International Journal of Biomedical Research

ISSN: 0976-9633 (Online)
Journal DOI:10.7439/ijbr
CODEN:IJBRFA

Research Article

Evaluation of Hepatoprotective activity of Ethanolic Extract of *Nymphaea alba Linn* Flower in experimental rats

Ashish K. Paharia[1*] and A. Pandurangan[2]

[1]*Department of Pharmaceutical Science, Shri Venkateshwara University, Gajraula (U.P.) India*
[2]*M. M. College of Pharmacy, MM University Mullana (Ambala) Haryana, India*

***Correspondence Info:**

Mr. Ashish K.Paharia
C/O Dr. Manoj Gupta,
16-B Tarun vihar colony, Near new Jiwaji Nagar
Thatipur Gwalior (M.P.) India -474011
Email: ashish04paharia@gmail.com

Abstract

Objective: In the present study Hepatoprotective activity of Ethanolic Extract of flower of Nymphaea alba Linn were investigated.

Material & Method: The hepatoprotective activity of Ethanolic Extract of flower of *Nymphaea alba* were evaluated by Carbon Tetra Chloride & Paracetamol induced hepatotoxicity in experimental rats. The Ethanolic Extract of *Nymphaea alba* (200 & 400 mg/kg) treat the hepatotoxicity and produced significant inhibition in the wet liver weight and wet liver volumes and the serum enzymes (SGOT, SGPT & ALP), bilirubin and cholesterol levels.

Result: Preliminary phytochemical analysis of Ethanolic Extract of *Nymphaea alba* revealed that the presence of various phytoconstituents Alkaloids, Carbohydrates (Polysaccharides), Glycosides, Steroids, Flavonoids and Tannin & Phenolic compound. The ethanolic extract (200 & 400 mg/kg) showed significant reduction in SGOT, SGPT, ALP, Bilirubin & cholesterol level as compared to control group. Maximum inhibition was obtained at dose 400 mg/kg of Ethanolic Extract of *Nymphaea alba* in both model.

Conclusion: The present study suggests that Ethanolic Extract of flower of *Nymphaea alba* Linn possess strong and potential Hepatoprotective activity in both model. So it has immense scope as an effective source to develop drug for the treatment of liver related diseases. These results may further suggest that ethanolic extract was found to possess hepatoprotective activity.

Keywords: Hepatoprotective activity, *Nymphaea alba* Linn, Carbon Tetra Chloride; Paracetamol

1. Introduction

Liver is the largest gland and heaviest gland of the body weighing about 1.4 kg in an average adult. The liver is the main site of metabolism for drugs and other exogenous compounds. The liver plays a central role in metabolism of large number of organic and inorganic chemicals and drugs. The main drug metabolizing system resides in the microsomal fraction of the smooth endoplasmic reticulum of the liver cells via p-450 cytochrome and cytochrome reductase enzyme system.

Nymphaea alba Linn (Nymphaeaceae) is Generally found in tanks and ponds throughout the warmer parts of India and Africa.. All parts of the plants are used in folk medicine. It grows in water from 30-150 centimeters deep and likes large ponds and lakes. The leaves may be up to thirty centimeters in diameter and they take up a spread of 150 centimeters per plant. It is an aquatic herb with perennial rhizomes or rootstocks anchored with mud. It is globally distributed in Europe, North Africa, Southwest Asia, India, China and Russia. It is rich in tannic acid, gallic acid, alkaloids, sterols, flavonoids, glycosides, hydrolyzable tannins and high–molecular-weight polyphenolic compounds. All the parts of the plant have medicinal uses in traditional system of medicine. It is used as an aphrodisiac, anodyne, antiscrophulatic, astringent,

cardiotonic, demulcent, sedative and anti-inflammatory. Further, it also produces calming and sedative effects upon the nervous system, and is useful in the treatment of insomnia, anxiety and similar disorders[3-8].

2. Material and Methods

2.1 Collection, identification and authentication of plant

The plant *Nymphaea alba Linn* (Flower) were collected from Sarasbag, Pune, Maharashtra, during the month of June-2012 The plant material was identified and authenticated by Prof. P. Jayaraman (Ph.D.), Director-Plant Anatomy Research Centre (PARC) Tambaram. The voucher specimen number is PARC/2012/1702 (a) and it was submitted to the laboratory of Department of Pharmaceutical Science, Shri Venkateshwara University Gajraula, Amroha (Uttar Pradesh) for future references.

2.2 Collection and maintenance of experimental animals

Wistar albino rats of either sex weighing between 150-250 gm of either sex were used. Institutional Animal Ethics Committee of Nagaji Institute of Pharmaceutical Science, Gwalior approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA Reg.No.-1498/PO/a/11/CPCSEA). The animals were housed in Poly propylene cages and maintained at 24˚C ± 2˚C under 12h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water.

2.3 Acute Toxicity Studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD_{50}) was taken as an effective dose. Acute toxicity study was done as per OECD, 2006 Guidelines. Acute oral toxicity tests found the LD_{50} of the Plant extract to be >2,000 mg/kg. The animals were observed for signs of toxicity such as hyperactivity, grooming, convulsions, sedation, and hypothermia continuously for 2 hours, and for mortality up to 24 hours, after administration of the doses [9-10].

2.4 Extraction Method

The flower of *Nymphaea alba* were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to successive extraction by Hot Percolation Method using petroleum ether, chloroform, and Ethanol solvents in a soxhelet extractor. The different extracts obtained were evaporated at 45˚C to get a semisolid mass. The extracts thus obtained were subjected to phytochemical analysis. The percentage yield of EENA was found to be 11 w/w %. [11]

2.5 Phytochemical Analysis of the Extracts

Preliminary phytochemical analysis of EENA revealed that the presence of various phytoconstituents Alkaloids, Carbohydrates (Polysaccharides), Glycosides, Steroids, Flavonoids and Tannin & Phenolic compound[12-13].

2.6 Evaluation of Hepatoprotective activity

2.6.1 Carbon tetrachloride induced hepatotoxicity [14]

In order to assess Hepatoprotective action of plant extract In Albino wistar rats, the rats were divided into the following groups each containing 6 rats (n=6):

Group-I and Group-II were served as Normal control and disease control respectively. Group-III, IV & V corresponded to reference standard (Silymarin-100 mg/kg/day), p.o. Ethanolic Extract of *Nymphaea alba Linn* (EENA) 200 mg/kg & 400 mg/kg respectively.

The treatment lasted for 7 days and on seventh day's night all the animals were fasted for 12 hrs. Then all the animals except those in group - I were treated with 1 ml of CCl_4 in liquid paraffin (1:1). 24 hrs after CCl_4 administration, blood sample were collected for the estimation of biochemical parameter (SGPT, SGOT, ALP, Total bilirubin and total cholesterol).

2.6.2 Paracetamol induced hepatotoxicity model[14]

In order to assess Hepatoprotective action of plant extract in Albino wistar rats, the rats were divided into the following groups each containing 6 rats (n=6):

Group-I and Group-II were served as Normal control and disease control respectively. Group-III, IV & V corresponded to reference standard (Silymarin-100 mg/kg/day), p. o. Ethanolic Extract of *Nymphaea alba Linn* (EENA) 200 mg/kg & 400 mg/kg respectively.

The treatment was carried out for 7 days and on seventh days night all the animal were fasted for 12 hrs. Then all the animals except those in group - I were treated with paracetamol (2 mg/kg, p.o.) in sucrose solution (40 % v/v) in three divided doses.48 hrs after paracetamol administration, blood sample were collected for the estimation of biochemical parameter (SGPT, SGOT, ALP, Total bilirubin and total cholesterol).

2.7 Assessment of hepatoprotective activity [15-16]

After 24 hours of the last treatment, the rats were anaesthetized with ether and blood samples from each animal of all groups were collected by retro-orbital plexus puncture in sterilized centrifuge tubes. The blood samples were then allowed to coagulate at 30° C for 45 minutes. Serum portion was Separated from each sample by centrifugation at 2500 rpm at 30°C for 10 minutes and subjected to biochemical investigation to assess liver function on the basis of determination of total bilirubin, Cholesterol, Alanine amino transferase (ALT/SGPT), Aspartate amino transferase (AST/SGOT), and alkaline phosphatase (ALP) level.

2.8 Histopathological studies [17]

The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Boucin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12h, then embeded in paraffin using conventional methods and cut into 5µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

2.9 Statistical analysis

The results were reported as Mean ± SEM of different observations. Experimental data were analyzed using one-way analysis of variance (ANOVA) to compare the difference between the control and treated values. Different value of P was considered significantly. Graph Pad Prism Version was used for statistical calculations

3. Results

3.1 Effect of Ethanolic Extract of *Nymphaea alba Linn* on CCl_4 induced hepatotoxicity

CCl_4 treated animal showed significant elevation of serum biochemical parameter such as SGPT, SGOT, ALP, total bilirubin and total cholesterol. The liver weight and the ratio of liver weight to body weight were increased compared with normal control group, and the pathological lesions of the liver were evident. Pre- treatment with silymarin-100 mg/kg p.o. and EENA at 200 mg/kg and 400 mg/kg p. o. for 7 days had produced significant protective effect on CCl_4 -induced hepatic damage by maintaining the morphological changes (liver weight and liver to body weight ratio) and normalizing the elevation of serum biochemical parameter and therefore inhibited the Histopathological abnormalities caused by CCl_4. EENA showed dose dependent protection against CCl_4 induced hepatic damage. (Table 1)

Table 1. Effect of EENA onEffect of EENA on Serum biochemical parameter (CCl_4 induced hepatotoxicity)

Group	Serum biochemical parameter			Total Bilirubin	Total cholesterol
	SGPT	SGOT	ALP		
G-I	53.55 ± 1.4	160.4 ± 20.0	430.6 ± 42.0	0.29 ± 0.01	19.30 ± 0.88
G-II	635.05 ± 24.4*	358.4 ± 21.50 *	667.9 ± 18.7 *	0.64 ± 0.02 *	101.3 ± 2.4*
G-III	192.3 ± 14.7 $^{\epsilon,b}$	586.9 ± 17.80 $^{\epsilon,b}$	500.0 ± 48.0 ^{a}	0.60 ± 0.05 *	213.8 ± 27*
G-IV	332.4 ± 9.4 $^{\epsilon,b,\psi}$	564.4 ± 18.2 $^{\epsilon,b,\psi}$	392.9 ± 18.0 ^{b}	0.68 ± 0.04 *	106.1 ± 6.9*
G-V	72.5 ± 3.4 $^{b,\psi}$	148.9 ± 17.80 $^{b,\psi}$	438.1 ± 42.5 ^{a}	0.62 ± 0.01 *	111.3 ± 10.48*

G-I: Normal control, G-II: Disease control (CCl_4), G-III: Silymarin (100 mg/kg) + CCl_4, G-IV & V: EENA doses (200 mg/kg & 400 mg/kg) + CCl_4. All values are express expressed as mean ± SEM of 6 observations, Comparation- Group I Vs II, III, IV & V. Significant at *p<0.05 compared to Normal control group. € P p<0.01 compared to Normal control group. a p<0.05 compared to Disease control group and. b p<0.01 compared to Disease control group, ψ p<0.01 compared to silymarin treated group.

3.2 Effect of Ethanolic Extract of *Nymphaea alba Linn* on Paracetamol induced hepatotoxicity

Administration of paracetamol at a dose of 2 gm/kg p. o. showed cetrilobular necrosis in Histopathological studies

in animal and its association with elevation of serum biomarkers for liver function such as SGPT, SGOT, ALP, total bilirubin and total cholesterol. The liver weight and the ratio of liver weight to body weight were increased. Pretreatment with Ethanolic Extract of *Nymphaea alba Linn* at 200 mg/kg & 400 mg/kg for 7 days offered significant protection against paracetamol induced hepatic damage by inhibiting the morphological changes (liver weight and the ratio of liver weight to body weight) and maintaining the serum biochemical parameter. Histopathological analysis demonstrated that the pathological lesions caused by paracetamol were very minimal in Ethanolic Extract of *Nymphaea alba Linn* pretreated group. The protective effect of Ethanolic Extract of *Nymphaea alba Linn* -400mg/kg p. o. was comparable with silymarin-100 mg/kg. (Table 2)

Table 2. Effect of EENA on Serum biochemical parameter (Paracetamol induced hepatotoxicity)

Group	Serum biochemical parameter			Total Bilirubin	Total cholesterol
	SGPT	SGOT	ALP		
G-I	53.55 ±1.4	160.4 ± 20.0	430.6 ± 42.0	0.29 ± 0.01	19.30 ± 0.88
G-II	385.05 ± 20.4 $^{€}$	651.4 ± 26.50 $^{€}$	767.9 ± 35.85 $^{€}$	1.69 ± 0.22 $^{€}$	102.30 ± 1.4 *
G-III	76.3 ± 5.3 ^{b}	187.2 ± 8.50 ^{b}	436.2 ± 8.30 ^{b}	0.25 ± 0.05 ^{b}	215.80 ± 17 *
G-IV	115.4 ± 11.24 ^{a}	237.4 ± 17.3 ^{a}	572.9 ± 21.0 ^{a}	0.61 ± 0.02 ^{b}	107.10 ± 7.9 *
G-V	68.05 ± 3.4 b, ^{ψ}	180.9 ± 21.80 b, ^{ψ}	412.1 ± 10.5 ^{b}	0.48 ± 0.09 ^{b}	114.3 ± 10.68 *

G-I: Normal control, G-II: Disease control (Paracetamol), G-III: Silymarin (100 mg/kg) + Paracetamol, G-IV & V: EENA doses (200 mg/kg & 400 mg/kg) + Paracetamol. All value are express expressed as mean ± SEM of 6 observations, Comparation- Group I Vs II, III, IV & V. Significant at *p<0.05 compared to Normal control group. € P p<0.01 compared to Normal control group. a p<0.05 compared to Disease control group and . b p<0.01 compared to Disease control group, ψ p<0.01 compared to silymarin treated group.

3.3 Wet liver weight and Wet liver volume

CCl_4 and paracetamol treatment in rats resulted in enlargement of liver which was evident by increase in the wet liver weight and volume. The groups were treated with Silymarin and Ethanolic Extract of *Nymphaea alba Linn* (flower) showed significant restoration of wet liver weight and wet liver volume nearer to normal. The EENA at 200mg/kg and 400mg/kg body weight showed reduction of wet liver weight and wet liver significantly at p<0.05. (Table 3 and Table 4))

Table 3. Effect of EENA on Wet liver weight and Wet liver volume (CCl_4 induced hepatotoxicity)

Group	Body weight		Liver weight	Liver weight to body weight ratio (%)
	Initial	Before sacrifice		
G-I	218.33± 13.2	222.17 ±14.5 ^{ns}	6.78 ±0.07	3.05± 0.06
G-II	217.37± 14.2	220.47 ±12.5 ^{ns}	8.85 ±0.17 $^{€}$	4.05± 0.08 $^{€}$
G-III	216.33± 14.2	218.37 ±12.5 ^{ns}	6.78 ±0.16 ^{b}	3.12± 0.08 ^{b}
G-IV	217.48± 17.5	220.51 ±16.5 ^{ns}	7.30 ±0.27 ^{b}	3.27± 0.13 ^{b}
G-V	218.0± 15.2	229.47 ±14.9 ^{ns}	7.13 ±0.22 ^{b}	3.25± 0.07 ^{b}

G-I: Normal control, G-II: Disease control (CCl_4), G-III: Silymarin (100 mg/kg) + CCl_4, G-IV & V: EENA doses (200 mg/kg & 400 mg/kg) + CCl_4. All values are express expressed as mean ± SEM of 6 observations, Comparation- Group I Vs II, III, IV & V. Significant at *p<0.05 compared to Normal control group. € P p<0.01 compared to Normal control group. a p<0.05 compared to Disease control group and. b p<0.01 compared to Disease control group, ψ p<0.01 compared to silymarin treated group.

Table 4. Effect of EENA on Wet liver weight and Wet liver volume (Paracetamol induced hepatotoxicity)

Group	Body weight		Liver weight	Liver weight to body weight ratio (%)
	Initial	Before sacrifice		
G-I	218.00 ± 13.2	223.17 ± 13.5	6.78 ± 0.07	3.05 ± 0.06
G-II	212.07 ± 14.2	215.07 ± 12.9 ^{ns}	8.35 ± 0.17 $^{€}$	3.88 ± 0.10 $^{€}$
G-III	215.33 ± 12.4	218.90 ± 12.5 ^{ns}	6.28 ± 0.16 ^{b}	2.99 ± 0.05 ^{b}
G-IV	217.48 ± 14.15	222.11 ± 12.5 ^{ns}	7.38 ± 0.17 ^{b}	3.34 ± 0.09 ^{b}
G-V	208.80 ± 13.5	213.47 ± 13.5 ^{ns}	6.78 ± 0.15 ^{b}	1.18 ± 0.02 ^{b}

G-I: Normal control, G-II: Disease control (Paracetamol), G-III: Silymarin (100 mg/kg) + Paracetamol, G-IV & V: EENA doses (200

mg/kg & 400 mg/kg) + Paracetamol. All value are express expressed as mean ± SEM of 6 observations, Comparation- Group I Vs II, III, IV & V. Significant at *p<0.05 compared to Normal control group. € P p<0.01 compared to Normal control group. a p<0.05 compared to Disease control group and . b p<0.01 compared to Disease control group, ψ p<0.01 compared to silymarin treated group.

4. Discussion

In the present study, CCl_4 & paracetamol was employed as toxic agents and the protective role of *Nymphaea alba* flower against the CCl_4 & paracetamol induced hepatotoxicity was studied. The extent of toxicity was estimated by histopathological studies and biochemical enzyme markers like SGOT, SGPT, ALP and Serum Bilirubin levels etc. The ethanolic extracts of flower at dose of 400 mg/kg demonstrated a significant reduction in the serum enzymes and bilirubin levels.

In case of toxic liver, Wet liver weight and Wet liver volumes are increased. In this case water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume. It is reported that liver mass and volume are important parameters in ascertaining the hepatoprotective effect of the drugs. So in this study treatment with ethanolic extract of *Nymphaea alba* flower significantly reduced the wet liver weight and wet liver volumes of animals and hence it possesses statistically significant($p<0.05$) hepatoprotective activity.

In the current study treatment of animals with EENA significantly ($p<0.05$ & $p<0.01$) decreased the levels of SGOT, SGPT, ALP in serum Enzyme which is an indicative of hepatoprotective activity[18-20].

5. Conclusion

The results of study demonstrate that EENA Flower possesses hepatoprotective property due to the decrease in the serum levels of these enzymes and recovery of hepatocyte shapes. Further studies are required to identify, isolate, characterize and evaluate the active principal responsible for hepatoprotective activity of plant. The hepatoprotective effect of EENA was confirmed by the following measures. The isolated livers from the toxicant (CCl_4 & paracetamol) treated animals exhibited increase in wet liver weight and wet liver volume. EENA showed marked change in level of serum marker enzymes such as SGPT, SGOT, SALP, total bilirubin and total cholesterol , due to its hepatoprotective effect.

Acknowledgement

The authors express their thanks to Dr. P. Jayaraman, Director at PARC, Chennai India, for authentication of the plant material and to Dr. Manish Maheshwari, Principal, Nagaji Institute of Pharmaceutical Science, Gwalior India for providing the facilities necessary to carry out the research work.

References

1. Doreswamy R. and Sharma D. "Plant Drug for Liver Disorder Management" *Indian Drugs,* 1995; 32(4): 139-140.
2. Harsh Mohan. “A Textbook of Pathology” Jaypee brothers medical publishers ltd. 5th edition-2005, pp.-563.
3. Eliana R, Ricardo T, Jose C, Galduroz F, Giuseppina N. Studies in Natural Products Chemistry. Vol. 35. Brazil: Elsevier; 2008. Plants with possible anxiolytic and/or hypnotic effects indicated by three brazilian cultures - indians, afrobrazilians, and river-dwellers; pp. 549–95.
4. Adnaik RS, Pai PT, Sapakal VD, Naikwade NS, Magdum CS. Anxiolytic activity of *Vitex Negundo* Linn. In experimental models of anxiety in mice. *Int J Green Pharm.*2009; 3:243–7.
5. Robin D. Nymphaea odorata: White pond lily. Medical Herbalism. *Materia Medica Pharm*. 2001; 11:231–3.
6. Vergeera LH, Vander VG. Phenolic content of daylight-exposed and shaded floating leaves of water lilies (Nymphaeaceae) in relation to infection by fungi. *Oecologia.* 1997; 112:481–4.
7. James AD. Duke’s Hand book of medicinal plants of the bible. USA: Taylor and Francis group; 2008. pp. 302–5.
8. Naghma K, Sarwat S. Anticarcinogenic effect of Nymphaea alba against oxidative damage and hyperproliferative response and renal carcinogenis in Wistar rats. *Mol Cell Biochem*. 2005;271:1–11.
9. Lipnick RL, Cotruvo JA, Hill RN, Bruce RD, Stitzel KA, Walker AP, *et al*. Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. *Food Chem Toxicol*.1995; 33: 223–31.

10. OECD Guidelines for Testing of Chemicals [internet]. France: OECD Publishing; 2006. Section 4, Health effects: Test No. 425: Acute oral toxicity: Up and down procedure.
11. Mukaherjee PK, Quality control of herbal drugs (an approach to evaluation of botanicals) New Delhi: Business Horizon's; 2002: p.380-421.
12. Ajayi IA, Ajibade O, Oderinde RA. Preliminary phytochemical analysis of some plant seeds. *Res.J.Chem.Sci.* 2011;1(3):58-62.
13. De S, Dey YN. Phytochemical investigation and chromatographic evaluation of the different extract of tuber of *Amorphaphallus paeonifolius*. International journal on Pharmaceutical and Biomedical Research 2010; 1(5): 150-157.
14. Chanchal KR, Jagadish V K, Mohammed A. Hepatoprotective activity of *Psidium guajava Linn* leaf extract. *Ind J Exp Biol* 2006; 44 : 305-311.
15. Kind P R & King E J, Estimation of plasma phosphatase by determination of hydrolysed phenol with amino Antipyrine*, J Clin Pathol,* 1954;7: 322.
16. Reitman S & Frankel S, A Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic Transaminases *Am J Clin Pathol* 1957; 28: 56.
17. Galighor, A.E., Kozloff, E.N., (1976): Essentials of practical micro technique 2nd edn, Lea and Febiger, NewYork.
18. Whitcomb D. C., and Block G.D. reported Association of acetaminophen hepatotoxicity with fasting and ethanol use. *JAMA* 1994:272(23):1845-1850.
19. Zimmerman H.J., and Seeff L.B., Enzymes in hepatic disease, in Goodly, E.L. ed, Diagnostic enzymology. *Med .Clin. North. Am.* 1959:43(2):371-400.
20. Sergent O *et al*, role for membrane fluidity in ethanol-induced oxidative Stress of Primary rat hepatocytes. *JPET* 2005:313:104-111.