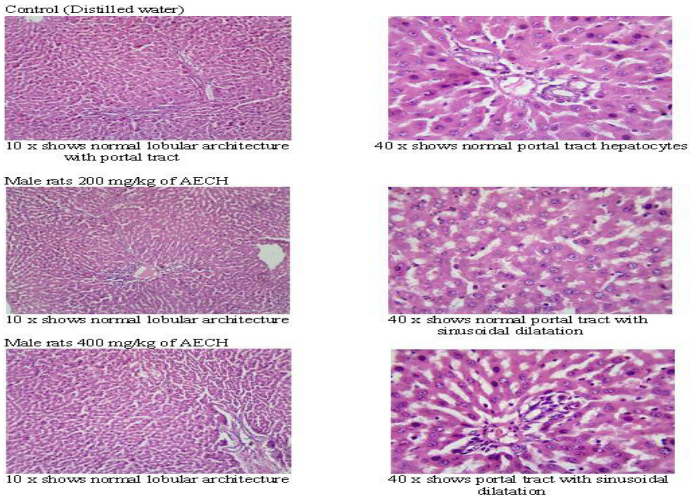


**Figure 14:** Histology of liver for control & treated male rats under 10 x and 40 x magnifications.

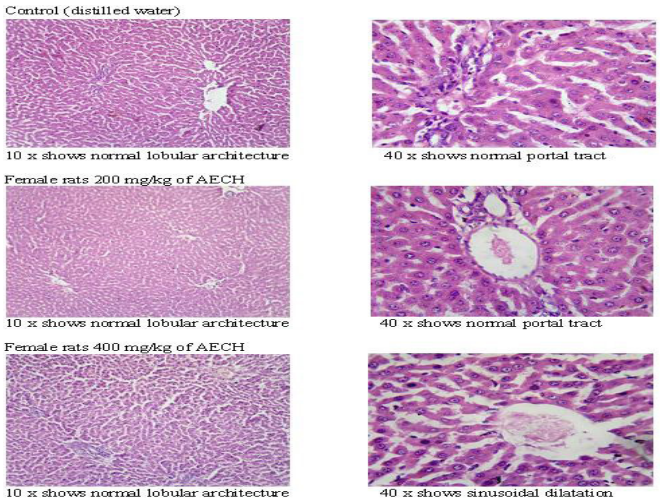


**Figure 15:** Histology of liver for control & treated female rats under 10 x & 40 x magnifications.

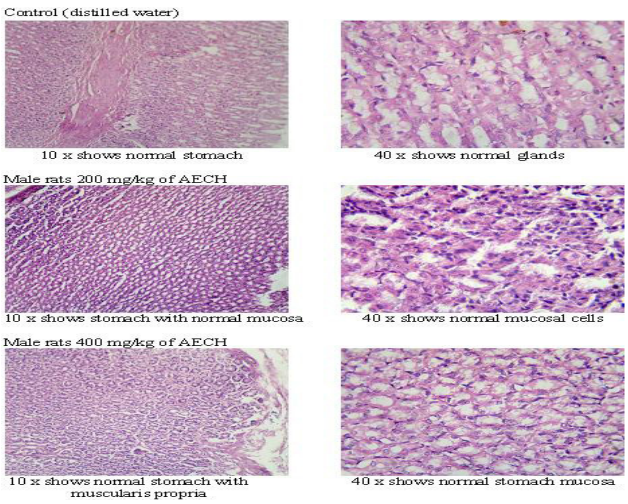


**Figure 16:** Histology of stomach for control& treated male rats under 10 x & 40 x magnifications.

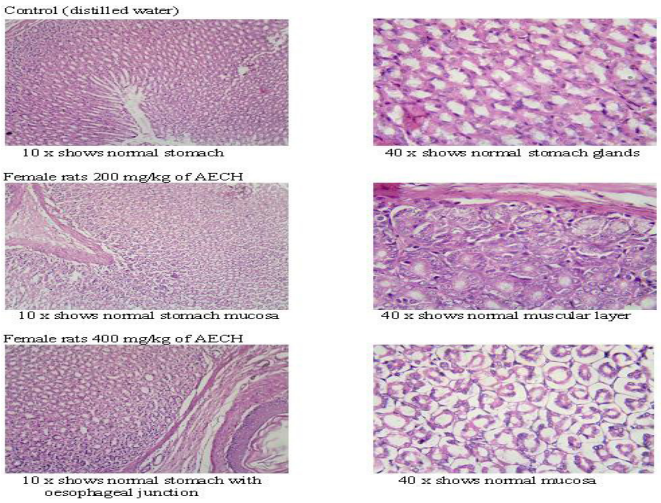


**Figure 17:** Histology of stomach for control& treated female rats under 10x & 40x magnifications.

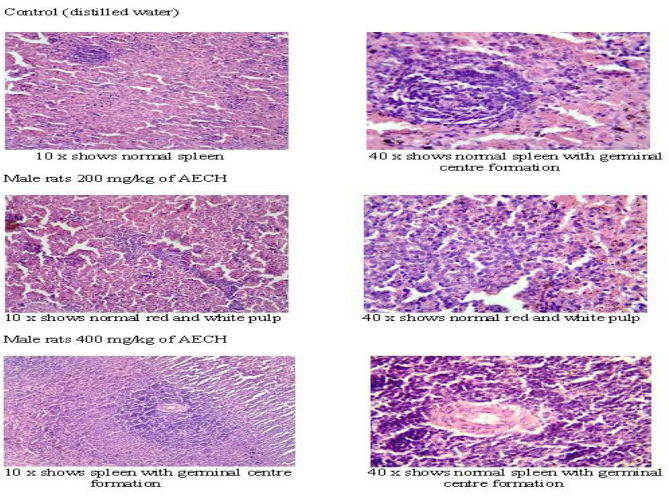


**Figure 18:** Histology of spleen for control & treated male rats under 10 x & 40 x magnifications.

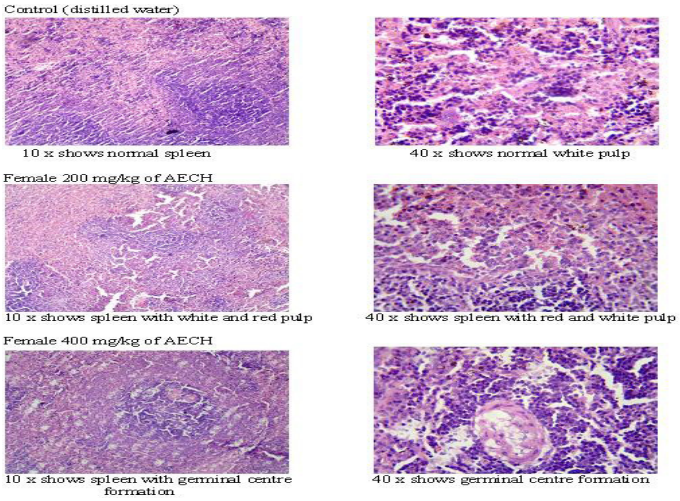


**Figure 19:**Histology of spleen for control & treated female rats under 10 x & 40 x magnifications

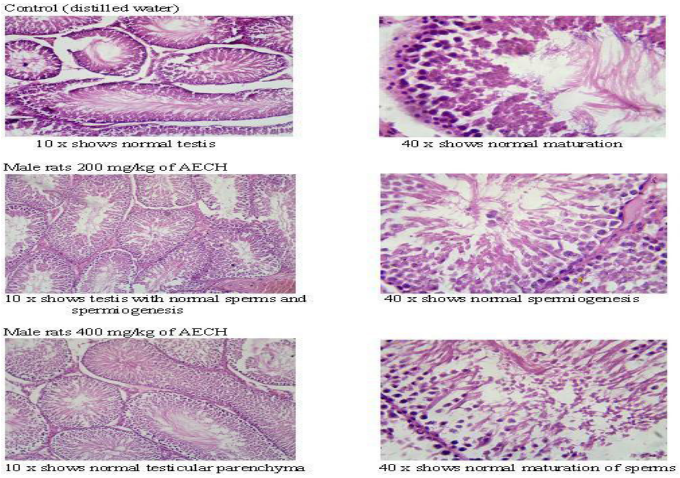


**Figure 22:** Histology of testes for control& treated male rats under 10 x & 40 x magnifications

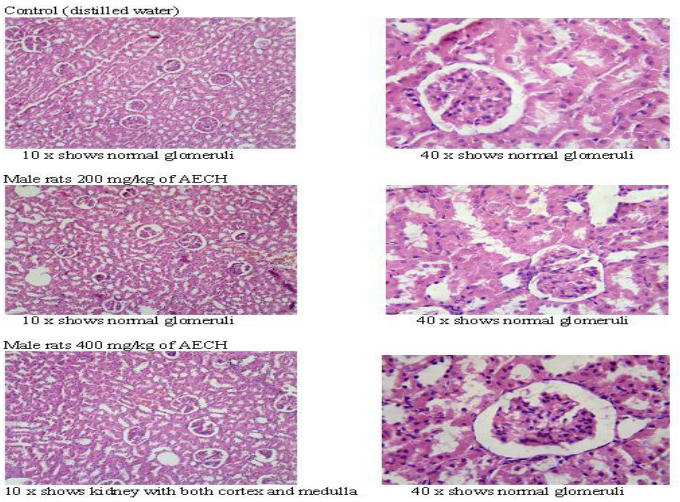


**Figure 20:** Histology of kidney for control& treated male rats under 10 x & 40 x magnifications

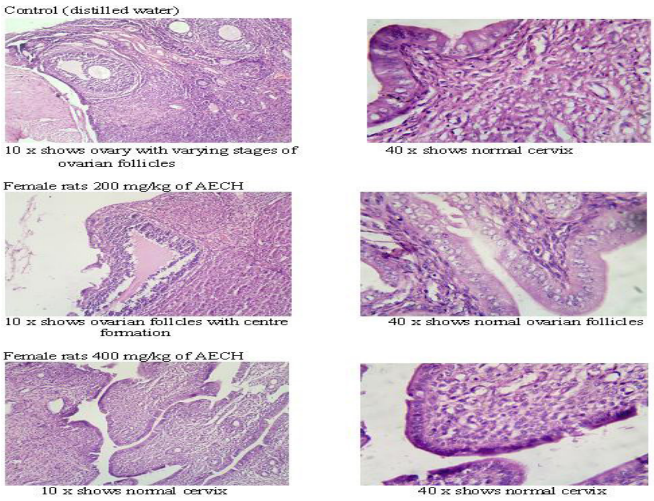


**Figure 23:** Histology of ovary for control& treated female rats under 10 x & 40 x magnifications

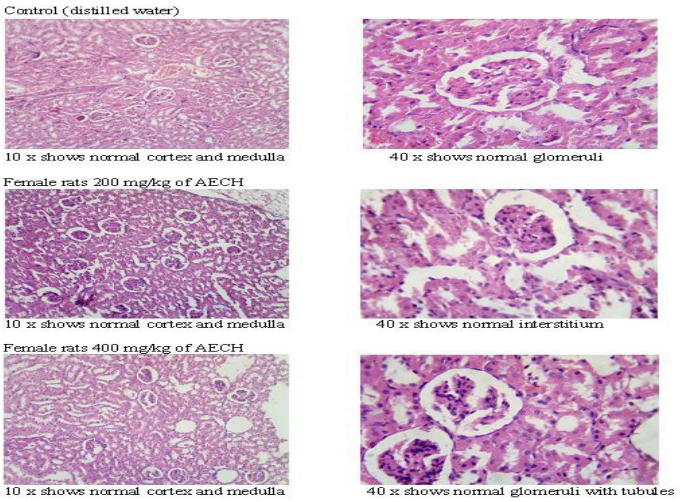


**Figure 21:** Histology of kidney for control& treated female rats under 10 x & 40x magnifications

### *Lungs*

Normal alveoli and normal bronchioles were observed in control group of rats and AECH treated male and female group of rats at a dose of 200 and 400 mg/kg (Figure 12 and 13).

### *Liver*

Histopathological section of liver in control group and AECH treated to male and female groups at a dose of 200 and 400 mg/kg showed normal lobular architecture. The portal tracts, hepatocytes, central veins, sinusoids are found to be normal. No evidence of toxic signs observed as there is no inflammation, fatty change or fibrosis (Figure 14 and 15).

### *Stomach*

Section from the stomach of control group and AECH treated to male and female group of rats at a dose of 200 and 400 mg/kg showed normal mucosa, muscle layer and stomach glands (Figure 16 and 17).

### *Spleen*

Section from the spleen of control group and AECH treated to male and female groups at a dose of 200 and 400 mg/kg showed normal spleen

**Table 1: Behavioural studies of AECH on mice in acute toxicity study**

| Gross activity | 30 Min | 1 h | 2 h | 3 h | 4 h | 24 h | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiration | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Writhing | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Tremor | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Convulsion | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Sense of touch/sound | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Salivation | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urination | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Defecation | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Diarrhoea | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Sedation | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Locomotor | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| edema | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Mortality | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

Indicates normal; – Indicates no effect.

**Table 2: Gross behavioural studies of rats after administration of AECH**

| Gross activity | 30 Min | 1 h | 2 h | 3 h | 4 h | 24 h | Day 3 | Day 5 | Day 7 | Day 9 | Day 11 | Day 13 | Day 15 | Day 17 | Day 19 | Day 21 | Day 23 | Day 25 | Day 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Respiration | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Writhing | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Tremor | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Convulsion | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Sense of touch/ sound | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Salivation | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urination | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Defecation | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Diarrhoea | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Sedation | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Locomotor | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| edema | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Mortality | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

Indicates normal; – Indicates no effect.

**Table 3: Body weight of mice in acute toxicity of AECH**

| Treatment | Body weight (g) | | | |
|---|---|---|---|---|
| | 0 day | 7th day | 14th day | Weight gain on 14th day |
| Control | 19.98 ± 0.5205 | 21.75 ± 0.36 | 23.91 ± 0.49 | 4.1 ± 0.26 |
| AECH 2000 mg/kg | 20.78 ± 0.4178 | 22.23 ± 0.86 | 24.08 ± 1.49 | 3.33 ± 1.29[a] |

Data provided as mean ± SEM (n=6); [a]p<0.05 treated groups Vs control.

**Table 4: Body weights of rats in sub acute toxicity of the AECH**

| Body weight (g) | Day 1 | Day 7 | Day 14 | Day 21 | Day 28 | Weight gain (g) on day 28 th day |
|---|---|---|---|---|---|---|
| Male control | 153.6 ± 1.49 | 182.16 ± 2.61 | 212.66 ± 2.94 | 231.66 ± 4.58 | 250.5 ± 1.62 | 96.83 ± 2.72 |
| Male AECH 200 mg/kg | 188.33 ± 1.52 | 213.0 ± 3.57 | 235.76 ± 3.66 | 255.83 ± 3.24 | 273.33 ± 3.90 | 85.00 ± 3.66[a] |
| Male AECH 400 mg/kg | 159.5 ± 0.67 | 169.83 ± 3.74 | 192.0 ± 4.42 | 211.33 ± 5.70 | 227.83 ± 3.08 | 68.33 ± 3.44[a] |
| Female Control | 124.66 ± 0.98 | 146.3 ± 0.71 | 164.33 ± 1.66 | 182.0 ± 2.35 | 199.5 ± 2.37 | 74.83 ± 2.45 |
| Female AECH 200 mg/kg | 143.83 ± 2.34 | 160.33 ± 2.37 | 182.66 ± 3.12 | 199.66 ± 3.01 | 218.0 ± 2.477 | 74.16 ± 3.04[a] |
| Female AECH 400 mg/kg | 125.66 ± 0.76 | 143.33 ± 1.68 | 162.5 ± 3.39 | 183.33 ± 4.66 | 195.5 ± 4.95 | 68.16 ± 5.34[a] |

Data provided as mean ± SEM (n=6); [a]p<0.05 treated groups Vs control.

**Table 5: Hematological studies on mice after administration of AECH**

| Groups | Total RBC ($X10^6$ cells/$mm^3$) | Total Hb (g/dl) | Total WBC ($X10^6$cells/$mm^3$) | Platelet Count ($X10^6$cells/$mm^3$) | PCV (%) | Differential Count | | | | MPV (fL) | MCV fL) | MCH (pg) | MCHC (g/dL) | RDW (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Polymorphs (%) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) | | | | | |
| Control | 14.40 ± 0.69 | 14.48 ±0.42 | 13.98 ± 0.75 | 789.5 ± 13.67 | 45.58 ± 1.49 | 5.16 ± 0.30 | 80.50 ± 2.23 | 3.66 ± 0.42 | 2.83 ± 0.30 | 8.05 ± 0.31 | 60.30 ± 0.43 | 19.15 ± 0.19 | 32.31 ± 0.54 | 18.9 ± 0.48 |
| AECH 2000 mg/kg | 16.83 ± 0.79[a] | 19.01 ±0.23[a] | 12.23 ± 0.55[a] | 811.7 ± 20.61[a] | 46.18 ± 1.45[a] | 5.5 ± 0.56[a] | 87.66 ± 9.66[a] | 3.83 ± 0.30[a] | 3.50 ± 0.22[a] | 7.75 ± 0.22[a] | 58.95 ±0.36[a] | 19.11 ± 0.22[a] | 30.38 ± 0.60[a] | 17.7 ± 0.54[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

with red pulp and germinal center formation. No abnormality was observed in histological sections of spleen (Figure 18 and 19).

### *Kidney*

Sections from kidney of control group and AECH treated to male and female groups at a dose of 200 and 400 mg/kg showed normal cortex and medulla. The cortex showed normal glomeruli. The interstitium and distal convoluted tubules are found to be normal. No inflammation or tubular necrosis was observed (Figure 20 and 21).

### *Testes*

Sections from kidney of control group and AECH treated to male groups at a dose of 200 and 400 mg/kg showed normal testes and spermatozoa (Figure 22).

### *Ovary*

Sections from kidney of control group and AECH treated to female groups at a dose of 200 and 400 mg/kg showed normal ovarian follicles (Figure 23).

## DISCUSSION

Safety of aqueous extract of whole plant of *C. heteroclita* by determining its potential toxicity after acute and sub-acute administration in mice and rats as per OECD guidelines 423 and 407 respectively as there were no earlier reports on the safety assessment of aqueous extract of *C. heteroclita*. In acute toxicity test, mortality was not observed in mice with the maximum dose of 2000 mg/kg throughout the 14 days of study. In sub-acute toxicity test also no mortality was observed in male and female rats with the maximum dose of 400 mg/kg throughout 28 days of study. Significant changes were not found in breathing, sense of touch/sound, central nervous systems, behaviour pattern and locomotor activity. Convulsion, tremor, excessive salivation, diarrhea, sedation and edema were not observed.

Body weight and internal organ weight changes is sensitive and indicative marker for the first sign of toxicity when exposed to toxic substances.[7] All animals were found to be active with increase in body weight both in acute and sub-acute toxicity study. Normal food and water consumption of animals clearly support the gain in body of animals throughout the study period.

Hematopoietic system is the most sensitive target for toxic substances and it is the important index of physiological and pathological status[8,9] and hence hematological investigation was carried out. No abnormality was observed in hematopoietic function indices for AECH treated groups compared with control groups indicating the extract is safe.

Lipid profile assessment was performed for the animals treated with AECH to verify any changes observed in serum levels of triglycerides, cholesterol and VLDL.[10] No significant variation in cholesterol, triglycerides and VLDL levels in AECH treated groups when compared with control groups.

SGOT, SGPT, ALP and bilirubin levels of the experimental animals were monitored as they are the specific markers for liver damage or injury.[11]

**Table 6:** Hematological studies on rats after administration of AECH

| Groups | Total RBC ($X10^6$cells/$mm^3$) | Total Hb (g/dl) | Total WBC ($X10^6$cells/$mm^3$) | Platelet Count ($X10^6$cells/$mm^3$) | PCV (%ia) | Differential Count | | | | MPV (fL) | MCV fL) | MCH (pg) | MCHC (g/dL) | RDW (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Polymorphs (%) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) | | | | | |
| Control Male | 4.98 ± 0.13 | 14.95 ± 0.59 | 11.55 ± 0.82 | 740.16 ± 40.67 | 46.48 ± 1.66 | 5.0 ± 1.06 | 84.83 ± 2.05 | 3.33 ± 0.21 | 5.66 ± 0.55 | 7.33 ± 0.20 | 63.95 ± 0.73 | 22.25 ± 0.68 | 31.88 ± 0.35 | 17.84 ± 0.41 |
| Male AECH 200 mg/kg | 5.35 ± .46[a] | 12.45 ± 0.89[a] | 13.45 ± 1.28[a] | 756.33 ± 46.98[a] | 38.5 ± 1.76[a] | 5.33 ± 1.45[a] | 85.66 ± 1.99[a] | 4.0 ± 0.44[a] | 4.83 ± 0.40[a] | 7.8 ± 0.16[a] | 62.68 ± 0.36[a] | 21.3 ± 0.52[a] | 29.75 ± 1.26[a] | 18.58 ± 0.26[a] |
| Male AECH 400 mg/kg | 4.86 ± 0.19[a] | 14.55 ± 0.49[a] | 11.90 ± 1.05[a] | 854.5 ± 22.37[a] | 45.55 ± 1.38[a] | 3.16 ± 0.60[a] | 86.5 ± 1.76[a] | 3.5 ± 0.22[a] | 5.5 ± 0.50[a] | 8.2 ± 0.17[a] | 62.96 ± 0.35[a] | 21.56 ± 0.29[a] | 32.85 ± 0.65[a] | 17.87 ± 0.20[a] |
| Control Female | 5.0 ± 0.10 | 15.36 ± 0.36 | 14.5 ± 0.35 | 777.3 ± 30.75 | 52.78 ± 0.96 | 5.00 ± 0.81 | 86.16 ± 1.60 | 3.76 ± 0.33 | 5.66 ± 0.33 | 8.1 ± 0.06 | 71.5 ± 1.54 | 32.88 ± 0.36 | 32.80 ± 0.36 | 18.45 ± 0.14 |
| Female AECH 200 mg/kg | 5.05 ± 0.27[a] | 15.33 ± 0.40[a] | 14.5 ± 0.35[a] | 815.83 ± 15.93 [a] | 53.26 ± 1.29[a] | 6.00 ± 0.63[a] | 87.33 ± 1.60[a] | 4.50 ± 0.42[a] | 5.35 ± 0.21[a] | 8.41 ± 0.19[a] | 73.66 ± 1.22[a] | 34.08 ± 0.40[a] | 34.28 ± 0.35[a] | 18.83 ± 0.15[a] |
| Female AECH 400 mg/kg | 5.27 ± 0.27[a] | 14.48 ± 0.53[a] | 11.9 ± 1.05[a] | 854.5 ± 22.37[a] | 49.1 ± 0.64[a] | 3.16 ± 0.60[a] | 86.5 ± 1.17[a] | 4.16 ± 0.30[a] | 5.66 ± 0.42[a] | 8.48 ± 0.14[a] | 70.91 ± 0.58[a] | 34.6 ± 0.50[a] | 34.25 ± 0.50[a] | 18.31 ± 0.21[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

**Table 7:** Effect of acute dose of AECH on liver function test on mice

| Groups | Dose mg/kg *(p.o.)* | SGOT (U/L) | SGPT (U/L) | ALP (U/L) | Total Bilirubin (mg/dl) |
|---|---|---|---|---|---|
| Control | Water (5 ml/kg) | 33.01 ± 1.01 | 29.08 ± 2.03 | 249.08 ± 6.83 | 0.80 ± 0.07 |
| AECH | 2000 mg/kg | 35.79 ± 1.56[a] | 31.66 ± 1.52[a] | 270.58 ± 6.40[a] | 1.03 ± 0.07[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

**Table 8: Effect of sub-acute dose of AECH on liver function test**

| Groups | Dose mg/kg *(p.o.)* | SGOT (U/L) | SGPT (U/L) | ALP (U/L) | Total Bilirubin (mg/dl) | Total protein (g/dl) | Albumin (g/dl) | Globulin (g/L) |
|---|---|---|---|---|---|---|---|---|
| Control Male | Water (5 ml/kg) | 54.29 ± 4.38 | 48.25 ± 2.07 | 237.35 ± 16.92 | 0.76 ± 0.090 | 7.23 ± 0.28 | 3.8 ± 0.18 | 3.08 ± 0.29 |
| Male AECH | 200 mg/kg | 57.21 ± 3.91[a] | 50.08 ± 1.95[a] | 247.6 ± 15.70[a] | 0.75 ± 0.17[a] | 7.61 ± 0.42[a] | 4.21 ± 0.44[a] | 4.41 ± 0.31[a] |
| Male AECH | 400 mg/kg | 50.31 ± 7.58[a] | 41.51 ± 3.74[a] | 264.18 ± 24.69[a] | 0.96 ± 0.13[a] | 7.4 ± 0.26[a] | 4.46 ± 0.16[a] | 3.76 ± 0.35[a] |
| Control Female | Water (5 ml/kg) | 51.33 ± 1.85 | 44.61 ± 1.25 | 272.75 ± 5.36 | 0.86 ± 0.04 | 7.16 ± 0.26 | 4.26 ± 0.11 | 2.86 ± 0.16 |
| Female AECH | 200 mg/kg | 57.83 ± 2.07[a] | 46.83 ± 2.12[a] | 291.0 ± 4.89[a] | 0.93 ± 0.03[a] | 7.21 ± 0.23[a] | 4.23 ± 0.10[a] | 3.23 ± 0.22[a] |
| Female AECH | 400 mg/kg | 53.33 ± 3.05[a] | 50.66 ± 1.28[a] | 286.33 ± 2.86[a] | 0.92 ± 0.04[a] | 7.28 ± 0.17[a] | 4.70 ± 0.11[a] | 3.36 ± 0.23[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

**Table 9: Effect of acute dose of AECH on kidney function test**

| Groups | Dose mg/kg *(p.o.)* | Urea (mg/dl) | Uric acid (mg/dl) |
|---|---|---|---|
| Control | Water (5 ml/kg) | 26.90 ± 2.81 | 4.81 ± 0.09 |
| AECH | 2000 mg/kg | 29.66 ± 1.49[a] | 4.76 ± 0.20[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

**Table 10: Effect of sub-acute dose of AECH on kidney function test**

| Groups | Dose mg/kg *(p.o.)* | Creatinine | Urea (mg/dl) | Uric acid (mg/dl) |
|---|---|---|---|---|
| Control Male | Water (5 ml/kg) | 0.7 ± 0.05 | 18.63 ± 1.29 | 2.3 ± 0.15 |
| Male AECH | 200 mg/kg | 0.75 ± 0.06[a] | 21.95 ± 2.28[a] | 1.81 ± 0.31[a] |
| Male AECH | 400 mg/kg | 0.71 ± 0.07[a] | 20.68 ± 3.23[a] | 2.45 ± 0.16[a] |
| Control Female | Water (5 ml/kg) | 0.80 ± 0.02 | 19.06 ± 0.42 | 2.34 ± 0.08 |
| Female AECH | 200 mg/kg | 0.80 ± 0.04[a] | 20.66 ± 0.49[a] | 2.35 ± 0.11[a] |
| Female AECH | 400 mg/kg | 0.76 ± 0.03[a] | 18.13 ± 0.42[a] | 2.63 ± 0.08[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

**Table 11: Effect of sub-acute dose of AECH on lipid profile**

| Groups | Dose mg/kg *(p.o.)* | Cholesterol (mg/dl) | Triglycerides (mg/dl) | VLDL(mg/dl) |
|---|---|---|---|---|
| Control Male | Water (5 ml/kg) | 95.05 ± 5.76 | 262.43 ± 34.98 | 52.58 ± 7.03 |
| Male AECH | 200 mg/kg | 107.45 ± 7.19[a] | 337.93 ± 12.20[a] | 67.58 ± 2.44[a] |
| Male AECH | 400 mg/kg | 111.1 ± 3.95[a] | 323.61 ± 18.27[a] | 64.72 ± 3.65[a] |
| Control Female | Water (5 ml/kg) | 89.03 ± 4.50 | 295.76 ± 14.55 | 59.15 ± 2.91 |
| Female AECH | 200 mg/kg | 95.66 ± 2.07[a] | 324.33 ± 4.01[a] | 64.86 ± 0.80[a] |
| Female AECH | 400 mg/kg | 96.66 ± 3.14[a] | 332.66 ± 5.69[a] | 66.53 ± 1.13[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

No significant changes in SGOT, SGPT, ALP and bilirubin level was observed in all the treated group animals. Thus animals treated with AECH were found to be non-hepatotoxic.

To assess whether treatment of AECH to rats causes any damage to kidney, kidney function test was performed. GLDH-UV Kinetic method, Jaffe method and Uricase POD method was adopted to evaluate the important markers of renal dysfunction viz. urea, uric acid and creatinine levels respectively.[12] AECH was found to be safe and nontoxic to kidney as there is no significant change observed in creatinine, urea and uric acid levels of AECH treated group of animals both in acute and sub-acute toxicity when compared with control group animals. When compared with control groups, no variation in relative organ weight was observed for AECH treated groups both in acute and sub-acute toxicity study. The above mentioned biochemical investigations were in correlation with the histopathological studies.

Section of the heart of the control and AECH treated animals showed no abnormality as shown in Figures 10 and 11. Alveoli and bronchioles were found to be normal both in control and treated group confirming that AECH did not cause any toxicity to lungs of the animals. Inflammation or cirrhosis or necrosis was not observed in the histological study of liver in mice and rats tested with AECH at a dose of 400 mg/kg. Normal cortex, medulla and normal glomeruli were observed indicating that AECH did not cause any damage to kidney. Cross section of stomach under low and high power magnification showed normal mucosal surface, normal stomach glands and normal propria indicated that AECH not caused any toxicity to rats. Histological sections of testes clearly implicated that no testicular toxicity was observed to the treated male group of rats for 28 days in sub-acute study. Observation of normal cervix, mucosa and follicles from histological sections of ovary confirmed that AECH was non toxic to ovary of female rats.

## CONCLUSION

Based on our results, we conclude that AECH were found to be safe up to a dose of 2000 mg/kg. Hematological, biochemical and histopathological investigations clearly demonstrates that single oral administration upto 2000 mg/kg in acute toxicity study and daily oral administration of the AECH for 28 days upto 400 mg/kg in sub-acute toxicity study caused no significant adverse changes in the organs like heart, lungs, liver, spleen and kidney. Abnormalities were also not observed in the organs stomach, testes and ovary of the animals tested at a dose of 200 and 400 mg/kg respectively. The study provided significant data on the

**Table 12: Relative organ weight in g/100 g (BW) of control and mice treated with AECH**

| Groups | Dose mg/kg *(p.o.)* | Heart (g) | Lungs (g) | Liver (g) | Spleen (g) | kidney (g) |
|---|---|---|---|---|---|---|
| Control | Water (5 ml/kg) | 0.12 ± 0.003 | 0.24 ± 0.012 | 1.16 ± 0.036 | 0.12 ± 0.004 | 0.331 ± 0.007 |
| AECH | 2000 mg/kg | 0.13 ± 0.001[a] | 0.25 ± 0.008[a] | 0.96 ± 0.283[a] | 0.15 ± 0.015[a] | 0.351 ± 0.014[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

**Table 13: Relative organ weight in g/100 g (BW) of control and rats treated with AECH**

| Groups | Heart (g) | Lungs (g) | Liver (g) | Spleen (g) | Left kidney (g) | Right kidney (g) | Thymus | TESTIS/ OVARY |
|---|---|---|---|---|---|---|---|---|
| Control Male | 0.352 ± 0.016 | 0.69 ± 0.02 | 2.65 ± 0.04 | 0.72 ± 0.01 | 0.32 ± 0.008 | 0.38 ± 0.013 | 0.29 ± 0.015 | 1.07 ± 0.022 |
| Male AECH 200 mg/kg | 0.355 ± 0.013[a] | 0.69 ± 0.06[a] | 2.72 ± 0.06[a] | 0.69 ± 0.01[a] | 0.31 ± 0.002[a] | 0.36 ± 0.020[a] | 0.27 ± 0.008[a] | 1.13 ± 0.016[a] |
| Male AECH 400 mg/kg | 0.340 ± 0.021[a] | 0.73 ± 0.02[a] | 2.72 ± 0.06[a] | 0.75 ± 0.01[a] | 0.33 ± 0.010[a] | 0.37 ± 0.010[a] | 0.29 ± 0.012[a] | 1.07 ± 0.035[a] |
| Control Female | 0.373 ± 0.010 | 0.758 ± 0.023 | 2.34 ± 0.036 | 0.628 ± 0.035 | 0.291 ± 0.007 | 0.367 ± 0.012[a] | 0.187 ± 0.013[a] | 0.499 ± 0.044[a] |
| Female AECH 200 mg/kg | 0.385 ± 0.012[a] | 0.796 ± 0.006[a] | 2.39 ± 0.048[a] | 0.678 ± 0.014[a] | 0.290 ± 0.006[a] | 0.330 ± 0.004[a] | 0.157 ± 0.012[a] | 0.478 ± 0.015[a] |
| Female AECH 400 mg/kg | 0.367 ± 0.017[a] | 0.771 ± 0.005[a] | 2.37 ± 0.034[a] | 0.655 ± 0.043[a] | 0.316 ± 0.016[a] | 0.353 ± 0.019[a] | 0.180 ± 0.007[a] | 0.584 ± 0.011[a] |

Data provided as mean ± SEM (n=6); [a]p>0.05 treated groups vs control.

sub-acute toxicity profile of AECH which may be valuable in the clinical study of medicinal herb *C. heteroclita* (L) Gilg. The aqueous extract of *C. heteroclita* (L) Gilg thus may be used as one of the ingredient in traditional formulation or any other pharmaceutical formulations. The study on the interaction of this plant with other herbs in multi herbal formulation may be useful for assessment of efficacy of the formulations.

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## CONFLICT OF INTEREST

Authors disclose no conflicts of interest for publication of the manuscript.

## ABBREVIATION USED

**CH:** *Canscora heteroclite* (L.) Gilg.**; AECH:** Aqueous extract of *Canscora heteroclita*; **OECD:** Organization for Economic Co-operation and Development; **RH:** Relative Humidity; **CPCSEA:** Committee for the Purpose of Control and Supervision on Experiments on Animals; **IAEC:** Institutional Animal Ethical Committee; **RBC:** Red blood cells; **WBC:** White blood cells; **Hb:** Hemoglobin; **PCV:** Packed cell volume; **MPV:** Mean Platelet Volume; **MCV:** Mean corpuscular volume; **MCH:** Mean corpuscular hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **RDW:** Red Blood Cell Distribution Width; **VLDL:** Very low density lipoprotein; **SGOT:** Serum glutamic oxaloacetic transaminase; **SGPT:** Serum glutamic-pyruvic transaminase; **ALP:** Alkaline phosphatase; **GLDH:** Glutamate dehydrogenase; **POD:** Peroxidase.

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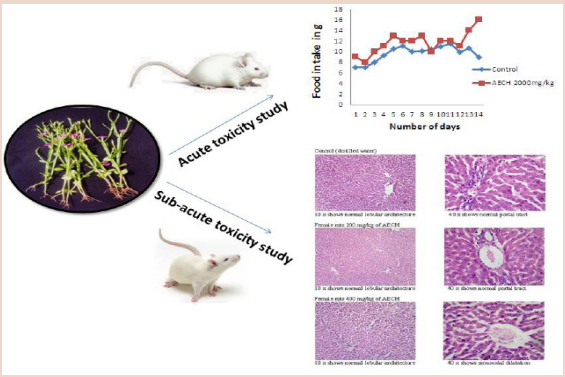
## PICTORIAL ABSTRACT




## SUMMARY

- No deaths or any sign of toxicity was observed in acute toxicity study after oral administration of AECH in mice upto a dose of 2000 mg/kg.
- Both male and female treated rodents showed no change in hematological, biochemical and histological investigations.
- AECH was found to be safe in subacute toxicity study, as no mortality was observed in rats treated with 400 mg/kg.

## ABOUT AUTHORS

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