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Research Article

HEPATOPROTECTIVE ACTIVITY OF *NELSONIA CANESCENS* (LAM.) SPRENG ON ACUTE HEPATOTOXICITY INDUCED BY PARACETAMOL

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ABSTRACT

The methanol extract (MLE) of the plant *Nelsonia* canescens (Lam.) Spreng at different doses (150, 300 and 500 mg/kg, b.w.) was tested for its efficacy against paracetamol induced acute hepatic damage in Wistar rats. The different groups of rats were administered with paracetamol (2 gm/kg, p.o.). The rats were monitored for morphological changes, biochemical changes of serum Glutamate Oxaloacetate Transaminase (GOT), serum Glutamate Pyruvate Transaminase (GPT), serum Alkaline Phosphatase (ALP), serum Gamma Glutamyl Transferase (GGT), serum Cholesterol, serum Bilirubin (Total and Direct) and for histopathological changes. Activity of the MLE on paracetamol induced lethal effect in mice was also studied. From the experimental results it was proved that the plant possesses hepatoprotective potency in a dose dependent manner and the dose 500 mg/kg has significant effect in reducing the damage caused by paracetamol; which was comparable to the protective effect of standard drug Silymarin (100 mg/kg, b.w.). The phytochemical screening revealed the presence of active phytoconstituents i.e. flavanoids and phenolics, which may offer hepatoprotection. The present work support the traditional claim of plant in the treatment of liver injury, may provide a new drug against a war with liver diseases.

Keywords: *Nelsonia canescens* (Lam.) Spreng, Hepatoprotective, Silymarin, Paracetamol

INTRODUTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful material. It's typical position and functions make it the most essential organ but also prone to number of diseases[1].

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. In India, numerous medicinal plants and their formulations are used in ethnomedical practices and traditional system of medicine for liver disorders. However, we do not have satisfactory remedy for serious liver diseases; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues. Liver disease has become a global concern worldwide[2].

Nelsonia canescens (Lam.) Spreng (Acanthaceae) (syn. *Justicia brunelloides* Lam.) is commonly known as Sunga-pat (Garo) and Paramul (Bengali). This plant is common in moist and shady forest floors. It is an erect or diffused villious herb. Decoction of the whole plant is used for the treatment of wounds, diarrhoea, syphilis, gastric problems, blister, and boils on tongue[3]. Fresh juice of the whole plant is taken orally in difficult delivery[4]. The whole plant is used as a pest protectant for the storage of maize and sorghum by farmers of tropical African zone[5]. Pharmacologically this plant has anti-inflammatory, analgesic and antioxidant properties[6]. The people of Garo community of Goalpara district use this plant in hepatic troubles. The present study aims on the investigation of the hepatoprotective properties of *Nelsonia canescens* (Lam.) Spreng to support the claim of the folk medicine.

MATERIALS AND METHODS

Plant materials

The plant was collected from Simlitola village of Goalpara district, Assam, India. The plant was identified in the Department of Botany, Gauhati University, Guwahati, Assam, and India. The whole plant was shade dried and exhaustively extracted in methanol (MLE, yield 7.45%). The extract was suspended in 0.5% Tween-80 in distilled water and administered p.o.

Experimental Animals

Wistar rats (180-210 gm body wt.) of both sexes and Swiss albino mice (20-25 gm body wt.) of both sexes were used. Animals were obtained from the animal house of Deptt. of Zoology, Gauhati University for experimental purpose. They were maintained on a standard normal diet, provided with water ad libitum and maintained at ambient room temperature (25°C ± 2°C). The study was approved by the animal ethics committee and all the ethical norms were strictly followed during the experiment.

Chemicals

Pure sample of Silymarin was obtained from sigma chemicals, USA, paracetamol was purchased from Remidex Pharma Pvt. Ltd. Tween-80 (Polyoxy methylene sorbitan monoleate) and diethyl ether was obtained from Merck India Ltd. The assay reagents of the GOT (Glutamate oxaloacetate transaminase), GPT (Glutamate pyruvate transaminase) and ALP (Alkaline phosphatase) was obtained from Merck India Ltd. and assay reagents for GGT (Gamma glutamyl transferase) and Cholesterol was obtained from Pointe Scientific, Inc. U.S.A. and lastly Direct bilirubin and Total bilirubin was obtained from Ranbaxy laboratory, Mumbai, India. Analytical grades of reagents were used.

Phytochemical screening

A number of qualitative phytochemical tests were carried out to detect the presence of volatile oils, alkaloids, tannins, saponins, flavanoids, glycosides, steroids, terpenoids and phenols utilizing standard methods of analysis[7, 8, 9]. The intensity of the colouration determines the abundance of the compound present.

Acute toxicity study in mice and evaluation of preliminary toxicity of plant extracts of *Nelsonia canescens* (Lam.) Spreng (LD-50 study)

Albino mice of both sexes were taken for this experiment. Mice were divided in six groups (n=6) and were given different doses of plant extract (p.o.) (150, 300, 500, 1000, 2000, 3000 mg/kg, b.w.) for four consecutive days and their mortality, loss of body wt. and general behaviour was recorded from the first dose up to 72 hours after the last administration of plant extract. One group was taken as the control group and was administered with normal saline (p.o.) [10].

Paracetamol (Pcml) induced lethality study in mice

Preliminary experiments were performed on mice to estimate the protective effect of the MLE (150, 300 and 500 mg/Kg b.w.) against a lethal dose of Pcml (1gm/Kg, p.o.)[11]. Pcml was suspended in 40% (w/v) aqueous sucrose soln. Mice were divided in 4 groups having 10 animals of both sexes in each. Three MLE treated groups (Group-I, II and III) were treated with MLE (150, 300 and 500 mg/Kg, p.o. respectively) followed after 1 hr by oral administration of Pcml. The fourth group (Group-IV) served as control and received the same treatment except that normal saline (0.9 % NaCl) was administered instead of MLE. The mortality was observed for 24 hr post-administration of Pcml.

Paracetamol (Pcml) induced toxicity study in rat

Rats were divided in six groups, with six animal per group (n=6). Pcml was suspended in 40% (w/v) aqueous sucrose soln. and administered (p.o.) at a dose of 2 gm/Kg[12]. Group-I (normal control) received single daily dose of 5% tween-80 (5 ml/Kg, p.o.) for 4 days & a single dose of 40% sucrose soln. (1 ml/rat, p.o.) on day 3. Group-II (paracetamol control) received single daily dose of 5% tween-80 (5 ml/kg, p.o.) for 4 days & a single dose of Pcml suspension (p.o.) on day 3. Test Groups (Group-III, IV, and V) received daily dose of extract suspension (p.o.) for 4 days (150, 300, 500 mg/Kg) & a single dose of Pcml suspension (p.o.) on day 3. Group-VI (reference group) received daily dose of silymarin (100 mg/Kg, p.o.) for 4 days & a single dose of Pcml suspension (p.o.) on day 3. Animals were sacrificed under mild ether anesthesia 48 hrs after Pcml administration on day 5. The body wt. of each animal before experiment start and just before sacrifice was recorded. After sacrifice the liver wt. of each animal was also recorded[13]. Blood and liver tissues were collected for further study.

Collection and histological study of liver

Livers of sacrificed animals were collected just after the sacrifice and thoroughly perfused in ice-cold saline. The livers were fixed in 10% Formosaline (10% v/v formaldehyde in normal saline) for 48 hrs. The livers were embedded in liquid paraffin following the standard microtechnique[14]. 5µ thick sections of paraffin embedded liver were used for staining (Delafield's hematoxylin and eosin stain) following routine histological procedure. The slides were examined under light microscope.

Collection of blood for biochemical analysis

Animals of all the groups of rats were sacrificed by cervical dislocation after mild Diethyl ether anesthesia on the stipulated time. Blood was collected from the carotid artery. Blood samples were kept for 30 min, and then centrifuged at 3000 rpm for 15 min. The serum was separated out for biochemical studies.

Statistical analysis of the data

Values are expressed as Mean ± SEM & significance of inter-group differences of each parameter was analyzed separately using the one way analysis of variance (ANOVA) and $P<0.05$ was considered to be significant. Significance within the group was analyzed using Student's t test and $P<0.01$and $P<0.001$ was considered to be significant[15].

Calculation of hepatoprotection (%)

Percentage of hepatoprotection for each biological parameter was calculated as follows assuming that there was no protection (100% damage) in Pcml control group[12].

% Hepatoprotection = 100 – [{(100/ (Toxin control – Normal control)} x (MLE or silymarin and toxin – Normal control)]

RESULTS

The results of the preliminary phytochemical analysis showed the presence of volatile oil, alkaloids, tannins, flavanoids, glycosides and phenols.

Methanolic extract of *Nelsonia canescens* (Lam.) Spreng did not produce any toxic symptoms or mortality upto the dose level of 3000 mg/kg body weight in mice, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

The study of paracetamol induced lethality is a preliminary study of the hepatoprotective properties of any substance. The substance which possesses hepatoprotective potentiality reduces the mortality rate by paracetamol. This may be due to direct protective influence on the liver cells caused by the substance. The 500 mg/Kg dose of MLE provided maximum protection in comparison the other two lower doses (70%).

Table 1: Preliminary phytochemical screening of the plant *Nelsonia canescens* (Lam.) Spreng

Plant sample	Phytochemical groups	Present = + , Absent = –
Nelsonia canescens (Lam.) Spreng	Volatile oils	+
	Alkaloids	+
	Tannins	+
	Saponins	-
	Flavanoids	+
	Glycosides	+
	Steroids	-
	Terpenoids	-
	Phenols	+

Table 2: Effect of *Nelsonia canescens* (Lam.) Spreng plant extracts on paracetamol induced lethality study in mice

Group	Treatment	Mortality (%)
I	MLE 150 mg/Kg + Paracetamol (1gm/Kg)	90
II	MLE 300 mg/Kg + Paracetamol (1gm/Kg)	70
III	MLE 500 mg/Kg + Paracetamol (1gm/Kg)	30
IV	Saline + Paracetamol (1gm/Kg)	100

The morphological results of the liver revealed that there was an increase in the liver wt. per 100 gm of final body wt. of the Pcml treated groups due to the blocking of secretion of hepatic triglycerides into the plasma[16]. The administration of the MLE significantly lowered down the elevation ($P<0.01$) in a dose dependent manner and the MLE (500 mg/kg) significantly ($P<0.1$, $P<0.01$ using Student's t-test) lowered down the liver wt. near to the reference drug silymarin.

The biochemical results showed a marked increase of all biochemical parameters i.e. GOT, GPT, ALP, GGT, Cholesterol, Total and Direct Bilirubin after administration of the given dose of Pcml. As shown in table-3 & 4 the concurrent treatment of MLE 500 mg/kg significantly decreased ($P<0.05$ and $P<0.01$) the elevation of enzymes by the toxin and thus provide satisfactory hepatoprotection in a dose dependent manner which was comparable to the effect of the reference drug Silymarin.

Table 3: Biochemical activity of MLE on serum enzymes (GOT, GPT, ALP, GGT) in rats acutely being toxicated with paracetamol

Group	Dose (mg/Kg)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	GGT (IU/L)
Normal control	-	26.47 ± 0.16	26.87 ± 0.5	47.92 ± 2.92	30.34 ± 2.66
Paracetamol control	2000	129.06 ± 0.25**	126.53 ± 7.3**	159.33 ± 1.77**	122.57 ± 5.85**
MLE [N.S.]	150	87.27 ± 0.2** (40.7%)	98 ± 4.1** (28.6%)	113.3 ± 3.96** (41.3%)	88 ± 1.52** (37.5%)
	300	60.25 ± 0.6** (67.07%)	58.67 ± 5.44** (68%)	82.3 ± 1.12** (69%)	56.7 ± 1.92** (71.4%)
	500	37.69 ± 0.19** (89.06%)	37.33 ± 3.8** (89.5%)	66 ± 3.49** (83.8%)	40.3 ± 2.53** (89.24%)
Reference	100	35.9 ± 0.3** (90.8%)	33.13 ± 1.54** (93.7%)	64.8 ± 2.82** (84.8%)	44.6 ± 2.85** (84.5%)
One way ANOVA	F	14944.86	83.9	206.5	118.99
	df	5, 30	5, 30	5, 30	5, 30
	P	0.05	0.05	0.05	0.05

** P<0.01 as paracetamol control group was compared with the normal control group and the rest other groups were compared with paracetamol control groups using Student's t-test. Values in parenthesis depict the percentage of hepatoprotection against paracetamol toxicity. (Values are Mean± SEM, n=6).

Table 4: Biochemical activity of MLE on serum (Cholesterol, Total and Direct Bilirubin) in rats acutely being toxicated with paracetamol

Group	Dose (mg/Kg)	Cholesterol (mg/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Normal control	-	58.83 ± 1.36	0.46 ± 0.01	0.32 ± 0.01
Paracetamol control	2000	154.22 ± 3.43**	2.4 ± 0.05**	1.67 ± 0.03**
MLE [N.S.]	150	88.15 ± 1.84** (69.3%)	1.1 ± 0.05** (67.07%)	0.81 ± 0.02** (63.7%)
	300	76.88 ± 3.75** (86.83%)	0.74 ± 0.03** (85.6%)	0.54 ± 0.01** (83.7%)
	500	71.39 ± 1.31** (89%)	0.62 ± 0.02** (91.75%)	0.49 ±0.01** (87.4%)
Reference	100	61.18 ± 4.46** (97.5%)	0.49 ± 0.01** (98.5%)	0.42 ± 0.01** (92.6%)
One way ANOVA	F	143.5	482.07	1103.07
	df	5, 30	5, 30	5, 30
	P	0.05	0.05	0.05

** P<0.01 as paracetamol control group was compared with the normal control group and the rest other groups were compared with paracetamol control groups using Student's t-test. (Values are Mean ± SEM, n=6). Values in parenthesis depict the percentage of hepatoprotection against paracetamol toxicity.

Table 5: Biochemical activity of MLE on body wt. and liver wt. (gm) in rats toxicated with paracetamol

Group	Dose (mg/Kg)	Liver wt. in gm/100 gm of body wt.
Normal control	-	2.85 ± 0.06
Paracetamol control	2000	3.45 ± 0.03**
MLE [N.S.]	150	3.38 ± 0.04*
	300	3.19 ± 0.02**
	500	3.14 ± 0.2**
Reference	100	3.11 ± 0.09**
One way ANOVA	F	4.9
	df	5,30
	P	0.05

*P<0.1, **P<0.01, by comparing all groups with the paracetamol control group using Student's t-test. (Values are Mean± SEM, n=6).

Histological studies also provided supportive evidence for the biochemical analysis. Normal control group showed a normal liver architecture, hepatocytes very well arranged, central vein without alterations (Figure 1A). Characteristic histopathological liver lesions produced after the administration of toxic dose of paracetamol were gross necrosis of centrilobular hepatocytes, nuclear pycnosis, karyolysis, spotty and diffuse hyaline necrosis with blood pooling in central vanules were evident. It was also viewed that many cells were at the end stage of death. Livers of animals treated with 500 mg/kg of MLE showed satisfactory protection because there near normal histology were seen. There was no inflammation or necrosis and the central vein architecture was about normal. The histopathological plates of the reference drug silymarin treated liver showed near normal state of liver.

DISCUSSION

Paracetamol or acetaminophen is the most commonly used analgesic and antipyretic drug and overdoses are a leading cause of liver failure today. Paracetamol is used for more than 20 years as a hepatotoxic agent[17]. It is suggested that Pcml may elicit a direct effect on the mitochondrial function before cell injury develops[18].

Toxicity from Pcml is not from the drug itself but from one of its metabolites, N-acetyl-p-benzoquinoneimine (NAPQ1) following metabolism by a number of isoenzymes of cytochrome P_{450} (CYPs), i.e., CYP 2E1[19], CYP 1A2[20], CYP 2A6[21], CYP 3A4 and CYP 2D6[22]. All these involved in drug metabolism. Hepatocyte apoptosis has also been shown to be a significant contributor to paracetamol induced liver injury and to precede necrosis[23]. Reactive oxygen species[24], nitric oxide[25], lipid peroxidation[26] and disordered calcium homeostasis[27] are mechanisms that may have a contributory role in the development of liver injury after Pcml overdoses.

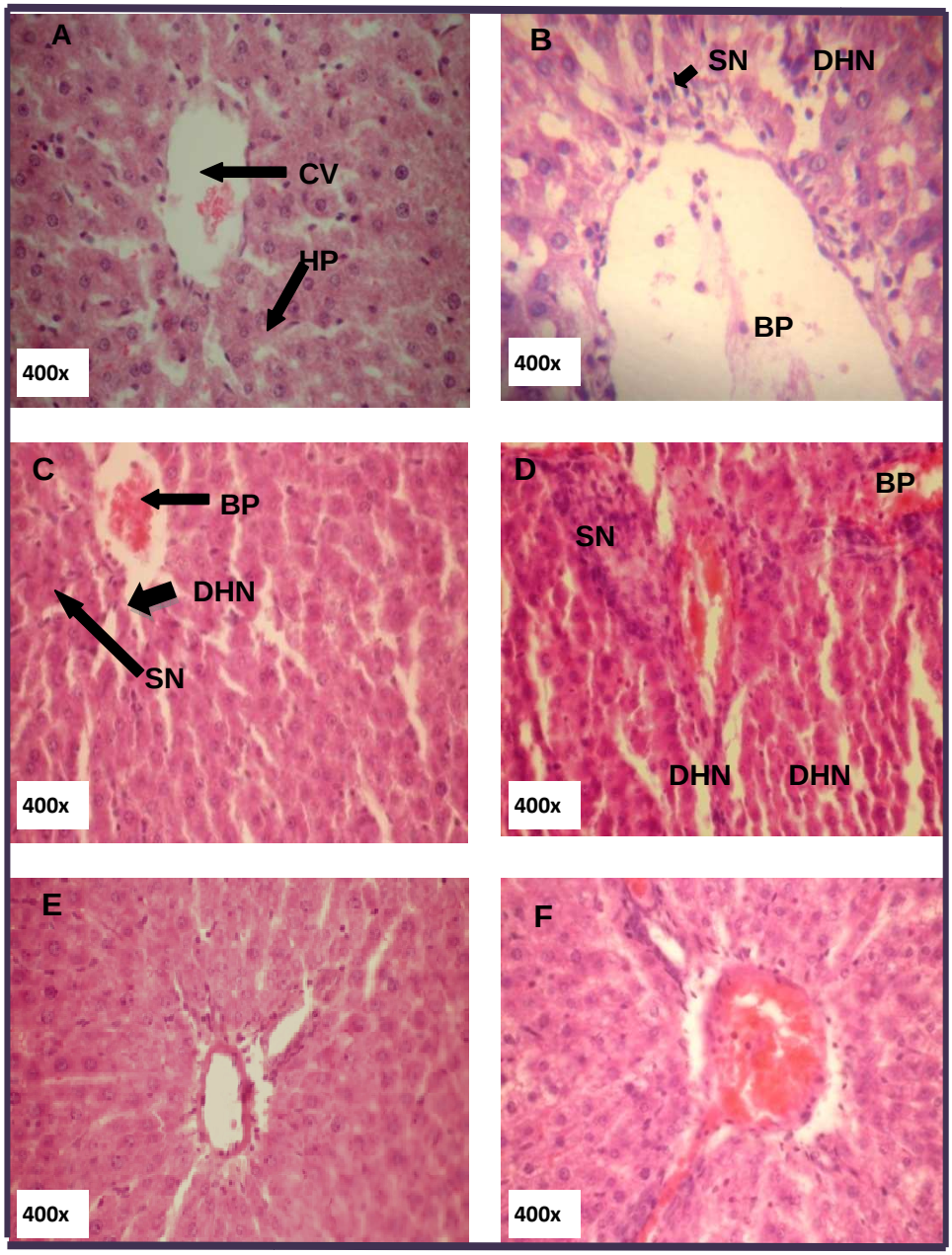

Fig. 1: Photomicrographs of liver slides of experimental animals. (A) Normal liver, (B) Pcml control liver, (C) Pcml toxicated and MLE 150 mg/Kg treated liver, (D) Pcml toxicated and MLE 300 mg/Kg treated liver, (E) Pcml toxicated and MLE 500 mg/Kg treated liver, (F) Pcml toxicated and silymarin treated liver,

CV ----------Central vein
HP ----------Hepatocytes
BP ----------Blood pooling
DHN ---------Diffused hyaline necrosis
SN ---------Spotty necrosis
PCS ---------Perilobular clowdy swelling

The hepatic injury caused by Pcml were manifested by quantitative elevation of serum GOT, GPT, ALP, GGT, cholesterol, direct and total bilirubin level along with histomorphological changes[28- 32]. In the assessment of liver damage by Pcml, the determination of enzyme marker levels such as GPT (ALT) and GOT (AST) is often used. In necrosis or membrane damage, the enzymes are released into circulation and it can be therefore measured in serum as markers of hepatic damage[33]. The GPT catalysis the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Therefore, GPT is more specific to the liver and is thus a better

parameter for detecting liver injury[34]. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver[35]. Similarly, serum ALP and bilirubin level on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis; in presence of increasing biliary pressure has been reported by[36]. The increase in level of serum bilirubin is an index of degree of jaundice. It could be possibly be as a result of increased production, decreased uptake by liver, decreased conjugation, decreased secretion from liver[37]. GGT and ALP are membrane bound enzymes, which are released unequally depending on the pathological phenomenon. The elevation of serum GGT concentrations is regarded as one of the most sensitive indices of hepatic damage. Liver is the organ and involved in the synthesis of lipoproteins and metabolism of cholesterol. In the present study administration of Pcml in rats showed an increase in the level of cholesterol when compared with control rats. The alteration in lipid profiles might result from accumulation of triglycerides, inhibition of bile acid synthesis from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in cholesterol levels[38]. Therefore, suppression of cholesterol levels by the extract suggests that the bile acid synthesis was reversed.

In our study, *Nelsonia canescens* (Lam.) Spreng have shown a good hepatoprotection at a dose of 500 mg/kg which was proved in biochemical studies as well as from histopathological studies. From literature it was found that this plant possess good percentage of total phenolic content and total flavanoids moreover, this plant also possess antioxidant properties. In our experiment also, it was found that this plant contains flavanoids and phenolic components. Earlier researchers also found a correlation between total phenolic content and antioxidant properties[39]. Hence it can be said that the hepatoprotective effects of *Nelsonia canescens* (Lam.) Spreng may be due to its antioxidant properties. The phenolic and flavanoid components present in this plant may play the hepatoprotection role[40].

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