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Research Article

THERAPEUTIC EFFECT OF *NYMPHAEA ALBA* LINN. FLOWERS AGAINST ISONIAZID-INDUCED HEPATOTOXICITY: AN EXPERIMENTAL STUDY

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ABSTRACT

Objective: This study was designed to evaluate the effect of ethanolic extract of *Nymphaea alba* (NAEE) Linn. flowers on liver marker enzymes, histology, and antioxidant tests against isoniazid (INH)-induced hepatotoxicity in rats.

Methods: Wistar albino rats were treated with INH (50 mg/kg) for 28 days to induce hepatotoxicity. Silymarin (100 mg/kg) and NAEE Linn flowers in 200 mg/kg and 400 mg/kg doses, respectively, were used as standard and test drugs. Liver marker enzymes and histological examination of livers were performed to demonstrate the effect of NAEE against INH-induced hepatotoxicity. Catalase [CAT], glutathione, and malondialdehyde (MDA) levels were estimated to evaluate the antioxidant property of the NAEE extract.

Results: The NAEE extract in 200 mg/kg and 400 mg/kg doses significantly decreased INH-induced elevation of liver marker enzymes as well as oxidative stress markers (CAT, glutathione, and MDA) in rats. Changes in biochemical parameters were supported by histological improvements of the liver.

Conclusion: The NAEE Linn flowers in 200 mg/kg and 400 mg/kg doses showed a significant reversal of hepatic damage which was induced by INH in rats.

Keywords: Ethanolic extract, *Nymphaea alba* Linn., Flowers, Isoniazid-induced hepatotoxicity.

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INTRODUCTION

Tuberculosis is one among the oldest diseases known to affect the human being and is one of the major causes of death worldwide [1]. Isoniazid (INH) is the preferred drug in both chemoprophylaxis and treatment of tuberculosis [2,3]. Daily intake can lead to moderate elevation in liver enzymes and severe hepatic damage (especially, hepatic necrosis) in 3-20% and 1-2% of patients, respectively [3,4]. Recent experimental studies have shown that one of the mechanisms responsible for INH-induced hepatic injury is oxidative stress. Free oxygen radicals may result in oxidative damage by lipid peroxidation. One of the aldehyde compounds generated from lipid peroxidation is malondialdehyde (MDA), which can be used to evaluate this damage [2]. The decrease of catalase (CAT) and glutathione (GSH) levels and increase of MDA level are closely related to oxidative stress, and the normalization of these levels by drugs or chemicals is extremely important for the attenuation of oxidative tissue damage [5-8].

Nymphaea alba Linn. (Nymphaeaceae) is mainly found in tanks and ponds throughout the 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 [9]. All parts of the plant have medicinal uses in the traditional system of medicine. It is employed as an anti-inflammatory, anodyne, astringent, antiscrophulatic, cardiotonic, demulcent, sedative, and aphrodisiac. It also produces sedative and calming effects on the nervous system and is useful in the treatment of anxiety, insomnia, and similar disorders [10-12]. The leaves and rhizomes of *N. alba* possess antioxidant activity [13,14].

The aim of the present study was therefore to investigate the therapeutic effect of ethanolic extract of *N. alba* (NAEE) Linn. flowers against INH-induced hepatotoxicity in rats.

METHODS

Institutional Animal Ethical Committee (IAEC) approval

The Institutional Animal Ethics Committee approved the experimental protocol (Registration No. 401/Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) dated 09.05.2012). All experiments were carried out in accordance with the rules and regulations of IAEC and CPCSEA.

Preparation of extract

The flowers of *N. alba* Linn. were procured from Dawakhana Tibbiya 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, Uttar Pradesh, India. Shade-dried flowers were coarsely powdered and then subjected to extraction. Extraction of powder in ethanol was done 72 h using Soxhlet apparatus. The extract was filtered using Whatman No. 1 filter paper, evaporated on a water bath at 50°C until it dried completely, and stored in the refrigerator for further use. The yield of NAEE was found to be 10.6%.

Experimental animals

Thirty-six healthy Wistar albino rats weighing 150–200 g of either sex procured from Central Animal House of the Institute were housed under standard condition (temperature 27 ± 2°C, Humidity 30-70%, and 12 h light/dark cycles) and fed with standard pellet diet and water *ad libitum*. All the animals were acclimatized to laboratory conditions for 1 week before the experimental procedure.

Chemicals and drugs

INH was acquired from Macleods Pharmaceuticals Ltd., Mumbai, Maharashtra, India. Silymarin was acquired from Micro Labs Ltd., Bengaluru, Karnataka, India. The aspartate aminotransferase (AST),

alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) kit were purchased from Siemens, Mumbai.

Experimental design

The animals were divided in 6 groups comprising of 6 animals each. All drugs were administered orally. The groups were treated as follows:

- Group-I: Normal saline (1 mL/kg) for 28 days.
- Group-II: INH (50 mg/kg) [2] for 28 days.
- Group-III: INH (50 mg/kg) for 28 days followed by normal saline (1 mL/kg) for 15 days.
- Group-IV: INH (50 mg/kg) for 28 days followed by silymarin (50 mg/kg) [15] for 15 days.
- Group-V: INH (50 mg/kg) for 28 days followed by NAEE Linn. flowers (200 mg/kg) for 15 days
- Group-VI: INH (50 mg/kg) for 28 days followed by NAEE Linn flowers (400 mg/kg) for 15 days.

Animals were sacrificed under sodium pentobarbitone (50 mg/kg intraperitoneally). Rats of Groups-I and II were sacrificed on the 29th day, while animals of Groups III–VI were sacrificed on the 44th day. Blood sample was taken by cardiac puncture for the 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 (AST, ALT, and ALP) [16,17].

Antioxidant tests

The homogenate of the liver (in 10% weight/volume of phosphate buffer [0.2 M, pH-6.6]) tissue was used to perform *in vivo* antioxidant tests such as CAT [18], reduced GSH [19], and MDA [20].

Histological examination of liver

The histological assessment of liver damage was done by scoring of structural changes described by the National Health Services, Maryland, USA [21]. The parameters were as follows: Degeneration (0 = no degeneration, 1 = few vacuolated cells per lesion, 2 = more than 10 vacuolated cells per lesion, 3 = one to two rows of vacuolated cells around necrotic zone per lesion, and 4 = more than two rows of vacuolated cells around necrotic zone per lesion), 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, and 4 = massive centrilobular necrosis with necrotic tissue bridging the central vein), fibrosis (0 = normal appearance of liver, 1 = central necrosis, hydropic degeneration, no fibrosis, 2 = fibrous tissue in periportal area only, 3 = fibrous tissues insinuating surrounding hepatic parenchyma, and 4 = formation of pseudo lobules), and regeneration (0 = no regeneration, 1 = mild, 2 = moderate, and 3 = excellent).

Statistical analysis

The data were analyzed using one-way analysis of variance with *post hoc* Tukey test for biochemical parameters and Mann–Whitney U-test for comparing histopathology score. The values are represented as mean ± standard error of mean. $p<0.05$ was considered statistically significant.

RESULTS

Effect of NAEE on biochemical tests

The therapeutic effect of *N. alba* Linn. flowers was measured against INH-induced hepatotoxicity as shown in Table 1. Oxidative stress damages the integrity of liver cells and causes the release of enzymes sucha s transaminases (AST and ALT) and ALP as shown in Group-II (INH only). There was not any improvement in any of the parameters when normal saline was administered after INH (Group-III). Group-IV (INH + silymarin) showed a significant decrease in AST ($p<0.001$), ALT levels ($p<0.001$), ALP ($p<0.01$), and TB ($p<0.01$) as compared to Group-II. Group-V (INH + NAEE 200 mg/kg) showed a significant decrease in AST ($p<0.001$), ALT levels ($p<0.001$), ALP ($p<0.05$), and TB ($p<0.01$) as compared to Group-II. Group-VI (INH + NAEE 400 mg/kg) showed a significant decrease in AST ($p<0.001$), ALT levels ($p<0.001$), ALP ($p<0.01$), and TB ($p<0.01$) as compared to Group-II (Table 1).

Effect of NAEE on antioxidant tests

In the experiment, the antioxidant activity was evaluated 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 INH 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 a decrease in MDA ($p<0.001$) levels in both NAEE 200 and NAEE 400 extracts treated groups as compared to negative control (Table 1).

Effect of NAEE on histological findings

INH administration in Group-II gave rise to necrosis, fibrosis, and degeneration of hepatic microstructure. In Group-III, there was degeneration along with bridging fibrosis of hepatic microstructure but no regenerating foci. Silymarin-treated group showed near normal hepatic microstructure. In NAEE-treated groups, there was increased regenerating foci (Fig. 1a-e). NAEE 200 and 400 mg/kg administrations resulted in a significant increase in regenerating foci and decrease in fibrosis and degeneration as compared to INH only group ($p<0.001$) (Table 2).

DISCUSSION

Drug-induced hepatotoxicity is the serious adverse effect of the currently used antitubercular chemotherapeutic agents containing INH (H), rifampicin, and pyrazinamide [2]. The serum levels of hepatic enzymes primarily reflect the degree of liver damage and have been

Table 1: Effect of NAEE Linn flowers on liver marker enzymes and oxidative stress markers against INH-induced hepatotoxicity in rats

Biochemical test (mean±SEM)	Groups (n=6)					
	Group-I (normal saline only)	Group-II (INH only)	Group-III (INH+normal saline)	Group-IV (INH+silymarin)	Group-V (INH+NAEE 200 mg/kg)	Group-VI (INH+NAEE 400 mg/kg)
AST (IU/mL)	38.4±1.5	151.1±6.3^{z}	149.9±6.9	60.2±6.6^{c}	84.4±7.9^{c}	68.7±6.8^{c}
ALT (IU/mL)	39.8±1.6	155.3±5.1^{z}	152.2±5.5	58.5±2.8^{c}	81.1±4.5^{c}	64.4±3.1^{c}
ALP (KAU/dL)	41.8±2.6	79.6±5.5^{z}	79.5±5.7	50.2±4.7 ^{b}	62.6±5.1^{a}	55.2±4.9 ^{b}
TB (mg/dL)	0.363±0.032	0.851±0.082^{z}	0.795±0.084	0.514±0.042^{b}	0.531±0.041^{b}	0.499±0.028^{b}
CAT (U/min/mg)	84.1±4.9	46.4±3.7^{z}	47.4±2.8	69.8±2.2^{c}	60.8±2.1^{c}	67.0±1.6^{c}
GSH (µmoL/mg)	5.29±0.29	2.18±0.26^{z}	2.30±0.25	4.58±0.13^{c}	4.22±0.19^{c}	4.60±0.11^{c}
MDA (nmoL/mg)	204.8±8.9	438.9±18.9^{z}	430.1±17.6	254.8±7.8^{c}	277.4±10.7^{c}	253.4±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 Groups IV-VI were compared with Group-II, NAEE: Ethanolic extract of *Nymphaea alba*, INH: Isoniazid, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, TB: Total bilirubin, MDA: Malondialdehyde, GSH: Glutathione, CAT: Catalase

Table 2: Effect of NAEE Linn. flowers on histopathology score against INH-induced hepatotoxicity in rats

Parameter (mean±SEM)	Groups (n=6)					
	Group-I (normal saline only)	Group-II (INH only)	Group-III (INH+normal saline)	Group-IV (INH+silymarin)	Group-V (INH+NAEE 200 mg/kg)	Group-VI (INH+NAEE 400 mg/kg)
Degeneration	0	2.9±0.4 [z]	2.7±0.3	0.6±0.01[c]	1.2±0.08[c]	0.7±0.02[c]
Necrosis	0	2.1±0.3 [z]	1.9±0.3	0[c]	0[c]	0[c]
Fibrosis	0	1.9±0.2 [z]	1.7±0.2	0.5±0.02 [c]	0.8±0.09 [c]	0.6±0.04 [c]
Regeneration	0	0	0	3.0±0.06 [c]	2.8±0.24 [c]	2.9±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 Groups IV-VI were compared with Group-II, NAEE: Ethanolic extract of *Nymphaea alba*, INH: Isoniazid

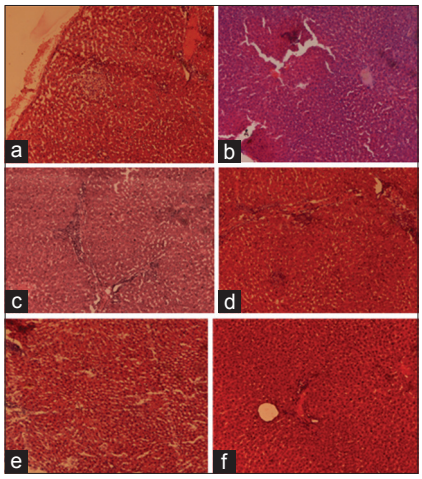

Fig. 1: (a) Photomicrograph of rat liver from Group-I (normal saline only) showing normal liver microstructure with intact hepatic cords and sinusoids. Hepatocytes show normal contour. (H and E stain, ×10), (b) photomicrograph of rat liver from Group-II (isoniazid [INH] only) showing necrosis, fibrosis, and degeneration of hepatic microstructure (H and E stain, ×10), (c) photomicrograph of rat liver from Group-III (INH + normal saline) showing degeneration of hepatic microstructure. There is also bridging fibrosis of hepatic microstructure (H and E stain, ×10), (d) photomicrograph of rat liver from Group-IV (INH + silymarin) group showing maintained hepatic microstructure. There are also regenerating nodules (H and E stain, ×10), (e) photomicrograph of rat liver from Group-V (INH + ethanolic extract of *Nymphaea alba* [NAEE] 200 mg/kg) showing occasional hepatocytic degeneration and fibrosis, along with a few regenerating nodules (H and E stain, ×10), (f) photomicrograph of rat liver from Group-VI (INH + NAEE 400 mg/kg) showing evidence of maintained hepatic microstructure. There are abundant regenerating foci (H and E stain, ×10)

frequently used as a diagnostic marker for hepatotoxicity [22,23]. INH (50 mg/kg) administration produced hepatotoxicity which was evident from the deranged biochemical and histological parameters.

INH is used extensively for prophylaxis as well as treatment of tuberculosis, but significant hepatotoxicity has often been reported. Hepatotoxicity after INH treatment is encountered in approximately 2% of cases, which if not recognized timely can have fatal outcome [24]. INH gets converted to acetylisoniazid by the enzyme N-acetyl transferase-2 which is eliminated by the kidney; acetylisoniazid is further transformed into acetylhydrazine and then to potential hepatotoxic metabolite acetyl diazine by the CYP enzymes which generates reactive acetyl onium ion, acetyl radical, and ketene, which causes irreversible damage to the liver tissue [25].

The NAEE Linn. flowers in 200 mg/kg and 400 mg/kg dose were therapeutically effective against INH-induced hepatotoxicity in rats. Both biochemical and histological parameters showed marked improvement with the restoration of liver functions. Both the doses decreased the derangement of liver function parameters, but this effect was more dominant in the high dose, i.e., 400 mg/kg dose group. Rats treated with NAEE showed significant reduction in the serum bilirubin, AST ALT, and ALP levels at both the doses. Since in Group-III there was no improvement in above parameters, it can be taken into account that the improvement in NAEE-treated groups was not due to self-healing process. There was further significant improvement in the antioxidants levels in NAEE-treated groups. This may be due to the prevention of lipid peroxidation as manifested by the decrease in MDA levels in NAEE-treated groups. The therapeutic benefit provided by NAEE was similar to that offered by the standard drug silymarin.

The findings of the biochemical analysis were further supported by histological examination of the liver tissues from the test group. NAEE in both the doses protected or maintained the liver tissue morphology evidenced by a reduction in the degeneration, necrosis, and fibrosis score in the histopathological examination. Regenerative nodules were likewise seen in the liver samples from both the test groups. Hence, the findings of the histological analysis also indicate that protection of the hepatocytes against damage induced by INH was significant in NAEE-treated groups. These findings were comparable to the prevailing drug silymarin.

One of the proposed mechanisms for INH-induced hepatic injury is oxidative stress [2]. Generation of free radicals damages the hepatocytes. The curative effect of extract can be explained by its antioxidant activity as demonstrated in the study. Previous studies demonstrate the presence of flavonoids and phenolic compounds in NAEE Linn [7]. The presence of these compounds might be responsible for the antioxidant activity.

CONCLUSION

The NAEE Linn. flowers in 200 mg/kg and 400 mg/kg doses showed therapeutically beneficial effect against INH-induced hepatotoxicity in rats.

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CONFLICT OF INTEREST

Nil.

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