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COMPARATIVE STUDY ON ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *Nyctanthus arber tristis* LEAVES USING LIVER SLICE MODEL

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ABSTRACT

Xenobiotic-induced injuries are very common to liver as it plays a central role in metabolism and biotransformation of xenobiotics. Liver disorders are very common in todays lifestyle. Medicines obtained from various herbs are most abundantly used to treat liver ailments. Nyctanthes arbortristis is one of well known medicinal plant and many of its part are used to treat different diseases/disorders.

In the present project efforts are made to study the differences in the biological activities of the aqueous and ethanolic extract of the Nyctanthes arbortristis leaves using the liver slice model. To study the biological activity and cytoprotective potency associated with the Nyctanthes arbortristis leaf extract CCl_4 toxicity model was used. Rat liver slices were incubated *in vitro* in presence of CCl_4 and Nyctanthes arbortristis aqueous and ethanolic leaf extracts for one, two and three hours. The leakage of liver specific enzymes that is aspartate transaminase and alanine transaminase was assessed in the medium. After statistical analysis of the data showed that the leaves of Nyctanthes arbortristis possess hepatoprotective activity however some differences were noted in the activities of aqueous and ethanolic extract mediated protective efficacy.

Key words: hepatoprotecitve *in vitro*, liver, CCl_4 *Nyctanthus arbertristis*

[I]INTRODUCTION

Nyctanthes arbortristis Linn. is popularly known as night flowering jasmine [1]. Harleen Kaur Sandhar and co-workers [2] reviewed that the leaves of *Nyctanthes arbortristis* Linn. are used extensively in Ayurvedic medicine for the treament of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic [3] . Leaves/leaf juice alongwith honey is used to treat cough and leaf paste together with honey is used for the treatment of fever, high blood pressure and diabetes [4]. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, and diuretic so also in enlargement of spleen [5, 6]. Loss of

appetite, piles, liver disorders, biliary disorders, intestinal worms, chronic fever, obstinate sciatica, rheumatism and fever with rigors are also treated with Nyctanthes arbor tristis leaves. [7]. The extracted leaf juice along with the sugar acts as a cholagogue, laxative and mild bitter tonic hence given to children as a remedy for intestinal ailments. Potential anti-malarial activity (against malarial fever has also been found associated with Nyctanthes arbortrisits leaves. [8]. Leaf decoction is extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative [1].

[II] Materials and Methods

2.1 Plant material: *Nyctanthus arbor tristis* plants were collected locally, from Koldoli a small village in Kolhapur district, MS India. The collection was in the month of November of the year 2010. The plant identification was done by an expert in Botany, Dr A. R. Jadhav, from Department of Botany, Yashavantarao Chavan College, Warananagar, Kolhapur, MS India.

Extract preparations:

Aqueous extract: Twenty grams of dried powered plant material (leaves & flower) was soaked in 200ml Double Distilled Water (DDW), kept on a rotary shaker for 24 hrs. Thereafter it was kept at slow heat for 8 hrs and then filtered through 8 layers of muslin cloth. The resultant liquid was then centrifuged at 7000 rpm for 15 minutes. The supernatant was collected and then concentrated by evaporation at 50°C to make the final volume one-twentieth of the original volume (10ml). The extract was then autoclaved at 121°C and 15 lbs pressure, and stored in sealed tubes at 4°C until use.

Ethanolic extract: For ethanolic extract preparation dried powered plant material was soaked in 100% pure ethanol through 8 layers of muslin cloth. The resultant liquid was then centrifuged at 7000 rpm for 15 minutes. The supernatant was collected and then concentrated by evaporating the ethanol at 35°C.

2.2 Chemicals: Highly pure and of analytical grade chemicals were utilized for the present studies. CCl_4 was purchased from S D fine chemicals, Mumbai while the culture media was obtained from Himedia (M199).

2.3 Experimental Animals: Healthy Wistar strain Albino male rats weighing 175 to 225 gm, bred and reared under standard housing conditions were obtained from the registered animal house of Tatyasaheb Kore College of Pharmacy, Waranangar, Dist. Kolhapur, Maharashtra, India. The animals were kept in standard plastic animal cages with a 12 hours light and dark cycle and fed on standard rat chow and provided pure water *ad libitum*. The protocol was sanctioned by local ethical committee. The experiments were carried out according to guidelines of 'Committee for Prevention and Control of Scientific Experimentation on Animals' (CPCSEA) New Delhi. Animals were sacrificed giving deep ether anesthesia.

2.4 Experimental Procedure: Surgical procedures were carried out on fed rats under deep ether anesthesia to obtain whole liver. The liver slices (LS) were prepared from the whole liver as described earlier [9]. The slices were transferred to experimental vials with combinations of hepatotoxin i.e. CCl_4 with/without aqueous or ethanolic *Nyctanthus arbor tristis* leaves Extract. Unsupplemented slices were used as control/s.

CCl_4 concentration used was 5 mM, based on results of earlier studies from various laboratories [10, 11]. The concentration of Nyctanthes arbortrisits used was 500µg/ml of medium based upon the preliminary studies from the laboratory.

Table 1 Experimental design for Comparative study on activity of aqueous and ethanolic extract of *Nyctanthus arber tristis* leaves using liver slice model against acetaminophen (AMP) and CCl_4

Sr. No	Designation	CCl_4	Aqueous Extract	Ethanolic Extract
1	Control	-	-	-
2	CCl_4	√	-	-
3	AqE	-	√	-
4	EtE	-	-	√
5	CCl_4 +AqE	√	√	-
6	CCl_4+EtE	√	-	√

2.5 Assessment by Biochemical parameters:
The vials in sets described in Table 1 representing experimental design, were incubated in standard conditions for 60, 90, 120 and 150 minutes. At the end of incubation, the medium was used for the assessment of leakage of Aspartate transaminase and Alanine transaminase [12]. Pathological diagnostic kits were procured from Pathozyme Diagnostics, India.

2.6 Statistical Analysis of the data: Statistical analysis of the results obtained from the experiments was carried out using ANOVA. The values with their respective units are expressed as mean of 6 sets ± SE. Value of $p < 0.05$ was considered as significant.

[III] RESULTS AND DISCUSSION: Statistical results of the above experiment are shown below graphically.

Fig. 1: Nyctanthes arbortristis mediated changes in Aspartate transaminase and alanine transaminase after 60 minutes of incubation of liver slices in presence of CCl_4

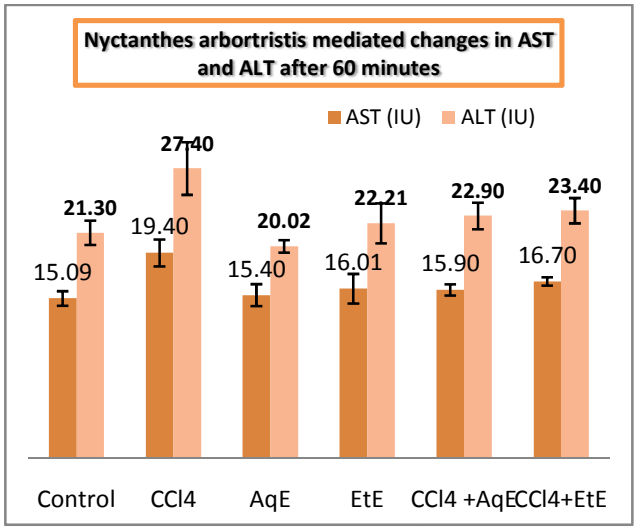


Fig. 2: Nyctanthes arbortristis mediated changes in Aspartate transaminase and alanine transaminase after 120 minutes of incubation of liver slices in presence of CCl_4

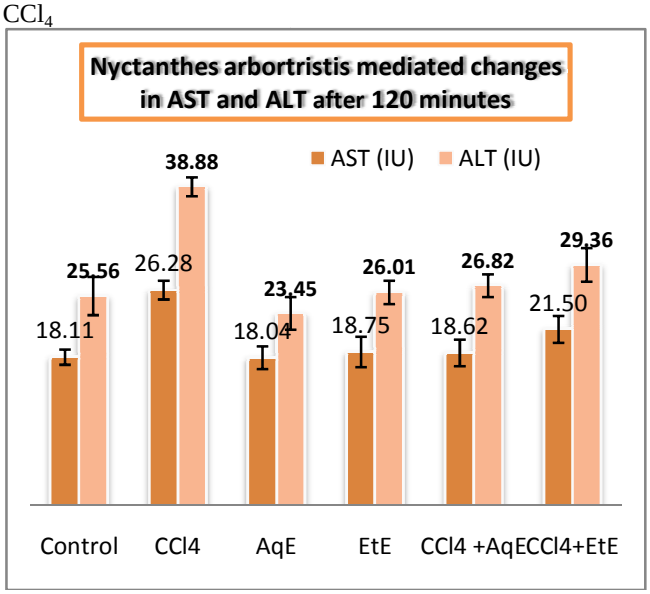
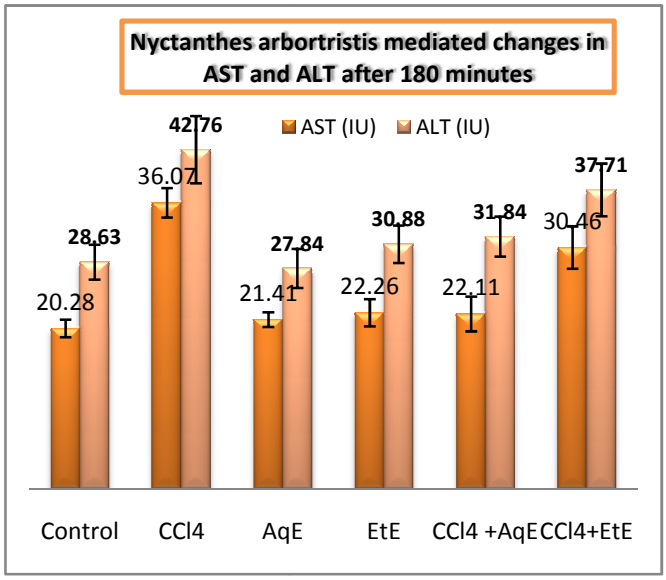


Fig. 3: Nyctanthes arbortristis mediated changes in Aspartate transaminase and alanine transaminase after 180 minutes of incubation of liver slices in presence of CCl_4



The control tests showed 15.09±0.69 units of AST per ml in the medium at 60 minutes, 18.11±25.56 units of AST at 120 minutes and 20.28±1.11 units of AST at 180 minutes. The ALT units found in per ml medium were 21.30±1.15, 25.56±2.33 and 28.63±2.19 respectively at 60, 120 and 180 minutes. The time

dependent slowly increasing levels of both AST and ALT may indicate the cell viability and metabolism. The levels of AST and ALT were significantly increased increased in presence of CCl_4 after 60 minutes, however highly significant increase was noted in these levels at 120 and 180 minutes. The elevated levels indicate damage of liver cell as AST and ALT are hepatospecific enzymes. The transaminases are cytoplasmic and are released into circulation after cellular damage [13]. These levels were declined when liver slices were co-incubated in the presence of aqueous and ethanolic extracts of Nyctanthes arbortristis. However it was noticed that the aquous extract is possessing more potency against protecting the cells against CCl_4 mediated damage when compared to that of the results of ethanolic extract of Nyctanthes arbortrisits leaves.

Measurement of ALT is more liver specific to determine hepatocellular damage than AST, however AST is still being used to assess liver function since it is considered to be a sensitive indicator of mitochondria damage [14]

The hepatotoxic effects of CCl_4 are largely due to the generation of free radicals [15]. CCl_4 is biotransformed by the cytochrome P450 system to produce the trichloromethyl free radicals, which in turn covalently bind to cell membranes and organelles to elicit lipid peroxidation [16]. Hence the possible mechanism underlying the protection of the hepatocytes by the Nyctanthes arbortristis extract could be scavenging of the CCl_3 and other free radicals and prevent the damage to the membranes, reducing the leakage of the membrane.

The leaves have been found to contain tannic acid, methyl salicylate, amorphous glucosides, mannitol, resin, ascorbic acid, carotene, and traces of a volatile oil. (Narendhirakannan RT) DPPH assay, free radical scavenging activity, reducing power assay, total antioxidant capacity concluded it to strongly possess antioxidant [17]. This supports the possibility of cytoprotection by antioxidant activity. The phytochemical composition studies of Nyctanthes arbortristis leaves shows that it contains phytosterol, phenolics, tannins,, flavonoids, steroids, glycosides and saponins. The phenolic components are found second most abundant among all other plant parts of Nyctanthes arbortristis. The antioxidant activity depends upon the phenolic components. The secondary metabolites present in *N. arbor-tristis* are known to be biologically active. The alkaloids the largest groups of chemicals produced by plants have many biological activities [18]. Possibly, the biologically active components may be contributing to the membrane stability, resulting in reduced leakage of transaminases in the medium indicating hepatocyte protection in these studies.

The differences observed in the activity exhibited by the aqueous and ethanolic extracts of Nyctanthes arbortristis leaves may be due to the loss of some bioactive ingredient during preparation of ethanolic extract from Nyctanthes arbortristis leaves. This loss may be full or partial, as the protective activity is not totally absent with ethanolic extract of Nyctanthes arbortristis, but appears limited. This may be due to low concentration of the active component in the ethanolic extract compared with the aqueous extract. The differences may be due to conformational changes in some active components during the process hindering the synergic bioactivity. There is possibility that the active component/s involved in the free rardical scavenging may have lost full/partial activity.

[IV]CONCLUSION:

The present study reveals that *Nyctanthes arbortristis* leaves possess hepatoprotective activity. However the ethanolic extract showed comparatively poor potency of cytoprotectivity when compared with aqueous extract. It may be due to full or partial loss of some active component / activity of component involved in hepatoprotective action during the ethanolic

extract preparation. Further studies are required to reveal the underlying mechanism.

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REFERENCES:

1. Narendhirakannan RT., Sameera T. (2010) " In vitro antioxidant studies on ethanolic extracts of leaves and stems of Nyctanthes arbortristis L. (Night flowering jasmine) *Int J of Biol and Medicla Res; (1) 188-192*
2. Sandhar Harleen Kaur, kaur M., Kumar B., Prasher S., (2011) "An update on Nyctanthes arbor-tristis Linn" *Int Pharmaceutica Sciencia* ,(*1*) 1
3. Tuntiwachwuttiku P, Rayanil K, Taylor WC., (2003) "Chemical constituents from the flowers of *Nyctanthes arbortristis*", *Science Asia, (29) 21-30*
4. Nawaz AHMM, Hossain M., Khan M., Jahan R., Rahmatullah M., (2009) "An ethnobotanicals survey of Jessore district in Khulna Division, Bangladesh" *Am-Eurasina J sustainable Agri., (3), 238-243*
5. Vats M., SharmaN, Sardana S., (2009) Antimicrobial activity of stem bark extracts of Nyctanthes arbortristis Linn (oleaceae), *Int j Pharmagnosy and Phytochemical Res, (1)* 12-14
6. Suresh V., Jaikumar S., Arunachalam G., (2010) Antidibetic activity of ethanolic extract of stem bark of Nyctanthes arbortristis Linn. (2010) *Res. J Pharma Biol and Chem Sci*, (*1*) 311-317
7. Nair R, Kalariya T., Chanda S., Antibacterial activity of some selected Indian Medicinal Flora (2005), *Turkish J of Biol.(29) 41-47*
8. Rout GR., Mahato A., Senapati SK, "In vitro clonal propagation of Nyctanthes arbortrisits Linn-a medicinal tree" (2007) *Horticulture Sci.* (34) 84-89
9. Patil Bhagyashree (2010) '*In vitro* study of cytoprotection by *Aloe vera* leaf gel against APAP (AAP) mediated oxidative stress in rat liver slices' *Int J Adv Biotech and Research*, 1 (*2*) 73-86
10. Dvorak, Z; Kopsina, P.; Walterova, D.; mSimanek, V.; Bachled, P. and Ulrichova, (2003) "Primary cultures of human hepatocytes as a tool in cytotoxicity studies; cell protection against model toxins by flaonolignans obtained from Silybum marianum" Toxicology J., 137, 201-212
11. Manal, Sh. Hussein, Osama, S. El-Tawil, Nour El-Hoda Yassin and Khalid, A. Abdou, (2010) "The protective effect of Morus Alba and Calandula officinalis plant extracts on Carbon Tetrachloride –Induced Hepatotoxic Isolated Rat Hepatocytes" *J. American Sci* , 6 (10), 762-773,
12. Reitman, S., and Frankel, S.A. (1957). A calorimetric method for the Determination of serum glutamate oxaloacetate and glutamate pyruvic transaminase. *Am J Clinical Pathology*, 28, 56
13. Sallie R. J., M. Tredger and R. William (1991) 'Drug and the liver' *Biopharm. Drug Disp*. 12, 251-259
14. Vishwanathan M., and Juvekar A.R. (2010) "Hepatoregenerative effect of Nyctanthes arbortristis Linn. On acetaminophen induced oxidative damage in rats", *Int j PharmTech Res 2* (*2)* 1291-1297
15. Shenoy; K. A., S. N. Somayaji, K. L. Bairy (2001) 'Hepatoprotective effects of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats' *Indian J. Pharmacol* 33, 260-266
16. Recknagel R. O., E. A. Glende, J. A. Dolak Jr and R. L. C. Waller (1989). Mechanism of carbon tetrachloride toxicity. Pharmacol. Ther. 43, 139-154.
17. Rathore B, Paul B, Chaudhary BP, Saxena AK, Sahu AP, Gupta YK (2007) "Comparative studies of different organs of *Nyctanthes arbortristis* in modulation of cytokines in murine model of arithritis", *Biomed and Envtl Sci.*, (*20*) 154-159
18. K. Priya and Deepak Ganjewala , 2007. Antibacterial Activities and Phytochemical Analysis of Different Plant Parts of *Nyctanthes arbor-tristis* (Linn.). *Research Journal of Phytochemistry, 1: 61-67.* DOI**:** 10.3923/rjphyto.2007.61.67