ANTIOXIDANT EFFECT OF *NYCTANTHES ARBOR TRISTIS* L. ON D-GALACTOSAMINE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

To investigate the antioxidant activity of *Nyctanthes arbor tristis*. In albino wistar rats. Rats were divided into four groups, groups 1 received 0.9% saline; groups 2 received control and ethanolic extract of *Nyctanthes arbor tristis* (300mg/kg BW); groups 3 received D-galactosamine (400mg/kg/BW) and group 4 received D-galactosamine and ethanolic extract of *Nyctanthes arbor tristis* for the total experimental period of 12 hrs. The results showed significantly elevated levels of serum AST (Aspartate aminotransferase), ALT (Alanine aminotransferase) and thiobarbituric acid reactive substances (TBARS), significantly lowered enzymic antioxidant activity of superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (Gpx) in D-galactosamine treated rats as compared to those of the experimental control rats. Ethanolic extract of *Nyctanthes arbor tristis* was administered at a dose of 300 mg/kg of body weight/day for the last 12hrs of the experiment to rats with D-galactosamine induced liver injury, which significantly decreased AST and ALT activities in serum, and also the hepatic level of TBARS, significantly elevated the activities of superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) in liver at the end of the experimental period as compared to those of untreated D Galactosamine - administered rats. Thus, our data indicate that treatment with *H. indicus* extract offers protection against free radical-mediated oxidative stress in serum and liver of animals with D-galactosamine - induced liver injury.

KEYWORDS: *Nyctanthes arbor tristis* (L); antioxidant activity; D-galactosamine.
INTRODUCTION
Liver toxicity due to poisons used in experimental model rarely occurs in human beings. It is, therefore, important to use hepatotoxic agents that or more relevant to human beings such as ethyl alcohol and D-galactosamine (D-GalN). Liver damage due to direct action of drugs is associated with D-GalN as the administration induces as inflammatory response in liver that resembles the reaction seen in viral hepatitis and infact D-GalN-induced hepatitis resembles viral hepatitis both biochemically and histologically.[1] Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense systems of an organism.[2] The formation of reactive oxygen species (ROS) is a naturally occurring intracellular metabolic process. But the concentration of ROS in the cell is kept fairly constant by enzymic (e.g. superoxide dismutase, glutathione peroxidase and catalase) and non-enzymic (e.g. reduced glutathione, ascorbic acid and α-tocopherol) antioxidants that are able to dispose the unwanted reactive oxygen species and generate non-toxic by-products. However, when the generation of ROS in the cells impairs antioxidant defences or exceeds the ability of the antioxidant defence system to eliminate them, oxidative stress results.[3] Many drugs and chemicals can induce oxidative stress in the body.

Lipid peroxidation is commonly used as an index for measuring the damage that occurs in cell membrane as a result of free radical generation. Many hepatotoxins intially injure the hepatocyte plasma membrane. Moreover, alteration of this membrane constitutes the irreversible step in the development of most forms of lethal hepatocytes damage.[4] In particular, the peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of D-GalN.[5] In the presence of molecular oxygen, such radicals attack unsaturated fatty acids in membrane and organelles to produce lipid epoxides and peroxides. Nyctanthes arbor tristis distributed in outer Himalayan ranges from the Chenab to Nepal, Assam, Burma, Bengal, Central India, Southwards to the Godavari cultivated in many parts of India.[6] Leaves give Mannitol, free glucose, fructose, benzoic acid, beta-amyrin, beta-sitosterol, hentri acontane, astragalin and nicotiflorin from leaves. Nyctanthoside, crocin-1, crocin-3 and D-mannitol from flowers. Beta-sitosterol and glycoside naringenis from stem.[7] The leaves are bitter, acrid, thermogenic, antibacterial, anodyne, anti-inflammatory, digestive, cholagogue, anthelmintic, depurative, sudorific, felorifuge, expectorant, diuretic, laxative, trichogenous and tonic. They are useful in vitiated conditions free glucose kapha and vatus obstinate sciatica, inflammations, dyspepsia, helmenthiasis, pruritus, dermatopathy, chronic fever, bronchitis, asthma, cough
strangury, constipation, hepatopathy, haemorrhoids, greyness of hair and baldness. The flowers are bitter, astringent, ophthalmic, stomachic, caminative and trichogenous and are useful in inflammations, ophthalmopathy, flatulence, colic, dydpepsia, and spleenomegaly. The seeds are very useful in baldness, scurvy and infections of the scalp.\cite{8}

MATERIALS AND METHODS

Plant materials

*Nyctanthes arbor tristis* was selected for the treatment of hepatotoxicity. These are the naturopathic herbs being grown locally and easily available. These herbs are used in mainly of the Siddha medicine preparation in treating number of disease.

The plant were collected and around Mayiladuthurai. From the plant, healthy leaves were harvested and cleaned by running tap water to remove the dirt eggs of insects.

Preparation of ethanolic extract

The leaves were dried and converted to powder form. *Nyctanthes arbor tristis* extract was prepared by soxhlet continuous extraction method.

Processing of blood and tissue sample

Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 40 min. Serum was separated by centrifugation at 2000 rpm for 10 min.

Immediately after sacrifice, the tissues were quickly excised, rinsed with saline, blotted dry on filter paper and weighed. Subsequently 10% (w/v) tissue homogenates with appropriate buffer using a tissue homogenizer was prepared and the supernatants were used for the various biochemical estimations.

Animals

A healthy swiss albino rats were housed in well ventilated hygienic atomosphere. Animals with 160-180g were used for our study. Animals were fed with commercial rate feed (Saidurga feeds & foods, Bangalore) and tap water adlibitum. After randomization in to various groups, the rats were acclimatized for a period of 2-3 days in the new environment before initiation of experiment.
Experimental induction of hepatotoxicity: Hepatotoxicity was induced by single intraperitoneal (I.P) injection of D-Galactosamine (400mg/kg BW), dissolved in physiological saline.

Chemicals
All the chemicals used in the experiment were of analytical grade.

Experimental Design
The animals were randomly divided into four groups of six animals in each. The *Nyctanthes arbor tristis* extract were dissolved in 0.9% saline vehicle solution and fed by intubation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>Control rats received 0.9% saline only</td>
</tr>
<tr>
<td>II</td>
<td>Control + <em>Nyctanthes arbor tristis</em> (300mg/kg BW.)</td>
</tr>
<tr>
<td>III</td>
<td>D-GalN control (400 mg/kg BW.)</td>
</tr>
<tr>
<td>IV</td>
<td>D-GalN + <em>Nyctanthes arbor tristis</em> (300mg/kg BW.)</td>
</tr>
</tbody>
</table>

After treatment, the animals were fasted for 12 h, and sacrificed by cervical dislocation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of various biochemical parameters. Tissue (liver) were surgically removed, washed with cold physiological saline, cleared off adherent lipids and immediately transferred to ice-cold containers.

Biochemical measurements
Serum aspartate aminotransferase (AST) (EC 2.6.1.1) and serum alanine aminotransferase (ALT) (EC 2.6.1.2) were assayed using a diagnostic kit based on the method of Reitman and Frankel. The enzyme activity is expressed as IU/L of plasma. Lipid peroxidation was assayed by measuring thiobarbituric acid-reactive substances (TBARS) in the tissues by the method of Ohkawa *et al.* and Yagi. The pink chromogen produced by the reaction of malondialdehyde, a secondary product of lipid peroxidation with thiobarbituric acid, was estimated at 532 nm. Values are expressed as mmoles/g tissue. SOD (EC 1.15.1.1) was assayed by the method of Kakkar *et al.* The assay was based on the 50% inhibition of the formation of NADH-phenazine methosulfate-nitro blue tetrazolium (NBT) formazan at 520 nm. The enzyme activity (SOD) was expressed as enzyme required for 50% inhibition of NBT reduction/min/mg/protein.minute/mg of protein. The activity of CAT (EC 1.11.1.6) was assayed by the method of Sinha based on the conversion of dichromate in acetic acid to perchromic acid and then to chromic acetate, when heated in the presence of hydrogen peroxide. The chromic acetate formed was measured at 620 nm. The enzyme activity (CAT)
was expressed as µmoles of H₂O₂ utilized/ minute/mg of protein. The activity of GPx (EC 1.11.1.9) was assayed by the method of Rotruck et al. A known amount of enzyme preparation was incubated with H₂O₂ in the presence of GSH for a specified time period. The amount of H₂O₂ utilized was determined by the method of Ellman. The enzyme activity was expressed as µg of GSH consumed/ minute/mg of protein.

Statistical analysis
Data were analysed by one way analysis of variance followed by Duncan’s multiple range test using SPSS for Windows (v. 11.0; SPSS Inc., Chicago, IL, USA). Results are presented as means ± SD of six rats in each group. Values of P < 0.05 were regarded as statistically significant and the data are represented as mean ± SD for the absolute values. The statistical significance of differential findings between the experimental groups and control was determined.

RESULT AND DISCUSSION
The present study was carried out to evaluate the hepatoprotective effect of ethanolic extracts of Nyctanthes-arbor-tristis against D-galactosamine induced liver injury.

Table 1. Effect of Nyctanthes arbor tristis and D-galactosamine on plasma aspartate transaminase (AST) and alanine transaminase (ALT) of control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aspartate transaminase (IU/L)</th>
<th>Alanine transaminase (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.50±7.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.52±1.97&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control + Nyctanthes arbor tristis</td>
<td>86.9±8.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.73±2.47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>D-galactosamine</td>
<td>132.80±12.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.42±4.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D-galactosamine + Nyctanthes arbor tristis</td>
<td>82.85±7.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.03±2.98&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± S.D. of six rats in each group. Values not sharing a common superscript letter differ significantly at p< 0.05 (Duncan's multiple range test). For AST and ALT 1 IU is defined as µmoles of pyruvate formed per minute under experimental conditions.

Table 1 shows the activities of plasma AST and ALT. D-GalN administration in rats disrupts the membrane permeability of the plasma membrane causing leakage of the enzymes from the cell, which leads to elevation in the levels of serum enzymes. Elevated serum enzymes are indicative of the cellular leakage and loss of functional integrity of the cell membrane in
liver.\(^{[17]}\) Hence significant rise in the transaminases levels could be taken index liver damage. The serum marker enzyme (AST and ALT) are cytoplasmic in nature, but upon liver injury these enzymes enter into the circulatory system due to the altered permeability of membrane.\(^{[18]}\) Hepatoprotective activity upon *Nyctanthes arbor-tristis*–treatment might be due to its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes. D-GalN induced rats treated with *Nyctanthes arbor-tristis* brought back the enzyme level to near normalcy indicating clearly the therapeutic value of *Nyctanthes arbor-tristis* in hepatotoxicity. Activities of both the enzymes were significantly increased in D-galactosamine rats as compared to the control rats. Supplementation with *Nyctanthes arbor tristis* to D-galactosamine rats (group 4) significantly decreased the liver marker enzymes as compared to the unsupplemented D-galactosamine rats (group 3; \(P < 0.05\)).

Table 2. Effect of *Nyctanthes arbor tristis* and D-galactosamine on tissue thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) of control and experimental rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Control + <em>Nyctanthes arbor tristis</em></th>
<th>D-galactosamine</th>
<th>D-galactosamine + <em>Nyctanthes arbor tristis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>LIVER TBARS (nmoles/g tissue)</td>
<td>20.28±1.95(^b)</td>
<td>19.14±1.8(^b)</td>
<td>50.66±4.87(^a)</td>
<td>22.88±2.0(^b)</td>
</tr>
<tr>
<td>LIVER SOD (50% inhibition of NBT reduction/min/mg protein)</td>
<td>6.8±0.65(^a)</td>
<td>7.03±0.67(^a)</td>
<td>2.55±0.24(^b)</td>
<td>6.52±0.62(^a)</td>
</tr>
<tr>
<td>LIVER CAT ((\mu)moles (H_2O_2) utilized/min/mg protein)</td>
<td>79.66±7.66(^a)</td>
<td>73.23±7.04(^a,c)</td>
<td>42.02±4.04(^b)</td>
<td>66.50±6.40(^c)</td>
</tr>
<tr>
<td>LIVER Glutathioneperoxidase ((\mu)g of GSH utilized/min/mg protein)</td>
<td>15.60±1.50(^a)</td>
<td>16.11±1.55(^a)</td>
<td>7.03±0.677(^b)</td>
<td>13.66±1.31(^c)</td>
</tr>
</tbody>
</table>

Values are means ± S.D. of six rats in each group. Values not sharing a common superscript letter differ significantly at \(P<0.05\) (Duncan's multiple range test).

Table 2 shows the activities of tissue TBARS, SOD, CAT and GPx. Lipid peroxidation is initiated by free radicals and is the oxidative deterioration of poly unsaturated fatty acids.\(^{[19]}\) There was an increase in the levels of lipid peroxidation in tissue after D-GalN which is in
In line with the findings of Mourella and Meza,\textsuperscript{[20]} Sakuguchi and Yokota.\textsuperscript{[5]} The increase in the level of TBARS indicate enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals.\textsuperscript{[21]} The peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of D-GalN\textsuperscript{[5]} (Sakuguchi and Yokota, 1995). Treatment with Nyctanthes arbor-tristis causes significant decrease in the level of TBARS in rats intoxicated with D-GalN suggesting that Nyctanthes arbor-tristis may exert a stabilizing action on liver cell membrane. In line with our findings Rathi et al.,\textsuperscript{[22]} also reported that Nyctanthes arbor-tristis inhibits the progress of lipid peroxidation in CCl\textsubscript{4} administered rats, Nyctanthes arbor-tristis demonstrating the potent antioxidant and antiperoxidative effects of Nyctanthes arbor-tristis. The level of tissue TBARS was significantly increased in D-galactosamine rats as compared to the control rats. Supplementation with Nyctanthes arbor tristis to D-galactosamine rats (group 4) significantly decreased the level of tissue TBARS as compared to the unsupplemented D-galactosamine rats (group 3; \(P < 0.05\)).

Free radical mediated oxidative damage can contribute to acute hepatitis.\textsuperscript{[23]} High levels and/or inadequate removal of reactive oxygen species may cause severe metabolic imbalance and oxidative damage to biological macromolecules.\textsuperscript{[24]} To prevent oxidative damage in the cell, a variety of antioxidants scavenge free radicals. The primary defense against oxidative stress in the tissue rests with antioxidants. Therefore, these antioxidants are expected to be consumed by enhanced radical reactions.\textsuperscript{[25]} Significant decrease in the activity of tissue defence system after intraperitoneal administration of D-GalN has already been reported.\textsuperscript{[26]} SOD which converts superoxide radicals to \(\text{H}_2\text{O}_2\) is widely distributed in cells having oxidative metabolism and is believed to protect such cells against the toxic effects of superoxide anion.\textsuperscript{[27]} Superoxide anions are known to exert destructive effects on cellular components with lipid peroxidation being one such consequence. CAT is a heme protein, which catalyses the direct degradation of hydrogen peroxide to water. It protects the cellular constituents against oxidative damage. The decreased activities of these antiperoxidative enzymes during D-GalN administered in our study are compatible with other studies.\textsuperscript{[28,29]} The decreased activity of these enzymatic antioxidants may be due to the accumulation of \(\text{H}_2\text{O}_2\) which in turn causes the inhibition of these enzymes.\textsuperscript{[30]} In this context, Nanu Rathod et al.,\textsuperscript{[31]} have reported that polyphenol present in Nyctanthes arbor-tristis possesses powerful free radical scavenging activity. The reduced activities of these enzymes were normalized upon treatment with Nyctanthes arbor-tristis. GPx catalyses the reduction of hydrogen
peroxide and hydroperoxide to non-toxic products and scavenges the highly reactive lipid peroxides in the aqueous phase of cell membrane. GPx and the cellular NADPH-generating mechanisms together form a system for removing hydroperoxides from the cell.\cite{32} The activities of both the tissue SOD, CAT and GPx were significantly increased in D-galactosamine rats as compared to the control rats. Supplementation with \textit{Nyctanthes arbor-tristis} to D-galactosamine rats (group 4) significantly decreased the activities of tissue SOD, CAT and GPx as compared to the unsupplemented D-galactosamine rats (group 3; $P < 0.05$). The decreased activity of GPx in D-GalN intoxicated group might be correlated to the decreased availability of its substrate GSH. After oral Treatment with \textit{Nyctanthes arbor-tristis} improved the GPx levels significantly to near normal.

\section*{CONCLUSION}
Our results suggest that administration of \textit{Nyctanthes arbor-tristis} during the entire experimental period significantly inhibited hepatotoxicity, decreased lipid peroxidation and enhanced enzymic antioxidant concentrations. Thus, this study primarily emphasized the effect of \textit{Nyctanthes arbor-tristis} against D-GalN induced hepatotoxicity and oxidative stress in experimental albino Wistar rats.

\section*{ACKNOWLEDGEMENT}
The authors are grateful thank to the principal, D.G.G.Arts College (W), Mayiladuthurai - 609001, Tamilnadu, India for providing necessary laboratory facilities to complete this manuscript and also to Dr. A. Malarvizhi (Head, Department of Biochemistry, DGGA College (W), Mayiladuthurai) for supporting this research work.

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