

HEPATOPROTECTIVE AND ANTIPYRETIC EFFECT OF BARK OF *NYCTANTHES ARBORTRISTIS* Linn.

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ABSTRACT

Traditionally the different parts of *N. Arboortristis* Linn like leaves, flower, seeds, stem are used for the treatment of different diseases like malarial fever, bronchitis, mouth ulcer, gum bleeding, throat pains, high blood pressure etc. In the present investigation the hepatoprotective and antipyretic activity of petroleum ether and methanol extracts of bark of *N. Arboortristis* (PeNa and MNa) were evaluated using CCl₄ induced hepatotoxicity and Brewer's Yeast induced pyrexia model in mice respectively. 100 mg/kg and 200 mg/kg of PeNa and MNa were considered for evaluation of hepatoprotective and antipyretic activity in the mice. The hepatic toxicity was induced in the liver of the mice by injecting CCl₄. The function of the liver was determined by measuring the level of Serum Glutamate Pyruvic Transaminase (SGPT), Serum Glutamate Oxaloacetic Transaminase (SGOT), Alkaline phosphatase (ALP), Direct Bilirubin (DB) and Total Bilirubin (TB) in the serum of the mice. The silymarin was used as standard drug. The dose of 200 mg/kg of MNa was found more effective in hepatoprotection than 100 mg/kg of PeNa, 200 mg/kg of PeNa and 100 mg/kg of MNa in mice. The rectal temperature of the mice was measured at 0, 1, 2, 3 hour after drug administration. Aspirin (150 mg/kg) was used as standard drug and it was found that the groups which received PeNa and MNa (100 mg/kg and 200 mg/kg) showed significant decrease in rectal temperature after its administration.

Keywords: *Nyctanthes arboortristis* Linn, Hepatoprotective, Antipyretic, Petroleum ether extract of bark of *N. Arboortristis*, Methanol extract of bark of *N. Arboortristis* Linn.

INTRODUCTION

Nyctanthes arboortristis Linn (Oleaceae) is well known medicinal plant used throughout the world. It is found growing in the forests of sub- Himalayan regions, from India to Nepal including Assam, Bengal, Madhya Bharat and Southwards to the Godavari and cultivated in many parts of India⁵. It is easily propagated by seeds and cuttings of the branches. This species is easy to cultivate in a very wide range of ecological circumstances.

MATERIALS AND METHODS

Plant material

The bark of *N. Arboortristis* was collected from Southern Assam, India. The plant was identified by the experts of Botanical Survey of India, Shillong, Meghalaya, India.

Preparation of plant extract

The bark of *N. Arboortristis* were air dried for 6 to 7 days and made into powder form with the help of a blender machine and kept in airtight containers. The powdered plant material was extracted in petroleum ether and methanol solvents using a Soxhlet Apparatus for 48 hours. The petroleum ether and methanol extracts of the barks of *N. Arboortristis* were made solvent free by using a rotary evaporator. The solvent free crude extract was collected and stored below 40C for biological and phytochemical screening.

Phytochemical screening

Alkaloid, Cardiac glycosides, Tannin, Saponin, Terpenoid, Phlobatannins, Fixed oils and fats and Flavonoid in the petroleum and methanol extracts of the bark of *N. Arboortristis* were identified by the standard methods^{6, 16, 18, 2 & 3} respectively and shown in table 1

Experimental animals

In this investigation healthy Swiss albino mice having weight of 25-30 gm were procured from Pasture Institute, Shillong, Meghalaya and were maintained at animal house of Department of Life Science and Bioinformatics, Assam University, Silchar. The animals were fed with standard pellet diet and supplied sterile water ad libitum. The

cleaning of the cages, animals and sterilization of the diet, water containers and disinfection of animal house was also undertaken periodically. The experiments and procedure used in study were approved by the Institutional Animal Ethics Committee (IAEC), Assam University, Silchar.

Chemicals

Silymarin (Sigma chemicals), 2,4 Dinitro-phenylhydrazine, Sodium hydroxide, Sodium pyruvate, Trichloroacetic Acid, Aminonaphtholsulphonic Acid, Stannous chloride, Ammonium molybdate, caffeine sodium benzoate, EDTA, Sulphanilic acid, Sodium nitrite, Ascorbic acid, . Alkaline tartrate Brewer's Yeast, Digital thermometer were purchased from Merk India Ltd, India.

Assessment of hepatoprotective activity

The mice were divided into 5 groups of 6 animals in each group (n=6). The group I served as normal control group which received olive oil (1ml/kg) on 2nd and 3rd day. Group II served as toxic group which was given CCl₄ (2ml/kg) in a ratio of 1:1 with olive oil on 2nd and 3rd day subcutaneously. Group III served as standard group, received Silymarin (100 mg/kg) all five days and a single dose of CCl₄ (2ml/kg) subcutaneously on 2nd and 3rd day after 30 minutes of silymarin administration. Group IV and group V received 100 mg/kg and 200mg/kg of petroleum ether extract of bark of *N. Arboortristis* orally all five days and a dose of CCl₄ (2ml/kg) subcutaneously on 2nd and 3rd day after 30 minutes of extract administration. Group VI and group VII received 100 mg/kg and 200mg/kg of methanol extract of bark of *N. Arboortristis* orally all five days and a dose of CCl₄ (2ml/kg) subcutaneously on 2nd and 3rd day after 30 minutes of extract administration respectively. The food was withdrawn on preceding night of the experiment^{9 & 17}.

Determination of enzyme level

Serum Glutamate Pyruvic Transaminase (SGPT), Serum Glutamate Oxaloacetic Transaminase. (SGOT), Alkaline phosphatase (ALP), Direct Bilirubin (DB) and Total Bilirubin (TB)^{14,7 & 4} respectively.

Assessment of antipyretic activity

Fever was induced by injecting 20% aqueous suspension (10ml/kg) of Brewer's Yeast (North -East chemicals, Guwahati) subcutaneously

into the dorsum region of the animals. The initial rectal temperature of the mice were recorded before Yeast injection using a digital thermometer and again measured the rectal temperature of the mice after Yeast injection (+18 hrs) using the same thermometer. Only mice that showed an increased in the rectal body temperature 0.70C were used for the further experiment to evaluate the antipyretic activity of the plant (bark of *N. Arboortristis*). The animals were divided into 10 Groups containing 6 animals (n=6) in each group. Group (i) served as control group (received only distilled water),

Group (ii) received as standard drug (Aspirin 150 mg/kg) orally, Group (iii) received the Petroleum ether extract of bark of *N. Arboortristis* (100 mg/kg) orally, Group (iv) received Petroleum ether extract of bark of *N. Arboortristis* (200mg/kg) orally, Group (v) received methanolic extracts of bark of *N. Arboortristis* (100mg/kg) orally, Group (vi) received methanolic extracts of bark of *N. Arboortristis* (200mg/kg) orally respectively. Again the rectal temperature of the animals were recorded by the digital thermometer at an interval of 1, 2, 3 hrs after drug administration.^{15&11.}

Table 1: It shows phytochemical screening of petroleum ether and methanol extracts of *N. Arboortristis* (PeNa & MNa).

Name of the plant	Extracts	Alkaloid	Flavonoid	Fixed oils and fats	Saponin	Tanin	Phlobatannins	Cardiac glycosi-des	Terpenoids
<i>Nyctanthes arboortristis</i> L	Petroleum ether	-	-	+	+	-	-	-	+
<i>Nyctanthes arboortristis</i> L	Methanol	-	+	-	-	+	-	-	+

+ = Present, - = Absent.

Statistical analysis

The mean value of Serum Glutamate Pyruvic Transaminase (SGPT), Serum Glutamate Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP), Direct bilirubin and Total bilirubin content in blood and rectal temperature of the animals shown by the plant extracts (PeNa & MNa) was estimated. The standard error mean was also calculated for control, standard and test groups. One way Analysis of Variance (ANOVA) of the results and post-hoc (SNK) test were carried out using a statistical package (SPSS version 17.0),

RESULTS

On preliminary phytochemical analysis of the petroleum ether extract of bark of *N.arboortristis* shown the presence of Fixed oil and fats, Saponin and Terpenoids. The methanol extract of bark of *N. Arboortristis* shown the presence of Flavonoid, Tannin and Terpenoid as represented in table 1. In table 2 the SGPT, SGOT, ALP, DB and TB level of the CCl4 treated animals were found

higher than the normal, silymarin and test extracts (PeNa and MNa) treatment groups indicating the liver cells damage of the animals. After administration of silymarin and PeNa and MNa (100mg/kg & 200 mg/kg) to the animals the serum marker enzymes (SGPT, SGOT, ALP, DB and TB) showed significantly decreased ($p < 0.001$ & $p < 0.05$). But the SGPT, SGOT, ALP, DB and TB level of the silymarin treatment animals were lower than the plant extract (PeNa and MNa) treatment groups. The minimum level of significance between the groups was found at $p < 0.05$. The SGPT level (U/L) of the PeNa and MNa (100 mg/kg & 200 mg/kg) treatment groups was shown in Fig. (1). The SGOT level (U/L) of PeNa and MNa (100 mg/kg & 200 mg/kg) treatment groups was shown in Fig. 2. The ALP level (U/L) of the PeNa and MNa (100 mg/kg & 200 mg/kg) treatment groups was shown in Fig. 3. The DB level (mg/dl) of PeNa and MNa (100 mg/kg & 200 mg/kg) treatment groups was shown in Fig. 4 and The TB level (mg/dl) of PeNa and MNa (100 mg/kg & 200 mg/kg) treatment groups was shown in Fig. 5

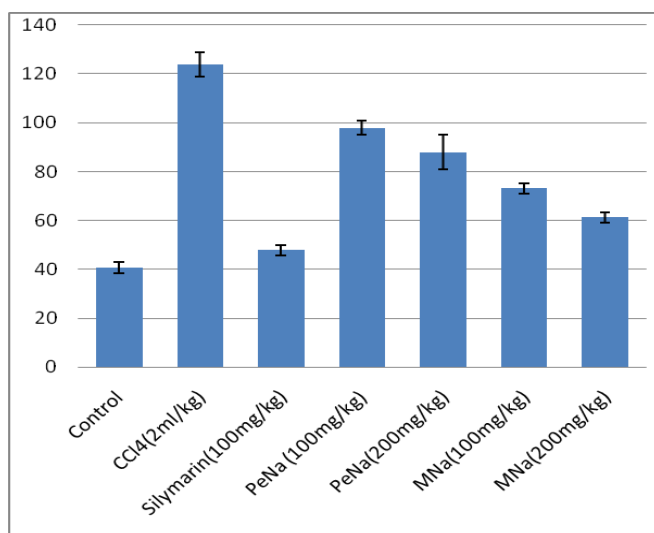


Fig. 1: It shows the Serum Glutamic Pyruvic Transaminase (SGPT) activity treated with bark of *Nyctanthes arboortristis* (PeNa & MNa)

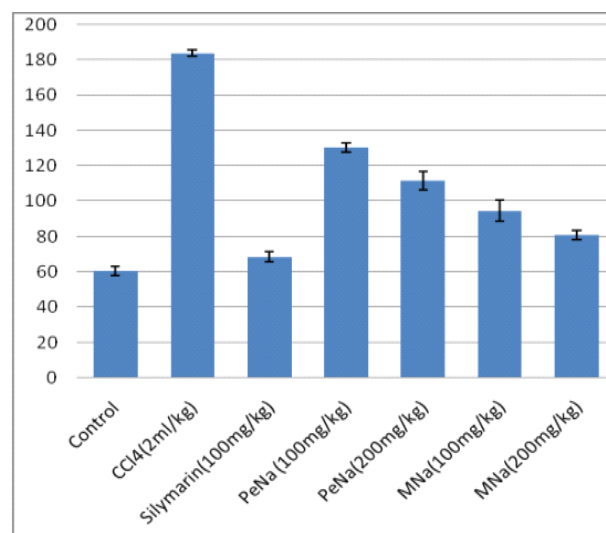


Fig. 2: It shows the Serum Glutamate Oxaloacetate Transaminase activity treated with bark of *Nyctanthes arboortristis* L (PeNa & MNa)

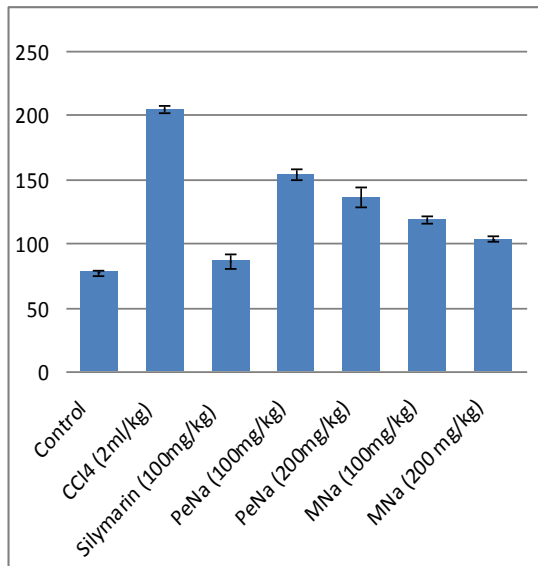


Fig. 3: It shows the Alkaline Phosphatase (ALP) activity treated with bark of *Nyctanthes arbortristis* L (PeNa & MNa)

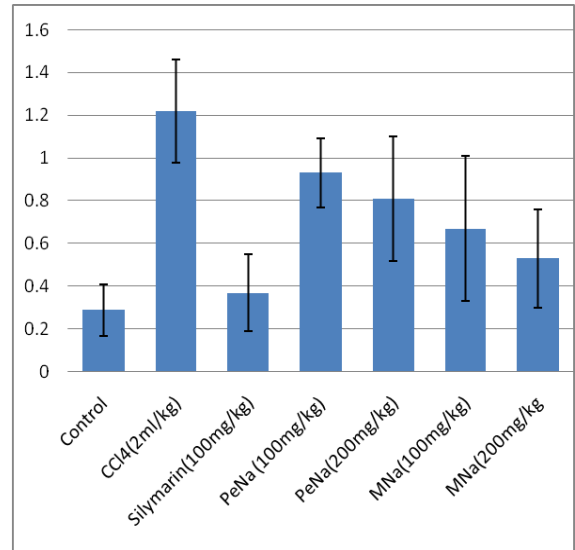


Fig.4: It shows the Direct bilirubin (DB) activity treated with bark of *Nyctanthes arbortristis* L (PeNa & MNa)

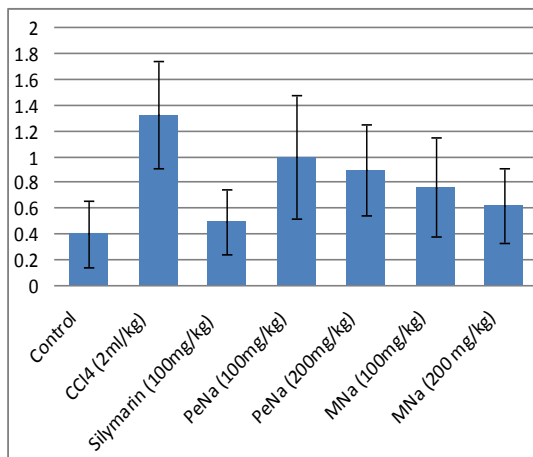


Fig. 5: It shows the Total bilirubin (TB) activity treated with bark of *Nyctanthes arbortristis* L (PeNa & MNa).

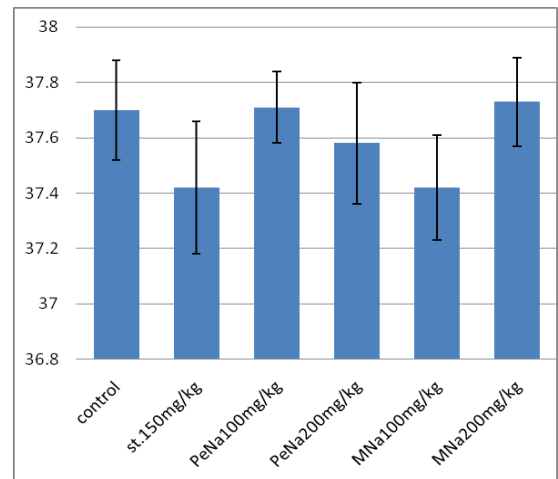


Fig. 6: It shows the initial rectal temperature of the animals before Yeast injection (-18)

In table 3 the rectal temperature of the animals was decreased significantly ($p < 0.001$ & 0.05) at a interval of time 1, 2 and 3 hours after administration of standard drug and plant extracts (PeNa & MNa). The difference of rectal temperature of the control, standard and plant extracts PeNa and MNa (100 mg/kg and 200 mg/kg) groups before pyrexia induced in the animals was shown in fig 6.

The rectal temperature of the control, standard and plant extracts PeNa and MNa (100 mg/kg and 200 mg/kg) groups after yeast injection was shown in fig 7 and the rectal temperature of the control, standard and plant extracts PeNa and MNa (100 mg/kg and 200 mg/kg) groups after 1, 2, 3 hours was shown in fig 8- 10 respectively,

Table 2: It shows the effect of petroleum ether and methanol extract of bark of *N. Arbortristis* on CCl4 induced hepatotoxicity in mice.

Name of the group	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	DB (mg/dl)	TB (mg/dl)
Control	40.72±2.21	60.42±2.78	77.21±2.41	0.29±0.12	0.40±0.26
CCl4 (2ml/kg)	123.96±5.01**	184.06±1.84**	204.93±2.55**	1.22±0.24**	1.32±0.42**
Silymarin (100mg/kg)	47.95±2.10*	68.62±2.65**	86.35±5.49*	0.37±0.18**	0.49±0.25**
PeNa (100mg/kg)	97.96±3.02**	130.37±2.63**	154.30±3.90*	0.93±0.16**	0.99±0.48*
PeNa (200mg/kg)	87.99±7.01*	111.56±5.35**	136.15±8.23**	0.81±0.29**	0.89±0.35**
MNa (100mg/kg)	73.17±2.15**	94.58±6.01**	118.14±2.77**	0.67±0.34**	0.76±0.38**
MNa (200 mg/kg)	61.19±2.04**	80.78±2.66**	103.54±2.48**	0.53±0.23**	0.62±0.29**

Value are mean ±SEM, *statistically $p < 0.05$ & **statistically $p < 0.001$

Table 3: It shows the effect of petroleum ether and methanol extract (PeNa & MNa) of bark of *N. Arborescens* on Brewer's Yeast induced pyrexia in mice.

Name of the group	Dose(mg.kg)	Rectal temperature before yeast injection	Rectal temperature after drug administration			
			0hr (After yeast injection)	1hr	2hr	3hr
Control	-	37.70±0.18	38.37±0.22	38.60±0.13	38.43±0.11	37.93±0.20
Standard	150	37.42±0.24**	38.67±0.15*	36.90±0.28**	36.60±0.24**	36.20±0.25**
PeNa	100	37.71±0.13**	38.25±0.21**	37.87±0.22**	37.68±0.27*	37.46±0.34**
PeNa	200	37.58±0.22**	38.48±0.18**	37.68±0.28**	37.48±0.21*	37.23±0.18*
MNa	100	37.42±0.19**	38.06±0.21**	37.42±0.31**	37.13±0.14**	36.90±0.15**
MNa	200	37.73±0.16**	38.61±0.19**	37.20±0.29**	36.88±0.25**	36.67±0.27**

Value are mean ±SEM, *statistically p<0.05 & **statistically p<0.001

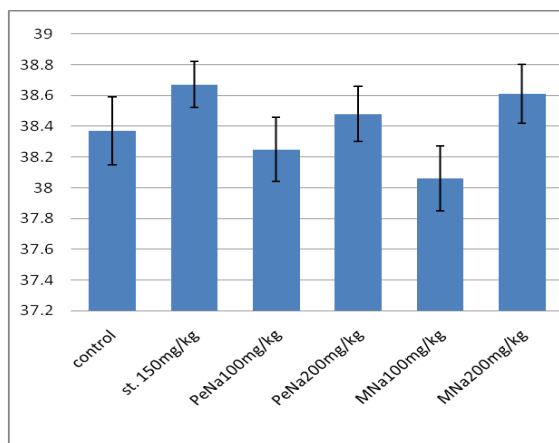


Fig. 7: It shows the rectal temperature of the animals before of the animals after Yeast injection yeast injection (-18). (+18hrs).

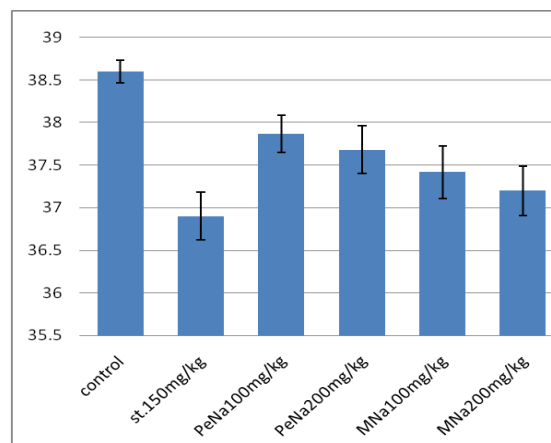


Fig. 8: It shows the rectal temperature of the animals after 1 hr of treated with bark of *Nyctanthes arborescens* L (PeNa & MNa)

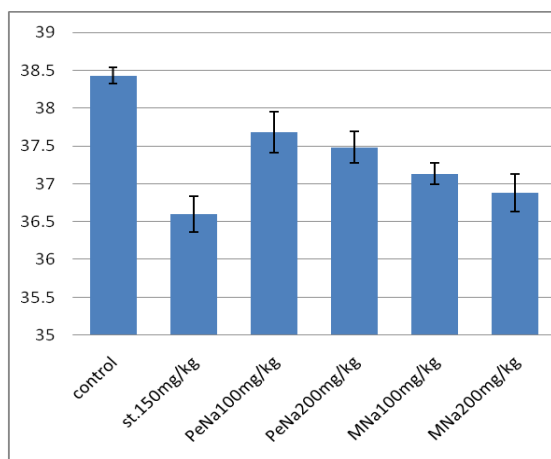


Fig.9: It shows the rectal temperature of the animals after 2 hrs of treated with bark of *Nyctanthes arborescens* L (PeNa & MNa).

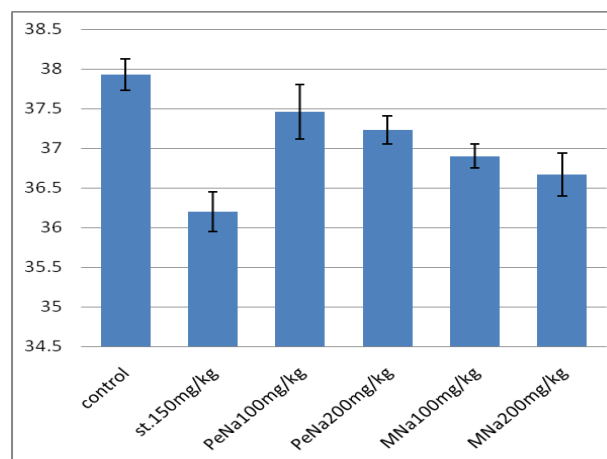


Fig. 10: It shows the rectal temperature of the animals after 3 hrs of treated with bark of *Nyctanthes arborescens* L (PeNa & MNa).

DISCUSSION

The petroleum ether and methanol extracts (PeNa & MNa) of bark of *N. Arborescens* have exhibited the hepatoprotective activity against carbontetrachloride (CCl₄) induced hepatotoxicity and antipyretic activity against yeast induced pyrexia model in mice. The assessment of the liver function were measured by estimating the biochemical parameters SGPT, SGOT, ALP, Direct bilirubin and Total bilirubin level in the blood of the experimental animals. Carbontetrachloride is the most common hepatotoxin used in the experimental study of liver diseases. Carbontetrachloride causes toxicity in liver cells by maintaining semi normal metabolic

functions of the cells and lead to the formation of trichloromethyl free radical which causes the lipid per oxidation of the liver cell, reduction of protein synthesis, elevation of serum transaminase and other liver related enzymes in the blood^{8 & 19}. The SGPT, SGOT, ALP, DB and TB level of the PeNa (100mg/kg & 200 mg/kg) treatment groups showed hepatoprotective activity as shown by the petroleum ether extract of *Jatropha gossypifolia*¹². Again the SGPT, SGOT, ALP, DB and TB level of the MNa (100mg/kg & 200 mg/kg) treatment groups showed hepatoprotective activity as shown by the methanol extract of *Tylophora indica* and *Casuarina equisetifolia*, *Cajanus cajan* and *Bixa orellana*, *Elettaria cardamomum*^{10,1 & 20} respectively.

After 1, 2 and 3 hours of oral administration of standard drug and PeNa and MNa (100 mg/kg & 200 mg/kg) the rectal temperature of the animals decreased significantly. The rectal temperature of the PeNa (100 mg/kg & 200 mg/kg) treatment groups was decreased as shown by the petroleum ether extract of *Centratherum anthelminticum* seed respectively¹³. Again the rectal temperature of the MNa (100 mg/kg & 200 mg/kg) treatment groups was decreased as shown by the methanol extract of methanol extracts of *Capparis zeylanica* L. respectively¹¹. It has been found that the PeNa and MNa (100 mg/kg & 200 mg/kg) possess both hepatoprotective activity against CCl₄ induced hepatotoxicity and antipyretic activity against yeast induced pyrexia in mice. However in both cases the 200 mg/kg showed more effective activity than the 100 mg/kg of the respective extracts.

CONCLUSION

In the above study it has been found that the petroleum ether and methanol extracts (PeNa & MNa) of bark of *N. Arbortristis* possessed the hepatoprotective and antipyretic activity in mice. This is due to presence of some active constituents in the respective extracts. Further study may be required for utility profile of this medicinal plant.

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