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FTIR & Acute, Subacte Toxicity Evaluation of Alli Chooranam (*Nympheanouchali) burm. f)*

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Abstract: ***Aim of the study: Present study was undertaken to evaluate the acute, subacute toxicity & FTIR of hydroalcoholic extract (HEE) of powder of rhizome & flower of the plant Alli (Nympheanouchaliburm. f.) in rats. Materials and methods: Acute toxicity test was performed on Female wister rats at a single oral dose of 1 - 10 g/kg for 14 consecutive days. General behavioral adverse effects, mortality, and latency of mortality were determined. In the subacute study, the Alli chooranam (Nympheanouchaliburm. f.) extract was administered orally at doses of 300, 1000, and 2000 mg/kg daily for 28 days to Wistar rats. Body weight and selected biochemical and hematological parameters were determined at the end of the experiment. Sections of livers and kidneys were removed for histological studies. Results: Acute toxicity study showed that LD_{50} value of hydroethanolic extract of Alli chooranam (Nympheanouchaliburm. f.) is superior to 2000mg/kg. The subacute toxicity study of Alli chooranam (Nympheanouchaliburm. f.) extract at doses 300, 1000, and 2000 mg/kg did not produce any observable symptoms of toxicity and no significant variation in body weight, organ weights, food, and water consumption or mortality in all treated rats. Conclusion: Results indicated no toxicity of hydroalcoholic extract of powder of rhizome & flower of the Alli chooranam (Nympheanouchaliburm. f However, further toxicity assessments should be done to ascertain the safety or the toxicity of this valuable plant species Nympheanouchaliin sub chronic treatments.***

Keywords: Alli, Nymphaea, FTIR, acute, subacute, Siddha

1. Introduction

According to the World Health Organization (WHO), more than 80% of the world's population trusts on traditional medicine for their primary healthcare needs. The usage of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine holds a wide range of substances that can be used to treat chronic as well as infectious diseases. Currently, this botanical medicine is increasingly becoming popular throughout the world, especially in developing countries, where medicinal plants are available, accessible, and are at the reach of the poor people. Even though the use of these plants has shown promising potential phototherapeutic effects with high global demand, but there are still concerns about not only their use but also their safety [LoubnaKharchoufa et al 2020]. It is proved that *N. nouchali* hydroalcoholic seed extract has DDPH scavenging activity nitric oxide scavenging activity & lipid peroxidation inhibitory activity. [Mable et al2014]

The Preparation and standardization of medicinal herbs are urgently need for future studies and therapies. The Alli (*NympheanouchaliBum, f*) large aquatic herb of the family Nymphaeaceae, commonly known as Water lily (Alli in Tamil). Aquatic perennial herb lactiferous rooted. Flowers bisexual floating &solitary. It is native to tempo rate& tropical Asia, Australia& tropical Africa. Siddha medicine recommended flower & rhizome of this plant has astringent & emollient action can be used in the treatment of Diabetes mellitus, urinary diseases, eye diseases& for healing ulcers. Although antidiabetic activity of Alli (*NympheanouchaliBum, f*) have been reported, lack of sufficient literature on flower & rhizome. This study was focused on evaluating FTIR & acute, subacute toxicity of hydroethanolic extract (HEE) of powder of rhizome & flower of the plant

2. Materials and methods

2.1. Collection and Authentication of Plant

The flower & rhizome of Alli (*NympheanouchaliBurm. f)* freshly collected from various places of Kerala. Identified and authenticated by the Medicinal Botanists at Government Siddha Medical College and Hospital, Palayamkottai. These herbal formulations purified according to the suitable procedure methods described in Siddha classical literature. The drug is dried and subjected to size reduction to get uniform coarse powder. The powdered material then subjected to excessive extraction using water & ethanol solvents in a Soxhlet extractor.

2.2. Selection and acclimatization of animals

Wistar strains of albino rats weighing between 180 - 200g are used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature (22 ± 5^0c) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period

2.3. FTIR spectra of Alli chooranam (*Nympheanouchali) burm. f)*

FTIR spectra were recorded on a Perkin Elmer Spectrum One equipped with an ATR - FTIR unit. A few milligrams of sub - fraction A3 sample were placed in the head of ATR. The spectra were obtained with a resolution of 4 cm - 1 and 16 co - addition scans in a wavelength range of 450 - 4000 cm - 1. For each spectrum, 16 scans were accumulated and

averaged. The spectra were collected and analyzed using Spectrum software (Perkin Elmer).

2.4. Evaluation of acute toxicity of Alli chooranam (*Nympheanouchali) burm. f)*

Acute toxicity study was carried out in the female 15 Wister rats arranged in five groups, each group contains 3 rats. . All the animals were feded by oral dose 5, 50, 300, 1000, 2000 mg/Kg/body weight of animal as suspension along with water for 14 days. Signs of acute toxicity such as changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self - mutilation, walking backwards etc. were observed. At the 14th day, sensory reactivity to stimuli of different types (e. g., auditory, visual and proprioceptive stimuli) was conducted. Body weight, hematological, biochemical and histopathological parameters were noted. At the end of the study animal were sacrificed and asessed the effect of Alli chooranam.

2.5. Evaluation of subacute toxicity of Alli chooranam (*Nympheanouchali) burm. f)*

The study was conducted on 5 male & 5 female Wister rats grouped in to four different groups.

1) Group - I: Normal control treated with of normal saline (10ml/Kg, PO)
2) Group - II: Low dose of HEE 300mg/kg
3) Group - III: Middle dose of HEE 1000mg/kg
4) Group - IV: High dose of HEE 2000mg/kg

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill - health or behavioral changes, clinical signs of toxicity daily for 28 days. Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats were calculated. Water intake was checked by visual observation during the study. The body weight of each rat was recorded one week before treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood was collected through retro - orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted overnight prior to the blood collection &hematological, biochemical and histopathological parameters were noted

2.6. Statistical Analysis

All the values were expressed as mean ± standard error of mean. The data were statistically analyzed by one - way ANOVA followed by Dennett's t - test, and value $P < 0.05$ was considered to be significant. Statistical analysis was performed using INSTAT - V3 Software programme.

3. Results

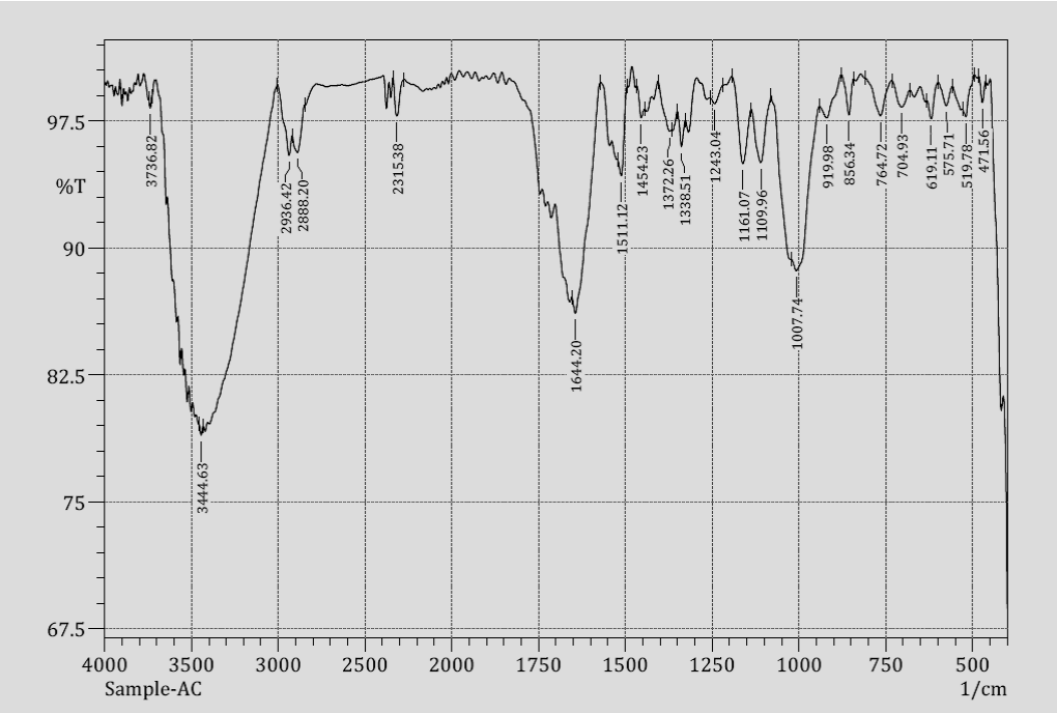

Figure 3.1: FTIR spectra of Alli chooranam (*Nympheanouchali) burm. f)*

Table 3.1: FTIR observed peak values of Alli chooranam (*Nympheanouchali) burm. F)*

Wave length	Functional groups	Structure
519.78	Misc	S - S disulfide
575.71	Alkyl halides	R - Br
619.11	Alkynes	RC#CH
704.93	Aromatics	Monosubst
764.72	Aromatics	1, 2, 3 - trisub
856.34	Aromatics	1, 2, 4, 5 - tetrasub
919.98	Misc.	P - OR esters
1109.96	Misc.	Si - OR
1161.07	Misc	P=O Phosphine oxide
1243.04	Misc.	Si - CH3
1338.51	Misc.	S=O sulfone
1372.26	Alkanes	RCH2CH3
1511.12	Misc.	N=O nitroso
1644.20	Alkenes	6 - rings
2315.38	Misc.	Si - H silane
2888.20	Carboxylic acid	RCO - OH
2936.42	Alkanes	RCH2CH3
3444.63	Amides	RCONHR'

FTIR spectroscopic studies revealed different characteristic peak values with various functional compounds in the extract of Alli chooranam (*Nympheanouchali) burm. f)* exhibits the FTIR spectra values and peak values at 3444.63 has the structure RCONHR, 2936.2 has the structure RCH2CH3, 2888.20 has the structure RCO - OH, 2315.38 has the structure Si - H silane. Extract mainly contains Alkyl halides, Alkynes, Carboxylic acid & Amides.

3.2. Evaluation of acute toxicity of Alli chooranam (*Nympheanouchali) burm. f)*

Table 3.2.1: Sign of acute toxicity and Motality ratio.

Group	Dose (mg/kg)	Physical and behavioral examinations (n=3)	Home cage activity (n=3)	Hand held observation (n=3)	Mortality ratio (n=3)
I	5 mg/kg	Normal	Normal	Normal	0 of 3
II	50 mg/kg	Normal	Normal	Normal	0 of 3
III	300 mg/kg	Normal	Normal	Normal	0 of 3
IV	1000 mg/kg	Normal	Normal	Normal	0 of 3
V	2000 mg/kg	Normal	Normal	Normal	0 of 3

There was no mortality or morbidity were observed in five groups of animals during14 days administered as singledose. Statistical significance (p) calculated by one way ANOVA followed by Dennett's (n=6); $p>0.05$, $p<0.05$, $p<0.01$, $p<0.001$, calculated by comparing treated groups with control group.

3.3. Evaluation of subacute toxicity of Alli chooranam (*Nympheanouchali) burm. f)*

3.3.1. Table Effect of sub - acute dose on body weight in gram

Group	Control	Low	Mid	High
1st day	162.06±0.70	175.15±0.80	182.80±0.80	190.75±0.84
7th day	165.13±0.72	178.21±0.73	185.29±0.82	191.75±0.84
14th day	168.11±0.69	179.17±0.80	188.98±0.84	192.81±0.76
21st day	169.03±0.76	185.09±0.707	190.42±0.83	195.95±0.71
28th day	170.15±0.77 *	188.14±0.70 *	191.05±0.74 *	195.86±0.75 *

Body weight significantly increase (*$p<0.05$) in all the treated animals compared to control.

3.3.2. Table Effect of sub - acute dose on haematological parameters

Dose	RBC 10^{12}/ltr	WBC 10^{9}/ltr	Haemoglobin gm /liter	Differential count %			
				Neutrophils	Eosinophils	Monocyte	Lymphocyte
Control	5.01±0.14	6.06±3.14	12.501±0.15	55.13±0.14	1.53±0.15	5.40±0.15	39.47±0.16
Low	5.05±0.18	7.25±3.14	12.546±0.16	56.45±0.16	1.56±0.26	5.46±0.17	38.47±0.16
Mid	5.101±0.13	7.15±3.16	13.241±0.14	56.75±0.14	1.57±0.24	6.56±0.16	37.45±0.13
High	5.10±0.17	6.18±3.14	13.821±0.25	57.86±0.15	1.60±0.28	6.58±0.15	36.85±0.18

The values are expressed as mean ± S. E. M. n=6. The results of group I were compared with other groups such as II, III, IV. Table [3.3.2] showed effect of HEE on haematological parameters of the rats White blood cell count, Hemoglobin, Monocyte were higher & Lymphocyte reduced in all the treated groups compared to control. There were no significant differences ($P>0.5$) in RBC, Neutrophils, Eosinophil's concentrations when compared to control.

3.3.3 Table Effect of sub - acute dose on biochemical parameters

Parameter	Control	Low	Medium	High
SGPT (U/L)	41.20±0.08	43.51±0.25	47.62±0.55	49.10±0.74
SGOT (U/L)	49.19±0.15	52.71±0.12	57.84±0.17	58.17±0.14
ALP (U/L)	123.29±0.15	127.77±0.17	133.16±0.18	139.89±0.16
Urea (mg/dl)	36.59±0.15	37.62±0.16	39.44±0.13	40.86±0.18
Creatinine	0.81±0.15	0.86±0.17	0.89±0.18	0.91±0.16
Totalbilirubin	0.35±0.15	0.47±0.14	0.52±0.17	0.65±0.15
Sodium	139.15±0.14	140.55±0.15	141.07±0.17	140.88±0.17
Chloride	100.24±0.18	101.01±0.16	100.20±0.16	100.31±0.15
Potassium	3.90±0.12	4.12±0.16	4.06±0.19	4.08±0.13

The values are expressed as mean ± S. E. M. n=6. No significant ($p<0.05$) changes were observed in SGPT, SGOT, ALP, urea, Creatinine, Sodium, Chloride & Potassium values of experimental rats.

4. Discussion

In acute toxicity evaluation there was no mortality or morbidity were observed in five groups of animals. Data obtained in this study indicated ($p>0.05$) no significance changes in hand held observation, physical & behavioral observation, home cage activity & signs of any toxicity such as tremors, convulsions, salivation, lethargy due to administration of HEE *at* the doses of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

In subacute study treatment with HEE White blood cell count, Monocyte were higher & Lymphocyte reduced in all the treated groups compared to control. The significant increase in the level of hemoglobin was found treatment with HEE with the higher dose of 2000mg/kg. There were no significant differences ($P>0.5$) in RBC, Neutrophils, Eosinophil's concentrations when compared to control. The haemopoietic system serves as important target for toxic chemicals and sensitive index of pathological conditions. Increase in WBC production is not deleterious to body as WBC play vital role in immune system. Assessment of the plant extract on body weight may be an indication of abnormal functioning of organs, reduction in body weight is simple and sensitive index of toxicity. Both the control and treated groups showed a progress in the body weight and organs over the duration of treatment. The increase in body weight could be attributed to the nutritive components in their feed and plant extract.

5. Conclusion

FTIR spectroscopic studies revealed different characteristic peak values with various functional compounds in the extract of Alli chooranam (*Nympheanouchali) burm. f)* mainly contains Alkyl halides, Alkynes, Carboxylic acid & Amides.

Acute and subacute toxicity were carried out in Wister albino rats according to OECD guidelines (423) This drug has no acute toxicity as there was no mortality seen. Sub - acute toxicity is carried by repeated dose of test drug for 28 days. Mortality, the functional observation, hematological and biochemical investigations were done. There were no significant changes in the biochemical and hematological profile. So, the toxicological study of this test drug Alli chooranam (*Nympheanouchali) burm. f)* establish the safety of the drug for long time administration. These effectswere statistically analyzed by ANNOVA & found to be significant. ($p<0.05$)

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Conflict of interest
The author declares no conflict of interest in the present work

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