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Acute toxicity study of hydroalcoholic extract of two Indian medicinal plants *Alternanthera ficoidea* and *Ludwigia octovalvis*

Research Article

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Abstract

Background: The ethano-pharmacological survey on two Indian medicinal plants *Alternanthera ficoidea* and *Ludwigia octovalvis* revels that both plants are biologically potential and effective in the management of various diseases. Despite the both the plants as whole are used traditionally by the local people in Indian subcontinent but the toxicity studies on whole plant extract of both plant has not explored scientifically. Objective: The toxicity study was designed to explore the toxicity potential associated to hydroalcoholic extract of whole plant of *Alternanthera ficoidea* and *Ludwigia octovalvis according OECD guidelines.* Material and methods: Healthy Charles Foster albino female rats of 8-12 weeks' old were divided in 3 groups. Group I, group II, and group III received vehicle (0.5% CMC, p.o., single dose), hydroalcoholic extract of whole plant of *Alternanthera ficoidea* (HAF) (Single dose of 2000 mg/kg, given orally), and hydroalcoholic extract of whole plant of *Ludwigia octovalvis* (HLO) (Single dose of 2000 mg/kg, given orally), respectively. After 14 days, all rats were anesthetized with chloroform and blood were collected by retro-orbital capillary puncture for the study of haematological and biochemical parameters. After blood collection, rats were sacrificed; organs were carefully collected, weighted and examined for changes. Results: The acute toxicity study on Charles foster female rats showed that there was no sign and symptoms of toxicity were seen in rats of all group. Haematological parameters, biochemical parameters and histopathological architectures of various organs of treated groups were found to be non-significant and normal in range when compared with normal control groups. Conclusion: The study found that no mortality occurred at a dosage of 2 g/kg of HAF and HLO, and it came to the conclusion that the IC_{50} of HAF and HLO was higher than that level.

Key Words: Toxicity, *Alternanthera ficoidea, Ludwigia octovalvis*, Hydroalcoholic extract.

Introduction

Many people around the world, primarily in developing nations, use medicinal plants instead of conventional medications to treat many diseases. Information regarding the toxicity of the most common herbs and plants is limited. The World Health Organization has suggested that additional research be done in order to determine whether traditional plants used for disease treatment have any hazardous side effects. Toxicological information aids in determining whether or not an unknown herbal medication is harmless for clinical use.

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In the amaranthaceae family, *Alternanthera ficoidea* Linn. (common name "green amaranth") is a perennial plant, which is known to contain a variety of phytochemicals, including volatile components, essential amino acids, flavonoids, glycosides, and steroids(1, 2). *A. ficoidea* is a monoecious plant that ranges in height from 100 to 300 cm. It was most likely grown in China and India. Tropical and subtropical parts of the world have a high prevalence of it. This weed's leaves can be eaten as greens to supplement nutrition, prevent or treat numerous ailments, and lower risk of different illnesses including cancer and heart disease (3, 4).

A common aquatic plant, *Ludwigia octovalvis* (Family- Onagraceae) (5) has been taken in treatment of a variety of infections, including urinary infections, diarrhoea and dysentery(6, 7). *L. octovalvis* has been shown to have many pharmacological properties, including antibacterial, antioxidant, antidiarrheal, anti-diabetic, anti-inflammatory, anticancer, and hepatoprotective properties. It is also used in the treatment of edema, swollen glands, nervous

diseases, leucorrhoea, orchitis, hypertension, headache, and nephritis (8,9,10,11) . It is generally drunk as a health beverage (6).

In the current study, we studied the acute oral toxicity of hydro-alcoholic extract of whole plant of *A. ficoidea* and *L. octovalvis* to boost confidence in their safety for treating a variety of human illnesses.

Materials and Methods

Collection and identification of plant materials

In March, whole plants of *A. ficoidea* and *L. octovalvis* were obtained from RGSC, BHU, Mirzapur, India. The botanist, Prof. NK Dubey, Department of Botany, BHU, Varanasi, India, identified and verified the plants. The plant's voucher specimen of *A. ficoidea* and *L. octovalvis*was deposited at the herbarium as Amarantha 2019/1 and Onarga 2019/1, respectively.

Plant Material and Extraction

A. ficoidea and *L. octovalvis* whole plants weighed each 500g as dried coarse powder were used separately for extraction by hot extraction method using solvent system of ethyl alcohol and water at a ratio of 70:30 for 72 hrs. After filtering with filter paper, the crude extract was obtained. A rotatory evaporator was used to evaporate the filtrate, producing a thick, dark, brownish residue. The yield value of the extracts of *A. ficoidea* and *L. octovalvis* was 9 percent (w/w) and 10 percent (w/w), respectively. For pharmacological testing, the hydro-alcoholic extract of *A. ficoidea* (HAF) and *L. octovalvis* (HLO) were separately suspended in 0.5 percent carboxymethyl cellulose (CMC) (12, 13).

Animals

Healthy Charles Foster albino female rats weighing 130–170 g, 8-12 weeks old, were included in the experiment. They had unrestricted access to regular food pellets and water as well as standard environment with conditions maintained as temp.22°C±3°C, RH55±5% and 12h day- 12h night cycles. Rats were used for study after acclimation for a week (14, 15). Rats were handled according to the OECD-423 guidelines and procedures have been authorized by the central animal ethics committee of the IMS, BHU, Varanasi, India (Reg. No. 542/GO/ReBi/S/02/CPCSEA; Dated 05/05/2019).

Acute Oral Toxicity study

The acute oral toxicity of HAF and HLO were tested in accordance with OECD norms (423, 17 December 2001).The rats were housed in polyethylene cages, chosen at random, and marked to allow for individual identification. Three groups of three rats each were formed. The volume of extract preparation shouldn't be greater than 1mL/100g of body weight in rats. The HAF (Single high dose of 2000 mg/kg), HLO (Single high dose of 2000 mg/kg), and vehicle (Single dose of 0.5% CMC) were administered orally to Group I (the normal control group), Group II (HAF treated group), and Group III (HLO treated group), respectively. Rats were monitored for 14 days (14, 15, 16).

Mortality and Changes in behavior

Throughout the entire experiment, animals were examined for the number of deaths, tremors, hair loss, allergies, changes in behavior, diarrhea, sleep, body weight, water and food intake (14).

Haematological and biochemical analysis

Once the toxicity experiment was finished, the animals were anesthetized with chloroform and blood was drawn by retro-orbital capillary puncture. An Auto-Haematology analyzer (Arkray Autocell Plus) was used to determine the haematological parameters. Standard kits (bought from Autospan Liquid Gold, Arkray Healthcare Pvt. Ltd.) and a UV-Visible spectrophotometer (Systronics Double Beam UV-VIS Spectrophotometer: 2202) were used to conduct the biochemical analysis (14).

Histopathological Studies

All animals were slaughtered at the completion of the 14-day experiment and organs were carefully obtained and weighed. The organs were kept for preservation in 10% formalin solution, including the liver, heart, lungs, and kidney. In contrast to the control group, the tissue sections (tissues stained with hematoxylin and eosin, followed by cut into 4µm thick pieces by the help of microtome) were examined and their pathological abnormalities were assessed under a light microscope (with a magnification of 40X) [Olympus Magnus Inverted (INVI) inverted microscope] (14).

Statistical analysis

The software GraphPad Prism 8.0.2 was used to analyze the statistical data collected and included the standard one-way and two-way analysis of variance in addition to the Dunnett's multiple comparisons test. Mean ± SEM was used to express the findings.

Results and discussion

As was already mentioned, *A. ficoidea* and *L. octovalvis* are both used to cure a number of illnesses in folk medicine. However, there is no information available regarding its toxicological effects with respect to hydro-alcoholic extract of whole part of both plants. In this context, we used in vivo experimental models to study the acute toxicity profile of whole plant of *A. ficoidea* and *L. octovalvis* hydroalcoholic extract.The primary effects of both extracts after a single oral intake was assessed using the acute toxicity studies.

The acute oral toxicity experiment did not find any evidence of toxicity related symptoms, including morbidity or mortality. Because there were no deaths noted in treated rats, the LD50 for HAF and HLO could be higher than 2000 mg/kg. The bodyweight of the HAF and HLO treatment groups and the normal control group were increased steadily in the acute toxicity experiment, but the rise in mean body weight was non-

significant difference (Table 1).All rats consumed normal water and food (Table 1). Rat's organ weight of HLO and HAF treated groups were non-significant when compared with normal control group. Normal gross pathological (morphology) findings were seen in rat's brain, liver, kidney, lungs, spleen, and heart of HAF and HLO treated groups when it was compared with compared group (Table 4). The statistical data of the normal control group as compared with the findings of the extract treated groups' haematological (Table 2) and biochemical parameters (Table 3) were non-significant and within range; and similar to research data that have already been published for the extract of other medicinal plants (17, 18, 19).

The sub-cortical white matter was seen to be normal in all histological section of the cerebral cortex. Compared to the more intensely stained sub-cortical white matter, the cortex has a significantly milder shade of pink (Figure 1A, 1B, and 1C). Cardiomyocytes are organized in pseudosyncytium in the heart's segment, and they are normal thickness and free of inflammation or myonecrosis (Figure 1D, 1E, and 1F). The kidney's histology revealed normal glomeruli, tubules, and vessels without any signs of deterioration or inflammation (Figure 1G, 1H, and 1I).The liver's histology revealed no inflammation, normal hepatic cells, and no signs of acute or chronic damage (Figure 1J, 1K, and 1L).In the lung parenchyma, there is no sign of inflammation or localized cell death, and the segment of lungs exhibited alveolar cell of normal shape and size (Figure 1M, 1N, and 1O).

Table 1. Bodyweight changes, food and water intake observed in Charles Foster albino female rat in acute oral toxicity study of HAF and HLO

Parameter	Normal control group	HAF treated group	HLO treated group
Body Weight Initial wt(g)	155.67±2.03	155.33±2.6 ns	156.33±1.76 ns
Final Weight after 2 week (g)	161.33±2.6	160.67±2.33 ns	162.67±1.76 ns
BWG(g)	5.67±0.67	6±0.58 ns	6.33±0.67 ns
Food Intake(g/day)	15.33±0.88	15.67±1.45 ns	16±0.58 ns
Water Intake (ml/day)	12.33±1.45	12.67±0.88 ns	13.33±1.2 ns

Values represent the mean ± SEM (n = 3); non-significant, ns P>0.05 compared with normal control group.

Table 2. Haematological parameters of Charles Foster albino female rat in acute oral toxicity study of HAF and HLO

Parameter	Normal control group	HAF treated group	HLO treated group
Haemoglobin (Hb; g/dL)	14.9±0.26	13.77±0.15 ns	14.73±0.41 ns
Packed cell volume (PCV; %)	45.83±1.7	45±1.14 ns	43.97±1.35 ns
Red Blood Cells COUNT(RBC; mill/mm3)	9.1±0.14	9.01±0.2 ns	9±0.21 ns
Mean corpuscular volume (MCV; fL)	50.37±1.99	49.93±0.68 ns	48.96±2.49 ns
Mean corpuscular haemoglobin (MCH; pg)	16.37±0.27	15.29±0.46 ns	16.40±0.76 ns
Mean corpuscular haemoglobin concentration (MCHC; g/dL)	32.64±1.79	30.63±0.85 ns	33.52±0.21 ns
Red cell distribution width (RDW; %)	21±0.68	22.78±1.03 ns	22.55±1.01 ns
Total leucocytes count (TLC; thousand/mm3)	8366.67±218.58	8333.33±185.59 ns	8766.67±296.27 ns
Segmented N(%)	69.67±1.2	68±2.52 ns	65.33±4.33 ns
Lymphocytes (%)	28±1.15	30±2.08 ns	32.67±4.33 ns
Eosinophils (%)	2±0.58	2±1 ns	2±0.1 ns
Monocytes (%)	0.33±0.33	0.00	0.00
Basophils (%)	0.00	0.00	0.00
Platelets(thousand/mm3)	858.67±17.37	868.33±10.14 ns	845±17.62 ns
Mean Platelet Volume(fL)	8.12±0.12	8.31±0.21 ns	8.23±0.09 ns

Values represent the mean ± SEM (n = 3); non-significant, ns P>0.05 compared with normal control group.

Table 3. Biochemical parameters of Charles Foster albino female rat in acute oral toxicity study of HAF and HLO

Parameter	Normal control group	HAF treated group	HLO treated group
Serum urea	33.33±0.33	27.67±1.45 ns	37±0.58 ns
Serum Creatinine	0.75±0.03	0.83±0.03 ns	0.93±0.03 ns
Serum Uric acid	3.57±0.17	4.57±0.32 ns	4.13±0.03 ns
Serum Calcium	8.47±0.26	8.43±0.23 ns	8.33±0.09 ns
Sodium (mEq/L)	131.1±8.56	143.2±1.43 ns	140.5±0.99 ns
Potassium (mEq/L)	6.73±1	5.97±0.12 ns	7.67±0.5 ns
Serum Bilirubin Total	0.74±0.03	0.75±0.03 ns	0.65±0.03 ns
Serum Bilirubin Direct	0.25±0.03	0.21±0.01 ns	0.2±0.01 ns
Serum Bilirubin Indirect	0.49±0.01	0.54±0.03 ns	0.44±0.03 ns
Alkaline Phosphatase (ALP)	110.33±0.88	107.67±6.49 ns	114.33±6.36 ns
Aspartate aminotransferase (AST; SGOT)	72.82±1.9	80.48±3.03 ns	83.13±2.49 ns
Alanine aminotransferase (ALT; SGPT)	43.78±2.97	38.05±3.11 ns	40.95±1.56 ns
Albumin	4.03±0.12	3.97±0.18 ns	3.9±0.12 ns
Globulin	3.7±0.15	3.53±0.12 ns	3.57±0.09 ns
Total Protien	7.73±0.27	7.5±0.29 ns	7.47±0.09 ns
Albumin/globulin ratio	1.09±0.01	1.12±0.02 ns	1.10±0.06 ns

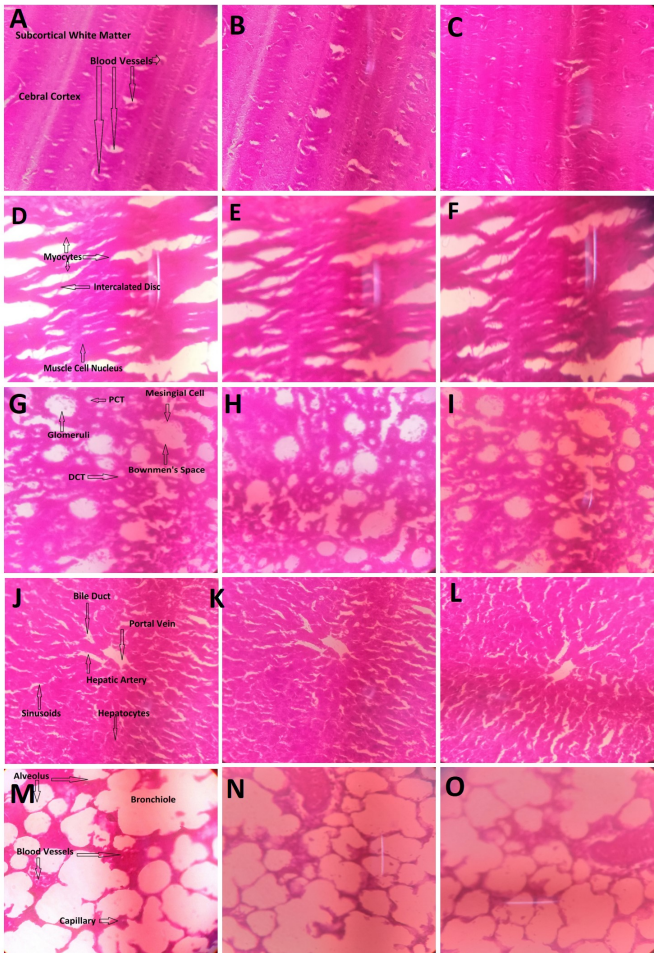
Values represent the mean ± SEM (n = 3); non-significant, ns P>0.05 compared with normal control group.

Table 4. Charles Foster albino female rat organ weights (g) of acute oral toxicity study

Organ	Organ weight		
	Normal control group	HAF treated group	HLO treated group
Liver	4.0202±0.0765	4.1638±0.0861 ns	4.1065±0.0818 ns
Right Kidney	0.4703±0.0106	0.4426±0.0206 ns	0.4254±0.0215 ns
Left Kidney	0.4429±0.0151	0.4504±0.0144 ns	0.4492±0.0186 ns
Right Adrenal Gland	0.0167±0.0044	0.0278±0.0016 ns	0.279±0.0057 ns
Left Adrenal gland	0.0261±0.0032	0.0223±0.0047 ns	0.0197±0.0013 ns
Heart	0.4625±0.0189	0.4809±0.0207 ns	0.4673±0.016 ns
Spleen	0.2916±0.0089	0.2995±0.0116 ns	0.2896±0.0236 ns
Brain	1.5122±0.0564	1.548±0.0571 ns	1.4924±0.0328 ns
lungs	0.9268±0.0241	0.9095±0.0361 ns	0.8685±0.0317 ns

Values represent the mean ± SEM (n = 3); non-significant, ns P>0.05 compared with normal control group.

Figure 1. Histology of Brain (A), heart (D), kidney (G), liver (J), lung (M) of the control group and Brain (B), heart (E), kidney (H), liver (K), lung (N)of HAF treated group with 2000 mg/kg dose and Brain (C), heart (F), kidney (I), liver (L), lung (O) of the HLO treated group with2000 mg/kg dose in acute oral toxicity.



Conclusion

The acute oral toxicity study revealed that both extracts HAF and HLO at dose of 2000 mg/kg, no mortalities were observed and it was concluded that IC_{50} of HLO and HAF were above the 2000 mg/kg dose. These results give us a clear conclusion that HAF and HLO are not harmful at single dose of 2000 mg/ kg and did not result in any observable symptoms when tested for acute oral toxicity. Both the control group and the treatment group's kidney, liver, lung, and heart, showed no notable abnormalities during histological analysis. Additionally, the results of investigations on this plant's acute toxicity were gathered to increase trust in its safety uses in the formulation of medicines. More experimental activities are needed, such as testing on subacute and subchronic toxicity, for the utility of HLO and HAF as therapeutic agent with high grade safety to avoid any toxic effect or side effect induce by plant derivates as medicine.

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