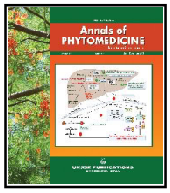
Annals of Phytomedicine 12(2): 713-719, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.84

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Oral toxicity study of *Nelumbo nucifera* Gaertn. rhizome extract in rats

Arvind Kumar Patel◆, Phool Chandra* and Neetu Sachan*,**

School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad-244102, Uttar Pradesh, India
**Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad-244 001, Uttar Pradesh, India*
***Maharana Pratap College of Pharmacy, Kothi, Mandhana, Kanpur-209217, Uttar Pradesh, India*

Article Info

Article history
Received 2 July 2023
Revised 22 August 2023
Accepted 23 August 2023
Published Online 30 December 2023

Keywords
Nelumbo nucifera Gaertn.
Rhizome
Hydroalcoholic
Acute
Oral toxicity
Rat

Abstract

Following OECD guidelines, the acute oral toxicity study of hydroalcoholic extract of *Nelumbo nucifera* Gaertn. (HNN) was conducted at 2000 mg/kg. Three rats received a single dose of the HNN at 48 h of interval, weighing 2000 mg/kg. After carefully monitoring the rats for four hours for any clinical symptoms, a careful clinical examination was performed on each animal once every day for the following fourteen days. Following OECD guidelines, a repeated dose oral toxicity study of HNN was conduct for 28 days with the following doses: 250 mg/kg, 500 mg/kg and 1000 mg/kg in comparison with control group which administered with 1% carboxymethyl cellulose (CMC) solvent at 10 mg/kg. We observed all rat groups for symptoms, abnormal behaviour, changes in food and water intake, changes in body weight, and parameters related to biochemical, haematological, and histopathological processes. The results showed that no animals exhibit acute oral toxicity with single dose administration of HNN at 2000 mg/kg of body weight. Almost, no clear harmful effects were found in any parameters after administration of HNN in the repeated dosage (28 days) oral toxicity investigation. According to this study, long term usage of high doses of HNN as a dietary or medical supplement, up to 1000 mg/kg/day was well tolerated

1. Introduction

Since the beginning of human history, plants have been used as a source of food and medicine (Warrier, 2021). The potential for the development of new medicines from medicinal plants is limitless, whether it takes the form of an extract, a pure component, or a derivative. There is scientific evidence for the biological activity of the majority of natural materials used in folk remedies, but there is limited information available regarding the potential toxicity of these medicinal plants to patients (Yuet Ping *et al.,* 2013). Researchers are concerned about both animal welfare and quick access to effective medications. It might be expected that traditional medicinal plants have a low level of harm when used for long-term human consumption. The most recent studies, however, revealed that several medicinal herbs used in conventional treatment showed adverse effects (Ertekin *et al.,* 2005; Koduru *et al.,* 2006). Therefore, the safety of a plant should not be inferred from its traditional use for therapeutic purposes. Because of, there is cause for concern regarding the possible harmful effects of both short and long term use of such medical plants. To strengthen the certainty of medicinal plants' safety for humans, data from studies on their acute and sub-acute severity in the form of extraction materials, pure compounds, or their derivatives should be collected (Ukwuani *et al.,* 2012). *N. nucifera* is a plant found on the flood surface of the water, this plant is related to the family of Nelumbonaceae. The plant is also famous as lotus (Duke, 2002). It is also the national flower of India and is widely distributed in central and northern India. This plant is adapted to thrive in flood fields, sluggish waterways, and delta regions (Mukherjee *et al.,* 2009).

The plant shows some therapeutic action. The rhizome, leaf, flower and seed are generally utilized to treat leucoderma, spermatorrhoea, pharyngopathy, pectoralgia, diarrhea, cough, smallpox, heamatemesis, haemoptysis, haematuria, metrorrhagia, fever, cholera, hepatopathy, hyperlipidemia. In Ayurveda system of medicine, it is useful as anthelmintic, antiemetic, diuretic also to treat strangury and rashes (Sridhar and Bhat, 2007). It is also utilized in the treatments of tissue irritation, malignant growth, leprosy, skin disease and as antidotes in poisoning (Chopra, 1956). Some chemical constituents which can produce therapeutic action are reported from rhizome, blossom, seeds, and leaves like steroids, alkaloids, flavonoids, triterpenoids, polyphenols and glycosides (Tomita *et al.,* 1965). Research on various part of *N. nucifera* has shown some pharmacological action like anticancer, antiviral, anti-ischemic, antioxidant, antidiarrhoeal, lipolytic, antiobesity, antipyretic, hypocholesterolaemic, anti-inflammatory, hypoglycaemic, antifungal, hepatoprotective, antibacterial, and diuretic action (Mukherjee *et al.,* 2009).

2. Materials and Methods

2.1 Drug and chemicals

The following chemicals were employed: Sterile water for injection (Nirlife Health Care, Mumbai), carboxyl-methyl cellulose (SD Fine Chemicals, Mumbai), ethyl alcohol and chloroform (CDH Ltd, New Delhi) was provided from IFTM University Moradabad.

Corresponding author: Mr. Arvind Kumar Patel
School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad- 244102, Uttar Pradesh, India
E-mail: akpatel4393@gmail.com
Tel.: +91-7392000101

2.2 Identification, collection and authentication of plant material

Plant material, rhizome of *Nelumbo nucifera* Gaertn. was taxonomically recognized, collected and authenticated by the Ayurveda Department, BHU, Varanasi, with reference No. DG/20-21/312/12. The collected part of the plant was dried in shade at 25ºC for 15 days. Then, the air-dried part of the plant was reduced to a coarse powder.

2.3 Extraction of plant material

The dried powder of the plant was passed through a 20-mesh sieve and then subjected to the extraction procedure. The extraction (Maceration) was done for rhizome of *N. nucifera* with 70% ethanol in water at 25ºC for 14 days to extract-out the plant material to be used. The hydroalcoholic extract of *N. nucifera* (HNN) was obtained by vacuum drying solid residues of the solvent after it had been evaporated under decreased pressure to obtain a semisolid mass. The dried extracts were stored in airtight container until the time of use.

2.4 Preliminary phytochemical screening

Prepared extract of the plant (HNN) was carried out for various chemical identification tests to determining the phytoconstituents presents in extracts such as alkaloids, carbohydrates, glycosides, proteins, amino acids, tannins, flavonoids, steroids, *etc*. (Evan and Trease; 2002; Kokate, 1999)

2.5 Experimental animals

Six-week-old wister rats were taken. The animals were kept in a room with air conditioning. The rats were given standard laboratory rodents and water. All procedures involving the animals were carried out by taking approval from CPCSEA (Reg. No. 837/PO/ReBiBt/S/04/CPCSEA) and following the committee's rules for animal care and use.

2.6 Acute oral toxicity Study

Female albino wistar rats were used to investigate the acute oral toxicity of hydroalcoholic extract of *N. nucifera* (HNN) in accordance with OECD test guideline No. 423. Three rats received a single dose of HNN at 48 h interval, at a concentration of 2000 mg/kg of body weight after the rats had been fasted, and assessed for physical characteristics, and body weight. After that, the rat was closely monitored for 4 h to look for any clinical signs and the weight of rats were recorded again after 6 h. A detailed clinical examination was performed once every day for the following 14 days after the extract has been administered (OECD/OCDE, 2008).

2.7 Repeated dose (28 days) oral toxicity study

Mostly used method for doing long-term toxicity testing on rodents was the, 28-day oral toxicity test. The highest dose administered is designed to induce some toxicity without causing fatality. Several clinical and histological evaluations have been made when the test is finished, including experimental findings and analyses of the entire body and individual organs.

2.7.1 Dose administration

According to OECD guidelines No. 407, repeated dose oral toxicity tests for HNN was carried out using albino wistar rats. Rats of both sexes with body weights ranging from 100 to 150 g were divided into four groups, each containing 10 animals (five males and five females). Group I served as the control group and was given 1% CMC vehicle orally every day for 28 days at a dose of 10 ml/kg. Test medications were administered to Groups II, III, and IV at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight.

2.7.2 Observation of animal

Animals were monitored for symptoms, abnormal behaviour, changes in food and water intake, and changes in body weight. Over the 28-day duration of the study, all animals were monitored twice a day to check for signs of mortality. The weight of each rat was recorded once before the start of dosing, once weekly intervals throughout the study. The group means body weights were calculated.

2.7.3 Haematological parameters

At the end of the 28 days, the animals fasted overnight. Fasted for 24 h after being heparinised, blood samples were collected from the orbital sinus. In heparinised EDTA tubes, blood samples were analysed. Blood samples were analyzed for characteristics such as hemoglobin, erythrocyte sedimentation rate, white blood cell count, red blood cell count, platelet count, and clot time.

2.7.4 Biochemical parameters

Whole blood was centrifuged for 15 min at 2400 rpm without any anticoagulants for separating the plasma, and then analysed biochemically. Blood was drawn and tested for a number of biomarkers including albumin, glucose, cholesterol, glutamate pyruvate transaminase (SGPT), and glutamate oxaloacetate transaminase (SGOT), as well as total bilirubin, urea, and total protein, and creatinine.

2.7.5 Histopathology

After blood collection, one animal from all groups was euthanized for pathological examinations, liver was collected for histopathology. Organs were weighed, preserved in 10% neutral buffered formalin, trimmed, and tissue slices stained for microscopic examination in haematoxylin and eosin (OECD, 2008; Potbhare *et al.,* 2022).

3. Results

3.1 Preliminary phytochemical screening

Different chemical analysis of the hydroalcoholic extracts of *N. nucifera* (HNN) showed the presence of the following compound (Table 1).

Table 1: Phytochemical compounds present in HNN

S. No.	Phytoconstituents	HNN
1	Alkaloids	+
2	Carbohydrates	++
3	Tannins	+
4	Phenolic compound	++
5	Flavonoids	+
6	Proteins	+++
7	Amino acids	++
8	Saponins	+
9	Triterpenoids	+++
10	Cardiac glycosides	+

+: present in low concentration, ++: present in moderate concentration, +++: present in high concentration.

3.2 Acute oral toxicity study

An acute oral toxicity study was carried out to find out the safety of test hydroalcoholic drug extract under the study according to OECD test guideline. Animals were observed for 14 days (post-administration) with special attention for the first 4 h after administration. It was observed that all animals were only slightly sedated within the first hour of administration, and were normal and active within two hours after the treatment. All of the animals survived 14 days after receiving the hydroalcoholic extract of *N. nucifera*, and no more toxic signs were seen throughout the study time. Observations revealed that the extract was safe up to a dosage level of 2000 mg/kg, therefore, LD_{50} value was observed as higher than this value.

3.3 Repeated dose (28 days) oral toxicity study

In both sexes of the rats, oral administration of *N. nucifera* hydroalcoholic extract (HNN) at doses of 250, 500, and 1000 mg/kg did not show any signs of toxicity during the 28-day research period.

3.3.1 Physiological parameter

Rats treated with HNN and those in the control groups were weighed at first and their final weights did not differ significantly from one another. The absence of harmful effects was also seen when food and water were consumed. Organ weight did not considerably differ between control and treatment groups (Tables 2-5).

Table 2: Effect of HNN on body weight of rats

Group	Sex	Body weight (g)				
		1st day	7th day	14th day	21st day	28th day
Control	Male	140 ± 3.4	142 ± 3.5	143 ± 3.2	144 ± 4.8	145 ± 3.6
	Female	126 ± 3.5	128 ± 2.5	131 ± 2.4	133 ± 2.7	135 ± 3.2
HNN (250 mg/kg)	Male	139 ± 4.2	141 ± 2.4	142 ± 4.3	143 ± 2.5	143 ± 4.3
	Female	135 ± 2.2	137 ± 2.4	138 ± 4.3	140 ± 3.5	141 ± 3.2
HNN (500 mg/kg)	Male	141 ± 2.4	142 ± 3.3	144 ± 3.3	145 ± 3.4	146 ± 4.2
	Female	133 ± 3.4	135 ± 3.3	136 ± 2.3	137 ± 3.2	139 ± 3.3
HNN (1000 mg/kg)	Male	142 ± 2.4	144 ± 3.2	145 ± 4.3	146 ± 3.3	148 ± 3.2
	Female	132 ± 4.2	134 ± 2.4	136 ± 3.2	137 ± 3.2	139 ± 2.4

Values are represent the mean ± SEM (n=5).

Table 3: Effect of HNN on feed intake in rats

Group	Sex	Feed intake (g)			
		1st week	2nd week	3rd week	4th week
Control	Male	40.23 ± 2.4	41.32 ± 4.3	40.24 ± 2.4	39.24 ± 4.2
	Female	35.34 ± 2.3	36.42 ± 2.4	36.34 ± 3.4	37.22 ± 2.3
HNN (250 mg/kg)	Male	38.24 ± 2.3	39.42 ± 2.4	40.25 ± 2.5	39.43 ± 2.3
	Female	37.32 ± 2.3	37.32 ± 3.3	38.22 ± 2.4	37.26 ± 3.4
HNN (500 mg/kg)	Male	38.23 ± 2.4	39.34 ± 3.4	39.53 ± 3.2	38.32 ± 3.4
	Female	34.32 ± 2.3	35.32 ± 2.3	35.35 ± 2.3	34.23 ± 2.3
HNN (1000 mg/kg)	Male	41.23 ± 2.4	41.23 ± 2.3	42.33 ± 3.2	42.23 ± 3.3
	Female	37.24 ± 2.2	38.42 ± 3.2	38.37 ± 2.3	37.32 ± 3.4

Values are represent the mean ± SEM (n=5).

Table 4: Effect of HNN on water intake in rats

Group	Sex	Water intake (ml)			
		1st week	2nd week	3rd week	4th week
Control	Male	47.43 ± 2.3	47.86 ± 2.3	48.23 ± 2.4	47.34 ± 3.2
	Female	43.34 ± 2.4	45.34 ± 1.4	46.37 ± 2.2	45.23 ± 2.4
HNN (250 mg/kg)	Male	50.36 ± 2.3	49.23 ± 2.4	49.34 ± 2.3	50.34 ± 3.4
	Female	44.44 ± 2.3	45.23 ± 2.2	45.34 ± 2.2	44.34 ± 3.2
HNN (500 mg/kg)	Male	49.23 ± 2.4	50.23 ± 2.5	50.43 ± 2.3	48.32 ± 2.3
	Female	46.34 ± 3.3	47.43 ± 2.2	46.23 ± 2.3	48.23 ± 2.3
HNN (1000 mg/kg)	Male	50.21 ± 2.3	50.32 ± 2.3	49.23 ± 1.4	49.23 ± 2.4
	Female	43.32 ± 2.2	44.24 ± 2.4	44.23 ± 2.3	43.32 ± 2.3

Values are represent the mean ± SEM (n=5).

Table 5: Effect of HNN on body organ weight of rats

Organ	Sex	Organ weight (g)			
		Control	HNN (250 mg/kg)	HNN (500 mg/kg)	HNN (1000 mg/kg)
Liver	Male	5.21 ± 0.20	5.42 ± 0.20	5.41 ± 0.60	5.44 ± 0.30
	Female	4.35 ± 0.30	4.60 ± 0.40	5.12 ± 0.40	4.84 ± 0.30
Kidney	Male	1.12 ± 0.20	1.13 ± 0.40	1.00 ± 0.20	1.10 ± 0.30
	Female	1.00 ± 0.30	1.11 ± 0.30	1.05 ± 0.30	1.11 ± 0.20
Stomach	Male	2.13 ± 0.30	2.23 ± 0.20	2.12 ± 0.20	2.35 ± 0.30
	Female	2.11 ± 0.30	2.21 ± 0.20	2.20 ± 0.30	2.23 ± 0.30
Brain	Male	1.43 ± 0.02	1.39 ± 0.03	1.38 ± 0.04	1.45 ± 0.03
	Female	1.40 ± 0.04	1.43 ± 0.02	1.46 ± 0.03	1.40 ± 0.02
Heart	Male	0.64 ± 0.01	0.65 ± 0.04	0.65 ± 0.05	0.65 ± 0.04
	Female	0.65 ± 0.04	0.62 ± 0.02	0.68 ± 0.03	0.64 ± 0.04
Spleen	Male	0.43 ± 0.03	0.45 ± 0.02	0.45 ± 0.01	0.48 ± 0.03
	Female	0.44 ± 0.03	0.45 ± 0.02	0.44 ± 0.04	0.45 ± 0.03

Values are represent the mean ± SEM (n=5).

3.3.2 Haematological parameters

During the study period, both the control and HNN treated groups' haematological measures including haemoglobin, erythrocyte sedimentation rate, white blood cell count, red blood cell count, platelet count, and clot time, all were found within normal range (Table 6).

Table 6: Effect of HNN on hematological parameters

Group	Sex	Haematological parameters					
		WBC $10^3/mm^3$	RBCs $10^6/mm^3$	Hb (gm/dl)	ESR (mm/hr)	Platelet (K/µl)	Clotting time (Sec)
Control	Male	9.23 ± 0.4	5.54 ± 0.3	14.8 ± 0.5	3.06 ± 0.03	589 ± 22	135 ± 1.3
	Female	8.93 ± 0.5	5.11 ± 0.2	14.6 ± 0.4	3.05 ± 0.04	598 ± 19	137 ± 2.4
HNN (250 mg/kg)	Male	8.82 ± 0.3	5.32 ± 0.3	15.9 ± 0.3	3.06 ± 0.05	603 ± 23	140 ± 2.2
	Female	9.23 ± 0.4	4.90 ± 0.2	15.3 ± 0.2	3.13 ± 0.03	596 ± 14	132 ± 2.4
HNN (500 mg/kg)	Male	9.12 ± 0.5	5.19 ± 0.3	16.2 ± 0.5	3.07 ± 0.11	623 ± 24	136 ± 1.3
	Female	8.87 ± 0.3	5.12 ± 0.4	14.8 ± 0.4	3.06 ± 0.07	612 ± 14	139 ± 1.4
HNN (1000 mg/kg)	Male	9.06 ± 0.4	5.53 ± 0.2	14.9 ± 0.3	3.08 ± 0.05	632 ± 21	141 ± 2.1
	Female	8.93 ± 0.3	4.95 ± 0.2	14.6 ± 0.2	3.07 ± 0.12	612 ± 23	139 ± 1.5

Values are represent the mean ± SEM (n=5).

3.3.3 Biochemical parameters

When compared to control groups, the administration of *N. nucifera* hydroalcoholic extract (HNN) did not result in any appreciable changes in biochemical parameters like blood sugar, albumin, cholesterol, glutamate pyruvate transaminase (SGPT), and glutamate oxaloacetate transaminase (SGOT), as well as total bilirubin, urea, total protein, and creatinine (Table 7).

Table 7: Effect of HNN on biochemical parameters

Biochemical parameter	Sex	Groups			
		Control	(250 mg/kg)	(500 mg/kg)	(1000 mg/kg)
Glucose (mg/dl)	Male	118.4 ± 5.00	116.3 ± 7.00	119.6 ± 5.00	122.3 ± 5.00
	Female	120.3 ± 5.00	119.3 ± 5.00	116.7 ± 5.00	120.5 ± 6.00
Cholesterol (mg/dl)	Male	79.53 ± 6.00	78.56 ± 7.00	76.54 ± 6.00	81.57 ± 5.00
	Female	80.52 ± 5.00	78.97 ± 4.00	79.52 ± 5.00	79.65 ± 4.00

SGPT (U/L)	Male	133.7 ± 1.40	130.4 ± 1.50	129.4 ± 3.40	131.4 ± 2.30
	Female	130.6 ± 3.10	129.3 ± 1.40	132.3 ± 2.10	128.2 ± 1.40
SGOT (U/L)	Male	62.43 ± 2.30	65.45 ± 2.30	61.34 ± 1.40	62.23 ± 1.30
	Female	60.32 ± 1.50	61.22 ± 1.60	61.72 ± 2.40	60.45 ± 1.50
Bilirubin (mg/dl)	Male	0.640 ± 0.13	0.620 ± 0.15	0.720 ± 0.15	0.650 ± 0.25
	Female	0.730 ± 0.15	0.730 ± 0.23	0.730 ± 0.11	0.680 ± 0.22
Total protein (g/dl)	Male	6.910 ± 0.23	6.680 ± 0.23	5.960 ± 0.13	6.140 ± 0.23
	Female	5.920 ± 0.24	6.230 ± 0.23	6.240 ± 0.22	5.860 ± 0.32
Albumin (g/dl)	Male	3.650 ± 0.14	3.760 ± 0.21	3.880 ± 0.24	4.120 ± 0.21
	Female	3.320 ± 0.12	4.120 ± 0.23	3.970 ± 0.23	4.230 ± 0.23
Creatinine (mg/dl)	Male	1.630 ± 0.21	1.540 ± 0.15	1.580 ± 0.22	1.620 ± 0.23
	Female	1.570 ±0.23	1.610 ± 0.24	1.610 ± 0.14	1.580 ± 0.19

Values are represent the mean ± SEM (n=5).

3.3.4 Histopathology

Histopathological examinations of liver did not show any significant abnormalities for both control and hydroalcoholic extract of *N. nucifera* (HNN) treated group (Figure 1).

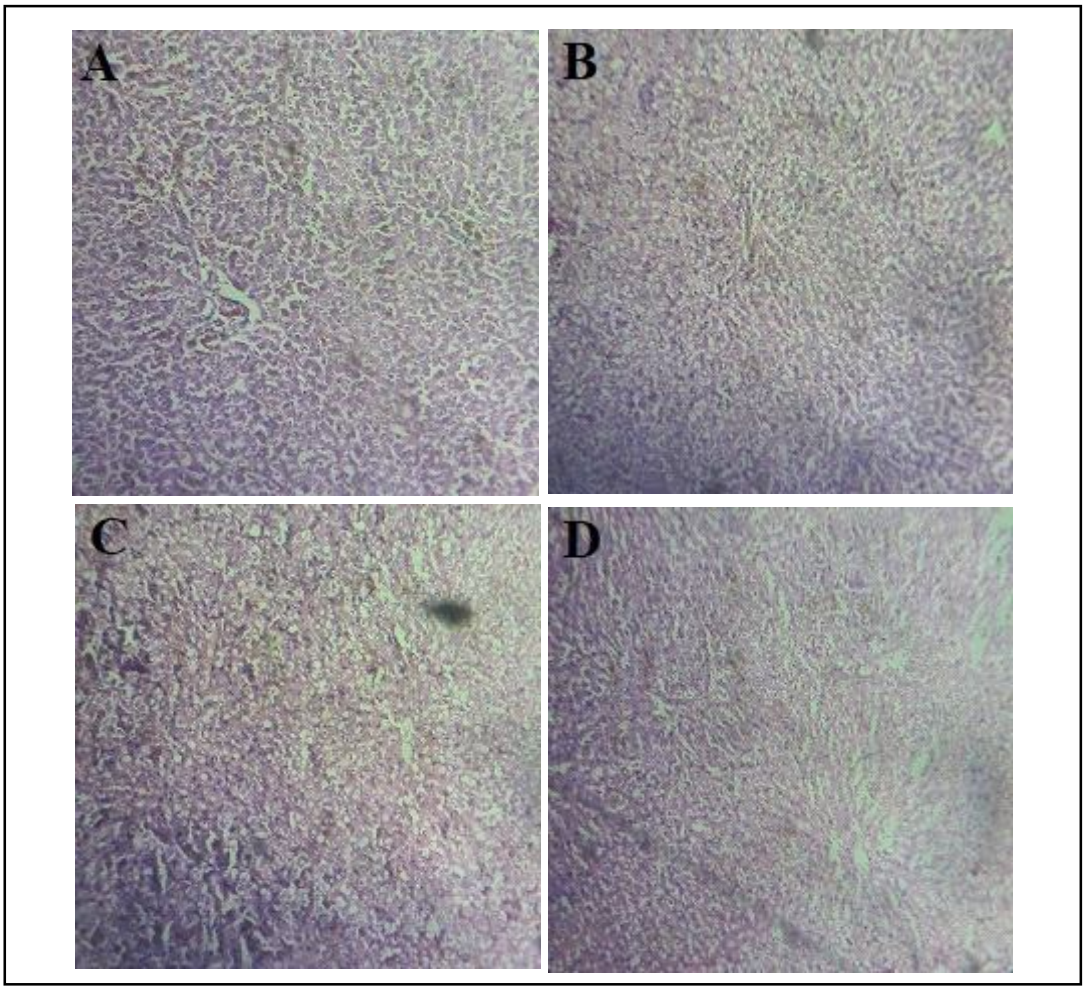


Figure 1: Histopathological assessment of liver. Repeated dose (28 days) oral toxicity studies; (A) section of liver treated with 1% CMC, (B) section of liver treated with HNN (250 mg/kg), (C) section of liver treated with HNN (500 mg/kg), (D) section of liver treated with HNN (1000 mg/kg).

No-observed adverse effect level (NOAEL) of this herbal drug (extract) was observed up to the dose level of 1000 mg/kg/day in rats. Therefore, it can be said that hydroalcoholic extract of *N. nucifera* (HNN) is safe for oral administration.

4. Discussion

Since herbal products come from nature sources and are widely used for self-medication, herbal medicines made from medicinal plants are well known in the healthcare industry and are regarded as safe (Vaghasiya *et al.,* 2011). In general, the majority of the natural products are biodegradable and have low toxicity (Chaves, 2020). Regarding these compounds', toxicological profile and adverse consequences, there is a dearth of information (Sharma, 2021). Therefore, acute and sub-acute toxicity study is required not only to identify the further range of doses in animal studies but also to explain the probable clinical signs evoked by the test compounds under investigation. Additionally, it plays a crucial role in the calculation of the therapeutic index of medications (Rang *et al.,* 2011). Examining the drug's toxicity profile was the goal of this investigation. To validate the safety and effectiveness of these natural sources, to promote the use of plant-based products, it is essential to examine the toxicological profile of these plants. Therefore, the present investigation was carried out to assess acute and repeated dose oral toxicity of *N. nucifera* extract (HNN).

No morbidity or mortality was seen in rats treated with (HNN) during the 14 day observation period of the acute oral toxicity test. Additionally, the findings indicated that there were no adverse reactions at a dose of 2000 mg/kg, indicating that the hydroalcoholic extract of *N. nucifera* (HNN) LD_{50} was higher than this value.

In the repeated dose (28 day) oral toxicity test, none of the rats groups experienced any fatalities or symptoms associated to the medication. Additionally, no discernible differences were seen between the control and HNN treated groups in terms of body weight changes, body organ weight, and food and water intake. Haematological markers like RBC, WBC, and haemoglobin were unaffected by HNN treatment, indicating that neither the abnormality nor the formation of blood cells was impacted. In assessing the toxicity brought on by medications, clinical biochemistry and haematological data play a key role (Petterino *et al.,* 2006). The transaminases (SGOT and SGPT) are useful biomarkers for predicting the potential toxicity of medicines and are good indications of liver health (El Hilaly *et al.,* 2004). Any increase in these enzymes indicates that the liver parenchymal cells have been damaged, which causes their release into the bloodstream (Thomas *et al.,* 2022). HNN had no impact on SGPT or SGOT levels, which can be seen as an evidence that, it had no effect on liver function. The liver's histopathological examination did not reveal any abnormalities in the cells or tissues, indicating that the liver is essentially harmless for prolonged treatment.

5. Conclusion

Rats did not exhibit acute oral toxicity with single dose administration of HNN at 2000 mg/kg of body weight to different animals. No clear harmful effects were observed in any parameters after administration of HNN in the repeated dosage (28 days) oral toxicity investigation up to the dose level of 1000 mg/kg/day. Therefore, in this toxicity research, the "no-observed adverse effect level" (NOAEL) for rats of both sexes was set at 1000 mg/kg/day. According to the current study, long term usage of high doses of HNN as a dietary or medical supplement, up to 1000 mg/kg/day was well tolerated.

Acknowledgments

I wish to express my warm regards and sincere thanks to Dr. Navneet Verma, Dean, Faculty of Pharmacy, IFTM University and Dr. Sushil Kumar, Director, School of Pharmaceutical Sciences, IFTM University, for all necessary support to carry out the work.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

References

Chaves, D.S.A. (2020). The importance of the pet market for the development of new products based on medicinal plants and their derivatives. Ann. Phytomed., **9(1)**:1-6.

Chopra, R.N. (1956). Glossary of Indian medicinal plants. Council of Scientific and Industrial Research, New Delhi, pp:174.

Duke, J.A. (2002). Handbook of medicinal herbs. CRC Press.

El Hilaly, J., Israili, Z.H. and Lyoussi, B.J.J. (2004). Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. Journal of Ethnopharmacology, **91**(1):43-50.

Ertekin, V., Selimoðlu, M.A. and Altinkaynak, S. (2005). A combination of unusual presentations of *Datura stramonium* intoxication in a child: Rhabdomyolysis and fulminant hepatitius. The Journal of Emergency Medicine, **28**(2):227-228, doi:10.1016/j.jemermed.2004.11.006.

Evans, W.C. and Trease, G.E. (2002). Pharmacognosy. Saunders London.

Koduru, S., Grierson, D. and Afolayan, A.J.P. (2006). Antimicrobial activity of *Solanum aculeastrum*. Pharmaceutical Biology, **44**(4):283-286.

Kokate, C.K. (1999). Practical Pharmacognosy. Vallabh Prakashan Publication, pp:115.

Mukherjee, P.K., Mukherjee, D., Maji, A.K., Rai, S. and Heinrich, M.J.J. (2009). The sacred lotus (*Nelumbo nucifera*): Phytochemical and therapeutic profile. Journal of Pharmacy and Pharmacology, **61**(4):407-422.

OECD/OCDE (2008). OECD guideline for the testing of chemicals. Acute oral toxicity; up-and-down rocedure. OECD Publishing.

OECD, O. (2008). For test and chemicals: repeated dose 28-day oral toxicity study in rodents, Guideline No. 407. OECD Publishing.

Petterino, C. and Argentino-Storino, A.J.E. (2006). Clinical chemistry and haematology historical data in control sprague-dawley rats from pre-clinical toxicity studies. Experimental and Toxicological Pathology, **57**(3):213-219.

Potbhare, M.S., Barik, R. and Khobragade, D.S. (2022). Preclinical appraisal of acute oral toxicity of combination of root extracts of *Saussurea lappa* (Decne.) Sch.Bip. and *Valeriana wallichii* (DC.). Ann. Phytomed., **11**(2):405-410.

Rang, H.P., Dale, M.M., Ritter, J.M., Flower, R.J. and Henderson, G. (2011). Rang and Dale's pharmacology. Elsevier Health Sciences.

Sharma, V. (2021). Ayurveda and remedial plants in medication. Ann. Phytomed., **10**(1):1-5.

Sridhar, K. and Bhat, R.J.J. (2007). Lotus: A potential nutraceutical source. Journal of Agricultural Technology, **3**(1):143-155.

Thomas, M., Meena, K.C., Shrivastava, A. and Tripathi, N. (2022). In vitro assessment of cytotoxic effects of guggul in L929 mouse skin fibroblast cells. Ann. Phytomed., **11**(2):332-338.

Tomita, M., Furukawa, H., Yang, T.H. and Lin, T.J. (1965). On the alkaloids of *Nelumbo nucifera* Gaertn. Studies on the alkaloids of loti embryo. Structure of isoliensinine, a new biscoclaurine type alkaloid. Chemical and Pharmaceutical Bulletin, **13**(1):39-43, doi:10.1248/cpb.13.39

Ukwuani, A., Abubakar, M., Hassan, S. and Agaie, B.J.I.J. (2012). Toxicological studies of hydromethanolic leaves extract of *Grewia crenata*. International Journal of Pharmaceutical Science and Drug Research, **4**(4):245-249.

Vaghasiya, Y., Shukla, V. and Chanda, S.J.J.P. (2011). Acute oral toxicity study of *Pluchea arguta* boiss extract in mice. Journal of Pharmacological and Toxicological Methods, **6**(2):113-123.

Warrier, R.R. (2021). Authentication of herbal products to attract global markets. Ann. Phytomed., **10**(2):1-3.

Yuet Ping, K., Darah, I., Chen, Y., Sreeramanan, S. and Sasidharan, S. (2013). Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. Bio. Med. Research International, **2013**:182064, doi:10.1155/2013/182064.

Citation **Arvind Kumar Patel, Phool Chandra and Neetu Sachan (2023). Oral toxicity study of *Nelumbo nucifera* Gaertn. rhizome extract in rats. Ann. Phytomed., 12(2):713-719. http://dx.doi.org/10.54085/ap.2023.12.2.84.**