Original Article

EVALUATION OF SAFETY PROFILE OF *ALPINIA OFFICINARUM* AND *HYMENOCRATER SESSILIFOLIUS* BY ACUTE TOXICITY STUDY IN ALBINO RATS

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Abstract:

Background: *Hymenocrater sessilifolius* and *Alpinia officinarum* extracts have known for their folkloric uses but no potential toxicity has been described yet. This project was aimed to find the safe dose range and biological response of both extracts in rats by analyzing hematological parameters, liver and renal function tests and histopathological study.

Material and Methods: *Hymenocrater sessilifolius* and *Alpinia officinarum* extracts were administered to albino rats orally. The sighting study was carried out with following doses of plant extract 5, 50, 300 and 2000 (mg/kg) of body weight. Furthermore, the highest dose, 2000 mg/kg body weight was selected for the main test of the acute oral toxicity experiment. Three groups (control group and two plants treated group) each having 5 rats were studied. After administration of (2000 mg/kg) to the rats, general behavior, untoward action, and death rates were checked 2 weeks. Blood was collected for hematological, liver and renal function tests and histological necroscopy after sacrificing the plants treated and the control group of rats. Data was statistically analyzed by Graph Pad Prism using one-way ANOVA, and for comparison Bonferroni was employed.

Results: Results showed no significant alterations in hematological, liver and renal function tests when compared against the control group. The vital organs did not reveal any gross and histological necroscopy.

Conclusions: *Hymenocrater sessilifolius* extract and *Alpinia officinarum* extract did not show any toxicological effects. However, sub-acute and chronic toxicity studies will provide the complete profiles of their safety.

Keywords: Acute toxicity, Albino Rats, *Hymenocrater sessilifolius* extract, *Alpinia officinarum*

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INTRODUCTION

One of the components of complementary and alternative medicines is herbal medicine and it is gaining privilege across the globe. It is generally considered that treatment with plants is natural and safe. Nevertheless, when natural remedies are not taken in correct manner, not only failure of treatment is experienced but hazardous results are also observed.[1] However, , in many areas around the world herbal plants are considered non-toxic and relatively safe. People using natural remedies from these areas have common belief that herbal treatment has less adverse effects actions as compared to synthetic medicines. However, the common belief that herbal treatment is free from all side effects is not completely true.[2] In fact, the administration of these enriched medicinal plants for prolonged periods will be a big problem for patients. Thus, this study is of utmost importance to assure the safety profile

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of valuable medicinal plants that are used traditionally. The family Lamiaceae; genus Hymenocrater; consists of almost 21 species. The only specie present in Pakistan is *Hymenocrater sessilifolius* (HS). *H. sessilifolius* mainly grows in Quetta. Herbs of genus Hymenocrater are taken as sedative, anti-inflammatory, antiallergy,[3] against bacterial infection[4] and fungal infection and as antioxidant agents.[4,5] However, HS (voucher no. GC.Herb.Bot.3691) is taken by residents for the treatment of cardiovascular disorder, gastrointestinal disorder, respiratory issues and urinary tract related problems. The major active chemical constituents of HS are aromatic oil and flavonoids.[6,7] HS is an ethno-medicinal herb that Iranians have used in the past to improve cardiac efficiency.[8] *Alpinia officinarum* (AO) grows in China and it is a pungent plant while its rhizome has an aromatic constituents whereas in Europe, its rhizomes have been added in foods as condiments for over thousands of years.[9] In traditional Chinese and Ayurvedic medicines, it has also been used for medicinal purpose for better functioning of stomach, pain relief and as an antiemetic.[10] This herb majorly exerts its effect on alimentary canal and its accessory organs such as stomach and spleen. Furthermore, it is also used for the treatment of gastric pain, cold, for revitalizing the blood circulation system and for decreasing swelling.[11] Although pharmacological effects of HS extract and AO extracts are beneficial and have been used by local people for prolonged period but a comprehensive scientific information on their toxicity potential is not available yet. Therefore, the objective of the acute toxicity study involved the safety evaluation of plant extracts, determination of their dose and finding particular untoward actions of these plant extracts in albino rats.

MATERIAL AND METHODS

Extract was prepared from aerial parts of *Hymenocrater sessilifolius* Benth (HS) (Sursandh) (20kg) which was purchased from Plant market in Quetta, Balochistan and a voucher of the specimen (GC.Herb.Bot.3691) was deposited. The whole plant of HS was shade dried and grounded into powder form and soaked in methanol: water (70:30) for 3 days. The mixture was filtered by means of a muslin cloth and afterwards with the help of Whatman filterpaper (qualitative grade 1). This procedure was performed two more times; then the filtrate was gathered and subjected to rotary evaporator (at 35 to 40ºC under reduced pressure of –760 mmHg), that gave a viscous brown colored material, called as crude extract of HS and its yield was 14.8%. The solubilization of HS crude extract was poor in water so it was suspended in carboxymethylcellulose sodium (CMC-Na) (1%).[12]*Alpinia officinarum* (AO) extract was purchased from Salus Company (China). AO with batch number 180720 was catered with small portion of extract along with analytical report to Pharmacy Department of Government College University, Faisalabad (GCUF), Pakistan. The experimental procedures involving animals were approved by the Institutional Review Board (IRB 761) and performed in compliance with relevant ethical and institutional guidelines. This 14 days pre-clinal study that was performed in GCUF by housing rats at temperature-controlled environment (23±2 ºC) and exposed to 12/12 hours of light/ dark cycle each 24-hour period. The rats were fed with standard laboratory rat food pellets and water ad libitum. Animals selected for study were 10-week-old and weight ranging from 130g to 170g. Being most sensitive gender, the female albino rats were selected to check the response of treatment.[13,14] Rats having any sign and symptoms of disease, pregnancy, lactating rats, animal with prior exposure to any drug or plant extract within last 30 days were excluded from the study. Rats were deprived of food 3 hour prior to dosing but water was allowed. After the completion of fasting period, rats were weighed and plants' extracts were given orally as single dose of 5, 50, 300 and 2000 (mg/kg) as preliminary test when it was observed that all rats survived then main experiment was conducted with dose of 2000mg/kg. Control group, HS (2000mg/kg) treated group and AO

(2000mg/kg) treated group were consisting of 5 rats per group. The volume for the extract administered was 1ml/kg body weight (BW) of the rats. The randomized study was chosen to avoid biasness of healthier and heavier rats or on the basis of ages of rats. During the first 24 h, after the administration of dose, all rats were observed individually at intervals of 30 min, for first 4 hours the rats were attended with particular care, and after then observed them daily, for a total of 14 days while symptoms of sickness, health or behavioral alterations were done twice a day. According to Organization for Economic Co-operation and Development (OECD) guidelines for Testing of Chemicals number 420 (OECD, 2001), acute oral toxicity study was performed which is an *in vivo* toxicity evaluation in rats. Following monitoring must be done in response to treatment, including deaths, moribund, sickness, alteration in skin, fur, eyes, mucus membranes, behavioral manner and tremors. Weekly observed the weight of each rat and the difference of the BW at the start of study and on 14th day of study was recorded. The quantity of food (200g) and water 200ml in the bottle that was sufficient for a week, was kept in the food tray and quantity of food and water remained were calculated at the end of the week to get the quantity of consumed food and water. At the end of study (14th day of experiment), under the effect of chloroform anesthesia, blood was collected via cardiac puncture before the autopsy. The blood was collected in vacutainer tube without EDTA for serum collection to perform the evaluation of hematological, liver function test (LFT) and renal function test (RFT). The hematological parameters that were measured included erythrocyte, leukocyte, platelets, hemoglobin, hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte %, and mean cell volume (MCV). For LFTs, the parameters were alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) while the RFT was based on the following parameters, urea and uric acid. Gross autopsy study was performed to see any modification in organ such as aorta, heart, liver, stomach and kidney. For histopathological evaluation, slices of each organ (aorta, heart and kidney) were fixed in buffered solution of formalin (10%) for 24 hours. The organs of rat were exposed to different concentrations of alcohol including absolute alcohol then the tissue was embedded in molten paraffin wax that became semi-solid block of paraffin wax on cooling. This block of paraffin having embedded tissue was mounted on rotary microtome to have section cutting of 4 µm thickness. The section cuttings obtained were shifted carefully to water bath and place in oven at 58 ° for overnight. The slides were then stained by using histological stains (hematoxylin & eosin). Xylene was added to avoid presence of bubbles and thereafter covered with cover slips. The prepared slides were placed in light microscope to observe any anomaly or structural variation in the tissue.

GraphPad Prism7.0 software was employed for calculation and statistics. All values were presented as mean± standard error of the mean (SEM). The values were statistically analyzed with the help of one- way analysis of variance (ANOVA) followed by Bonferroni test. Values with $p< 0.05$ were considered statistically significant. The ethical approval of this study was taken from GC University Faisalabad under IRB number 761 dated 09-09-2020.

RESULTS

The visual observation did not show any signs of toxic response in preliminary study performed with the following doses 5, 50, 300 and 2000 mg/kg BW of rats, for both plants HS and AO extracts. As all the rats survived so the main test was carried out with the dose of 2000 mg/kg BW. The acute toxicity study neither showed any death of treated groups of rats nor toxic responses were found throughout the duration of test (14 days). The BW of the plant extracts-treated and untreated rats (Table 1). It was observed that there was gradual rise in BW in all three groups. HS extract treated and AO extract treated groups revealed that the

variation in BW and food and water consumption were not statistically significant when compared against rats of the control group. The utilization of food and consumption of water of the HS extract treated and AO extract treated groups were also not changed significantly in comparison to the rats of control group (Table 1).

Table 1: Body weight (g), utilization of food (g) and usage of water (ml) of control and treated groups of rats given HS extract and AO extract

Groups	Body weight (g)		Utilization of food (g)		Usage of water (ml)	
	Day7	Day 14	Day 7	Day 14	Day 7	Day 14
Control	145.0 ±9.97	156.8 ±11.12	91.2 ±9.18	94.0 ±6.32	246.8 ±7.08	251.4 ±5.22
HS 2000mg/kg	137.6 ±7.27	151.6 ±6.58	90.0 ±6.28	98.0 ±6.12	238.8 ±7.22	244.4 ±8.20
AO 2000mg/kg	158.2 ±14.3	169.6 ±16.45	87.2 ±7.43	92.8 ±6.53	231.0 ±14.74	239.2 ±7.66

Results of n=5 are shown as mean ± standard deviation

Results related to hematology, LFT and RFT are revealed in Table 2. Hematological parameters revealed a non-significant rise in leukocytes, erythrocytes, hemoglobin, HCT and platelets of HS extract and AO extract treated groups as compared to control group of rats. Similarly, insignificant decrease in MCV, MCH and MCHC of HS extract and AO extract when compared to control group and it was found that they were within normal range (control limits). The clinical values of ALP, AST, ALT, urea and uric acid of the HS extract and AO extract treated groups were not remarkably varied when compared against the control (Table 2). All observed parameters were normal at 2000mg/kg of rat. This revealed the both extracts, HS plant and AO plant, did not cause toxicity. Thus, the median lethal dose (LD_{50}) of both extracts exceeds 2000 mg/kg body weight. Therefore, the administration of a single dose of HS extract or AO extract had no adverse effects. Substances having LD50 > 2,000mg are considered as relatively safe according to Globally Harmonised Classification System (GHS). Hence, HS extract or AO extract can be classified as Category 5 according to GHS

Table 2: Hematological values of control and treated rats with HS extract and AO extract

Parameters of hematology	Control	HS extract (2000mg/kg)	AO extract (2000mg/kg)
Leukocyte	4.04±1.25	4.14±1.04	4.44±1.04
Hemoglobin	13.58±0.51	14.22±0.68	14.60±0.57
Erythrocyte	6.58±0.69	7.50±0.43	7.97±0.44
Hematocrit	35.72±3.17	39.66±4.35	43.78±4.14
MCV	65.47±4.44	62.29±2.49	57.02±2.26
MCH	26.22±0.97	25.44±1.27	22.57±2.72
MCHC	37.74±1.96	34.36±1.03	33.29±2.61
Platelets	1133.56 ±247.80	1162.46 ±261.98	1179.27 ±271.48
Values of liver profile and renal profile of control and rats treated with HS extract and AO extract			
Serum analysis	**Control**	**HS extract (2000mg/kg)**	**AO extract (2000mg/kg)**
Liver profile			
ALP (U/L)	281.70 ±32.60	297.85 ±41.88	304.00 ±20.78
AST (U/L)	163.27± 28.47	168.23 ±23.98	174.87 ±9.62
ALT (U/L)	52.64 ±5.98	59.18 ±4.60	65.18 ±7.13
Renal profile			
Urea (mmol/L)	5.23 ±1.18	6.38 ±0.90	7.16 ±1.16
Uric acid (umol/L)	144.35 ±25.32	152.23 ±28.96	166.30 ±19.33

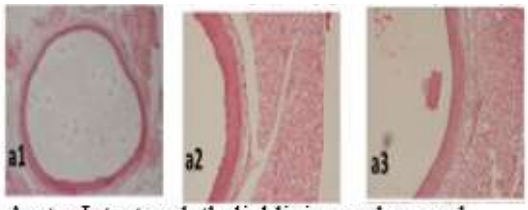
Results are showed as mean ± standard deviation (n = 5)

MCV – Mean corpuscular volume
MCH – Mean corpuscular hemoglobin
MCHC – Mean corpuscular hemoglobin concentration

No physical signs of toxicity have been observed during the study period in rats of HS treated group and AO treated group.

Gross study of various organs inside the body showed normal texture and no apparent sign of abnormality. The aorta sections from rats treated with HS extract and AO extract showed normal histological architecture, with intact endothelial lining and no signs of inflammation, necrosis, or vascular damage (Figure 1). The tunica intima, media, and adventitia layers were well-preserved, indicating no adverse effects of the extract on aortic tissue. The cardiomyocytes appeared healthy, with no signs of degeneration or vacuolization, indicating that HS extract and AO extract did not induce cardiac toxicity in treated rats (Figure 1). Renal histopathology showed preserved glomerular and tubular structure, with no signs of tubular necrosis,

inflammation, or glomerular damage (Figure 1). The renal corpuscles and tubules appeared normal, indicating that HS extract and AO extract did not cause nephrotoxicity in treated rats. Thus, no significant treatment related histopathological abnormality was noted in internal organs such as aorta, heart and kidney.



Aorta: Intact endothelial lining and normal wall architecture.

Heart: Normal cardiac muscle fibers with no signs of necrosis or inflammation.

Kidney: Preserved renal structure with intact glomeruli and tubules, indicating normal histology.

Figure 1. Representative photomicrographs of hematoxylin and eosin-stained rat organ sections. (1) Aorta: Intact endothelial lining and normal wall architecture. (2) Heart: Normal cardiac muscle fibers with no signs of necrosis or inflammation. (3) Kidney: Preserved renal structure with intact glomeruli and tubules, indicating normal histology.

DISCUSSION

As mentioned before, HS extract or AO extract have traditional uses so are used by locals as decoction or spices as well. Although many studies have been carried out on AO extract and very few studies have been performed on HS extract, there is a huge study gap for their toxicity safety profile. This study showed that all groups of animals remained alive in acute toxicity study performed with dose of 2000mg/kg on HS extract or AO extract. According to GHS classification of toxic substances, HS extract or AO extract with oral LD_{50} ranging between 2000–5000 (mg/kg BW) have relatively low toxicity.[15] Both extracts have no effect on body weight and utilization of food and water of extracts treated groups when compared to control group. The rise in BW of all three groups is nominal and normal supported by Halim *et al*.[16] Normally, on exposure of toxic material there is reduction in the body weight which is a sensitive index of toxicity. Variations in BW is indicator of adverse action of medicine or chemical substance. This indicator becomes significant when the reduction of body weight is 10% from initial value.[17] This rise in BW is directly proportional to increase in consumption of food and water. Blood parameters gives the idea about the response of human body to trauma, wounds, inflammation and starvation. In this study, the doses of both extracts did not affect differentiation of leukocytes so non-significant variation of number of leukocytes was found when control group was compared against HS-extract and AO-extract. The mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, hemoglobin, hematocrit and erythrocytes are observed as they are important parameters to evaluate anemia[18,19] and in this study, data indicate that HS- or AO- extract do not cause anemia. Though the blood parameters were high in HS- or AO- extract treated groups but it was insignificant variation as compared to control group of rats. This rise may be due to the synthesis of growth factors.[20] Changes in serological and biochemical parameters are important to estimate effect of toxic substance.[21] Toxic effects of xenobiotics on liver and kidney are assessed by liver function test and renal function test as both organs are responsible for the metabolism and excretion of

xenobiotics.[22] The most frequently studied liver enzymes AST, ALT and ALP showed insignificant variation when HS extract- or AO extract – treated groups were compared to control and showed no significant variations between treated and control groups supported by study of Balaji and Ganesan.[23] The urinary system is sensitive to xenobiotics like medicines or active constituents of ethnomedicinal plants which may result in kidney failure.[24] Insignificant variation in the amount of urea and uric acid suggested no kidney injury occurred in HS extract- or AO extract – treated groups. No gross lesions were found in the aorta, heart and kidney after the extract was given for 14 days supported by study of Akintimehin *et al*.[25]

CONCLUSION

Hymenocrater sessilifolius extract and *Alpinia officinarum* extract did not show any toxicological effects. However, sub-acute and chronic toxicity studies will provide the complete profiles of their safety.

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CONFLICT OF INTEREST

None

SOURCE OF FUNDING

None

AUTHOR'S CONTRIBUTION

FJ: Performed experiment, writing the manuscript, statistical analysis,
SFF: Experimental planning and statistical evaluation, critical evaluation of manuscript,
KS: Statistical evaluation, layout of manuscript,
AS: Data evaluation, editorial assessment of Manuscript

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