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Preclinical Toxicological Profile of Hydroalcholic Leaf Extract of *Ipomoea aquatica* Forsk an Indian Medicinal Plant

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ABSTRACT

Objective: The present investigation was carried out to evaluate the safety of an hydroalcholic extract of *Ipomoea aquatica* Forsk (HAEIA) leaves by determining its potential toxicity after acute and sub-acute administration in rodent. **Materials and Methods:** For the acute study HAEIA was administered in single doses of 500, 1000 and 2000 mg kg^{-1} (p.o). General behavior, adverse effects and mortality were determined for up to 14 days. The sub-acute toxicity test was based on the daily administration of two doses of HAEIA (200 and 400 mg kg^{-1} body weight) for four weeks, 0.1% CMC served as control group. **Results:** In the acute and sub-acute study hydroalcoholic (Water plus ethanol) extract of *Ipomoea aquatica* leaves doesn't caused any change in general behavior, adverse effects and mortality. Throughout the study period no sign of toxicity was registered. Conversely, the sub-acute doses stimulated slight increase in body weight of the animals treated test drug. Further it was observed that HAEIA at the dose of 200 and 400 mg kg^{-1} did not modify the weight index. It was evident that daily oral dose of HAEIA at at both the dose level for 28 day days did not shown any significant change in hematological parameters and also no change observed in biological analysis of serum Aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), bilirubin, creatinine, Blood Urea Nitrogen (BUN) levels. **Conclusion:** In view of the results obtained through acute toxicity and sub-acute study of *Ipomoea aquatica* (Water Spinach) which is consumed as a traditional medicine of india and china. No significant changes were observed in organ weights and histopathological results showed normal profile suggesting no morphological alterations there is a wide margin of safety for the therapeutic use of this plant was proved through this study which enables the research to carry out the lead invention from the natural resources.

Key words: *Ipomoea aquatica,* acute toxicity, sub-acute toxicity, hematological parameters, biological analysis, histopathology

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INTRODUCTION

A Word Health Organization survey indicated that about 70-80% of the world's populations rely on non-conventional medicine, mainly of herbal source, in their primary healthcare. This is especially the case in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the means of most people[1,2]. Although, medicinal plants may produce several biological activities in humans, generally very little is known about their toxicity and the same applies for *Ipomoea aquatica* Forsk.

A large number of plants belonging to the convolvulaceae are used in the traditional medicine of certain countries[3] .The convolvulaceae represent one of the largest families of flowering plants of about 1750 species and Ipomoea has about 500 species in particular. *Ipomoea aquatica* Forsk commly known as water spinach belongs to the family Convolvulaceae grows wild and is cultivated throughout Southeast Asia and is a widely consumed vegetable in these regions[4]. Many of the waters where IAF grows serve as recipients for domestic and other types of waste water.

Water spinach is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka[5]. Only a very few scientific studies have been conducted on its medicinal aspects. These include the inhibition of effects on liver diseases[6], constipation[7]. IAF is considered a tonic the species contains several vitamins, including A, B, C, E and "U"

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(S-methyl-methionine) and is used to treat gastric and intestinal disorders[8,9,10]. The species also contains aliphatic pyrrolidine amides, carotenoids, hentriacontane, β-sitosterol and its glycosides, N-trans- and N-cis feruloyl tyramines[11,12,13,14,15]. It is runner type plant with numerous small flowers[16]. The present study was carried out to evaluate the safety of a hydroalcholic extract of *Ipomoea aquatica* forsk (HAEIA) leaves by determining its potential toxicity after acute and sub-acute administration in rodent.

MATERIALS AND METHODS

Plant material: The fresh leaves of *Ipomoea aquatica* (IA) were collected from (Perambur region of chennai, Tamilnadu, India). The plant was identified and authenticated by Dr. Sasikala Ethirajulu, Captain srinivasa moorthy Research Foundation, Chennai, Tamil Nadu, India. The specimen voucher was deposited in the Department of Pharmacology and toxicology, C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India.

Preparation of the Hydroalcholicic Extract of IAF: The fresh leaf of IAF was collected and washed with running water. It was shade dried at room temperature and 1 kg of the dried leaf was made in to coarse powder. The powder was passed through a 60 No. mesh sieve. Air dried Powdered drug was extracted with mixture of hydroalcholic:water (6:4) (hydro-alcoholic extract) by using soxhlet extraction apparatus and allowed to stand at room temperature for about 24 h. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The weight of extract obtained was 20.6%.

Animals: Healthy swiss albino of either sex weighing 25-30 g, obtained from the C.L. Baid Metha college of pharmacy animal house. Animals were segregated according to the gender and housed five per plastic cage in a well-ventilated room with 12 h cycle of day and night light conditions and temperature maintained at around 25°C.

Acute toxicity testing[17]: Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co-operation and Development, Guideline-423, adopted on 17th December, 2001. Animals were divided into four groups of 10 animals each five female and five male in each group. All animals were deprived of food, but not water 16 h prior to the administration of the test drug. The first group (control group) received vehicle (0.1% CMC) orally. Groups 2-4 were orally treated with hydroalcholic leaf extract of *Ipomoea aquatica* Forsk aseptically suspended in 0.1% CMC solution and administered in single oral dose of 500, 1000 and 2000 mg kg^{-1} (p.o.) by gavage . Doses were increased progressively so that each dose was 50% higher than the preceding one. The general behavior of the and observations of toxic symptoms were made and recorded systematically at one, two, four, six up to 24 h after drug administration. Briefly, measurements were first carried out in the home cage. The observer recorded each animal's posture, activity and palpebral closure. The presence or absence of tremors and convulsions was noted and, if present, described. The presence or absence of spontaneous vocalizations and biting was also noted. The presence or absence of hind limb flexor resistance was also noted. Palpebral closure and any lacrimation or salivation was rated. Other abnormal clinical signs were also recorded.

The animal was next placed in an open field arena having a piece of clean absorbent paper on the surface and allowed to freely explore for 3 min. During that time, the observer ranked the arousal, gait score, activity level and rears as well as any abnormal postures, unusual movements and stereotypy. At the end of the 3 min, the number of fecal boluses and urine pools and presence or absence of diarrhea on the absorbent paper was recorded. Sensorial responses were ranked according to a variety of stimuli (click stimulus using a metal clicker and touch rump with a blunt object, pinch of the tail using forceps, constriction of the pupil to a penlight stimulus and touch of the corner of the eye and the inside of the ear with a fine object).

Motor activity[18]: An open field of 50×50×60 cm whose floor was divided into 12×12 cm squares by black lines was used. The number of squares entered with all four paws, rearings, groomings and line crossing were scored for 20 min. After each animal was removed, the open field was carefully cleaned with a damp cloth.

Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days, signs of toxicity and mortality were observed once daily for 14 consecutive days.

Subacute toxicity[19]: Sub-acute toxicity tests were also performed in mice of both sexes, in order to evaluate the toxic effect of the HAEIA on repeated dosage for 28 days. The animals were divided into three groups of ten animals each five females and five male's totaling of 30 animals. HAEIA was suspended in 0.1% CMC was administered orally by gavaging at two dose levels of 200 and 400 mg kg^{-1} daily to two groups for a period of 28 days. The control group received an equal volume of the 0.1% CMC vehicle. All animals were weighed and observed daily for food and water intake, physiological

and behavioral changes. Whole blood samples were collected by cardiac puncture after 24 h of the last dose HAEIA for haematological studies such as Hb, RBC and total WBC and differential WBC. The serum was analyzed for Blood Urea Nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatases (ALP), bilirubin, creatinine and cholesterol.

Vital organs like liver, brain, kidney, spleen, heart, lungs, testis and ovary were dissected, collected, weighed and visually inspected for any histopathological changes followed by preservation in 10% formalin for histopathalogical studies.

Blood analyses: The hematological and the biochemical analyses were performed at Research and Development Unit of C.L. Baid metha College of pharmacy. Full blood cell counts (red blood cells, hemoglobin, white blood cell and platelets) were determined on a fully automated analyzer. Bio chemical test were performed for analyzine the following Blood Urea Nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatases (ALP), bilirubin, creatinine and cholesterol.

Histopathological evaluation: Appearances of organs were observed and the index of each organ was calculated. These organs included of liver, brain, kidney, spleen, heart, lungs, testis and ovary. Histological slides of organs were made and observed under the microscope. The pathological observations of these tissues were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

Statistical analysis: The statistical analysis was carried by one way ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as Mean±standard error. A statistical comparison was carried out using the Duncan Multiple Range Test for the control and treatment group. p-values less than 0.05 were set as the level of significance.

RESULTS

Effect of HAEIA on acute oral toxicity study: The maximum dose used for acute toxicity was 2000 mg kg^{-1} .Animals did not cause any mortality or any clinical signs of acute toxicity in observed for a short period (24-48 h) and a long period (14 days). At the dose of 500, 1000 and 2000 mg kg^{-1} animals also did not show any mortality and Fig. 1 shows effect of HAEIA on open field habituation.

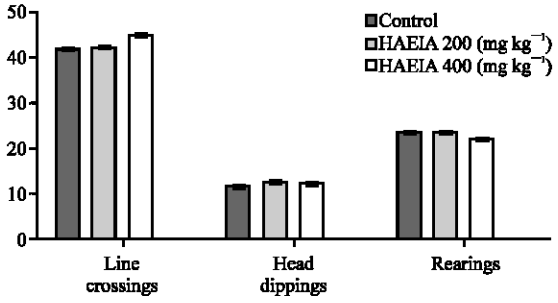

Fig. 1: Effect of HAEIA on open field exploratory behavior

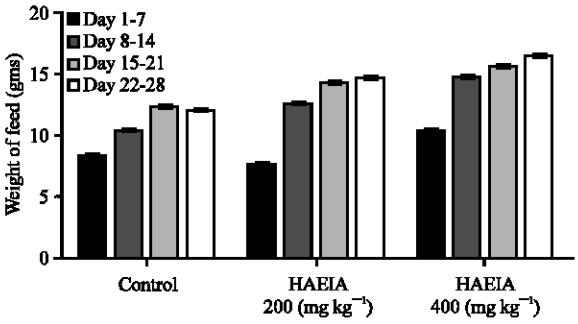

Fig. 2: Effect of HAEIA on feed consumption by mice

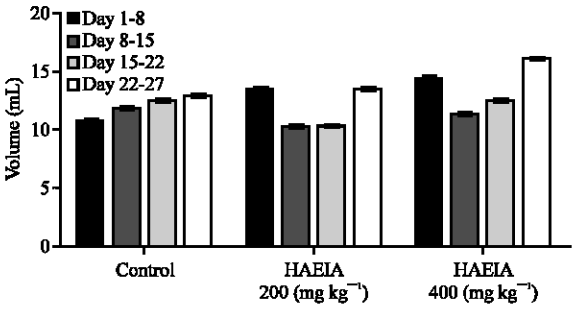

Fig. 3: Effect of HAEIA on water consumption by mice

No significant change was observed in line crossing, Head dipping and rearing.

Effect of HAEIA on sub-acute toxicity study: No toxicity signs (such as piloerection, convulsion and alteration in the locomotor activity or diarrhea) or deaths were recorded during the 14 consecutive days of treatment via oral route with HAEIA at doses of 200 and 400 mg kg^{-1}. No significant differences were found between the initial and final body weight of the animals treated with HAEIA and control. A similar absence of toxic effect was observed in the case of food and water consumption as per Fig. 2 and 3.

Gross necropsy findings did not show any adverse effects in any organ. No statistically significant differences in organ weights were present in any of the male and female mice receiving the extracts of HAEIA as compared to the control group (Table 1). Moreover, no lethality was recorded for any dose up to the maximum of 400 mg kg^{-1} body weight during the 28 days of treatment. Histopathological examination was performed on animals in the control, low and high-dose groups. No significant histopathological change was noted in the high-dose group as compared to control.

Effect of HAEIA on organ weight changes in sub-Acute oral toxicity in mice: The effects of HAEIA on wet weight changes of mice organs i.e., liver, brain, kidney, spleen, heart, lungs, testis and ovary are summarized in Table 2 and 3. The chronic oral ingestion of HAEIA (200 and 400 mg kg^{-1}, p.o. for 28 days) caused no significant changes in the weights of the vital organs as compared to normal control group.

Effect of HAEIA on blood analyses: Hematological and biochemical parameters of the treated and control groups are presented in Table 4. No statistically significant differences were recorded in any of the parameters examined.

Effect of HAEIA on histopathological study: The macroscopic analysis of the target organs of the treated animals did not show significant changes in color and texture when compared with the control group. In addition, the microscopical analysis did not suggest histological alterations in any of the organs examined. Histopathology images represented as figure (Fig. 4-11).

Table 1: Quantitative data on the body Weight of mice treated with HAEIA for 28 days

	Pre-treatment		Post-Treatment	
Treatment	Male	Female	Male	Female
Control	25.46+0.22	29.47±0.29	31.2±0.40	32.19±0.20
HAEIA 200 (mg kg^{-1})	27.57±0.36	32.97±0.40	34.03±0.35*	33.23±0.32*
HAEIA 400 (mg kg^{-1})	28.72±0.35	34.26±0.33	34.77±0.11*	33.50±0.22*

Values are Mean+SEM, Tabular value represents *p<0.05. The average weight calculated on the number of surviving animals (n=10). *Significant at p<0.05

Table 2: Quantitative Data on organ weight (g) of mice treated with HAEIA for 28 days

Treatment	Heart	Lungs	Live	Kidney
Control	0.513±0.07	0.59±0.01	5.16±0.05	1.46±0.01
HAEIA 200 (mg kg^{-1})	0.449±0.06	0.55±0.06	4.73±0.16	1.456±0.01
HAEIA 400 (mg kg^{-1})	0.52±0.07	0.63±0.05	0.56±0.05	1.43±0.04

Values are Mean±SEM (n = 10/group)

Table 3: Quantitative data on organ weight of mice treated with HAEIA for 28 days

Treatment	Spleen	Testis	Ovary	Brain
Control	0.49±0.07	0.65±0.03	0.27±0.13	0.46±0.05
HAEIA 200 (mg kg^{-1})	0.48±0.06	0.63±0.08	0.27±0.02	0.47±0.02
HAEIA 400 (mg kg^{-1})	0.51±0.06	0.65±0.02	0.28±0.02	0.47±0.03

Values are Mean±SEM (n = 10/group)

Table 4: Selected hematological parameters following 28 days of exposure to HAEIA in sub-acute study

Parameters	Control	HAEIA 200 (mg kg^{-1})	HAEIA 400 (mg kg^{-1})
RBC (10^6 mL^{-1})	7.5±0.12	8.29±0.10	8.48±0.05
WBC (10^3 mL^{-1})	5.32±0.08	5.45±0.007	5.28±0.05
Platelets ($\times10^3$ μL^{-1})	563.7±1.07	542±2.11	547±1.25
HGB (g dL^{-1})	12.16±0.07	13.28±0.09	13.34±0.05
MCV (fL)	54.7±0.61	54.5±0.63	56.31±0.44
MCH (pg)	15.58±0.10	17.37±0.08	15.98±0.06
Differential count (%)			
Eosinophils	0.38±0.01	0.63±0.02	0.74±0.02
Neutrophils	66.46±0.42	70.49±0.45	64.69±0.17
Basophils	0.00	0.00	0.00
Lymphocytes	34.5±0.54	34.10±0.48	36±0.39
Monocytes	3.1±0.2	3.4±0.12	3.8±0.21

Values are mean±SEM (n = 10/group)

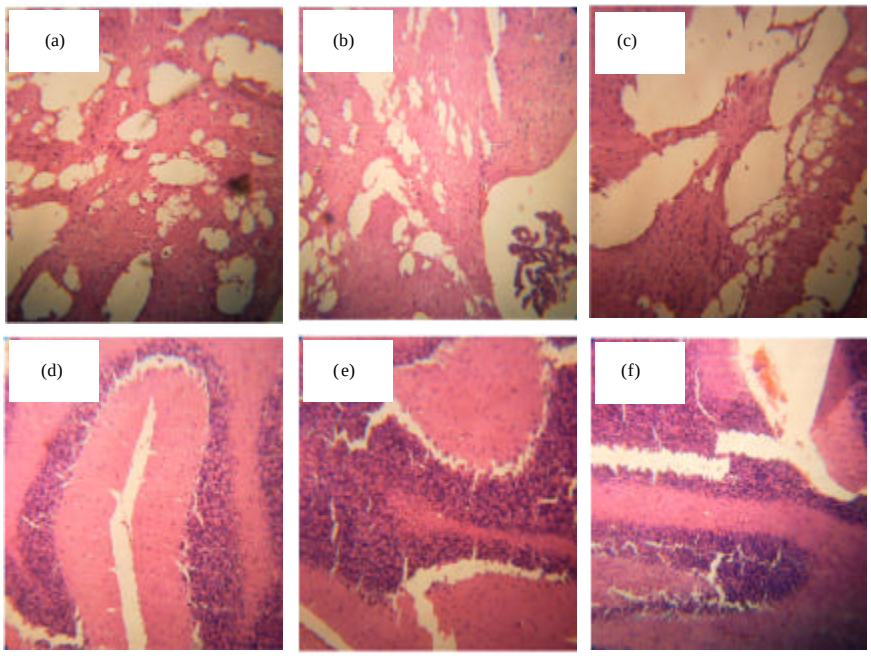

Fig. 4(a-d): Histopathology of kidney shows normal glomerulus and interstitium, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

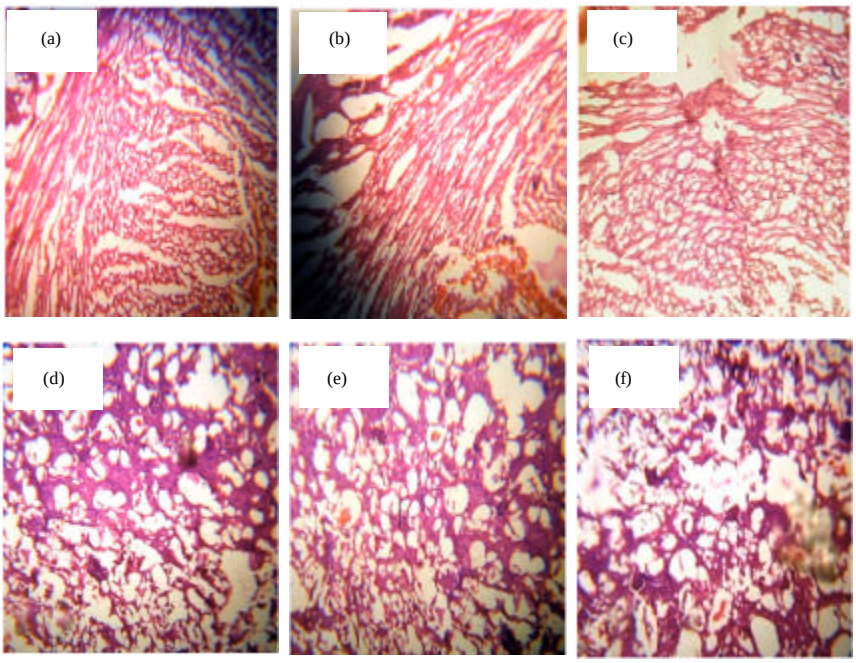

Fig. 5(a-d): Histopathology of heart shows normal cardiac muscle fibers, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

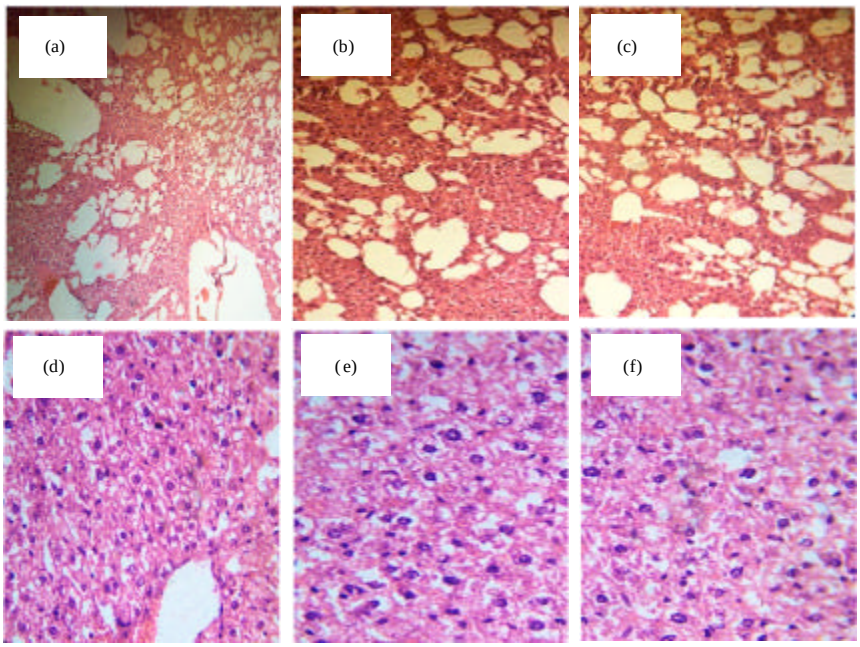

Fig. 6(a-d): Histopathology of liver shows normal architecture with noraml heptatocytes and portal tracts, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

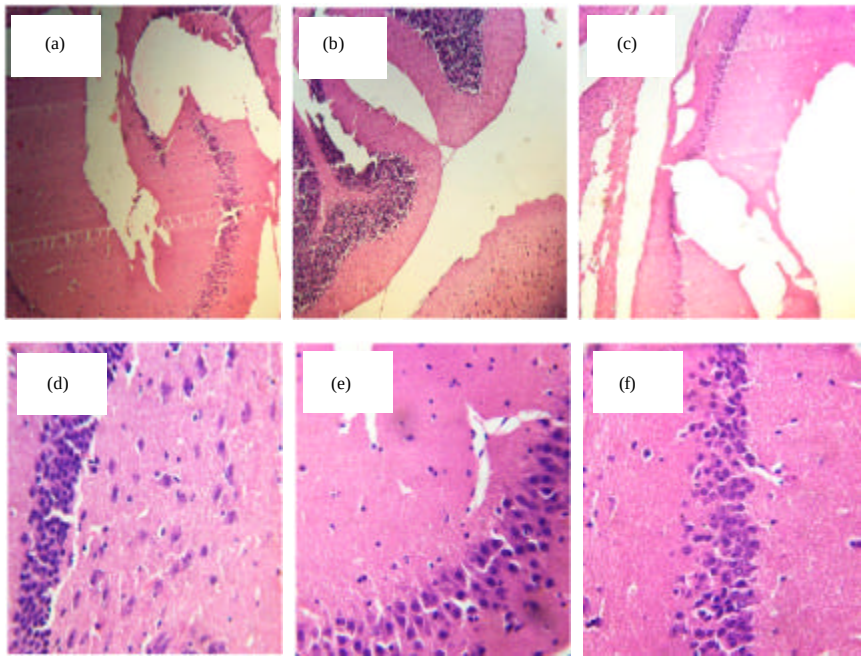

Fig. 7(a-d): Histopathology of brain shows normal neuronal architecture with no signs of degeneration, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

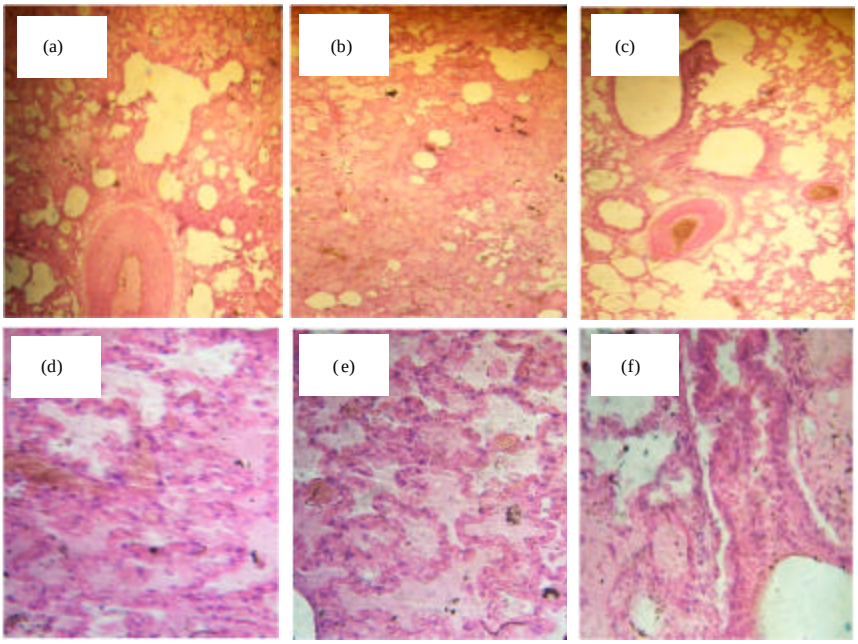

Fig. 8(a-d): Histopathology of lungs shows normal peri bronchial and intertitial cells, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

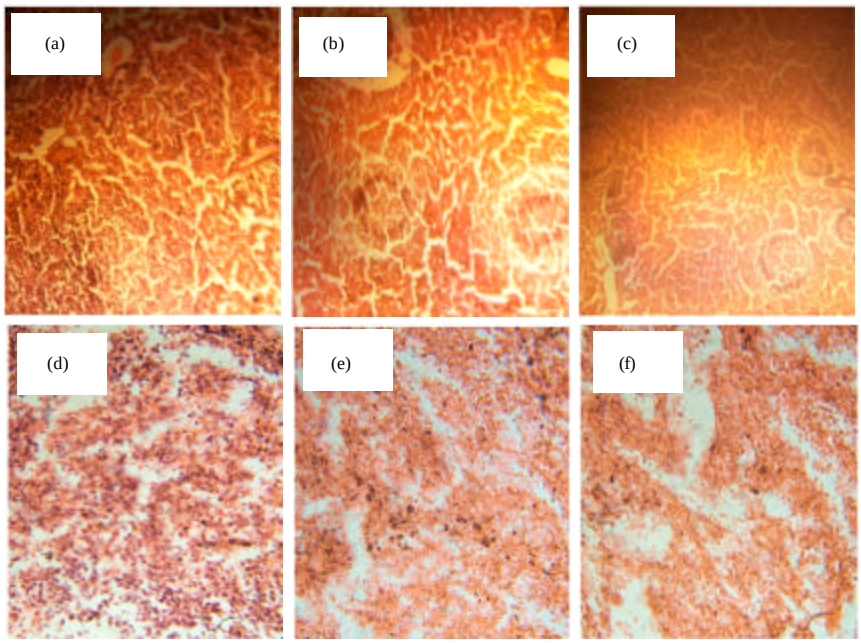

Fig. 9(a-d): Histopathology of sleepn shows normal histology, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

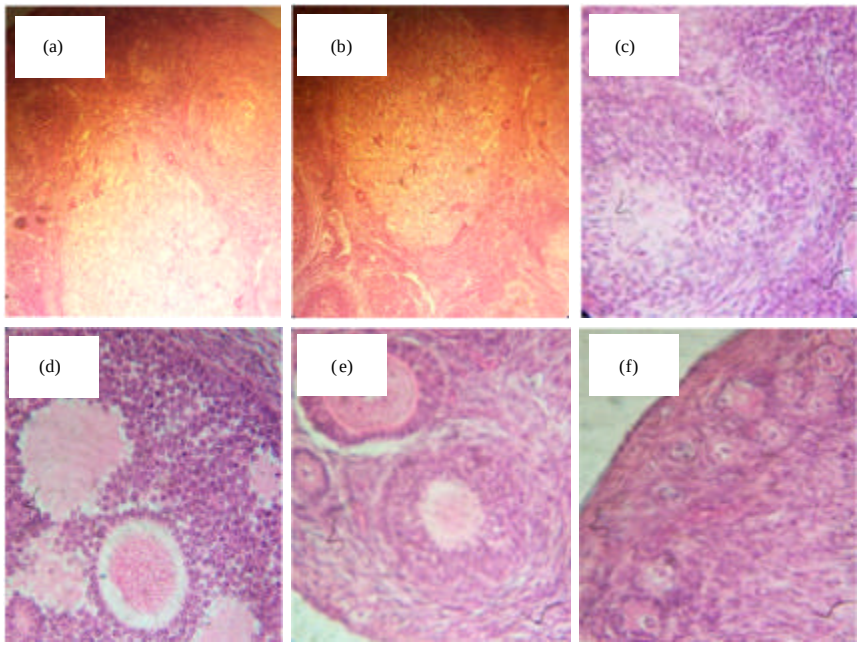

Fig. 10(a-d): Histopathology of ovary shows normal histology, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

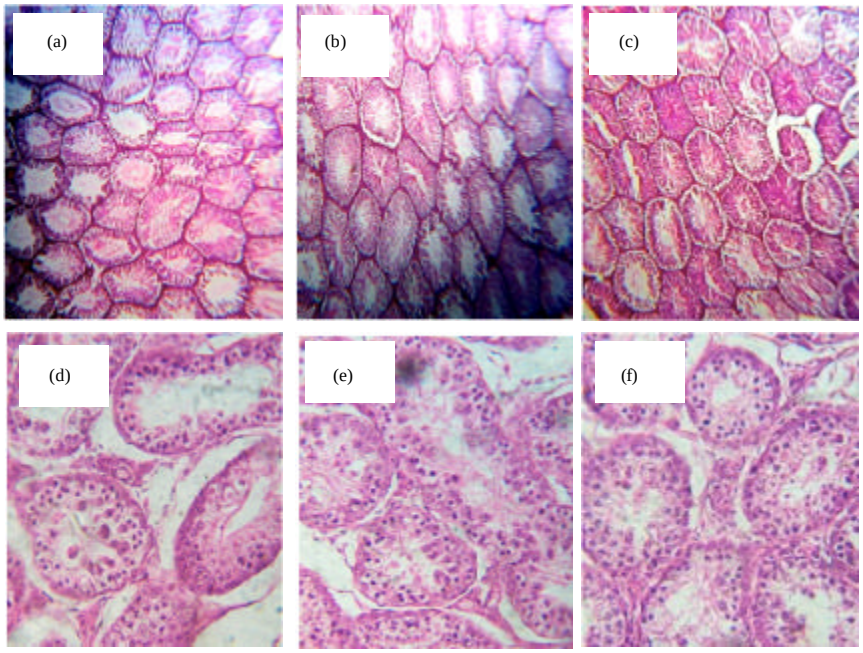

Fig. 11(a-d): Histopathology of testis shows presence of mature stomach cells project the perfect histo morphology of testicular cells, (a-c) Low power, (d-f) High power, (a) Control group, (b) HAEIA 200 (mg kg^{-1}), (c) HAEIA 400 (mg kg^{-1}), (d) Control group, (e) HAEIA 200 (mg kg^{-1}) and (f) HAEIA 400 (mg kg^{-1})

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[20]. 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.

Because safety should be the overriding criterion in the selection of medicinal plants for use in healthcare systems[21]. In this study, the hydroalcholic extract of *Ipomoea aquatica*Forsk was found to be non-toxic in at the dose of 2000mg kg^{-1}, p.o. The results on the acute toxicity has proven that HAEIA was well tolerated in mice up to oral dose of 2000 mg kg^{-1}.

Body weight changes are marker of adverse effects of drugs and chemicals sub-acute administration for 28 consecutive days of HAEIA (200 and 400 mg kg^{-1}, p.o.) have shown no significant changes in the body weight, general behavior as well as mortality of mice. This implicate that long term administration of HAEIA could be safe and it shall be used for chronic ailment.

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[22,23]. The transaminases (AST, ALT, APT) are well known enzymes used as biomarkers predicting possible toxicity[24]. Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases[25]. HAEIA at high dose level of 400 mg kg^{-1} doesn't causes any alteration in liver enzyme and also no alteration were observed in renal function it was further supported by histological studies on these vital organs. It is important to note that no significant difference in the weights of the vital organs, body weight and color of the organs.

The results of hematological and biochemical analyses have shown that sub-acute oral administration of HAEIA at doses of 200 and 400 mg kg^{-1} did not show any significant effect on hematological and biochemical parameters. This indicates that HAEIIA does not have any toxic effects on hematological (circulating blood cells), renal and cardiac functions etc.

CONCLUSION

Overall, this study provides valuable preliminary data on the toxicity profile of *Ipomoea aquatica* Forsk that should be useful for the planning of future pre-clinical and clinical studies of this plant medicine. The HAEIA appears to be relatively non-toxic, causes no apparent organ damage. Further studies to determine the effects of this plant on the foetus in pregnant animals, on the reproductive capacity of animals, on the genetic system. Collectively the findings of the present study would suggest a very low potential of this plant to produce adverse effects. But more studies are needed in animals and mainly in humans in order to have the possibility of safe use in humans.

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