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Evaluation of Hepatoprotective Activity of the Leaves of *Nyctanthes arbor-tristis* Linn.

Mayee R[1], Thosar A[2*], Kondapure A[1]

[1]*Dr. Ved Prakaash Patil College of Pharmacy, Georai Tanda, Aurangabad, Maharashtra, India*
[2]*MGM Institute of Bioscience, Aurangabad, Maharashtra, India*

ABSTRACT
The decoction of the leaves of *Nyctanthes arbor-tristis* Linn. (Family- *Oleaceae*) is widely used in Ayurvedic system of medicine for the treatment of sciatica, arthritis, fevers, various painful conditions, liver disorders and as laxative & diuretic. The aim of the present study was to evaluate the alcoholic and aqueous extracts of the leaves of *Nyctanthes arbor-tristis* for hepatoprotective effect against galactosamine-induced liver damage in rats. Administration of alcoholic and aqueous extracts of the leaves of *Nyctanthes arbor-tristis* was observed to protect the liver from toxic effects of galactosamine by reducing the elevated levels of Serum Glutamate Pyruvate Transaminase, Serum Glutamate Oxaloacetate Transaminase and serum Bilirubin (total). Liver section of standard treated and from *Nyctanthes arbor-tristis* treated groups show moderate protection in galactosamine-induced liver damage. Results revealed that both the alcoholic and aqueous extracts showed significant hepatoprotective activity by reducing the elevated levels of biochemical parameters at a dose of 500 mg/kg body weight.

Keywords: *Nyctanthes arbor-tristis,* galactosamine, hepatoprotective activity.

INTRODUCTION

The liver is the largest glandular organ in the body, and has more functions than any other human organ. A person's entire blood supply passes through the liver several times a day. The Liver has a pivotal role in human metabolism. Liver produces and secretes bile; it also produces prothrombin, fibrinogen, and heparin, those helps keeps blood from clotting within the circulatory system. The liver converts sugar into glycogen. Liver diseases have become one of the major causes of morbidity and mortality in man and animals all over globe and hepatotoxicity due to drugs appears to be the most common contributing factor. Among the many diseases that can affect the liver the most common is 'viral hepatitis' (Inflammation of liver caused by viral infection). Hepatitis can be caused by drugs, viruses, bacteria, mushrooms, parasites like amoebas or giardiasis. About 20,000 deaths occur every year due to liver disorders. The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or the other for this purpose. Scientific evaluation of plants has often shown that active principles in these are responsible for therapeutic success. A large number of medicinal plants have been tested and found to contain active principles with curative properties against a variety of diseases. [1-2]

Liver protective plants contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes. [3] Recent experiments have shown that plant drugs are relatively non-toxic, safe and even free from serious side effects. [4]

Nyctanthes arbor-tristis Linn. (Family: *Oleaceae*), commonly known as Harsingar or Night Jasmine, is a common wild hardy large shrub or small tree. It is a native of India, distributed wild in sub-Himalayan regions and southwards to river Godavari. It is also found in Indian gardens for ornamental purposes. Its different parts are known to possess different pharmacological activities in Indian systems of medicines. Several phytochemical and pharmacological investigations have also been done on this plant. [5-12] The antispasmodic and anthelmintic [5] activities of the leaves of *Nyctanthes arbor-tristis* Linn. have been reported. The objective of this study is to evaluate the hepatoprotective potential of the alcoholic and aqueous extracts of the leaves of *Nyctanthes arbor-tristis* Linn against hepatotoxicity induced by galactosamine.

The following are the various chemicals reported from various parts of the *Nyctanthes arbor-tristis* Linn.

1) Leaves: D-mannitol, β-sitosterole, flavanol glycosides-astragaline, nicotiflorin, oleanolic acid, nyctanthic acid, tannic acid, ascorbic acid, methyl salicylate, an amorphous glycoside, an amorphous resin, traces of volatile oil,

***Corresponding author: Mr. Thosar A.,**
10, Gurukunj Housing society, Tilak Nagar, Aurangabad 431005; **Mob.:** +91-9579421970
E-mail: ambi0210@gmail.com, rahul.mayee@rediffmail.com

carotene, friedeline, lupeol, mannitol, Glucose and fructose, iridoid glycosides & benzoic acid.
2) Flowers: Essential oil, nyctanthin, d-mannitol, tannin and glucose, carotenoid, glycosides Viz β-monogentiobioside ester of α - crocetin (or crocin-3), β-monogentiobioside -β-D monoglucoside ester of α-crocetin & β-digentiobioside ester of α-crocetin (or crocin-1).
3) Seeds: Arbortristoside A & B, glycerides of linoleic oleic, lignoceric, stearic, palmitic and myristic acids, nyctanthic acid, 3-4 secotriterpene acid, a water-soluble polysaccharide composed of D-glucose and D mannose.
4) Bark: Glycosides and alkaloids.
5) Stem: Glycoside-naringenin-4'-0-β-glucapyranosyl-α-xylopyranoside and β-sitosterol.
6) Flower oil: α-pinene, p-cymene, 1- hexanol methyl heptanone, phenyl acetaldehyde, 1-deconol and anisaldehyde.
7) Plant: 2, 3, 4, 6-tetra-0-methyl-D-glucose, 2, 3, 6 tri-0-methyl-D-glucose, 2, 3, 6-tri-0-methyl-D-mannose, 2, 3,-di-0-methyl-d-mannose, arbortristoside A, B, C and iridoid glycoside.

MATERIALS AND METHOD

Plant material

The plant of *Nyctanthes arbor-tristis* was collected from the roadside locations of Aurangabad (Maharashtra) region and was authenticated by department of Botany, BAMU, Aurangabad. Plant material was preserved in pharmacognosy department of Dr. Ved Prakash Patil, College of Pharmacy, Aurangabad. The leaves were shade dried and powdered in mixer grinder and stored in tightly closed container.

Preparation of plant extract

The dried powdered leaves were exhaustively extracted with 95% ethanol in a Soxhlet apparatus and also macerated with chloroform water for 7 days. The extracts were further concentrated in vacuum under pressure using rotary flash evaporator and dried in desiccator. Both the extracts were suspended in water using 1% Tween-80 and subjected for hepatoprotective activity in galactosamine-induced hepatotoxicity.

Experimental animals

Wistar rats (150-200 g) of either sex were used for Hepatoprotective activity. The animals were fasted over night prior to the experimental procedure. The method of Up and Down or 'Staircase' was used to determine the dose. [13] Tween-80 (1%) was used as a vehicle to suspend the extracts. The animals were grouped into five groups of six animals each and maintained on standard diet and water. All the animal experimental protocol has been approved by the Institutional Animal Ethics Committee.

Hepatoprotective activity

Hepatoprotective activity [14] was carried out using wistar rats (150-200 g) of either sex. The animals were divided in to five groups of six animals each and maintained on standard diet and water. Tween-80 (1%) was given to groups 1 and 2 as a vehicle for 10 days by oral route. Liv-52 was administered to group 3 at the dose of 1 ml per kg body weight by oral route for 10 days. Ethanol and aqueous extracts were administered to groups 4 and 5, respectively at a dose of 500 mg/kg by oral route for 10 days. Galactosamine at a dose of 0.7 ml per kg body weight was injected to animals of groups 2, 3, 4 and 5 on 3rd, 6th and 10th day by intraperitoneal route. On 10th day, 1 h after the last dose of galactosamine injection, the blood was collected from the carotid artery; serum was separated and used for the estimation of various biochemical parameters.

Statistical analysis

Biochemical parameters [15] such as glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase (ALP) and serum bilirubin (total) were determined (TABLE). Liver was excised quickly fixed in 10% formalin and then fixed in bovine solution, they were processed for paraffin embedding following the standard micro technique. Sections of liver were stained with haematoxylin-eosin and were observed microscopically for any histopathological changes. The mean value±SEM was calculated for each parameter, each parameter was analyzed separately using ANOVA followed by Dunnets' test. It is revealed that the alcoholic and aqueous extract contains flavanoids, sterols, tannins, carbohydrates and glycosides (flavonoidal). Results revealed that both the alcoholic and aqueous extract of leaves of *Nyctanthes arbor-tristis* Linn exhibited an ability to counter act the galactosamine -induced hepatotoxicity by decreasing the elevated enzyme levels in the blood.

RESULTS

Histopathology of liver from normal control group shows prominent central vein, normal arrangement of hepatic cells. Microscopical examination of galactosamine treated liver section shows various degrees of pathological changes starting from centrilobular necrosis of hepatic cells and central lobular fatty regeneration. Liver section of standard treated and from *Nyctanthes arbor-tristis* treated groups show moderate protection in galactosamine-induced liver damage. Since the results of hepatoprotective activity showed a significant decrease in the elevated levels of serum enzymes and histopathological results showed a significant regeneration of hepatocytes.

DISCUSSION

Aminotransferases are group of enzymes that catalyze reversible transfer of the amino acid group from an α- amino acid to an oxo acid .The largest pool of ALT is found in cytosol of hepatic parenchyma cells, whereas, AST is found in cytosol and mitochondria of hepatocytes and also found in cardiac muscle, skeletal muscle, pancreas and kidney. Therefore, measurement of ALT is more liver specific to determine hepatocellular damage. Nevertheless, AST is still being used to assess liver function since it is considered to be a sensitive indicator of mitochondria damage particularly in the centrilobular regions of liver. Treatment with NAT extract suppresses galactosamine induced AST and ALT elevations. Recovery towards normalization of the enzymes following NAT treatment suggested that the plant extract have some roles in preserving structural integrity of hepatocellular membrane, thus prevented enzymes leakage into the blood circulation. Due to treatment with NAT extract the enzyme levels return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes.

CONCLUSION

Thus, from these studies we may conclude that the ethanolic and aqueous extracts of the leaves of *Nyctanthes arbor-tristis* Linn. can be used as hepatoprotective.

Table 1: Effect of *Nyctanthes arbor-tristis* Linn. on galactosamine-induced hepatotoxicity in rats

Group	Dose mg/kg	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
Control	-	130.48±0.58	197.25±0.46	10.29±0.20	0.64±0.04
Galactosamine	0.7	240.45±0.70	257.24±0.25	11.26±0.12	1.36±0.30
Alcoholic Extract	500	151.8±0.28	209.26±0.25	10.51±0.33	0.62±0.02
Aqueous Extract	500	150.58±0.44	216.4±0.33	10.51±0.30	0.62±0.02

All the values expressed as mean ±SEM, (n=6), when compared with control and galactosamine.

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