

Evaluation of Hepatoprotective Activity of Aqueous Extract of *Nyctanthes Arborescens* Bark against Paracetamol-Induced Liver Damage

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Abstract

Herbal drugs aimed to determine the hepatoprotective activity of aqueous extract of *Nyctanthes arborescens* bark (AQENA) against paracetamol-induced liver damage (PILD) in rat models. Four groups of rats (n=6) were given one daily administration of 1gm/kg (negative control), 25mg/kg silymarin (positive control) and AQENA (500mg/kg) for 7 days followed by induction of hepatotoxicity using of paracetamol. Various parameters, such as physical parameters, biochemical parameters and microscopic analysis were used to evaluate the hepatoprotective activity. Results indicate that bark extract would a significant (p<0.05) hepatoprotective activity against inducers. These observations were supported by the histological finding. Conclusions: Findings indicate that AQENA possesses a potent hepatoprotective activity against paracetamol.

Keywords: Bark of *N. arborescens*, aqueous extract, and hepatoprotective activity, paracetamol

Introduction

The liver is the most essential visceral organ which detoxify of diverse exogenous and endogenous substances such as drugs [1]. Causative agents include diseases that interfere with liver function such as chemicals (ethanol, CCl₄, thioacetamide, D-galactosamine; environmental toxins) and drugs such as paracetamol[2]. Drug- induced liver damage accounts for more than 50% of acute liver damage according to the United States Acute Liver Failure Study Group [3]. Natural resources such as herbal plants and their formulations are utilized for protection of liver [4]. Example of silymarin extracted from the seeds of milk thistle (*Silybum marianum*) are utilized globally as hepatoprotective agent [5] [6].

Nyctanthes arborescens is a medicinal plant possesses various therapeutic characteristics belonging to the family Oleaceae .It is small, shrubs that grow up to 10m in heights [8]. It has various pharmacological activities, including antioxidant and hepatoprotective mechanisms has been established elsewhere [7].

I. Material and Methods

Collection and Authentication of the Stem Bark

Nyctanthes arborescens bark were collected from local area of village from Basti and Ambedkar Nagar and authentication by National Botanical Research Institute and under guidance of Dr. Manmeet Singh Saluja, Professor SunRise University, Alwar, Rajasthan, India.

Preparation of Crude Drug for Extract

Nyctanthes arbortristis bark were kept and dried under shade and grounded. The powder were passed through sieve No. 40 and stored in an airtight container for the extraction [8].

Preparation of extracts of Nyctanthes arbortristis

The grounded and sieved stem barks about 500gm were sequentially extracted using petroleum ether, chloroform, acetone and ethanol and distilled water in soxhlet apparatus. Materials were concentrated after about forty siphons of each solvent extraction step [9] and aqueous extract (the percentage yielded being approximately 19.2%).

Pharmacological Evaluation:

Animals

The female wistar albino rats (150-200 g) of approximately the same age were procured from CSIR-CDRI, Lucknow, Uttar Pradesh. Polypropylene cages were used for housing of animals, standard rodent nutrition along with water ad libitum and an alternate cycle of twelve hours of darkness and light were accommodated their sustenance. The medication given orally by methods of orogastric cannula. Creatures were exposed to fasting for least 12 hours, before any of the investigation performs, techniques for try were presented for the assessment of the institutional Animals Ethical Committee were passed by the equivalent. Giving to CPCSEA rules for care of research facility of creatures and the moral rule for examinations of exploratory agony in cognizant creatures, tests were acted in morning.

Acute toxicity study

This study were carried out the basis OECD423 guidelines, aqueous extract of Nyctanthes arbortristis stem bark was found to nontoxic up to 5000mg/kg hence the LD₅₀ was 5000mg/kg and ED₅₀ 500mg/kg was selected as dose for the study.

Experimental Design

The animals' Female Wistar albino rats weighing (150-200 g) were divided into four groups consisting of 6 animals in each. Animals were fed with basal diet and water throughout the experimental period [10] [11] [12].

- **Group I:** Received water (5 ml/kg, p.o.) for 9 days once a daily, and served as normal control.
- **Group II:** Received water (5 ml/kg, p.o.) for 9 days once a daily and paracetamol (1 g/kg, p.o.) on the 7th day.
- **Group III:** Received standard drug silymarin (25 mg/kg, p.o.) for 9 days once a daily and paracetamol (1 g/kg, p.o.) on the 7th day.
- **Groups IV:** Received all extract (500 mg/kg) for 9 days once a daily and paracetamol (1 g/kg, p.o.) on the 7th day.

Histopathological study

On the last day, after 24h of dose, 6 mice from each group were dissected and studies histological change occurs due to hepatohepatotoxicant [16]. For fixation of tissues, ten percent formalin buffer was used. This sample was then fixed in paraffin wax. After cooling, tissues were cut into 5µm section. By using hematoxylin and eosin stains, the cut tissues sections were stained. Cover tissue with cover slip and by using light microscope tissues were investigated.

Statistical analysis

All the values were expressed as mean ± SEM (standard error of mean) for six mice. Statistical implication of contrasts between the control and investigational sets was evaluated by One-way ANOVA. The value of probability values (P < 0.05) as compared to the control group [14].

II. Results and Discussion

Effect of Nyctanthes arbortristis extracts on physical parameters:

The results of the research work reveal a hepatoprotective effect of AQENA in rat. The extract exhibited significant (p < 0.05)

hepatoprotective activity against the paracetamol induced liver models of toxicity by improving liver function, as indicated by the restoration of physical and biochemical parameter of liver compared with the control group.

In paracetamol treated rats, enlargement of liver was observed, which were evident of increase in

the liver weight. The groups treated with **AQENA** (500 mg/kg, p.o) and silymarin showed significant restoration of liver weight nearer to normal and significant reduction in liver volume, shown in Table 1.

Table 1: Effect of *N. arbortristis* extracts on physical parameters paracetamol induced hepatotoxic rats.

S.No.	Treatment/ Dose	Liver weight (wt/100gm bw)	Liver Volume
1	Normal	6.84 ±0.06	6.97 ± 0.05
2	Induced(Paracetamol)	8.84 ± 0.48*	9.02 ± 0.49*
3	Standard (Silymarin)	7.02 ±0.46***	7.36 ± 0.49***
4	AQENA(500mg/kg)	7.38±0.80***	7.59 ± 0.83***

Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with paracetamol induced group and * P<0.05, when compared with normal group.

Effect of *Nyctanthes arbortristis* extracts serum marker enzymes parameter

Table 2: Effect of *N. arbortristis* extracts on serum marker enzyme parameters paracetamol induced hepatotoxic rats.

S.No.	Treatment/ Dose	SGPT U/L	SGOT U/L	ALP U/L
1	Normal	62.0 ± 3.71	168.04 ± 2.80	190.0 ± 8.01
2	Induced (Paracetamol)	154.8 ± 8.64*	248.4 ± 9.24*	360.20 ± 8.82*
3	Standard (Silymarin)	86.86 ± 8.63***	176.16 ± 8.17***	166.35 ± 4.27***
4	AQENA(500mg/kg)	92.24±8.24***	186.48±8.52***	193.0±6.14***

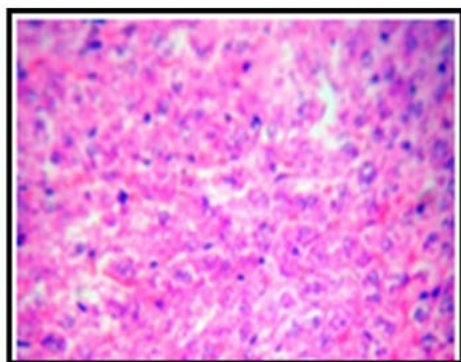
Values are mean ±SEM, n= 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with paracetamol induced group and * P<0.05, when compared with normal group.

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and ALP in hepatotoxicated animals. In treat group with **AQENA** (500 mg/kg, po) and silymarin (25 mg/kg, po) exhibited an ability to counteract the hepatotoxicity by decreasing the elevated level of serum marker enzymes, shown in Table 2.

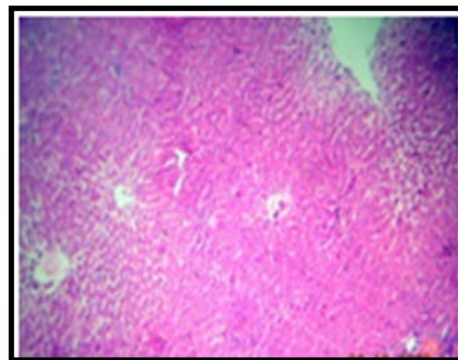
Histopathological Analysis

Histopathologic studies of liver were done by using haematoxylin and eosin staining and development of paracetamol-induced necrosis of hepatocytes in rats. In groups treated with extracts and standard drug, hepatocytes become normal in size with normal portal area observed and show hepatoprotective effect of extracts shown in Figure.

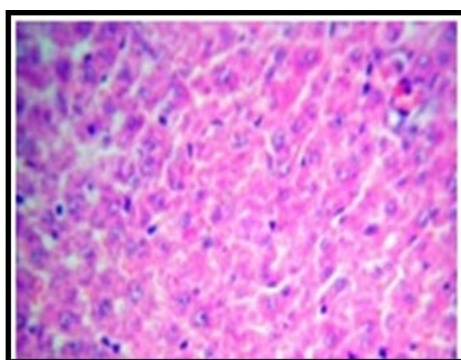
Figure: Effect of selected plant extracts on histopathological diagram of liver tissue in paracetamol induced hepatotoxic rats.



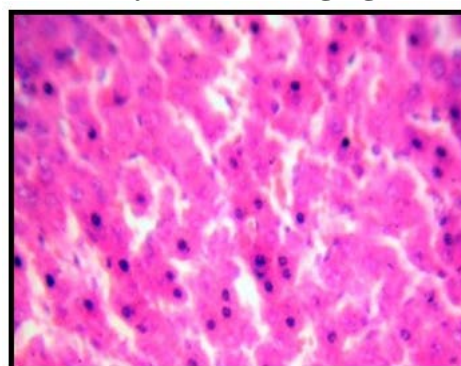
Normal Control



Silymarin (25 mg/kg)



Paracetamol Induced



Aqena (500mg/Kg)

V. Conclusion

The present study demonstrated that the *N.arbortristis* stem bark possesses Hepatoprotective activity against to the paracetamol induced liver toxicity, which requires further extensive studies.

References

1. Kohen, R., Nyska, A., 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.* 30 (6), 620–650.
2. Reed, J.C., 2000. In: Reed, JC (Ed.), *Apoptosis, Method in Enzymology*, 322.
3. Kaplowitz, N., 2001. Drug-induced liver disorders: implications for drug development and regulation. *Drug Saf.* 24 (7), 483–490.
4. Dash, D.K., Yeligar, V.C., Nayak, S.S., Ghosh, T., Rajalingam, D., Sengupta, P., Maiti, B. C., Maity, T.K., 2007. Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. *Trop. J. Pharm. Res.* 6 (3), 755–765.
5. Fraschini, F., Demartini, G., Esposti, D., 2002. Pharmacology of silymarin. *Clin. Drug Investig.* 22 (1), 51–65.
6. Sharma, S.K., Ali, M., Gupta, J., 2002. Plants having hepatoprotective activity. *Phytochem. Pharmacol.* 2, 253–270.
7. Chattopadhyay RR., 2003 Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract. Part II. *J Ethnopharmacol*; 89:217-219.
8. Farnsworth NR. (1966). Biological and phytochemical screening of plants. *J PharmaceutSci.*, 55(3): 225 276.

9. Kokate CK and Gokhale SB., 2008. Practical Pharmacognosy (12th ed). In: Physical and Chemical testes for evaluation of crude drugs, Nirali Prakashan, Pune, India, 14 19, 107 111 and 121.
10. Balakrishnan N, Balasubaramaniam A, Sangameswarn B and Bhaskar VH. (2011). Hepatoprotective activity of two Indian medicinal plants from western ghats, TamilNandu. J Nat Pharm, 2(2): 92 98.
11. Sangameswaran B, Deshraj C, Balakrishnan RB and Jaykar B. (2008). Hepatoprotective effects of *Thespesia lampas* Dalz and Gibs in CCl₄ induced liver injury in rats. J Pharma Sci, 7(1): 11 15.
12. Fleurentin J, Hoefler C, Lexa A, Mortier F and Pelt JM. (1986). *Anisotes trisulcus*: Two traditional medicinal plants or Hepatoprotective properties of *Crepis rueppellii*. J Ethnopharmacol., 16(1): 105 111.
13. Kind PRN and King EJ. (1954). Estimation of Plasma Phosphates by Determination of Hydrolysed Phenol with Antipyrine. J. Clin. Pathol., 322 30.
14. Amin A, Lotfy M, Shafiullah M and Adeghate E. 2006., The Protective Effect of *Tribulus Terrestris* in Diabetes. Annals of the New York Academy of Sciences; 1084: 391-401
15. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951). Protein measurement with the Folin phenol reagent, J Biol Chem., 193: 265 275.
16. Yue M, Yu CH, Ren K, Chen W and Li Y. (2006). Transient elevation of hepatic enzymes in volunteers after intake of alcohol. Hepatobiliary Pancreat Dis Int., 5(1): 52 55.