

Research Article**Hepatoprotective activity of *Pentatropis nivalis* against Carbon tetrachloride induced hepatic damage in rats**Nilesh P. Babre^{1*}, T. Shivaraj Gouda², Narayanswamy Lachmanan Gowrishankar³¹Department of Pharmacology, VIVA Institute of Pharmacy, Virar (E), Palghar, Mumbai, 401303 India²Department of Pharmacology, NET Pharmacy College, Raichur - 584103, Karnataka, India³Department of Pharmacognosy, Prime College of Pharmacy, Erattayal, Kodumbu, Palakkad - 678551, Kerala, India

Received: 8 June 2020

Revised: 28 June 2020

Accepted: 2 July 2020

Abstract

Objective: To study the hepato protective activity of aerial parts of *Pentatropis nivalis* in CCL₄ induced rats. **Material and methods:** Hepatotoxicity was induced by CCL₄ (2 ml/kg i. p) in rats. Silymarin was used as a standard drug and Methanol extract of *Pentatropis nivalis* 200 mg and 400 mg /kg b. wt. were taken for the study. The degree of protection was determine biochemical parameter like SGOT, SGPT, ALP, Cholesterol, Total Bilirubin and Total Protein level in blood serum. **Results and Conclusion:** The present study revealed that the methanol extract of aerial part of *Pentatropis nivalis* contained alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, tannins, steroids and terpenoids. A significant increase in serum SGPT, SGOT and ALP, Cholesterol, total bilirubin and marked decrease in total protein level was observed in the animals treated with CCL₄ (2 ml/kg i. p.) as compared to normal. Treatment with MEPN (200mg/kg b. wt.) and (400mg/kg b. wt.) for the five days decrease the all the above parameter significantly ($P < 0.001$) Silymarin pretreatment produces significantly ($P < 0.001$) decreases all the parameter when compared with positive control group. The MEPN at the dose of 400 mg/kg b. wt. exhibited significant activity as compared to MEPN at the dose of 200 mg/kg b. wt.. Histopathological study also marked that the improvement in hepatocytes is dose dependent manner. Further study need to isolation and characterization of bioactive molecules in the *Pentatropis Nivalis*.

Keywords: *Pentatropis nivalis*, hepatoprotective, Carbontetrachloride, Silymarin

Introduction

Liver is essential organ of the human body which performs the detoxification of exogenous xenobiotic, drugs, viral infections and chronic alcoholism. The liver is involved with almost all the biochemical pathways to growth, fight against diseases, nutrient supply, energy provision and reproduction (Ward and Daly, 1999). Damage to hepatic parenchymal cells may contribute to physiological roles having a lethal effect (Wolf, 1999). Carbon tetrachloride [CCL₄] is the most commonly used hepatotoxin to study on experimental animals (Johnston and Korening, 1998). CCL₄ is a chemical toxin, which causes free radical mediated toxicity. (Singh et al., 2008) Free radicals induced an oxidative state that can lead to cellular injury with the consequent

alteration in metabolic process. Reactive oxygen species (ROS) plays an important role in the pathogenesis of numerous degenerative diseases have been arises like atherosclerosis, liver damage, kidney and lungs damage, diabetes mellitus & aging (Johnston and Korening, 1998). Hepatic damage induced by CCL₄ is an increase in Serum Glutamate oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase, Alkaline Phosphatase & total bilirubin concentrations. Increases in concentration of SGPT and SGOT are considered to be important markers for hepatic damage (Rabea et al., 2011; Bigoniya et al., 2009).

Herbal drugs plays an important role in the management of various liver disorder and get heals by naturally with speed up. Many medicinal plants and their formulations are used for liver disorder in ethno medicinal practice (Gedara et al., 2003). The *Pentatropis nivalis* (J. F. Gmel) D. V. Field & J. R. I. Wood belonging to the family Asclepiadaceae. In folklore medicine it is has been documented that *Pentatropis nivalis* is used for curing piles, inflammation, leucoderma, biliousness

***Address for Corresponding Author:**

Nilesh P. Babre

Late Shri Vishnu Waman Thakur charitable Trust's

VIVA Institute of Pharmacy, At. Shirgaon, Veer Sawarkar Road, Virar (E) Taluka: Vasai, Dist. Palghar, Mumbai, Maharashtra, India

Email: nileshbabre@gmail.com

DOI: <https://doi.org/10.31024/ajpp.2020.6.6.3>2455-2674/Copyright © 2020, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

(Ayurveda), gonorrhoea, emetic, kapha (Kirtikar and Basu, 1987). This plant extract was evaluated for its antioxidant property (Babre et al., 2018) and other species of plants shows anti-inflammatory, analgesis, anti-ulcers were reported. Keeping this view the present study was carried out on methanolic extracts of aerial part of *Pentatropis nivalis* (Asclepiadaceae) for its hepatoprotective effect.

Material and methods

Plant collection and authentication

Aerial part of *Pentatropis nivalis* was collected from Keshavshrushsti, Bhayandar, Mumbai, The collected plant material was authenticated by Dr. Rajendra D. Shinde, Head, Department of Botany & Director, Blatter Herbarium, St. Xavier's college, Mumbai. (Herbarium Specimen number NI-1922) The collected plant material was washed with water & then samples were air-dried at room temperature with dehumidifier. Dried samples were ground to powder using a mechanical grinder, and stored in a sealed plastic container.

Extraction method

In brief, 2200 g coarse powder of the sample was extracted in soxhlet extractor with methanol. Then the extract was evaporated by using rotatory vacuum evaporator to get semisolid mass. The concentrated extracts of aerial part of *P. nivalis* were stored in air tight close container in dark place (Harbone, 1973).

Phytochemical analysis

Phytochemical study was carried out for Methanolic extract of Aerial part of *P. nivalis* to detect the presence of different class of phytoconstituents like flavonoids, tannins, saponins, glycosides etc. by using standard procedure (Thripati et al., 2010; Ayoola et al., 2008).

Experimental animals

Healthy Wistar rats (150-180 g) were used from our animal house facility. Animals were housed separately in polycarbonate cage (29 cm × 22 cm × 14 cm) under standard laboratory conditions with Food and water *ad libitum*, and lights were on from 07:00 to 19:00 h. Experimental protocol was approved by the institutional animal ethics committee, approval no. (IAE/SKIPS/2016/03) and conformed to the Indian National Science Academy guidelines for the use and care of experimental animals in research.

Acute toxicity study and dose findings studies: (OECD Guidelines-423, 2004)

Acute toxicity study for the methanolic extract *P. Nivalis* was carried out on Mice according to OECD guidelines. Three mice were fasted overnight and maintained with water *ad libitum*. Each animal received single dose of MEPN (2000 mg/kg, p.o.)

was given to each animal. The animals were under observation for 1h and then 6 h and eventually 24 h up to 14 days for any toxicological symptoms and mortality.

Hepatoprotective activity

In the present activity, the dose dependent study of the MEPN was evaluated in albino rats, the rats were randomly assigned in to five groups and each group consists of six individuals' animals.

Group I: Normal control where the animals were administered 1ml of 2 % acacia solution of P.O. for five days and olive oil 2ml/kg on 2nd and 3rd day.

Group –II: Positive control (+ ve Control) where the animals were administered 1ml of 2 % acacia solution P.O. for five days.

Group –III: Where the animals were administered MEPN 200 mg/kg b. wt. P.O. for five days.

Group-IV: Where the animals were administered MEPN 400 mg/kg b. wt. P.O. for five days.

Group -V: Standard control, where the animals were administered with Silymarin 50 mg/kg. b. wt for five days.

Induction of Hepatotoxicity

Carbon tetrachloride was administered by Intra peritoneal route by mixing with olive oil (1:1). Upon this 2nd and 3rd day, group II, III, IV and V received carbon tetra chloride at a dose 2ml/kg b. wt. i. p. (Bagban et al., 2012).

Collection of blood Sample

After 30 minute's drugs treatment. Blood samples were collected on 6th day by retro orbital plexus route for evaluating various biochemical parameters (Yadav et al., 2008) for the assessment of various biochemical parameters, blood was centrifuged at 4000 rpm for 15 min and serum was collected. Serum were used to determine the biochemical parameters like serum glutamic oxalo-acetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), alkaline Phosphatase (ALP), total bilirubin, cholesterol and total protein using commercially available kits (Span Diagnostics Ltd., Surat, India). Overnight rats were fasted. The animals were then sacrificed by cervical dislocation and the liver was removed for histopathological studies.

Histopathology

The animal were sacrificed and liver was collected and wash with normal saline water & stored in 10% of formalin solution for histology to evaluate the hepato-architecture. Study was done by dissecting the liver and observed cellular damage to the liver cells and hepatic veins for microscopy. Sections were prepared and stained with hematoxylin (H) and eosin (E) dye for photo microscopic

evaluation (100X) including cell necrosis, fat migration, ballooning, degeneration, and central vein infiltration.

Statistical analysis

All the results were Statistical analyzed as Mean \pm SEM (n=6) were performed with one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test.

Results

Preliminary qualitative phytochemical analysis

The present study revealed that the methanolic of aerial part of *Pentatropis nivalis*. The percentage yield of MEPN was found 6.4 g% w/w. The phytochemical analysis of extract contained alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, saponins, tannins, steroids and terpenoids.

Acute toxicity studies

The methanol extract of *P. nivalis* did not cause any mortality when the dose is administered 2000 mg /kg. b. wt. and hence 1/10th and 1/5th of the maximum dose were chosen for the present study.

Hepatoprotective Study

Effect of MEPN on liver enzymes level in CCL₄ induced hepatotoxicity rats

A significant increase in serum SGPT, SGOT and ALP level was

Table 1. Phytochemical evaluation of methanol extract of aerial parts of *Pentatropis nivalis*

S. NO	Plant Constituents	MEPN
1	Alkaloids	+
2	Cardiac glycosides	++
3	Flavonoids	++
4	Glycoside	+++
5	Phenols	++
6	Saponins	++
7	Steroids	+++
8	Tannins	++
9	Terpenoids	++

Table 2. Effect of MEPN on enzyme SGOT, SGPT and ALP level in blood serum of CCL₄ induced hepatotoxicity

Groups	Treatment	Parameters		
		SGOT (U/L)	SGPT (U/L)	APL (U/L)
Group I	Normal Control	113.0 \pm 6.923	82.33 \pm 2.171	64.00 \pm 2.436
Group II	Positive Control(CCL ₄)	375.0 \pm 4.313	238.0 \pm 5.132	184.0 \pm 4.258
Group III	MEPN 200 mg/kg	265.0 \pm 5.477**	183.2 \pm 2.587***	175.5 \pm 3.096**
Group IV	MEPN 400 mg/kg	170.8 \pm 4.729***	122.7 \pm 5.840***	136.2 \pm 1.600***
Group V	Standard control (Silymarin)	122.5 \pm 5.439***	100.0 \pm 2.595***	89.50 \pm 3.160***

All groups were compared with positive control group. All the values were expressed Mean \pm SEM, Values were represented with statically significance *** P <0.001, ** P <0.01, * P <0.05 (where n=6 animals per group) All the values were analyzed by one way ANOVA followed by Bonferroni's multiple comparisons test.

observed in the animals treated with CCL₄ (2 ml/kg i.p) as compared to normal. Pretreatment with MEPN (200mg/kg b.wt.) and (400mg/kg b.wt.) for the five days decrease the all the above parameter significantly (P <0.001) as elicited in table 2 and figure 1. Silymarin pretreatment produces significantly (P <0.001) decreases all the parameter when compared with positive control group.

Effect of MEPN on measurement of Cholesterol, total bilirubin and total protein level in CCL₄ induced hepatotoxicity rats

From the observed results a significant increase in serum level Cholesterol, total bilirubin and marked decrease in total protein was observed in the animals treated with CCL₄ (2 ml/kg i. p.) as compared to normal. Pretreatment with MEPN (200mg/kg b. wt.) and (400mg/kg b. wt.) for the five days decrease the all the above parameter significantly (P <0.001) as elicited in Table 3 and figure 1. Silymarin pretreatment produces significantly (P <0.001) decreases all the parameter when compared with positive control group. The MEPN at the dose of 400 mg/kg b. wt. exhibited significant activity as compared to MEPN at the dose of 200 mg/kg b.wt.

Histopathological observations

Histopathological studies were represented in the **figure 2**. In the observation group I (A) the liver section of normal rats shows the normal liver lobular structure and no pathological changes were observed in the normal control group. Group II (B) in the positive control i.e. CCL₄ treated group shows that there was a degeneration of hepatocytes, necrosis and loss of cellular boundaries. Group III and IV (C, D) after the administration MEPN at the dose of 200 and 400 mg/kg b. wt. these histopathological changes were remarkably reversed and seen with lesser degeneration of hepatocytes. Group V (E) Standard Control (Silymarin) significantly normalized these effects. Silymarin and MEPN 400 mg/kg b. wt. treated group were correlates al the serum biochemical results.

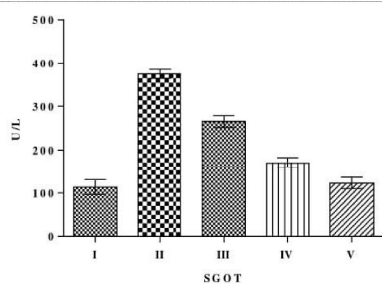


Figure A: SGOT

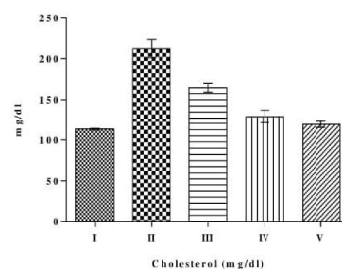


Figure D: Cholesterol

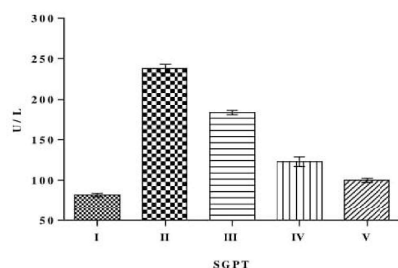


Figure B: SGPT

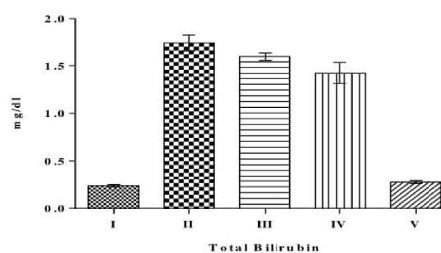


Figure E: Total Bilirubin

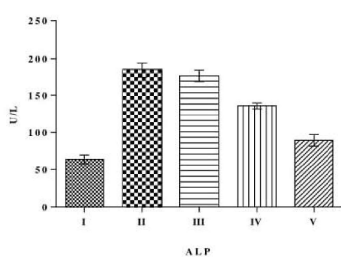


Figure C: ALP

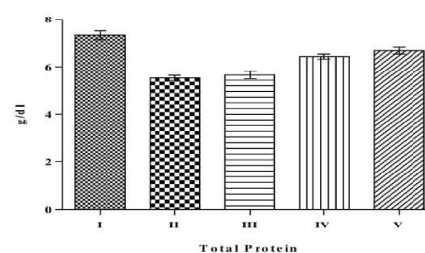


Figure F: Total protein

Figure 1. Effect of MEPN on enzyme level of SGOT, SGPT, ALP and measurement of cholesterol, total bilirubin and total protein level in blood serum of CCL₄ induced hepatotoxicity: Group –I: Normal control, Group –II: Positive control, Group –III and IV: Where the animals were administered MEPN 200 and 400 mg/kg b. wt. Group -V: Standard control

Table 3. Effect of MEPN on Cholesterol, total bilirubin and total protein level in blood serum of CCL₄ induced hepatotoxicity

Groups	Treatment	Parameters		
		Cholesterol(mg/dl)	Total Bilirubin(mg/dl)	Total Protein (g/dl)
Group I	Normal Control	113.2±0.9098	0.2345± 0.01159	7.322±0.1823
Group II	Positive Control(CCL ₄)	212.2±4.622	1.720±0.08198	5.533±0.1085
Group III	MEPN 200 mg/kg	164.5±2.217***	1.597±0.03981 ^{ns}	5.660±0.1590 ^{ns}
Group IV	MEPN 400 mg/kg	129.3±3.373***	1.423±0.1124**	6.435±0.1042**
Group V	Standard control (Silymarin)	119.0±1.633***	0.2712±0.01522***	6.683±0.1470***

All groups were compared with positive control group. All the values were expressed Mean ± SEM, values were represented with statically significance *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ (where n=6 animals per group) All the values were analyzed by one way ANOVA followed by Bonferroni's multiple comparisons test

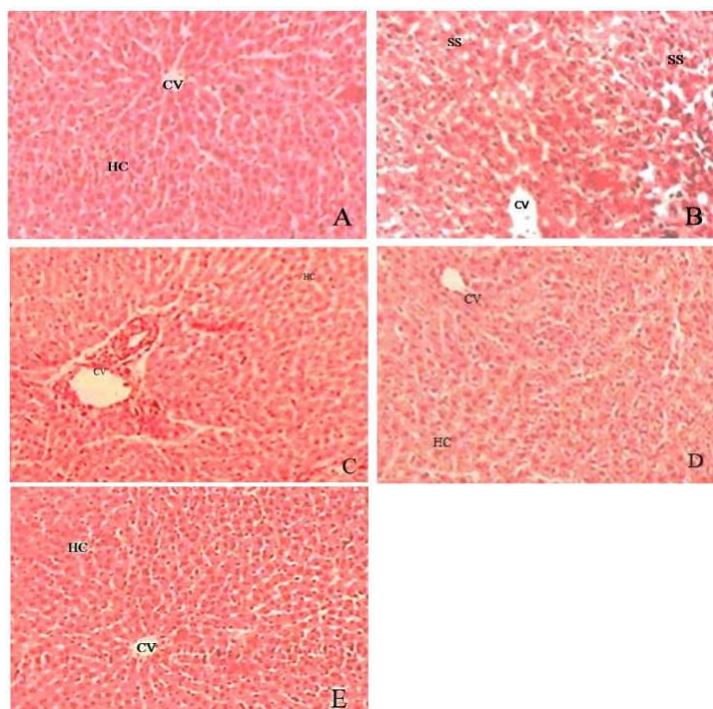


Figure 2. Histopathological architecture in the liver of control and experimental groups (A) Normal Control, (B) Positive control (CCL₄), (C) MEPN 200 mg /kg b.wt. (D) MEPN 400 mg /kg b. wt and (E) Standard Control (Silymarin); where CV: Central Vein, HC: Hepatic Cell and SS: Sinusoidal Space

Discussion and Conclusion

Aerial part of *Pentatropis nivalis* was collected, authenticated, dried and ground to powder. Further plant material was extracted with methanol by soxhlet method. Phytochemicals screening of was performed which showed the presence of Glycoside, steroids, saponins, flavonoids, phenols and terpenoids. The extract was subjected to acute toxicity study according to guide line of OECD 423, no lethality was observed at 2000mg / kg b.wt. Based upon the review of literature finding that *P. Nivalis* possesses good antioxidant property (Babre et al., 2018). The primary Phytochemical extract TLC (Thin layer Chromatography) shows the presence of flavonoids, terpenoids and phenol class of compound (Babre et al., 2018). The presence of flavonoids and phenol is known for its antioxidant and hepatoprotective capacity (Di Carlo et al., 1999). The hepatoprotective study was conducted on CCL₄ induced hepatotoxic in rats. The result was observed in dose dependent manner methanol extract of *Pentatropis Nivalis* 400 mg/kg b. shows significant results as compared to 200mg /kg b.wt. these finding indicate that the plant possesses good hepatoprotective property when compared to Positive control (CCL₄) Histopathological architecture also support to all the biochemical parameter.

Conflict of interest

None

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