Research Article

AMELIORATIVE POTENTIALS OF *NYMPHAEA ALBA* ON HEPATOTOXICITY AND OXIDATIVE DAMAGE INDUCED BY CHRONIC EXPOSURE TO ISONIAZID IN RATS

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ABSTRACT

Objective: Evaluation of hepatoprotective and antioxidant potentials of ethanolic extract of *Nymphaea alba* against chronic exposure to isoniazid in rats. **Material and Methods:** Chronic administration of isoniazid (50 mg/kg) orally for 28 days lead to hepatotoxicity in rats. Silymarin (50 mg/kg) and ethanolic extract of *Nymphaea alba* flowers and leaves (200 mg/kg and 400 mg/kg) were given orally for 31 days. Isoniazid was given to all rats except normal control from 4th to the 31st day of the study. On the 32nd day, all animals were sacrificed, blood samples were collected for liver function tests and antioxidant study. Liver tissues were subjected to histopathological examination. **Results:** Chronic administration of isoniazid resulted in a significant elevation in liver enzymes and oxidative stress markers in rats. The extracts in 200 mg/kg and 400 mg/kg doses significantly decreased the isoniazid-induced elevation of liver enzymes (AST, ALT, ALP and serum bilirubin) as well as oxidative stress markers (catalase, GSH, and MDA) in rats. Histological findings supported the changes in biochemical parameters. **Conclusion:** Ethanolic extract of *Nymphaea alba* (NAEE) in 200 mg/kg and 400 mg/kg dose possess hepatoprotective and antioxidant potential. NAEE has ameliorative potentials against chronic isoniazid exposure induced hepatotoxicity in rats.

Key Words: Ethanolic extract, *Nymphaea alba*, Isoniazid induced hepatotoxicity, antioxidant.

INTRODUCTION

The currently used anti-tubercular chemotherapeutic regimens containing Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PZA) are associated with serious adverse effects such as drug-induced hepatotoxicity.¹ All these drugs are potentially hepatotoxic, and toxic effects are enhanced synergistically when given together. This may lead to interruptions in the treatment and compromise efficacy, leading to grave consequences. The monoacetyl hydrazine, a metabolite of INH, gets converted to a toxic metabolite by cytochrome P450 which leads to hepatotoxicity. RIF may also increase the metabolism of INH to isonicotinic acid and hydrazine, which are hepatotoxic metabolites. Oxidative stress-induced hepatic injury is one of the important mechanisms in hepatotoxicity produced by antitubercular drugs.²

Nymphaea alba belongs to the Nymphaeaceae family and is found in warmer parts of India and Africa. The flowers and leaves contain various phytoconstituents such as alkaloids, carbohydrates (polysaccharides), glycosides, steroids, flavonoids, tannin, and phenolic compound.³ Previously it has been reported to be used as an anti-inflammatory, demulcent, analgesic, astringent, cardiotonic, sedative and aphrodisiac. It is also useful in the treatment of anxiety, insomnia, and similar disorders.^{4,5,6} The leaves and rhizomes of *N. alba* have been reported to possess antioxidant activity.^{7,8}

Therefore, the present study aimed to evaluate hepatoprotective and antioxidant potentials of ethanolic extract of *Nymphaea alba* against chronic exposure to isoniazid in rats.

MATERIAL AND METHODS

Preparation of Extract

The *Nymphaea alba* flowers and leaves were obtained from Dawakhana Tibbiya College, A.M.U., Aligarh and identified by Prof. S. H. Afaq of Pharmacognosy Section. Shade-dried flowers and leaves were powdered and subjected to extraction. The extraction in ethanol was done for 72 hours in the Soxhlet apparatus. The extract was filtered and evaporated on a water bath at 50°C and stored in the refrigerator for further use. The yield was found to be 18.3 %.

Chemicals and drugs

- Isoniazid was purchased from Macleod's Pharmaceuticals Ltd. Mumbai, Maharashtra, India.
- Silymarin was purchased from Micro Labs Ltd. Bengaluru, Karnataka, India.
- Aspartate aminotransferase [AST], alanine transaminase [ALT], alkaline phosphatase [ALP], and total bilirubin (TB) kits were purchased from Siemens, Mumbai.

Ethical Clearance

The Institutional Animal Ethics Committee (IAEC) on 09.05.2012 approved the study protocol (Registration No. 401/CPCSEA). All the animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA.

Experimental Animals

Adult Wistar albino rats of either sex (150-200g) were housed under standard conditions (temperature $27 \pm 2^\circ\text{C}$, Humidity 30-70% & 12 hour light/dark cycles) and fed with standard pellet diet and water ad libitum. They were procured from Central Animal House of the institute. The rats were acclimatized for one week before the experiments.

Experimental Design

Hepatotoxicity was induced in the animals by using isoniazid 50 mg/kg orally for 28 days.^{9,22,23} The animals were divided into five groups containing six animals each as per table 1. The standard and test drugs were given 30 minutes before the administration of isoniazid. All drugs were given orally.

Biochemical investigations

On the 32nd day, animals were sacrificed under Sodium pentobarbitone (50 mg/kg i.p). Blood samples were collected for estimation of liver function tests. The liver was dissected out for antioxidant tests and histological examination in each animal.

The serum separated from blood was used for biochemical analysis of liver function (aspartate aminotransferase [AST], alanine transaminase [ALT] and alkaline phosphatase [ALP]).^{11,12} The homogenate of liver (in 10% w/v of phosphate buffer [0.2 M, pH-6.6]) tissue was used to perform in vivo antioxidant tests such as catalase (CAT),¹³ reduced glutathione (GSH)¹⁴ and malondialdehyde (MDA).¹⁵

Histological examination of liver

The liver samples were preserved in 10% formalin for 48 h, fixed in paraffin. The tissue samples were processed according to standard histological techniques and stained with hematoxylin and eosin.

STATISTICAL ANALYSIS

The data were analyzed using one-way analysis of variance (ANOVA) with post hoc Tukey test for biochemical parameters. The values are represented as mean $\pm$ S.E.M. $p < 0.05$ was considered statistically significant.

RESULTS

The hepatoprotective effect of ethanolic extract *Nymphaea alba* was measured against isoniazid-induced hepatotoxicity, as shown in Table 2. The oxidative stress damages the integrity of liver cells and causes the release of enzymes like transaminases (AST and ALT) and ALP, as shown in Group-II (Isoniazid only). Group-III (Silymarin + Isoniazid) showed a significant decrease in AST ($p < 0.001$) ALT levels ($p < 0.001$), ALP ($p < 0.01$) and total bilirubin ($p < 0.01$) as compared to group-II. Group-IV (NAEE 200 mg/kg + Isoniazid) showed significant decrease in AST ($p < 0.001$) ALT levels ($p < 0.001$), ALP ($p < 0.05$) and total bilirubin ($p < 0.01$) as compared to group-II. Group-V (NAEE 400 mg/kg + Isoniazid) showed significant decrease in AST ($p < 0.001$) ALT levels ($p < 0.001$), ALP ($p < 0.01$) and total bilirubin ($p < 0.01$) as compared to group-II (Table 2).

In the experiment, the antioxidant activity was measured as the amount of CAT consumed per minute and total GSH present in liver tissue. There was a significant decrease in the levels and activity of GSH and CAT in isoniazid alone treated group. NAEE 200 and 400 mg/kg showed an increase in the levels of CAT ($p < 0.001$), thereby suggesting a correction in oxidative stress. There was a significant increase in the levels of GSH ($p < 0.001$) and decrease in MDA ($p < 0.001$) levels in both NAEE200 and NAEE 400 extracts treated groups as compared to the negative control (Table 3).

The microscopic architecture of the liver in the normal control group (Group I) showed no degeneration, necrosis, or fibrosis. In the isoniazid-only group (Group II) the liver exhibited severe degeneration of hepatic microstructure along with bridging fibrosis and necrosis of hepatocytes. Rats treated with silymarin (Group III) showed near normal hepatic microstructure with very few fibrotic foci and abundant regenerating nodules. The NAEE 200 mg/kg showed degeneration and fibrosis in some hepatocytes with a few regenerating nodules while NAEE 400 mg/kg treated group had well maintained hepatic microstructure with only a few occasional hepatocytes showing degenerative changes with numerous regenerating foci. (Figure 1-5)

Table 1. Grouping of animals

Group	Treatment given (Day 1-3)	Treatment given (Day 4-31)
1.	Normal Saline (1 ml/kg)	Normal Saline (1 ml/kg)
2.	-	Isoniazid (50 mg/kg)
3.	Silymarin (50 mg/kg)	Isoniazid (50 mg/kg) + Silymarin (50 mg/kg) ^[10]
4.	NAEE (200 mg/kg)	Isoniazid (50 mg/kg) + NAEE (200 mg/kg)
5.	NAEE (400 mg/kg)	Isoniazid (50 mg/kg) + NAEE (400 mg/kg)

Table 2: Effect of ethanolic extract of *Nymphaea alba* on different liver function parameters in isoniazid-induced hepatotoxicity.

Biochemical test (mean $\pm$ SEM)	Groups (n=6)				
	Group-I (Normal saline only)	Group-II (Isoniazid only)	Group-III (Silymarin + Isoniazid)	Group-IV (NAEE 200 mg/kg + Isoniazid)	Group-V (NAEE 400 mg/kg + Isoniazid)
AST (IU/ml)	38.4 $\pm$ 1.5	150.1 $\pm$ 6.9 ^z	64.1 $\pm$ 6.8 ^c	78.1 $\pm$ 7.8 ^c	67.3 $\pm$ 6.7 ^c
ALT (IU/ml)	39.1 $\pm$ 1.8	155.1 $\pm$ 5.5 ^z	63.5 $\pm$ 2.9 ^c	76.1 $\pm$ 4.5 ^c	66.1 $\pm$ 3.1 ^c
ALP (KAU/dl)	41.9 $\pm$ 2.4	81.8 $\pm$ 5.1 ^z	48.2 $\pm$ 4.7 ^b	59.6 $\pm$ 5.3 ^a	54.2 $\pm$ 4.8 ^b
T.Bilirubin (mg/dl)	0.35 $\pm$ 0.03	0.86 $\pm$ 0.08 ^z	0.50 $\pm$ 0.041 ^b	0.53 $\pm$ 0.04 ^b	0.49 $\pm$ 0.02 ^b

Values are Mean $\pm$ SEM. ^z $p < 0.001$ when group II was compared with group I; ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$ when group III to group V was compared with group II.

Table 3: Effect of ethanolic extract of *Nymphaea alba* on the antioxidant level in isoniazid-induced hepatotoxicity.

Biochemical test (mean±SEM)	Groups (n=6)				
	Group-I (Normal saline only)	Group-II (Isoniazid only)	Group-III (Silymarin + Isoniazid)	Group-IV (NAEE 200 mg/kg + Isoniazid)	Group-V (NAEE 400 mg/kg + Isoniazid)
Catalase (U/min/mg)	85.1±4.8	45.5±3.8 ^z	71.8±2.4 ^c	60.7±2.4 ^c	68.1±1.9 ^c
GSH (μmol/mg)	5.22±0.28	2.19±0.25 ^z	4.55±0.14 ^c	4.23±0.18 ^c	4.60±0.12 ^c
MDA (nmol/mg)	200.6±8.8	438.9±19.8 ^z	240.8±7.9 ^c	262.4±10.5 ^c	255.4±7.1 ^c

Values are Mean± SEM. ^zp<0.001 when group II was compared with group I; ^ap< 0.05, ^bp<0.01 and ^cp<0.001 when group III to group V was compared with group II.

Picture 1: Photomicrograph of rat liver from Group I (Normal Control) showing normal liver microstructure with intact hepatic cords, sinusoids, and normal contour. (40X. H & E stain).

Picture 2: Photomicrograph of rat liver from Group II (Negative Control) showing severe degeneration of hepatic microstructure. There is also bridging fibrosis and necrosis of the hepatocytes. (40X H & E Stain).

Picture 3: Photomicrograph of rat liver from Group III (Positive control group) showing near normal hepatic microstructure with very few fibrotic foci and abundant regenerating nodules. (40X. H & E stain).

Picture 4: Photomicrograph of rat liver from Group IV (NAEE 200 mg/kg) showing, degeneration, and fibrosis in some hepatocytes with a few regenerating nodules. (40X.H & E stain).

Picture 5: Photomicrograph of rat liver from the prophylactic study, Group V (NAEE 400 mg/kg) showing well maintained hepatic microstructure. Only a few occasional hepatocytes show degenerative changes with numerous regenerating foci. (40X. H & E stain).

DISCUSSION

The present study was planned to explore the hepatoprotective and antioxidant activity of *Nymphaea alba* against isoniazid-induced toxicity in experimental rats.

The hepatic enzymes (AST, ALT, and ALP) are cytoplasmic, but following injury they enter into circulation. This is due to the altered permeability of the membrane.¹⁶ Results showed that INH administration resulted in a significant elevation of serum levels of ALT, AST, and ALP in rats. The level of the enzymes was markedly low in rats pre-treated with NAEE. The hepatic microscopic structure was protected in groups pre-treated with NAEE. Therefore, the hepatoprotective effects of NAEE might be due to its ability to protect biomembrane against free radical injury.

MDA is synthesized from peroxidation of polyunsaturated fatty acid (PUFA) present in the biological membrane.¹⁷ It is a secondary product of lipid peroxidation, and a useful indicator of tissue damage.¹⁸ GSH plays a central role in coordinating the body's antioxidant defense process.¹⁹ The reduced GSH forms the first line of defense against free radicals.²⁰ NAEE at doses 200 and 400 mg/kg prevented elevation of liver MDA content and increase of GSH content that resulted from rat liver intoxication with INH administration. The hepatoprotective ability of NAEE might be due to its ability to stabilize liver cell membrane. Thus, the MDA production and the consumption of GSH were decreased.

CAT is an important antioxidant present in our body. Inhibition of such protective mechanisms results in enhanced free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity. Homogenized liver CAT activities in NAEE treated groups were significantly higher than those in the INH group. In this study, CAT was increased by administration of NAEE, suggesting that it can restore the CAT enzyme.

The hepatoprotective activity of NAEE may be due their antioxidant property. Previous studies have shown the presence of flavonoids and phenolic compounds in ethanolic extract of *Nymphaea alba*.³ The presence of these compounds might be responsible for the antioxidant activity. Hepatoprotection offered by *Nymphaea alba* ethanolic extract could be attributed to these constituents, since antioxidants have shown hepatoprotective activity in previous studies.^{21,23} To confirm their antioxidant potential and to identify various enzymes involved in generating oxygen, free radicals, further studies are essential. These shortcomings of the present studies open a new arena for future research. Considering the efficacy of the plant, their phytoconstituents (fractions) need to be isolated to explore their hepatoprotective activity. Further activity guided chemical studies of the fractions may help in developing new leads that would be useful for the treatment of presently untreatable hepatotoxicities.

CONCLUSION

The ethanolic extract of *Nymphaea alba* (NAEE) in 200 mg/kg and 400 mg/kg dose possess hepatoprotective and antioxidant potential. NAEE has ameliorative potentials against chronic isoniazid exposure induced hepatotoxicity in rats.

REFERENCES

- Petri WA. Drugs use in chemotherapy of tuberculosis, *Mycobacterium avium* complex disease, and leprosy. In

- Goodman & Gilman's The Pharmacological Basis of Therapeutics, Hardman JG, Limbird LE, Gilman AG (eds), 10th edn. McGraw-Hill, Medical Publishing Division: New York, 2001: 1273–1294.
- Vishal R, Tandon V, Khajuria B, Kapoor D, Kour S, Gupta. Hepatoprotective activity of Vitexnegundo leaf extract against anti-tubercular drugs induced hepatotoxicity. *Fitoterapia* 2008; 79: 533–538.
- Paharia AK and Pandurangan A. Evaluation of Hepatoprotective activity of Ethanolic Extract of *Nymphaea alba* Linn Flower in experimental rats. *International Journal of Biomedical Research* 2013; 04 (07) 349-54.
- Adnaik RS, Pai PT, Sapakal VD, Naikwade NS, Magdum CS. Anxiolytic activity of Vitexnegundo Linn in experimental models of anxiety in mice. *International Journal of Green Pharmacy* 2009; 3:243-47.
- Robin D. *Nymphaea odorata*: White pond lily. *Medical Herbalism* 2001; 11:6-7.
- 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.
- Madhusudhan N, Lakshmi T, Gowtham KS, Ramakrishnan, Konda VGR, Roy A, Geetha RV. Invitro antioxidant and free radical scavenging activity of aqueous and ethanolic flower extract of *Nymphaea alba*. *International Journal of Drug Development and Research* 2011; 3:23-6.
- Bose A, Ray SD, Sahoo M. Evaluation of analgesic and antioxidant potential of ethanolic extract of *Nymphaea alba* rhizome. *Oxidants and Antioxidants in Medical Science* 2012; 1(3):217-223.
- Attri S, Rana SV, Vaiphei K, Sodhi CP, Katyal R, Goel RC et al. Isoniazid- and rifampicin-induced oxidative hepatic injury — protection by N-acetylcysteine. *Human & Experimental Toxicology* 2000;19(9):517-522
- Singh G, Goyal R, Sharma PL. Pharmacological potential of Silymarin in combination with Hepatoprotective plants against experimental Hepatotoxicity in rats. *Asian Journal of Pharmaceutical and Clinical Research* 2012; 5:Suppl 1.
- Reitman S, Frankel S. A colorimetric method for the determination of serum levels of glutamic oxaloacetic acid and pyruvic acid transaminases. *American Journal of Clinical Pathology*.1957;10:394-9.
- Marsh WH, Fingerhut B, Kirsch E. Adaption of alkaline phosphatase method for automatic colorimetric analysis. *Clinical Chemistry* 1959;5:119-26.
- Beers RF, Sizier IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry* 1952;195(1):133-40.
- Ellman GC. Tissue sulphydryl groups. *Archives of Biochemistry and Biophysics* 1959;82(1):70-7.
- Niehans WG, Samuelsson D. Formation of malondialdehyde from phospholipids arrachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry* 1968;6(1):126-30.
- Hu HL, Chen RD. Changes in free radicals, trace elements, by D-galactosamine. *Biological Trace Element Research* 1992; 34:19–25.
- Vaca CE, Wilhelm J, Harms-Rihdsahl M. Interaction of lipid peroxidation product with DNA. A review. *Mutation Research-Reviews in Genetic Toxicology*.1998;195:137–49.
- Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry* 1979;95:351–58.
- Boyd SC, Sasame, HA, Boyd MR. Gastric glutathione depletion and acute ulcerogenesis by diethylma-leate given subcutaneously to rats. *Life Science* 1981;28:2987–92.
- Raja S, Ahamed, K.F.H.N, Kumar V, Mukherjee K,

- Bandyopadhyay A, Mukherjee PK. Antioxidant effect of *Cytisus coparius* against carbon tetrachloride treated liver injury in rats. Journal of Ethnopharmacology 2006;109:41–47.
21. Oz HS, McClain CJ, Nagasawa HT, Ray MB, de Villiers WJ, and Chen TS. Diverse antioxidants protect against acetaminophen hepatotoxicity. Journal of Biochemical and Molecular Toxicology 2004;18(6):361-68.
 22. Nasiruddin M, Khan IA, Arif SH. Therapeutic Effect of *Nymphaea alba* Linn Flowers against isoniazid induced hepatotoxicity: An Experimental Study. Asian Journal of Pharmaceutical and Clinical Research May 2018;11(5):333-36.
 23. Paul J, Nasiruddin M, Khan IA, Khan RA, Arif SH. Therapeutic Effect of *Nigella sativa* Oil in Hepatotoxicity Induced by Isoniazid in Rats. Indian Journal of Pharmaceutical Education and Research 2019;53(2):242-9.

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