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Hepatoprotective and antioxidant potential of *Nyctanthes arbor-tristis* L. leaves against antitubercular drugs induced hepatotoxicity

[Potencial hepatoprotector y antioxidante de hojas de *Nyctanthes Arbor-tristis* L. contra la hepatotoxicidad inducida por fármacos antituberculosos]

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Abstract

Context: *Nyctanthes arbor-tristis* L. (Oleaceae) leaf are used in treatment of malaria, rheumatoid arthritis, chronic fever and enlargement of spleen; however, there is paucity of information on its hepatoprotective and antioxidant potential.

Aims: To evaluate hepatoprotective and antioxidant potentials of ethanolic leaf extract of *Nyctanthes arbor-tristis* L.

Methods: After collection and authentication of the vegetal material, ethanolic extract was collected. The combination of antitubercular drugs (isoniazid, rifampicin and pyrazinamide) was used to induce hepatotoxicity in Wistar rats. Hepatoprotective effect was evaluated at doses 125, 250 and 500 mg/kg, body weight by estimating the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and levels of total bilirubin (TBL). The effects on lipid peroxidation (LPO), reduced glutathione (GSH) and catalase (CAT), superoxide dismutase (SOD) were estimated. Docking study was conducted to anticipate the probable biological targets associated with its hepatoprotective effect.

Results: The plant extract dose of 500 mg/kg, body weight significantly declined the levels of AST, ALT, ALP and TBL at ($p < 0.001$), which is approximately corresponding to the dose of reference compound silymarin and reversed the levels of LPO, GSH, SOD, CAT as compared to silymarin dose. Histopathological studies revealed regeneration of hepatocytes. The docking results suggested that some active constituents of plant leaves potentially interact with human pregnane X receptor, human constitutive androstane receptor and the farnesoid X receptor.

Conclusions: The extract of *Nyctanthes arbor-tristis* L. remarkably possesses hepatoprotective and antioxidant effect and evinced its traditional claim. Future studies should be done to isolate active phytoconstituents for use in drug discovery.

Keywords: antioxidant; docking; hepatoprotective; *Nyctanthes arbor-tristis*.

Resumen

Contexto: Las hojas de *Nyctanthes arbor-tristis* L. (Oleaceae) se utilizan en el tratamiento de la malaria, artritis reumatoide, la fiebre crónica y el bazo agrandado; sin embargo, existe escasez de información sobre su potencial hepatoprotector y antioxidante.

Objetivos: Evaluar el potencial hepatoprotector y antioxidante del extracto etanol de hoja de *Nyctanthes arbor-tristis* L.

Métodos: Después de la colección y la autenticación del material vegetal fue realizado el extracto etanólico. La combinación de fármacos antituberculosos (isoniacida, rifampicina y pirazinamida) fue utilizada para inducir hepatotoxicidad en ratas de Wistar. El efecto hepatoprotector se evaluó en dosis 125, 250 y 500 mg/kg de peso corporal mediante la estimación de la actividad de alanina aminotransferasa (ALT), aspartato aminotransferasa (AST), fosfatasa alcalina (ALP) y los niveles de bilirrubina total (TBL). Se estimaron los efectos sobre la peroxidación lipídica (LPO), glutatión reducido (GSH), catalasa (CAT), y superóxido dismutasa (SOD). Además, se llevó a cabo un estudio de acoplamiento para anticipar los probables blancos biológicos asociados con su efecto hepatoprotector.

Resultados: La dosis de 500 mg/kg de extracto de planta disminuyó significativamente los niveles de AST, ALT, ALP y TBL ($p < 0.001$), que se corresponde aproximadamente a la dosis del compuesto referencia silimarina y revertió los niveles de LPO, GSH, SOD, CAT, en comparación con la dosis de silimarina. Los estudios histopatológicos revelaron regeneración de hepatocitos. Los resultados del acoplamiento sugirieron que algunos componentes activos de las hojas de la planta potencialmente interactuarán con el receptor X de pregnano humano, el receptor androstano constitutivo humano y el receptor de farnesoid X.

Conclusiones: El extracto de *Nyctanthes arbor-tristis* L. posee efecto hepatoprotector y antioxidante notable y valida su uso tradicional. Estudios futuros deben llevarse a cabo para aislar fitoconstituyentes activos para su uso en el descubrimiento de medicamentos.

Palabras Clave: antioxidantes; antituberculosos; hepatoprotector; *Nyctanthes arbor-tristis*.

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INTRODUCTION

Liver is a vibrant organ that produces and secretes bile; it also generates fundamental blood anticoagulation factors like prothrombin, fibrinogen, and heparin. Liver transfigures sugar into glycogen (Rani and Lakshmi, 2012; Kannappan et al., 2014). Tuberculosis commonly known as (TB), is prominent infectious disease leading to death of 1.4 million patients around the globe in 2015. and many human identities are infected by TB due to their compromised immunity, as well as due to elevated rates of AIDS/HIV infection, co-infection with HIV increases the risk of TB six- to 50-fold (Lawn and Zumla, 2011; Ellis et al., 2017). Antitubercular drugs like isoniazid, rifampicin, and pyrazinamide have been used since many years for the treatment of TB. Prolonged use and high dose administration of anti-TB drug regimen develop adverse/toxic effects resulting in hepatotoxicity (Jeong et al., 2015).

Nyctanthes arbor-tristis L. (Oleaceae), commonly known as harsingar or night jasmine is a distinguished medically efficient plant. In Ayurveda, harsingar leaves are used in chronic fever, obstinate sciatica, coughs, malaria, constipation, intestinal worms (Bhosale et al., 2009; Dutta, 2015; Jain and Pandey, 2016). Leaves of *N. arbor-tristis* are recommended as hypnotic, tranquilizing, local anesthetic, and anti-asthmatic (Nirmal et al., 2011; 2012). Fresh leaves juice is antimalarial, antifungal. Traditionally, the powdered bark has been used in treatment of rheumatoid arthritis, malarial infection, enlargement of spleen (Karnik et al., 2008; Agrawal et al., 2013; Kumari and Charya, 2017). The chemical constituents of *Nyctanthes arbor-tristis* L. that were isolated from the plant leaves include; the steroid β -sitosterol, the flavonol glycoside-astragaline, nicotiflorin, the iridoid glycosides arborside (A, B and C) as well as nyctanthic acid (Srivastava et al., 1990; Paul and Saxena, 1997; Saxena et al., 2002; Bansal and Suri, 2015).

Some authors, reported on hepatoprotective effect of plant against galactosamine and carbon tetrachloride induced liver damage in rats (Hukkeri et al., 2006; Jayachitra and Rubavahini, 2015). Therefore, present investigation was designed to demonstrate the antioxidant and hepatoprotective effect of *N. arbor-tristis* L. in hepatotoxicity induced by anti-tubercular drugs in rats.

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MATERIAL AND METHODS

Chemicals

All the chemicals used were of analytical grade and procured from Qualigens fine chemicals, Mumbai, India. The assay kits were purchased from Sigma Chemicals Co., USA.

Leaves collection, authentication and extraction

Fresh leaves of *Nyctanthes arbor-tristis* L. were collected from the herbal garden of Moradabad Educational Trust, Group of Institutions, Faculty of Pharmacy, Moradabad, India (28.8706 °N, 78.7569 °E). The plant was authenticated by Dr. A.K Wahi, Dean, Moradabad Educational Trust, Group of Institutions, Faculty of Pharmacy, Moradabad, Uttar Pradesh and voucher specimen number (HGFOPII0) was submitted in the institutional herbarium. The leaves were dried under shade and powdered. The powdered plant material (500 g) was macerated with petroleum ether (1 L); the marc was exhaustively extracted with absolute ethanol (500 mL) using Soxhlet apparatus for three days. The solvent was evaporated using rotatory flash evaporator (IKA, Germany) and procured in desiccators. The percentage yield was discovered to be 0.62%. Tween-80, (1%) was used to prepare extract suspension of desirable concentration needed for pharmacological studies.

Animals

Albino Wistar male rats (150 - 250 g) were accommodated at temperature (22 \pm 2°C), relative humidity (55 \pm 5%) and light (12 h light/dark cycles). They were administered with standard pellet diet and water *ad libitum*. Research protocols were approved by Institutional Animal Ethics Committee (352/CPCSEA) and study was conducted according to guidelines of Committee for the purpose of control and supervision of experiments on animals.

Acute toxicity study

The plant extract was evaluated for acute toxicity at doses dose of 5, 50, 300, 2000 mg/kg, as per OECD423 guidelines and dose of 2000 mg/kg

showed the toxic symptoms. So, it is considered as LD₅₀ cutoff value. Doses selected for pharmacological studies by fixed dose methods were 125, 250 and 500 mg/kg, body weight (Chaudhary et al., 2016).

Arraying of animals for pharmacological studies

Wistar male rats (150 - 200 g) were apportioned into six groups of six animals each and study protocols were followed as: Group I numbered as normal control received distilled water orally only. Group II numbered as toxicant control received anti-tubercular agents (isoniazid 7.5 mg/kg, rifampicin 10 mg/kg and pyrazinamide 35 mg/kg body weight) for 35 days by intra-gastric administration in normal saline (Hussain et al., 2012). Group III received 125 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min initial to anti-tubercular drug challenge for 35 days. Group IV received 250 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min prior to anti-tubercular drug challenge for 35 days and Group V received 500 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min prior to anti-tubercular drug challenge for 35 days. Group VI served as standard control received silymarin, orally, 100 mg/kg *p.o.*, daily for 35 days, 45 min prior to anti-tubercular drug challenge as a reference. After the experimental period, the blood was collected from inner canthus of the eye, animals were humanly sacrificed, and liver samples were collected.

Hepatoprotective activity

From the collected blood samples (3 - 4 mL), serum was separated by centrifugation in a refrigerated tabletop centrifuge at 2500 rpm for 15 min and used for the estimation of marker enzymes like aspartate aminotransferase (AST, U/L), alanine transaminase (ALT, U/L), alkaline phosphatase (ALP, U/L), total bilirubin (TBL, mg/dL) were assayed using span diagnostics assay kits (Hussain et al., 2012).

Estimation of antioxidant parameters

Lipid peroxidation (LPO)

The dissected-out liver samples were washed using ice-cold 0.9% saline solution and portioned into two groups. One portion was used to prepare 10% w/v homogenate in 0.9% NaCl solution. An aliquot

of the homogenate was centrifuged at 900 rpm for 15 min, the supernatant was again centrifuged (Sorvall Legend XT centrifuge, Thermo Fisher Scientific, USA) at 15,000 for 10 min, and the obtained mitochondrial fraction was consumed for the estimation of LPO (Jamall and Smith, 1985). The quantitative determination of LPO was performed by estimating the concentration of TBA-reactive substances in the liver. The amount of malondialdehyde (MDA) formed was quantified by reaction with TBA and used as an index of LPO. The results were expressed as nanomole of MDA per gram of wet tissue using the molar extinction coefficient of the chromophore and 1, 1, 3, 3-tetra ethoxypropane was used as standard. 0.2 mL of homogenate was poured into a vial and 0.2 mL of a 8.1% (w/v) sodium dodecyl sulphate solution, 1.5 mL of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.5 mL of a 0.8% (w/v) solution of thiobarbituric acid (TBA) was added to it and finally the volume was adjusted to 4.0 mL using distilled water. Vials were placed on water bath for 1 h and cooled to room temperature. TBA (10%) was taken in a centrifuge tube and after adding test samples tubes were centrifuged at 1000 rpm for 15 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrophotometer, USA). Same protocols were followed for control experiment except that in place of TBA solution distilled water was used.

Catalase and superoxide dismutase

The liver tissue was homogenized, and mitochondrial fraction was prepared as described above. Decomposition of H₂O₂ in presence of catalase (CAT) was followed at 240 nm (Aebi et al., 1984). One unit (U) of catalase was considered as the amount of enzyme needed to decompose 1 μ mol of H₂O₂ per min, at 25°C and pH 7.0. Results are expressed as units (U) of CAT activity/mg protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide reduced-phenazine methosulphate-nitroblue-tetrazolium reaction system as expressed in reference literature (Kakkar et al., 1972; Nishikimi et al., 1972). One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25 \pm 2°C) and the results have been expressed as unit (U) of SOD activity/mg protein.

Reduced glutathione (GSH)

The concentration of GSH was determined by the method of Anderson based on the progression of a yellow color when 5,5-dithiobis (2-nitrobenzoic acid) is added to compounds containing sulfhydryl groups (Anderson, 1985). The reaction mixture contained equal volumes of 4% sulfosalicylic acid and tissue samples homogenized in 4 volume of ice cold 0.1 mL phosphate buffer (pH 7.4). The method used for estimating GSH in this study also measures non-protein sulfhydryl concentration inclusive of GSH. However, 80 - 90% of the non-protein sulfhydryl content of the cell represents free endogenous GSH.

Histopathological studies

For histopathological inspection, the liver tissues were affixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50 - 100%) alcohol and embedded in paraffin. Fine sections (5 μ M) were cut employing rotatory microtome (AMR 400, Amos Scientific, Australia) and stained with routine hematoxylin and eosin (H&E). The liver sections were evaluated for photo microscopic assessment (Nikon Eclipse E400 microscope with digital camera, USA).

Docking protocol

Docking study was carried-out employing the program Autodock Vina (Trott and Olson, 2010). The crystal structures for the human pregnane-X receptor (PDB-code 1ILH) (Watkins et al., 2001), nuclear factor- κ B (PDB-code 1VKX) (Chen et al., 1998), human constitutive androstane receptor (PDB-code 1XVP) (Xu et al., 2004) and the farnesoid X receptor (PDB-code 3FLI) (Flatt et al., 2009) were retrieved from the protein data bank. The complexed ligands were extracted from the initial X-ray crystal structures followed by removal of water molecules. Polar hydrogens and Gastieger charges were added and the corresponding charge files were generated using the Autodock Tools. The compounds were drawn using Chem Draw Ultra 8.0 software and were optimized for energy and geometry using MMFF94 force field. All the compounds were treated employing the previous preparation procedure mentioned earlier.

Grid boxes were established to cover the active sites of the macromolecules under study, with a spacing of 1.0 Å between the grid points. The exhaustiveness and the number of poses were set to 12 and 10 respectively. The docking results were visualized utilizing Discovery Studio 4.1 software (Biovia, San Diego, USA) and presented in Table 3.

Statistical analysis

The data were exhibited as mean \pm S.E.M. for six rats. ANOVA test was followed by individual comparison by Newman-Keuls test using Prism Pad software (Version 5.0) (GraphPad software, Inc., USA) for the estimation of level of significance. The values of $p < 0.05$ was considered statistically significant.

RESULTS

Acute toxicological outcome

Ethanollic extract of *N. arbor-tristis* at doses of 125, 250 and 500 mg/kg body weight does not produce any toxic outcome. Therefore, these doses were used for hepatoprotective studies.

Effect of plant extract on serum AST, ALT, ALP, TBL levels

Hepatic abrasion due to intake of antitubercular drugs generate significant alterations in marker enzyme as AST by 99.55%, ALT by 189.74%, ALP by 104.66% and TBL by 106.9% when compared to normal control (Group I). The percentage defense in marker enzyme of treated groups at 125, 250 mg/kg as AST 5.87 ($p < 0.05$), 8.87 ($p < 0.01$) ALT 6.95 ($p < 0.05$), 13.79 ($p < 0.01$), ALP 3.41 ($p < 0.05$), 16.64 ($p < 0.001$), and TBL 11.66 ($p < 0.05$), 15.83 ($p < 0.01$) when compared to toxicant control (Group II), while maximum percentage protection in marker enzyme at the dose of 500 mg/kg and silymarin (100 mg/kg) as AST 37.97 ($p < 0.001$), 42.99 ($p < 0.001$), ALT 20.35 ($p < 0.001$), 32.10 ($p < 0.001$), ALP 30.24 ($p < 0.001$), 36.69 ($p < 0.001$), total bilirubin 39.16 ($p < 0.001$), 46.66 ($p < 0.001$) which were approximately corresponding to the standard control (Group VI). Results are expressed in Table 1.

Table 1. Effect of *Nyctanthes arbor-tristis* extract on serum aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin (TBL) levels against antitubercular drugs induced hepatotoxicity in rats.

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	TBL (mg/dL)
I	121.59 ± 3.5	42.60 ± 2.1	141.50 ± 2.1	0.58 ± 0.06
II	242.64 ± 4.7 [†]	123.43 ± 2.8 [†]	289.60 ± 2.8 [†]	1.20 ± 0.03 [†]
III	228.38 ± 3.8 ^a	114.85 ± 2.8 ^a	279.70 ± 2.8 ^a	1.06 ± 0.02 ^a
IV	221.10 ± 3.1 ^b	106.40 ± 2.8 ^b	241.40 ± 3.1 ^c	1.01 ± 0.02 ^b
V	150.50 ± 4.1 ^c	98.30 ± 3.1 ^c	202.00 ± 3.2 ^c	0.73 ± 0.04 ^c
VI	138.32 ± 3.8 ^c	83.80 ± 2.6 ^c	183.34 ± 3.2 ^c	0.64 ± 0.02 ^c

Values are mean ± S.E.M. of 6 rats in each group. [†] $p < 0.001$ compared with respective control Group I and ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ compared with Group II.

Group I: normal control received distilled water orally. Group II: toxicant control received anti-tubercular agents (isoniazid 7.5 mg/kg, rifampicin 10 mg/kg and pyrazinamide 35 mg/kg body weight) for 35 days by intra-gastric administration in normal saline. Group III received 125 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min initial to anti-tubercular drug challenge for 35 days. Group IV received 250 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min prior to anti-tubercular drug challenge for 35 days and Group V received 500 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min prior to anti-tubercular drug challenge for 35 days. Group VI: standard control received silymarin, orally, 100 mg/kg *p.o.*, daily for 35 days, 45 min prior to anti-tubercular drug challenge as a reference.

Estimation of LPO, GSH, SOD, CAT

The remarkable percentage alteration in LPO level was observed among toxicant control as 69.85% ($p < 0.001$) compared to normal control. Treatment with *N. arbor-tristis* extract at the doses of 125, 250 and 500 mg/kg significantly reversed the levels and the percentage protection in LPO were 43.90 (ns) at dose of 125 mg/kg, 65.85% ($p < 0.05$) at dose of 250 mg/kg and 97.56% ($p < 0.01$) at dose of 500 mg/kg. A significant increase in level of GSH, SOD and CAT was observed in rats treated with *N. arbor-tristis* extract whereas toxicant control (Group II) demonstrated significant decline in these parameters compared to normal control. The percentage alterations of GSH, SOD, CAT in toxicant control were as 67.80 ($p < 0.001$), 60.81 ($p < 0.001$), 42.36% ($p < 0.001$) respectively. The percentage defense in GSH as 53.19 ($p < 0.05$), 78.72 ($p < 0.01$), 110.63% ($p < 0.001$), SOD 47.19 (ns), 89.88 ($p < 0.05$), 123.59% ($p < 0.01$), CAT 28 ($p < 0.05$), 45.36 ($p < 0.01$) and 60.69% ($p < 0.001$) at doses of 125, 250 and 500 mg/kg, respectively. *N. arbor-tristis* extract 500 mg/kg has shown utmost defense, which was almost comparable to those of the normal control and standard. Results are expressed in Table 2.

Histopathological studies

The liver section of control group (Fig. 1A) showed normal histology with no significant alterations in cellular architecture. The liver section of toxicant control (Fig. 1B) showed distortion in parenchymal architecture followed by cellular necrosis, inflammation and swelling. *N. arbor-tristis* extract (125 mg/kg) treated group rats liver section (Fig. 1C) showed mild hepatocellular degeneration. *N. arbor-tristis* extract (250 mg/kg) treated group rats liver section (Fig. 1D) showed less inflammation, swelling and absence central veins congestion. *N. arbor-tristis* extract (500 mg/kg) treated group rats liver section (Fig. 1E) showed dilated central veins, regeneration of hepatocytes with least hepatocellular degeneration. Silymarin treated rat liver section (Fig. 1F) showed almost normal cellular histology, structure and architecture. The cellular architecture of rats treated with plant extract (500 mg/kg) was more approaching towards normal as compared to rats treated at dose of 125 mg/kg and 250 mg/kg body weight.

Molecular docking studies

The docking results, illustrated in Table 3,

showed that many of the active constituents of *Nyctanthes arbor-tristis* leaves would affect the human pregnane-X receptor as indicated by their high binding scores. Those include β -sitosterol, arbor-side (A, B and C) as well as nyctanthic acid. Careful investigation of the best-docked poses suggested that both of β -sitosterol and nyctanthic acid would adapt binding modes similar to each other (Fig. 2A) and different from those adapted by the iridoid glycosides (arborside A, B and C) (Fig. 2B). Furthermore, the result showed that β -sitosterol would have an additional preferential binding with the constitute androstane receptor (CAR) active site (Fig. 2C). The farnesoid X receptor (FXR) was found to be affected by the arborside-B glycoside (Fig. 2D) more than the other constituents. In summary, the results evinced that the chemical components of the leaves extract would exert their hepatoprotective effect primarily through targeting the human pregnane-X receptor and to a lesser extent through targeting constitute androstane receptor and the farnesoid X receptor. No significant binding were seen in the case of NF- κ B.

DISCUSSION

Undoubtedly, drug-induced hepatic failure is a noteworthy cause of increased demise worldwide.

Hepatic toxicity due to ingestion of antitubercular drugs like; isoniazid, rifampicin, and pyrazinamide were well documented in the literature, however combination these drugs would potentially exert their toxic effects synergistically (Padma et al., 1998; Jeong et al., 2015). The magnitude of liver damage could be confirmed by estimation of marker enzymes like AST, ALT and ALP (Deepa and Varalakshmi, 2003). Treatment with *N. arbor-tristis* extracts in three respective doses as well as the reference drug silymarin significantly abbreviates liver enzymes level influencing that its hepatoprotective influence might be due to its impact against cellular leakage and promoting functional integrity of the cell membrane in hepatocytes.

Destruction of RBC leads to the formation of bilirubin that is removed by the action of conjugation and its subsequent secretion into bile. The level of it is usually promoted because of declined uptake by liver, less conjugation or blockage of bile duct which eventuate in hepatic destruction (Okokon et al., 2017). Toxicant control group rodents in the research, expressed an elevation in serum bilirubin content. Co-administration of *N. arbor-tristis* extract restore bilirubin content to near normal by its cytoprotective and cytochrome P-450 restraining potential.

Table 2. Effect of *Nyctanthes arbor-tristis* extract on liver peroxidation (LPO), reduced glutathione (GSH) superoxide dismutase (SOD) and catalase (CAT) levels against antitubercular drugs induced hepatotoxicity in rats.

Group	LPO (MDA nmol/min/mg of protein)	GSH (nmol/mg of protein)	SOD (unit/mg of protein)	CAT (units/mg of protein)
I	1.36 \pm 0.12	1.46 \pm 0.12	22.71 \pm 1.2	53.56 \pm 2.2
II	0.41 \pm 0.01 [†]	0.47 \pm 0.01 [†]	8.9 \pm 1.1 [†]	28.75 \pm 2.1 [†]
III	0.59 \pm 0.02 ⁿ	0.72 \pm 0.03 ^a	13.1 \pm 2.1 ⁿ	36.8 \pm 2.1 ^a
IV	0.68 \pm 0.04 ^a	0.84 \pm 0.03 ^b	16.9 \pm 1.5 ^a	41.8 \pm 3.8 ^b
V	0.81 \pm 0.07 ^b	0.99 \pm 0.06 ^c	19.9 \pm 2.1 ^b	46.2 \pm 2.1 ^c
VI	0.98 \pm 0.08 ^c	1.24 \pm 0.08 ^c	21.9 \pm 2.5 ^c	53.8 \pm 2.5 ^c

Values are expressed as mean \pm S.E.M. of 6 rats in each group. [†] $p < 0.001$ compared with respective control Group I and ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ compared with Group II.

Group I: normal control received distilled water orally. Group II: toxicant control received anti-tubercular agents (isoniazid 7.5 mg/kg, rifampicin 10 mg/kg and pyrazinamide 35 mg/kg body weight) for 35 days by intra-gastric administration in normal saline. Group III received 125 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min initial to anti-tubercular drug challenge for 35 days. Group IV received 250 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min prior to anti-tubercular drug challenge for 35 days and Group V received 500 mg/kg dose of *N. arbor-tristis* extract *p.o.*, 45 min prior to anti-tubercular drug challenge for 35 days. Group VI: standard control received silymarin, orally, 100 mg/kg *p.o.*, daily for 35 days, 45 min prior to anti-tubercular drug challenge as a reference.

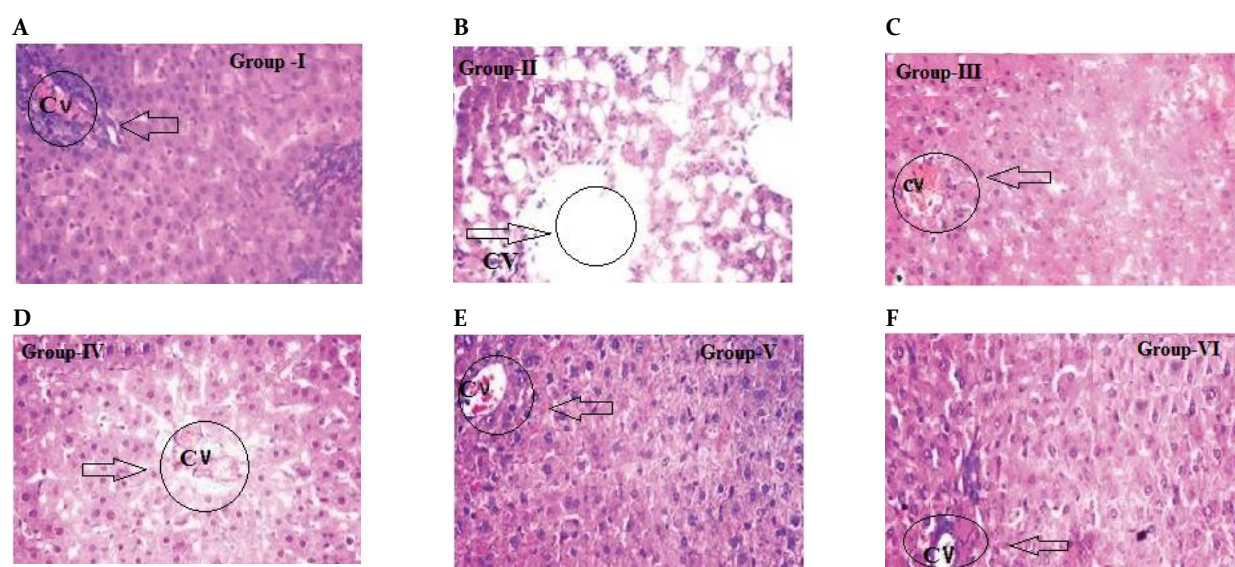


Figure 1. Histopathological injury induced by antitubercular drugs and the protection of *N. arbor-tristi* leaf ethanolic extract and silymarin orally administered.

(A) The liver section of Group I (control group, distilled water only administered) showed normal histology with no significant changes in cellular structure and architecture. (B) The liver section of Group II (toxicant control group, anti-tubercular agents in form of isoniazid-7.5 mg/kg, rifampicin-10 mg/kg and pyrazinamide-35 mg/kg, body weight for 35 days were administered) showed parenchyma with flaccid architecture, central veins showed congestion of sinusoids along with cloudy swelling, loss of cellular boundaries and necrosis with inflammation. (C) Liver section of Group III rats (*N. arbor-tristis* extract 125 mg/kg, *p.o.*, 45 min initial to anti-tubercular drug challenge for 35 days) showed moderate granular degeneration and mild central veins congestion. (D) Liver section of Group IV rats (*N. arbor-tristis* extract 250 mg/kg, *p.o.*, 45 min prior to anti-tubercular drug challenge for 35 days) exhibited less inflammatory cells, less hepatic necrosis and absence of central veins congestion. (E) Liver section of Group V rats (*N. arbor-tristis* extract 500 mg/kg, *p.o.*, 45 min prior to anti-tubercular drugs challenge for 35 days) represents minimal diffuse granular degeneration and regeneration of hepatocytes around central veins. Histoarchitecture of group V rats was more towards normal as compared with Group IV and III rats. (F) Liver section of group VI (silymarin, 100 mg/kg, *p.o.*, 45 min prior to combination of antitubercular drugs for 35 days), exhibited almost normal histology, cellular structure and architecture. Histopathology was performed by mean of H&E stained sections of liver at 250X. CV: Central veins

Table 3. Docking score of *Nyctanthes arbor-tristis* active constituents against the different hepatoprotective-related molecular targets.

Compound	1VKX (NF- κ B) (Kcal/mol)	1ILH (PXR) (Kcal/mol)	1XVP (CAR) (Kcal/mol)	3FLI (FXR) (Kcal/mol)
D-Mannitol	-5.6	-5.1	-4.0	-4.9
Oleanolic acid	-7.0	-8.6	-2.1	-4.1
Ascorbic acid	-6.1	-5.3	-5.1	-6.1
β -Sitosterol	-6.4	-9.6	-11.4	-8.7
Nicotiflorin	-8.3	-8.3	-6.4	-7.9
Astragaline	-8.4	-9.0	-7.9	-7.9
Arborside-A	-7.0	-10.6	-7.9	-6.1
Arborside-B	-7.7	-9.9	-8.1	-9.1
Arborside-C	-8.0	-10.2	-8.7	-7.2
Nyctanthic acid	-6.5	-10.9	-3.0	-6.1

NF- κ B: nuclear factor- κ B; PXR: pregnane-X receptor; CAR: constitute androstane receptor; FXR: farnesoid-X receptor.

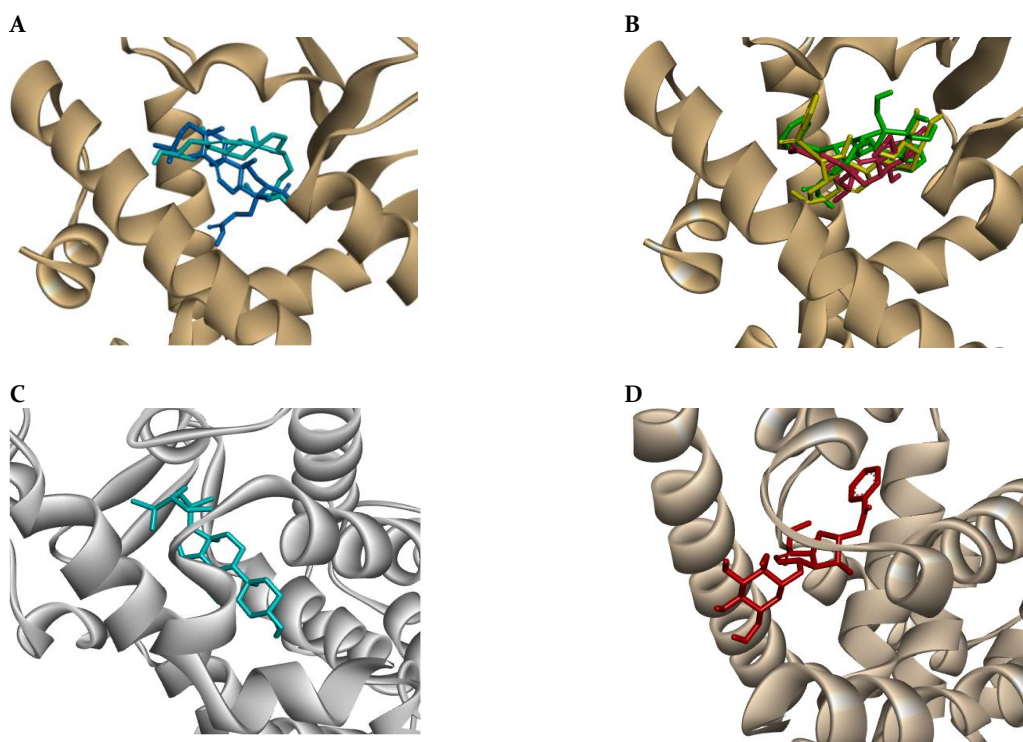


Figure 2. Binding modes for the active constituents of *Nyctanthes arbor-tristis* L.

(A) Overlay of the best-docked poses of β -sitosterol (cyan) and nyctanthic acid (blue) within the human pregnane X receptor LBD (PDB-code 1LH). (B) Overlay of the best-docked poses of arborside-A (green), arborside-B (red) and arborside-C (yellow) within the human pregnane X receptor LBD (PDB-code 1LH). (C) The best-docked pose of β -sitosterol (cyan) within the human constitutive androstane receptor active site (PDB-code 1XVP). (D) The best-docked pose of arborside-B (red) within the farnesoid X receptor (FXR) active site (PDB-code 3FLI).

Antitubercular drugs lead to cellular damage due to their induction for the generation of highly reactive oxygen species and the accompanying oxidative stress. The reactive oxygen species would act as stimulators for LPO and as a stimulator for hepatic toxicity (Adhvaryu et al., 2007; Evan et al., 2010). Treatment with *N. arbor-tristis* extracts would significantly reverse such changes. Hepatoprotective action of *N. arbor-tristis* is related to its antioxidant activity. The deleterious metabolites of antitubercular drugs would damage a number of cellular components in the liver containing antioxidant enzymes such as SOD, CAT. Conventionally SOD, CAT establishes a team of antioxidant enzymes that provide a safeguard module against reactive oxygen species. The remarkable decrease in SOD and CAT activity express liver damage in the rodents after ingestion of antitubercular drugs (Hussain et al., 2012). Moreover, treatment with 125, 250 and 500 mg/kg

of *N. arbor-tristis* extracts groups showed remarkable magnification in level of these enzymes constituting the antioxidant potential of *N. arbor-tristis*.

GSH is non-enzymatic antioxidant exist in the tissues. It is accountable for elimination of free oxygen species. In addition, it works as substrate for GSH S-transferase (Townsend et al., 2003). In the current study, the decrease in the GSH levels in tubercular drug induced rodents is speculated. GSH levels depletion was significantly restored by plant extract dose, which might be due to increased synthesis of GSH. *N. arbor-tristis* extracts at different dose levels offers hepatoprotection, but 500 mg/kg was more effective than all other lower doses. Liver histopathology images evidenced that *N. arbor-tristis* extracts attenuated the hepatocellular necrosis and led to reduction in inflammatory cells infiltration, may be attributed to its hepatoprotective effects.

In an attempt to elucidate the probable biochemical mechanism and the biological targets involved in leaves extract hepatoprotective activity; we decided to carry out a reverse docking study against number of biological macromolecule that are reported to be in direct relation with the hepatoprotective potential, thus we have selected the nuclear factor- κ B (NF- κ B), the farnesoid-X receptor (FXR), the constitute androstane receptor (CAR) and the pregnane-X receptor (PXR) as templates for our study. NF- κ B command many genes responsible in the inflammatory operation and cancer development and it is found active in many inflammatory events (Sethi et al., 2008; Sun and Karin, 2008). Thus, inhibiting NF- κ B signaling via herbal extracts would furnish potential therapeutic benefit in such cases (Paur et al., 2010; Rozema et al., 2012; Vogl et al., 2013). On the other hand, the ligand-regulated nuclear receptors namely; FXR, CAR and PXR were found to be highly expressed in the liver and intestine supervising essential steps in the metabolism of xenobiotics as well as endobiotics in hepatic tissues. Modulators of such targets reported to provide therapeutic value in cases such as drug-induced hepatotoxicity.

CONCLUSIONS

The present study results confirm that ethanol extract of *Nyctanthes arbor-tristis* leaves in 500 mg/kg dose represented the most potent antioxidant and hepatoprotective potential in a rat model of hepatotoxicity induced by anti-tubercular agents. This may be due to the presence of active constituents; nyctanthic acid, β -sitosterol and arborside A, B and C. The exact comprehensive molecular mechanism remains to be investigated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contribution:

Contribution	Chaudhary S	Gupta RK	Kumar A	Tarazi H
Concepts or ideas	X			
Design	X	X		
Definition of intellectual content	X			
Literature search	X			
Experimental studies	X	X	X	X
Data acquisition	X			
Data analysis	X			
Statistical analysis	X			
Manuscript preparation	X	X	X	X
Manuscript editing	X			
Manuscript review	X	X	X	X

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