© 2018 Journal of Pharmacy & Pharmacognosy Research, 6 (3), 205-215, 2018
ISSN 0719-4250
http://jppres.com/jppres

*Original Article | Artículo Original*

# 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]

**Sachin Chaudhary[1, 2*], Ramesh K. Gupta[2, 3], Amit Kumar[4], Hamadeh Tarazi[1]**

[1]College of Pharmacy, University of Sharjah, Sharjah-27272, United Arab Emirates.
[2]Moradabad Educational Trust Group of Institutions, Faculty of Pharmacy, Moradabad-244001, Uttar Pradesh, India.
[3]Sherwood College of Pharmacy, Barabanki-225001, Uttar Pradesh, India.
[4]College of Pharmacy, Neelkanth Group of Institutions, Meerut-250110, Uttar Pradesh, India.
*E-mail: schaudhary@sharjah.ac.ae

**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 androstane 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*.

ARTICLE INFO
Received: December 2, 2017.
Received in revised form: January 18, 2018.
Accepted: March 23, 2018.
Available Online: April 29, 2018.
Declaration of interests: The authors declare no conflict of interest.
Funding: The authors confirm that the project has not funding or grants.

## 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 antiasthmatic (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 antitubercular drugs in rats.

## 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 (HGFOP 110) 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 ± 2˚C), relative humidity (55 ± 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 $H_2O_2$ 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 µmol of $H_2O_2$ 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 ± 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 µM) 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-*kappa* 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 ± 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**

Ethanolic 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$^{\dagger}$ | 123.43 ± 2.8$^{\dagger}$ | 289.60 ± 2.8$^{\dagger}$ | 1.20 ± 0.03$^{\dagger}$ |
| 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. $^{\dagger}p<0.001$ 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. arbortristis* extract (125 mg/kg) treated group rats liver section (Fig. 1C) showed mild hepatocellular degeneration. *N. arbortristis* extract (250 mg/kg) treated group rats liver section (Fig. 1D) showed less inflammation, swelling and absence central veins congestion. *N. arbortristis* 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, arborside (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-κB.

## 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 ± 0.12 | 1.46 ± 0.12 | 22.71 ± 1.2 | 53.56 ± 2.2 |
| II | 0.41 ± 0.01$^{\dagger}$ | 0.47 ± 0.01$^{\dagger}$ | 8.9 ± 1.1$^{\dagger}$ | 28.75 ± 2.1$^{\dagger}$ |
| III | 0.59 ± 0.02$^{n}$ | 0.72 ± 0.03$^{a}$ | 13.1 ± 2.1$^{n}$ | 36.8 ± 2.1$^{a}$ |
| IV | 0.68 ± 0.04$^{a}$ | 0.84 ± 0.03$^{b}$ | 16.9 ± 1.5$^{a}$ | 41.8 ± 3.8$^{b}$ |
| V | 0.81 ± 0.07$^{b}$ | 0.99 ± 0.06$^{c}$ | 19.9 ± 2.1$^{b}$ | 46.2 ± 2.1$^{c}$ |
| VI | 0.98 ± 0.08$^{c}$ | 1.24 ± 0.08$^{c}$ | 21.9 ± 2.5$^{c}$ | 53.8 ± 2.5$^{c}$ |

Values are expressed as mean ± S.E.M. of 6 rats in each group. $^{\dagger}p<0.001$ 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.

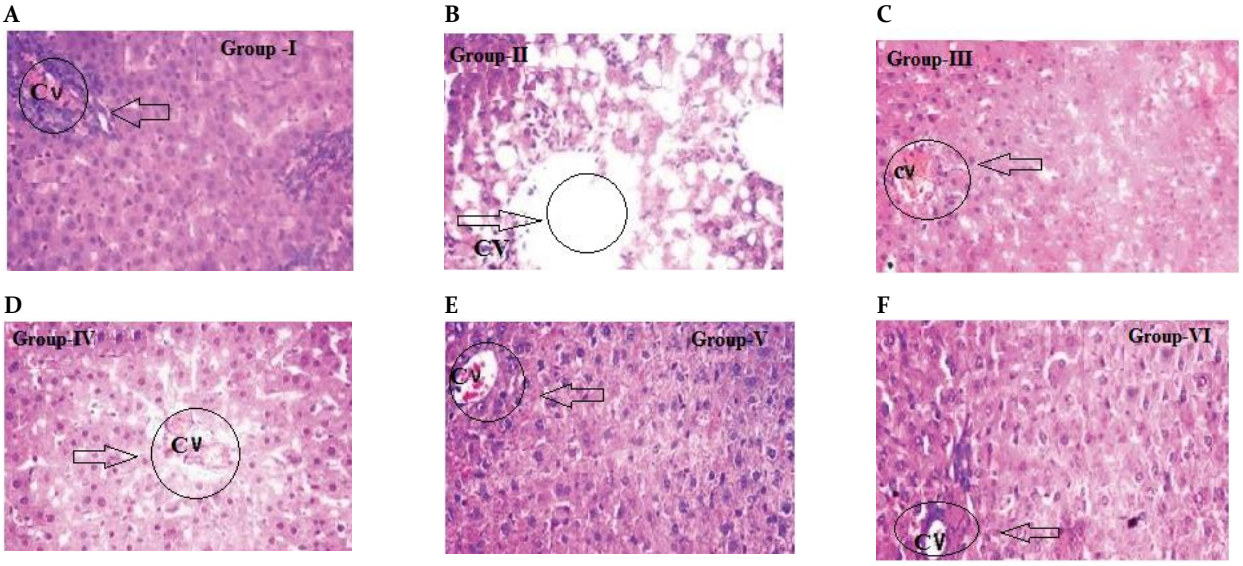


**Figure 1.** Histopathological injury induced by antitubercular drugs and the protection of *N. arbor-tristi* leaf ethanolic extract and silymarin orally administered.

**(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 dugs 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-κB) (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-κB: nuclear factor-*kappa* B; PXR: pregnane-X receptor; CAR: constitute androstane receptor; FXR: farnesoid-X receptor.

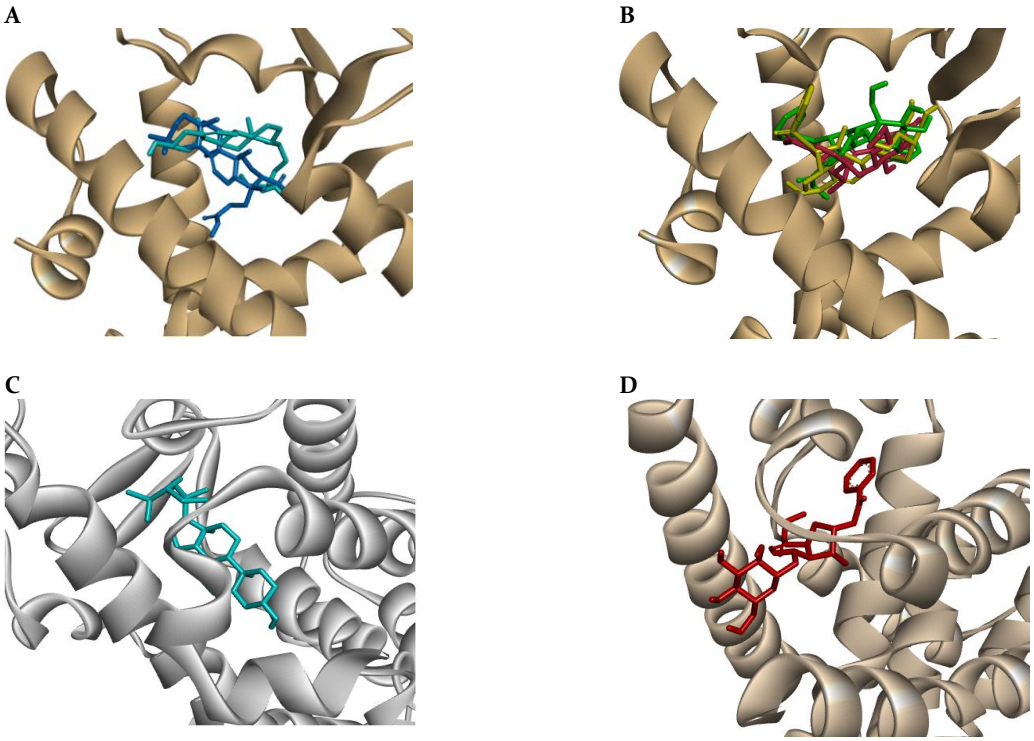


**Figure 2.** Binding modes for the active constituents of *Nyctanthes arbor-tristis* L.

**(A)** Overlay of the best-docked poses of β-sitosterol (cyan) and nyctanthic acid (blue) within the human pregnane X receptor LBD (PDB-code 1ILH). **(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 1ILH). **(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-*kappa* B (NF-κB), the farnesoid-X receptor (FXR), the constitute androstane receptor (CAR) and the pregnane-X receptor (PXR) as templates for our study. NF-κB 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-κB 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.

## ACKNOWLEDGMENT

The authors confirm that the project has no funding or grants. The authors thank to Prof. A.K. Wahi, for his kind assistance during entire research work

## REFERENCES

Adhvaryu MR, Reddy N, Parabia MH (2007) Effects of four Indian medicinal herbs on isoniazid, rifampicin and pyrazinamide-induced hepatic injury and immunosuppression in guinea pigs. World J Gastroenterol 13(23): 3199–3205.

Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105: 121–126.

Agrawal J, Shanker K, Chanda, D, Pal A (2013) *Nyctanthes arbor-tristis* positively affects immunopathology of malaria-infected mice prolonging its survival. Parasitol Res 112: 2601–2609.

Anderson ME (1985) Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 113: 548–555.

Bansal G, Suri KA (2015) A comprehensive review on *Nyctanthes abrortristis*. Int J Drug Dev Res 7: 183–193.

Bhosale AV, Abhyankar MM, Pawar SJ, Khan S, Patil N (2009) *Nyctanthes abrortristis*: A pharmacognostic review. Res J Pharmacog Phytochem 1: 91–97.

Chaudhary S, Semwal A, Kumar H, Verma HC (2016) In-vivo study for anti-hyperglycemic potential of aqueous extract of Basil seeds (*Ocimum basilicum* Linn) and its influence on biochemical parameters, serum electrolytes and haematological indices. Biomed Pharmacother 84: 2008–2013.

Chen FE, Huang DB, Chen YQ, Ghosh G (1998) Crystal structure of p50/p65 heterodimer of transcription factor NF-κB bound to DNA. Nature 391: 410–413.

Deepa PR, Varalakshmi P (2003) Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chem Biol Interact 146: 201–210.

Dutta S (2015) Medicinal application of different parts of *Nyctanthes abrortristis*. J Chem Pharm Res 7: 226–227.

Ellis PK, Martin WJ, Dodd PJ (2017) CD4 count and tuberculosis risk in HIV positive adults not on ART: a systematic review and meta-analysis. Peer J 5: 1–15.

Evan IS, Sahar ME, Mabrouka OS, Azza EB (2010) Role of oxidative stress and nitric oxide in the protective effects of α-lipoic acid and aminoguanidine against isoniazid-rifampicin induced hepatotoxicity in rats. Food Chem Toxicol 48: 1869–1875.

Flatt B, Martin R, Wang TL, Mahaney P, Murphy B, Gu XH, Foster P, Li J, Pircher P, Petrowski M, Schulman I, Westin S, Wrobel J, Yan G, Bischoff E, Daige C, Mohan R (2009) Discovery of XL335 (WAY-362450), a highly potent, selective, and orally active agonist of the farnesoid X receptor (FXR). J Med Chem 52: 904–907.

Hukkeri VI, Akki KS, Sureban RR, Gopalakrishna B, Byahatti VV, Rajendra SV (2006) Hepatoprotective activity of the leaves of *Nycthanthes arbortristis* Linn. Indian J Pharm Sci 68: 542–543.

Hussain T, Gupta RK, Sweety K, Khan MS, Hussain MS, Arif M (2012) Evaluation of antihepatotoxic potential of *Solanum xanthocarpum* fruit extract against antitubercular drugs induced hepatopathy in experimental rodents. Asian Pac J Trop Biomed 2: 454–460.

Jain PK, Pandey A (2016) The wonder of ayurvedic medicine-*Nyctanthes arbortristis.* Intl J Herbal Med 4(4): 9–17.

Jamall IS, Smith JC (1985) Effects of cadmium on glutathione peroxidase, superoxide dismutase, and lipid peroxidation

in rat heart: a possible mechanism of cadmium cardiotoxicity. Toxicol Appl Pharmacol 80: 33–42.

Jayachitra J, Rubavahini V (2015) Antioxidant effect of *Nycthanthes arbortristis* L. on D-galactosamine induced hepatotoxicity in rats. World J Pharm Pharm Sci 4: 1009–1018.

Jeong I, Park JS, Cho YJ, Yoon HI, Song J, Lee CT, Lee JH (2015) Drug-induced hepatotoxicity of anti-tuberculosis drugs and their serum levels. J Korean Med Sci 30: 167–172.

Kakkar P, Das B, Viswanathan PN (1972) A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem and Biophys 197: 588–590.

Kannappan P, Palanisamy CP, Velliyur KG (2014) Protective effect of ethanolic extract of *Tabernaemontana divaricate* (L) R. Br. against DEN and Fe NTA induced liver necrosis in Wistar albino rats. Biomed Res Int 2014: 1–9.

Karnik SR, Tathed PS, Antarkar DS, Gidse CS, Vaidya RA, Vaidya ADB (2008) Antimalarial activity and clinical safety of traditionally used *Nyctanthes arbor-tristis* Linn. Indian J Tradit Know 7: 330–334.

Kumari TDS, Charya MAS (2017) Phytochemistry, anti-cancer and anti-inflammatory activities of solvent leaf extracts of *Nyctanthes arbor-tristis*. Int J Pharm Sci Res 8(4): 1654–1663.

Lawn SD, Zumla AI (2011) Tuberculosis. Lancet 378 (9785): 57–72.

Nirmal SA, Pal SC, Mandal SC (2011) Antiasthmatic activity of *Nyctanthes arbortristis* leaves. Lat Am J Pharm 30: 654–660.

Nirmal SA, Pal SC, Mandal SC (2012) Pharmacognostic evaluation of *Nyctanthes arbortristis* bark. Asian Pac J Trop Biomed 2: S494–S500.

Nishikimi M, Rao NA, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46: 849–854.

Okokon JE, Simeon JO, Umoh EE (2017) Hepatoprotective activity of the extract of *Homalium letestui* stem against paracetamol-induced liver injury. Avicenna J Phytomed 7: 27–36.

Padma V, Suja V, Shyamala Devi CS (1998) Hepatoprotective effect of Liv.52 on antitubercular drug-induced hepatotoxicity in rats. Fitoterapia 49: 520–522.

Paul BN, Saxena AK (1997) Depletion of tumor necrosis factor-alpha in mice from *Nycthanthes arbor-tristis*. J Ethnopharmacol 56: 153–158.

Paur I, Balstad TR, Kolberg M, Pedersen MK, Austenaa LM, Jacobs DR, Blomhoff R (2010) Extract of oregano, coffee, thyme, clove and walnut inhibits NF-*kappa* B in monocytes and in transgenic reporter mice. Cancer Prev Res 3: 653–663.

Rani MJ, Lakshmi SM (2012) Hepatoprotective role of *Yucca gloriosa* L. extract against $CCl_4$ induced hepatotoxicity in rats. Int J Exp Pharmacol 2: 26–31.

Rozema E, Atanasov AG, Fakhrudin N, Singhuber J, Namduang U, Heiss EH, Reznicek G, Huck CW, Bonn GK, Dirsch VM, Kopp B (2012) Selected extracts of Chinese herbal medicines: Their effect on NF-κB, PPARα and PPARγ and the respective bioactive compounds. Evid Based Complement Alternat Med 2012: 1–10.

Saxena RS, Gupta B, Lata S (2002) Tranquilizing, antihistaminic and purgative activity of *Nyctanthes abrortristis* leaf extract. J Ethnopharmacol 81: 321–325.

Sethi H, Sung B, Aggarwal BB (2008) Nulcear factor-*kappa* B activation: from bench to bedside. Exp Biol Med 233: 21–31.

Srivastava V, Rathore A, Ali SM, Tandon JS (1990) New benzoic esters of loganin and *6*β-hydroxyloganin from *Nyctanthes arbor-tristis*. J Nat Prod 53: 303–308.

Sun B, Karin M (2008) NF-*kappa*B signaling, liver disease and hepatoprotective agents. Oncogene 27: 6228–6244.

Townsend DM, Tew KD, Tapiero H (2003) The importance of glutathione in human disease. Biomed Pharmacother 57: 145–155.

Trott O, Olson AJ (2010) Auto dock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31: 455–461.

Vogl S, Picker P, Mihaly-Bison J, Fakhrudin N, Atanasov AG, Heiss EH, Wawrosch C, Reznicek G, Dirsch VM, Saukel J, Kopp B (2013) Ethnopharmacological *in vitro* studies on Austria's folk medicine- an unexplored lore *in vitro* anti-inflammatory activities of 71 Austrian traditional herbal drugs. J Ethnopharmacol 149: 750–771.

Watkins RE, Wisely GB, Moore LB, Collins JL, Lambert MH, Willson TM, Kliewer SA, Redinbo MR (2001) The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. Science 292: 2329–2333.

Xu RX, Lambert MH, Wisel, BB, Warren EN, Weinert EE, Waitt GM, Williams JD, Collins JL, Moore LB, Willson TM, Moore JT (2004) A structural basis for constitutive activity in the human CAR/RXR alpha heterodimer. Mol Cell 16: 919–928.

**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 |

**Citation Format:** Chaudhary S, Gupta RK, Kumar A, Tarazi H (2018) Hepatoprotective and antioxidant potential of *Nyctanthes arbor-tristis* L. leaves against antitubercular drugs induced hepatotoxicity. J Pharm Pharmacogn Res 6(3): 205–215.