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# BIOEFFICACY OF NYCTANTHES ARBOR TRISTIS LINN., ON $CCL_4$ INDUCED HEPATOTOXICITY IN SWISS ALBINO RATS

**V. Bharathi, J. Jayachitra and *P. Venkatalakshmi**

PG and Research Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Tamilnadu-614001.

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***Correspondence for Author**
**P. Venkatalakshmi**
PG and Research Department of Biochemistry, S.T.E.T Women's College, Mannargudi, Tamilnadu.

## ABSTRACT

The present study has been carried out to evaluate the bioefficacy of *Nyctanthes arbor tristis* leaves on $CCl_4$ induced hepatotoxicity in swiss albino rats. Preliminary phytochemical analysis revealed the presence of tannins, saponins, alkaloids, flavanoids, steroids, phenolic compounds, reducing sugars and carbohydrates. Elevation of LDH, GGT and decline in NADH-dehydrogenase, Glucose-6-phosphatase, $Na^+/K^+$ dependent ATPase, Total ATPase was noted in $CCl_4$ administred rats. Total reversal of all the above said parameters was noted in both ethanolic extract treated and Silymarin treated rats. The efficacy of the plant drug has been found to be more than the standard drug Silymarin.

**KEYWORDS:** Biochemical analysis, HPLC, *Nyctanthes arbor tristis*, Phytochemical screening.

## INTRODUCTION

Drug induced liver disease is becoming more common at present. An individual drug may cause more than one type of reaction. More than 600 medical agents, chemicals recognized as hepatotoxic agents [1]. Chronic alcohol intake is the very most frequent cause of liver diseases today and accounts for the majority of the estimated 100,000 related deaths happens each and every year in USA [2]. $CCl_4$ induces successive hepatic damage, the changes consisting of hepatic steatosis, fibrosis, massive infiltration and cirrhosis [3]. The ability of a hepatoprotective drug to reduce, the injurious effects or to preserve the normal hepatic physiologic mechanisms which have been disturbed by a hepatotoxin is the index of its protective effects. *Nyctanthes arbor tristis* is a large shrub or small tree rough all over with

stiff whitish hairs, young branches sharply quadrangular, hairy. The leaves are bitter, acrid, thermogenic, antibacterial, cholestatic, hypocholestemic [4], radicus-ulna fracture [5], depression of spontaneous motor activity [6], antipyretic, anti-inflammatory activities [7] and also possess cell regeneration effect commonly known as antioxidant activity ie., scavenging of free radicals [8].

The seeds are useful in baldness, scurvy and infections of the scalp [9]. In the present study, steps were taken to evaluate the hepatoprotective effect of *Nyctanthes arbor tristis* leaves in $CCl_4$ intoxicated rats.

## MATERIALS AND METHODS

### Collection of Plant Materials

The leaves of *Nycatanthes arbor tristis* were collected from Mannargudi, Thiruvarur district. The collected plant materials were authenticated by the botanist Dr. K. Madhavan, S.T.E.T Women's college, Mannargudi.

### Preparation of the Extract

The collected leaves were washed in tap water and distilled water to remove impurities. The leaves were shade dried (28±2˚C), ground and sieved to get fine powder from which the extract was prepared. Ethanol extract of the plant material was obtained by taking 50g of dried leaf powder in a container, to which 250ml of ethanol was added and kept for 24hours with periodic shaking, then filtered and the filtrate was collected. This procedure was repeated thrice with fresh volumes of ethanol. The pooled extract was concentrated in a rotatory vaccum evaporator at 40˚C and evaporated to dryness. It was stored at 4˚C in an air tight bottle.

### Experimental Design

The rats were divided into 4 groups,

**Group I**: Control

**Group II**: $CCl_4$ treated (intraperitoneal administration of $CCl_4$ at a dosage of 1.5ml/kg body weight for 14 days).

**Group III**: $CCl_4$ and Silymarin treated (intraperitoneal administration of $CCl_4$ as the above mentioned dose along with oral administration of 25mg of Silymarin/ml of paraffin/kg body weight for 14 days).

**Group IV**: $CCl_4$ and *Nyctanthes arbor tristis* treated (intraperitoneal administration of $CCl_4$ as the above mentioned dose along with oral administration of *Nvctanthes arbor tristis* in 1ml of paraffin/kg body weight for 14 days).

After 14 days of herbal treatment, the liver was taken out by means of anaesthetizing the rats. Then the rats has undergone dissection, and the liver was taken out carefully and allowed to clean the liver in order to remove the flushing blood in the formalin solution prepared by means of dissolving 9ml of formaldehyde in 100ml of distilled water. The liver sample was homogenized in Tris-HCl buffer and used for estimation process.

**Biochemical Analysis**

Analysis of enzymes such as NADH dehydrogenase, Lactate dehydrogenase, Glucose-6-phosphatase,γ-Glutamyl transferase, Total ATPase,$Na^+/K^+$ dependent ATPase was carried out by means of standard laboratory procedures. The amino acid profile was obtained by HPLC. The phytochemical screening of the sample was also carried out qualitatively using standard laboratory procedures.

**RESULTS AND DISCUSSION**

The hepatotoxicity induced by $CCl_4$ is due to its metabolite CCl3*, a free radical that damages liver cell. It's generally accepted that the toxicity of $CCl_4$ depends on the cleavage of the carbon-chlorine bond to generate the trichloro methyl peroxy radicals. This free radical reacts rapidly with oxygen to form trichloro methyl peroxy radicals which may contribute to the hepatotoxicity [10].

In the present study bioefficacy of ethanolic extract of *Nyctanthes arbor tristis linn.*, on $CCl_4$ induced hepatotoxicity in swiss albino rats was determined. The phytochemical analysis revealed the presence of secondary metabolites such as tannins, saponins, alkaloids, flavanoids, steroids, phenolic compounds, reducing sugar and carbohydrate in *Nyctanthes arbortristis* crude leaf powder(Table 1). NADH dehydrogenase is a membrane bound enzyme involved in electron transport chain. The level of NADH dehydrogenase was decreased in $CCl_4$ induced rats and the level was restored in the plant drug and Silymarin treated rats. The effect of plant drug was found to be more than the standard drug Silymarin (Table 2). Plasma LDH activity typically is in the normal range within 24-48 hours of the onset of function and peaks in 3-6 days. Thereafter it gradually declines to reach normal values within 10-14 days. It increases during coronary heart diseases, hemolysis, megaloblastic anemia, leukaemia,

acute and chronic liver diseases. In the present study, there was an elevation in the level of LDH in $CCl_4$ treated rats. In the plant drug treated group, the elevated level of LDH was brought back to normal which might have been due to the protective effect exerted by the plant drug on liver cells. The efficacy of the plant drug was comparatively higher than the standard drug Silymarin (Table 3).

Glucose-6-phosphatase catalyses the final step in glycogenolysis, leading to the release of glucose on to the blood stream by the liver. The symptoms of type I glycogen storage disease (Von-Gierke's disease) which includes massive liver enlargement, severe hypoglyceamia [11] In the present study, group II rats show decreased of glucose-6-phosphatase activity than that of the normal. This is due to the fact that $CCl_4$ inhibits gluconeogenesis process. Herbal extract was found to be effective in restoring the levels of glucose-6-phosphatase (Table 4).
Serum GGT activity is increased with the administration of enzyme inducing drugs, such as phenobarbitone, phenytoin or glutathimide and also increases in case of liver damage produced by ethanol. It tends to revert to normal level over 2-3 weeks, but if a chronic liver disease is present it may be in elevated levels [12]. In the present study the GGT was increased than the normal level in $CCl_4$ induced rats and regained its normal level after the plant extract treatment (Table 5).

$Na^+/K^+$dependent ATPase is a transmembrane protein, involvesd in pumping $Na^+$ out and $K^+$ into the cell with concomitant hydrolysis of intracellular ATP. This extrusion of $Na^+$ enables animal cell to control their water content. In the present study $Na^+K^+$dependent ATPase was diminished in $CCl_4$ treated rats. The altered level was brought back to normal in plant drug treated rats (Table 6).

Total ATPase acts as a second messenger in a manner similar to AMP. Release of neurotransmitters from plasma membrane and endoplasmic reticulum, each containing $Ca^{2+}$ ATPase that actively pumps $Ca^{2+}$ out at the expense of ATP hydrolysis [13, 14] In the present study, the enzyme level got decreased it restored to normal than the group I rats in group II rats which was restored after plant drug treatment (Table 7).

HPLC is performed in order to determine the level of amino acids and the results are recorded in Table 8.The amino acids are found to be decreasing in group II rats and restored to normal in plant drud treated rats proving the efficacy of the plant drug.

**Table 1: Qualitative analysis of Phytochemicals in *Nyctanthes arbor tristis.***

| S. No | Compounds | Result |
|---|---|---|
| 1. | Tannins | + |
| 2. | Saponins | + |
| 3. | Alkaloids | + |
| 4. | Flavanoids | + |
| 5. | Steroids | + |
| 6. | Phenolic compounds | + |
| 7. | Reducing sugars | + |
| 8. | Carbohydrates | + |

+ Presence

**Table 2: Effect of *Nyctanthes arbor tristis* on NADH dehydrogenase (µmole/min/mg of protein) on $CCl_4$ induced hepatoxicity.**

| Group | Particulars | NADH dehydrogenase (µmole/min/mg of protein) |
|---|---|---|
| Group-I | Normal | 1216.00±90.99 |
| Group-II | $CCl_4$induced | 969.16±305.20*** |
| Group-III | Silymarin | 1138.32±157.60*** |
| Group-IV | Herbal treated | 1200.18±98.65*** |

Values are expressed as mean±SD for 4 rats in each group.

Students "t" test followed ***P<0.001.

**Table 3: Effect of *Nyctanthes arbor tristis* on LDH (m mole/min/mg of protein) on $CCl_4$ induced hepatotoxicity.**

| Group | Particulars | LDH(m mole/min/mg of protein) |
|---|---|---|
| Group-I | Normal | 106.4±3.28 |
| Group-II | $CCl_4$ induced | 189.7±1.24*** |
| Group-III | Silymarin | 109.4±2.72*** |
| Group-IV | Herbal treated | 104.6±3.12*** |

Values are expressed as mean±SD for 4 rats in each group.

Students "t" test followed ***P<0.001.

**Table 4: Effect of *Nyctanthes arbor tristis* on glucose-6-phosphatase (z mole/min/mg of protein) on $CCl_4$ induced hepatotoxicity.**

| Group | Particulars | Glucose-6-phosphatase (z mole/min/mg of protein) |
|---|---|---|
| Group-I | Normal | 10.42±0.31 |
| Group-II | $CCl_4$induced | 7.13±0.22*** |
| Group-III | Silymarin | 9.31±0.27*** |
| Group-IV | Herbal treated | 9.42±0.29*** |

Values are expressed as mean±SD for 4 rats in each group.

Students "t" test followed ***P<0.001.

**Table 5: Effect of Nyctanthes arbor *tristis* on GGT(IU/L) on $CCl_4$ induced hepatotoxicity.**

| Group | Particulars | GGT(IU/L) |
|---|---|---|
| Group-I | Normal | 3.37±0.14 |
| Group-II | $CCl_4$ induced | 14.27±0.98*** |
| Group-III | Silymarin | 4.43±0.78*** |
| Group-IV | Herbal treated | 4.13±0.21*** |

Values are expressed as mean±SD for 4 rats in each group.

Students "t" test followed ***P<0.001.

**Table 6: Effect of *Nyctanthes arbor tristis* on Total ATPase (m mole/ mg protein) on $CCl_4$ induced hepatotoxicity**.

| Group | Particulars | Total ATPase (m mole/ mg protein) |
|---|---|---|
| Group-I | Normal | 9.52±0.699 |
| Group-II | $CCl_4$ induced | 7.79±0.906*** |
| Group-III | Silymarin | 9.3±1.93*** |
| Group-IV | Herbal treated | 9.68±0.785*** |

Values are expressed as mean±SD for 4 rats in each group.

Students "t" test followed ***P<0.001.

**Table 7: Effect of *Nyctanthes arbor tristis* on $Na^+$ $K^+$ dependent ATPase(y mole/min/mg of protein) on $CCl_4$ induced hepatotoxicity**.

| Group | Particulars | $Na^+k^+$ dependent ATPase (y mole/min/mg of protein) |
|---|---|---|
| Group-I | Normal | 3.450±0.465 |
| Group-II | $CCl_4$ induced | 1.980±0.435*** |
| Group-III | Silymarin | 2.250±0.380*** |
| Group-IV | Herbal treated | 3.250±0.288*** |

Values are expressed as mean±SD for 4 rats in each group.

Students "t" test followed ***P<0.001.

**Table-8: Estimation of amino acid profile from different group of rats.**

| | | Amino acid concentration (mg/g of cells) | | | |
|---|---|---|---|---|---|
| S. No | Name of the amino acid | Group-I | Group-II | Group-III | Group-IV |
| 1. | Glycine | 1.54 | 1.25 | 1.50 | 1.49 |
| 2. | Cystine | 1.62 | 1.62 | 1.61 | 1.60 |
| 3. | Arginine | 1.64 | 1.63 | 1.62 | 1.64 |
| 4. | Glutamine | 183.4 | 126.96 | 179.9 | 180.3 |
| 5. | Alanine | 3.64 | 3.42 | 3.03 | 3.63 |
| 6. | Histidine | 0.24 | - | 0.29 | 0.20 |
| 7. | Methionine | 4.59 | 4.30 | 4.17 | 4.25 |
| 8. | Serine | 6.17 | 5.92 | 5.90 | 6.01 |
| 9. | Aspartate | 11.73 | 10.72 | 9.16 | 9.26 |
| 10. | Proline | 0.83 | - | 0.91 | 0.82 |

## CONCLUSION

It can be concluded that the present study showed that some indigenous plant based products are very promising against hepatotoxic activities. They offer a safer alternative to synthetic chemicals and can be obtained at a very low cost. *Nyctanathes arbor tristis* can be used for effective protection of hepatic disorders, their potential under field conditions needs to be evaluated. Further investigation regarding the hepatoprotective principles of *Nyctanathes arbor tristis* should be carried out in future.

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