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Original Article

Protective Effect of *Nymphaea alba* Linn Flowers Against Isoniazid Induced Toxicity in Experimental Rats

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ABSTRACT

Objective: To evaluate the protective effect of ethanolic extract of Nymphaea alba Linn flowers against isoniazid induced hepatotoxicity in rats.
Material and Methods: Hepatotoxicity in rats was induced by isoniazid (50mg/kg). Silymarin (50mg/kg) was used as standard drug in the study. Silymarin and two doses of ethanolic extract of Nymphaea alba Linn flowers (200 mg/kg and 400 mg/kg) were given for 31 days. Isoniazid was given in standard and test groups from 4th to 31st day of the study. All drugs were given orally. After 31 days blood samples were collected from the animals for biochemical analysis and liver tissues were subjected to histopathological examination.
Results: Administration of isoniazid caused significant elevation in the levels of liver marker enzymes and oxidative stress markers in rats. Thetest extract in 200 mg/kg and 400 mg/kg doses significantly decreased isoniazid induced elevation of liver marker enzymes (AST, ALT, ALP and serum bilirubin)as well as oxidative stress markers(catalase, GSH and MDA)in rats. The changes in biochemical parameters were supported by histological profile.
Conclusion: The ethanolic extract of Nymphaea alba Linn flowers (NAEE) in 200 mg/kg and 400 mg/kg dose showed protective effectagainst isoniazid induced hepatotoxicity in rats.
Key Words: Ethanolic extract, Nymphaea alba Linn, Flowers, Isoniazid induced hepatotoxicity.

1. INTRODUCTION

Drug-induced hepatotoxicity is a potentially serious adverse effect of the currently used anti-tubercular chemotherapeutic regimens containing Isoniazid (INH), Rifampicin (RIF) and Pyrazinamide (PZA). [1] All these drugs are potentially

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hepatotoxicity independently, when given in combination their toxic effects are enhanced in a synergistic manner. It interrupts the treatment regime and compromises its efficacy, leading to grave consequences. The conversion of monoacetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P450 leads to hepatotoxicity. RIF can also increase the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. In addition to these mechanisms; oxidative stress induced hepatic injury is one of the important mechanisms in hepatotoxicity produced by anti- tubercular drugs.[2]

Nymphaea alba Linn (Nymphaeaceae) is mainly found in warmer parts of India and Africa. The flowers are white and they contain various phytoconstituents such as alkaloids, carbohydrates (polysaccharides), glycosides, steroids, flavonoids, tannin and phenolic compound.[3] It is used as an anti-inflammatory, anodyne, astringent, antiscrophulatic, cardiotonic, demulcent, sedative and aphrodisiac. It also produces sedative and calming effects upon nervous system and is useful in the treatment of anxiety, insomnia and similar disorders.[4-6] The leaves and rhizomes of N. alba possess antioxidant activity.[7-8]

Therefore, the aim of present study was to investigate the protective effect of ethanolic extract of *Nymphaea alba Linn* flowers against isoniazid induced hepatotoxicity in rats.

2. MATERIAL AND METHODS

Experimental Animals

Adult wistar albino rats of either sex (150-200g) were obtained from Central Animal House of the institute. They were housed under standard conditions (temperature 27 ± 2°C, Humidity 30-70% & 12 hour light/dark cycles). They were fed with standard pellet diet and water ad libitum. The rats were acclimatized to the laboratory condition for 1 week prior to the experiments. 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.

Preparation of Extract

The flowers of *Nymphaea alba Linn* were procured from DawakhanaTibbiya College,A.M.U., Aligarh and identified by Prof. S. H. Afaq, Pharmacognosy Section, Department of Ilmul Advia, A.K.T.C., A.M.U., Aligarh, U.P., India. The shade-dried flowers were coarsely powdered and then subjected to extraction. The extraction of powder in ethanol was done 72 hours using Soxhlet apparatus. The extract was filtered using Whatman No. 1 filter paper, evaporated on water bath at 50ºC until it dried completely and stored in refrigerator for further use. The yield of *Nymphaea alba Linn*e thanolic extract (NAEE) was found to be 10.6 %.

Chemicals and drugs

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

Induction of hepatotoxicity

Hepatotoxicity was induced in the animals by using isoniazid 50mg/kg orally for 28 days. [9]

Experimental Design

The animals were divided into 5 groups containing 6 animals each. Group I was given Normal Saline (1ml/kg) for 31 days. Group III was administered silymarin, the standard drug orally in a dose of 50mg/kg for 31 days.[10] The ethanolic extract of Nymphaea alba Linn flowers (NAEE) was administered orally in a dose of 200mg/kg and 400mg/kg for 31 days in group IV and V respectively. Isoniazid (50mg/kg) was administered orally from 3rdday to 31stday of treatment in groups II, III, IV and V. The standard and test drugs were given 30 minutes before the administration of isoniazid. On the 32ndday, animals were sacrificed under Sodium pentobarbitone (50 mg/kg i.p). Blood sample was taken by cardiac puncture for estimation of liver function tests and liver was dissected out for antioxidant tests and histological examination in each animal.

Biochemical investigations

The serum separated from blood was used for biochemical analysis of liver function (aspartate amino transferase [AST], alanine transaminase [ALT] and alkaline phosphatase [ALP]).[11,12]

Antioxidant tests

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 histological assessment of liver damage was done by scoring of structural changes described by National Health Services Meryland, USA.[16]

The parameters were as followed:

a. Degeneration

(0-No degeneration, 1-few vacuolated cells perlesion, 2-more than 10 vacuolated cells per lesion, 3-one to two rows of vacuolated cells around necrotic zone per lesion, 4- more than two rows of vacuolated cells around necrotic zone per lesion)

b. Necrosis

(0-No necrosis, 1-Focal necrosis of one or two cells per lesion, 2-focal necrosis of more than two cells per lesion, 3-massive centrilobular necrosis,4-massive centrilobular necrosis with necrotic tissue bridging the central vein)

c. Fibrosis

(0-normal appearance of liver, 2- central necrosis, hydropic degeneration, no fibrosis, 2- fibrous tissue in periportal area only, 3- Fibrous tissues insinuating surrounding hepatic parenchyma, 4- formation of peudolobules)

d. Regeneration

(0-no Regeneration, 1- Mild, 2- Moderate,3- Excellent)

Statistical Analysis

The data was analyzed using one way analysis of variance (ANOVA) with post hoc Tukey test for biochemical parameters and Mann-Whitney U test for comparing histopathology scores. The values are represented as mean ± S.E.M. $p<0.05$ was considered statistically significant.

3. RESULTS

A. Effect of NAEE on liver function tests

The proective effect of *Nymphaea alba Linn* flowers was measured against isoniazid induced hepatotoxicity as shown in Table 1. 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 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 1).

B. Effect of NAEE on antioxidant tests

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 significant decrease in the levels and activity of GSH and CAT in isoniazida lone treated group. NAEE 200 and 400 mg/kg showed an increase in the levels of CAT ($p<0.001$) thereby suggesting correction in oxidative stress. There was 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 negative control(Table 1).

Table 1: Prophylactic Effect of Ethanolic extract of *Nymphaea alba Linn* Flowers on different biochemical parameters 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)
AST (IU/ml)	37.5±1.6	152.1±6.4^{z}	61.2±6.7^{c}	77.1±7.7^{c}	64.3±6.6^{c}
ALT (IU/ml)	38.9±1.7	154.3±5.2^{z}	59.5±2.7^{c}	76.1±4.5^{c}	63.4±3.0^{c}
ALP (KAU/dl)	42.0±2.5	80.9±5.5^{z}	48.2±4.7^{b}	57.6 ±5.1^{a}	53.2±4.9^{b}
T.Bilirubin (mg/dl)	0.363±0.032	0.871±0.081^{z}	0.501±0.041^{b}	0.508±0.040^{b}	0.497±0.027^{b}
Catalase (U/min/mg)	84.3±4.9	46.5±3.7^{z}	70.8±2.2^{c}	61.8±2.1^{c}	68.0±1.6^{c}
GSH (µmol/mg)	5.29±0.29	2.18±0.26^{z}	4.68±0.13^{c}	4.32±0.19^{c}	4.70±0.11^{c}
MDA (nmol/mg)	202.8±8.9	440.9±18.9^{z}	245.8±7.8^{c}	264.4±10.7^{c}	251.4±6.9^{c}

Values are expressed as Mean± 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 groupII.

C. Effect of NAEE on histological findings

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 histopathological score exhibited significant degeneration ($p<0.001$), necrosis ($p<0.001$) and fibrosis ($p<0.001$) with no regeneration. The degeneration ($p<0.001$), necrosis ($p<0.001$) and fibrosis ($p<0.001$) scores were significantly reduced in the rats treated with silymarin (Group III) and the regeneration ($p<0.001$) scores showed commendable improvement. The NAEE 200 mg/kg and 400 mg/kg treated groups (Group IV& V) also showed significantly reduction in degeneration ($p<0.001$), necrosis ($p<0.001$) and fibrosis ($p<0.001$) scores and the regeneration ($p<0.001$) scores showed commendable improvement (Table 2).

Table 2: Prophylactic Effect of Ethanolic extract of *Nymphaea alba Linn* Flowers (NAEE) on histopathology score in isoniazid induced hepatotoxicity

Parameter (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)
Degeneration	0	2.8±0.4^{z}	0.7±0.12^{c}	1.2±0.08^{c}	0.7±0.02^{c}
Necrosis	0	2.1±0.3^{z}	0^{c}	0^{c}	0^{c}
Fibrosis	0	2.8±0.2^{z}	0.5±0.02^{c}	0.8±0.10^{c}	0.6±0.07^{c}
Regeneration	0	0	3.0±0.06^{c}	2.7±0.24^{c}	2.8±0.09^{c}

Values are expressed as Mean± SEM. $^{z}p<0.001$ when group II was compared with group I; $^{c}p<0.001$ when group III to group V was compared with groupII.

4. DISCUSSION

The present study was planned to explore hepatoprotective activity of *Nymphaea alba Linn* flowers against isoniazid induced toxicity in experimental rats.

The serum marker enzymes (AST, ALT and ALP) are cytoplasmic in nature, but upon liver injury these enzymes enter into the circulatory system due to altered permeability of membrane. [17] In our study, results showed that INH caused a significant elevation of serum levels of ALT, AST and ALP in rats. These effects were markedly reduced when the rats were pre-treated with NAEE. The microscopic architecture of the liver was protected in the groups pre-treated with NAEE. Together these evidences suggest that the hepatoprotective effects of NAEE might be in part due to its ability to protect biomembrane against free radicals.

MDA is a major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (PUFA).[18] MDA, a secondary product of lipid peroxidation, is a useful indicator of tissue damage involving a series of chain reactions. [19] GSH, an important protein thiol in living organisms plays a central role in coordinating the body's antioxidant defense process.[20] Reducing GSH

constitutes the first line of defense against free radicals. [21] 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 challenge. 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 a key component of the antioxidant defense system. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. Homogenated liver CAT activities in NAEE treated groups were significantly higher than those in INH group. In this study, CAT was increased by administration of NAEE, suggesting that it can restore CAT enzyme.

The hepatoprotective activity of NAEE may be by the virtue of their antioxidant property. Previous studies demonstrate the presence of flavonoids and phenolic compounds in ethanolic extract of *Nymphaea alba Linn*.[3] The presence of these compounds might be responsible for the antioxidant activity.In order to confirm their antioxidant potential and to identify various enzymes involved in generating oxygen free radicals further studies are essential.

Hepatoprotection offered by *Nymphaea alba Linn* ethanolicextract could be attributed to these constituents, since antioxidants have been reported to posses hepatoprotective activity. [22] In order 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 the future research. Considering the efficacy of the plant, their phytoconstituents (fractions) need to be isolated in order 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.

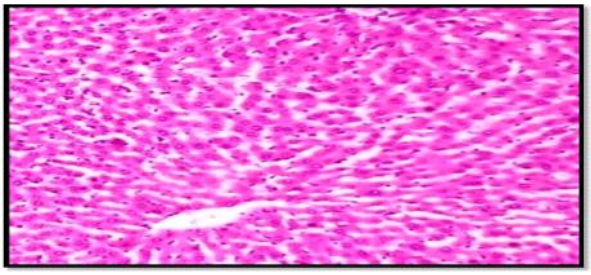

Fig 1:Photomicrograph of rat liver from Group I(Normal Control) showing normal liver microstructure with intact hepatic cords, sinusoids and normal contour. (40X. H & E stain).

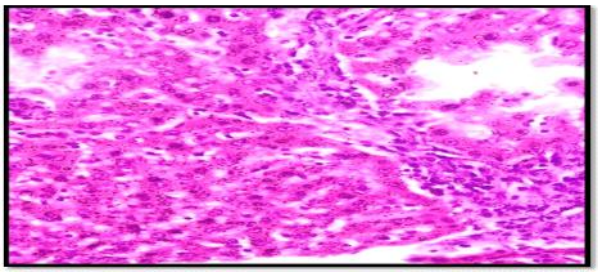

Fig 2: Photomicrograph of rat liver from Group II (Negative Control)showing severe degeneration of hepatic microstructure. There is also bridging fibrosis and necrosis of the hepatocytes. (40X H & E Stain).

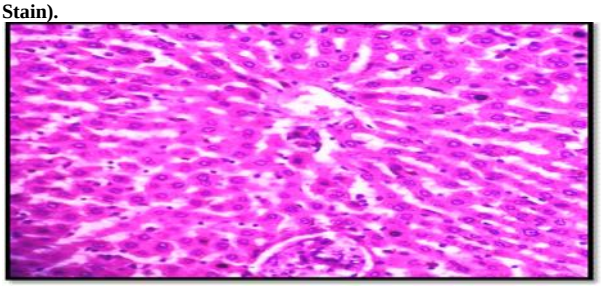

Fig 3: Photomicrograph of rat liver from GroupIII(Positive control group)showing near normal hepatic microstructure with very few fibrotic foci and abundant regenerating nodules. (40X. H & E stain).

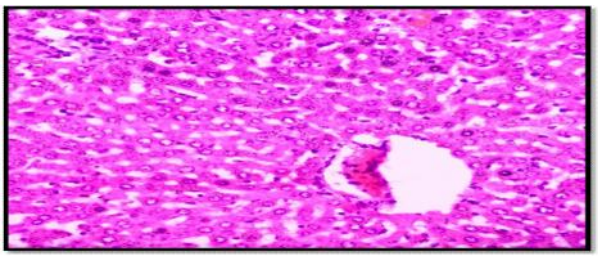

Fig 4: Photomicrograph of rat liver from Group IV (NAEE 200 mg/kg) showing, degeneration and fibrosis in some hepatocytes with a few regenerating nodules.(40X.H & E stain).

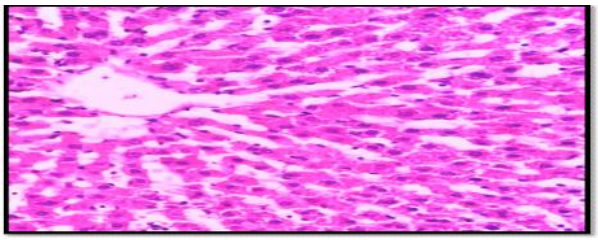

Fig 5: Photomicrograph of rat liver from prophylactic study, Group V (NAEE 400 mg/kg) showing well maintained hepatic microstructure. Only few occasional hepatocytes show degenerative changes with numerous regenerating foci. (40X. H & E stain).

5. CONCLUSION

The ethanolic extract of *Nymphaea alba Linn* flowers (NAEE) in 200 mg/kg and 400 mg/kg dose showed hepatoprotective effect against isoniazid induced hepatotoxicity in rats.

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