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Hepatoprotective Activity of Stem Bark Aqueous Extract of Nyctanthes Arbortristis

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Conflicts of Interest: Nil

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ABSTRACT

The present study aimed to determine the hepatoprotective activity of aqueous extract of Nyctanthes arbortristis bark (AQENA) using one established rat models. Four groups of rats (n=6) were givens one daily administration of 1ml/kg in 50% of v/v olive oil (negative control), 25mg/kg silymarin (positive control) and AQENA (500mg/kg) for 7 days fallowed by induction of hepatotoxicity using of carbon tetrachloride. Various parameters, such as physical parameters, biochemical parameters and microscopic analysis ware used to evaluate the hepatoprotective activity. Results indicate that bark extract would significantly support hepatoprotective activity and antioxidants activity, therefore requires in further in depth studies.

Keywords: Bark of N. arbortristis, aqueous extract, and hepatoprotective activity

Introduction

The human liver is the most essential visceral organ in body concerned with synthesis excretion, metabolism and detoxification of diverse exogenous and endogenous substances such as drugs [1]. Diseases and other causative agents such as chemicals (ethanol, CCl4, thioacetamide, D-galactosamine; environmental toxins) and drugs that interfere the biochemical parameters of liver [2]. Liver diseases are a problem worldwide, and the conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects, thus, interest and effort have shifted toward herbal medicine to protect liver [3].The plant based herbal formulations containing herbal extracts are executive to liver disorders and sold in the market especially in countries like India, China and Malaysia [4] [5].

Nyctanthes arbortristis is a medicinal plant possesses various therapeutic characteristics belonging to the family Oleaceae. Small shrubs that grow up to 10m in heights [7]. It has various properties including antioxidant and hepatoprotective [6].

Material and Methods

Collection and Authentication of the Stem Bark Nyctanthes arbortristis bark ware 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 ware keep and dried under shade and grounded. The grounded stem bark were sieved through sieve no.40 and stowed in a sealed vessel for extraction [7].

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 [8] 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 libitumand 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 ware carried out the basis OECD423 guidelines, aqueous extract of stem bark was found to non-toxic 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 [9] [10] [11].

Group I: Received water (5 ml/kg, p.o.) for 9 days once daily, and served as normal control.

Group II: Received water (5 ml/kg, p.o.) for 9 days once daily and carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) on the 7 th day.

Group III: Received standard drug silymarin (25 mg/kg, p.o.) for 9 days once daily and carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) on the 7 th day.

Groups IV: Received AQENA extract (500 mg/kg) for 9 days once daily and carbon tetrachloride (1 ml/kg in 50% v/v olive oil, s.c.) on the 7 th 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 [12]. 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 rats. 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 [13].

Results and Discussion

Effect of Nyctanthes arbortristis extracts on physical parameters in CCl4 induced hepatotoxicrats.

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 CCl4 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 Table 1 and Table 2.

In CCl4 treated group, and treated group with silymarin(25 mg/kg, p.o.) and AQENA (500 mg/kg, p.o) liver wt and liver valume ware observed to be 9.12 ± 1.28 w/100 gm b.w, and 7.04 ±1.48w and 7.56 ±0.21w/100 gm b.w. and liver valume to be 9.52 ± 1.18 ml, 7.02±1.49 and 7.66±0.28 ml respectively. The extract and std drug treated groups showed significant restoration of liver weight and volume nearer to normal.

Table 1: Effect of selected Plant extracts on Physical Parameters in CCl4 induced Hepatotoxicrats.

S.No.	Treatment/ Dose	Liver weight (wt/100gm bw)	Liver Volume
1	Normal	6.84 ±0.06	6.85±0.07
2	Induced(CCl4)	9.12 ± 1.28	9.52±1.18
3	Standard (Silymarin)	7.04 ±1.48	7.02±1.49
4	AQENA (500mg/kg)	7.56±0.21	7.66±0.28

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 CCl4 induced group and P<0.05, when compared with normal group.

Effect of *Nyctanthes arbortristis* extracts on biochemical parameters in CCl4 induced hepatotoxicrats.

In CCl4 treated group, and treated group with silymarin(25 mg/kg, p.o.) and AQENA (500 mg/kg, p.o) total bilirubin and total protein content were obverted to be 9.20 ± 0.24mg/dl, 0.54 ± 0.20mg and 0.60 ± 0.60mg/dl respectively and total protein found to be 6.02 ± 1.46gm/dl, 9.24 ± 1.26gm and 8.71 ± 1.24gm/dl respectively. The extract and std drug treated groups showed significant increase in total bilirubin and significant reduction in total protein content.

Table 2: Effect of selected plant extracts on biochemical parameters CCl4induced hepatotoxic rats

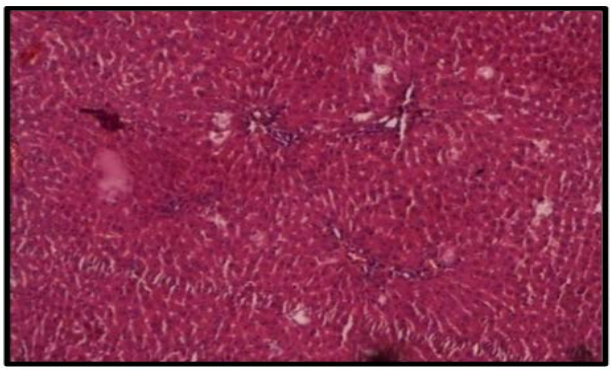
S.N.	Treatment/ Dose	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
1	Normal	0.38 ±0.06	9.57 ± 0.24
2	Induced(CCl4)	9.20 ± 0.24	6.02 ± 1.46
3	Standard (Silymarin)	0.54 ± 0.20	9.24 ± 1.26
4	AQENA (500mg/kg)	0.60±0.60	8.71±1.24

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 CCl4 induced group and P<0.05, when compared with normal group.

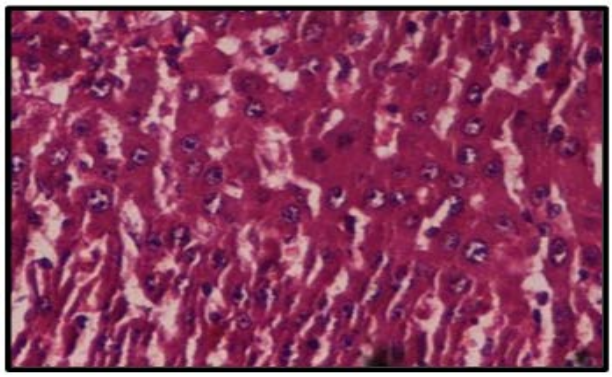
Histopathological Analysis

Histopathologic studies of liver ware done by using haematoxylin and eosin staining and development of CCl4-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. Figure.3, 4.

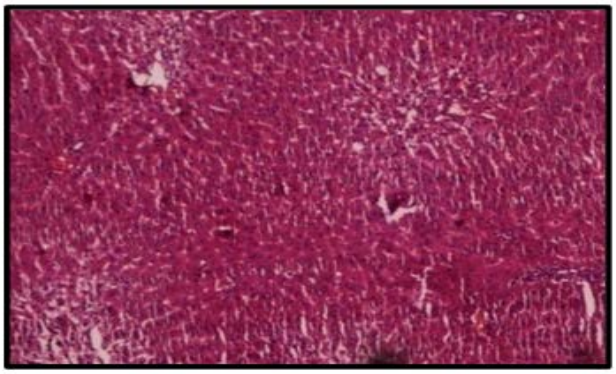
Effect of selected plant extracts on histopathological diagram of liver tissue in CCl4 induced hepatotoxic rats.



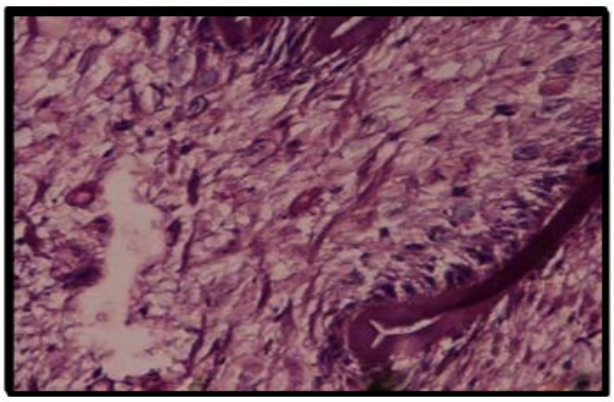
Normal: The architecture is normal. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei. There is no periportal inflammation.



Silymarin(25mg/kg): Hepatocytes have shown normal size with normal portal area. There is mild increase in fibrous connective tissues with minimal sign of hepatotoxicity. Regenerative activity is maximum.



Silymarin(25mg/kg): Hepatocytes have shown normal size with normal portal area. There is mild increase in fibrous connective tissues with minimal sign of hepatotoxicity. Regenerative activity is maximum.



AQENA (500mg/kg): The central veins show mild dilatation and congestion. The hepatocytes are normal. The portal triads appear normal.

Conclusion

The Present Study Demonstrated That The *N.Arbortristis* Stem Bark Possesses Hepatoprotective Activity Against To The Ccl4 Induced Liver Toxicity, Which Requires Further Extensive Studies.

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