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Research Article

HEPATOPROTECTIVE ACTIVITY OF THE METHANOLIC EXTRACT OF *NELSONIA CANESCENS* (LAM..) SPRENG ON CARBON TETRACHLORIDE INDUCED HEPATIC DAMAGE IN ALBINO RATS

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ABSTRACT

Methanol extract (MLE) of the plant *Nelsonia* canescens (Lam..) Spreng at different doses (150, 300 and 500 mg/kg, b.w) were tested for its efficacy against carbon tetrachloride (CCl_4) induced acute hepatic damage in Wistar rats. The different groups of rats were administered with CCl_4 (1ml/kg, s.c.). 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 histopathological changes. From the experimental results it was proved that the plant showed a very good hepatoprotection in a dose dependent manner and the dose 500 mg/kg has significant effect in reducing the damage caused by CCl_4 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. flavonoids 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, carbon tetrachloride.

INTRODUCTION

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 principles. It is the second largest organ after skin and largest internal organ of the human body. 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 vital role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethno-medical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; 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 Blue pussyleaf (Eng), 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 in 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. So here 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, India. One voucher specimen was deposited there for future reference. The whole plant was shade dried and exhaustively extracted in methanol (MLE, yield 7.45%). The extracts were 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 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. Tween-80 (Polyoxy methylene sorbitan monoleate), CCl_4 and Diethyl ether were obtained from Merck India Ltd. Olive oil (Bertoli) was purchased from local market in Guwahati, Assam, India. 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 qualitative phytochemical test was carried out to detect the presence of volatile oils, alkaloids, tannins, saponins, flavonoids, 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. Qualitative phytochemical analysis of the powder of the plant was determined as follows: for tannins one gm of plant grinded, then sample was boiled in 20 ml of 70% ethanol for 2 min on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. Blue-black precipitate indicated the presence of tannins. For phenol 2 ml of extract was added to 2 ml of ferric chloride solution ($FeCl_3$); a deep bluish green solution was formed with presence of phenols. The test for alkaloids was carried out by subjecting 5 g ground plant material extracted with 10 ml ammoniacal chloroform and 5 ml chloroform. After filtration, the solution was shaken with 10 drops aqueous sulphuric acid 0.5 M. Creamish precipitate indicated the presence of respective alkaloids. For steroids Liebermann-Burchard reaction was applied. Two hundred milligram plant material boiled in 10 ml chloroform and the mixture was filtered; a 2 ml filtrate was added to 2 ml acetic anhydride and concentrated H_2SO_4. Blue-green ring indicated the presence of steroids and red colour indicated the presence of terpenoids. The alcoholic extract (15 ml, corresponding to 3 g of plant material) was treated with a few drops of concentrated HCl and magnesium Ribbon (0.5 g). Pink-tomato red colour indicated the presence of flavonoids. Froth test for saponins was used. The test for saponin was carried out by subjecting 5 gm of the plant powder extracted with 15 ml methanol. After evaporation, residue was shaken vigorously with ethyl ether and 5 ml HCl 2N. Precipitate indicated the presence of saponin. For detection of volatile oils, 1 gm fresh plant sample was boiled in 10 ml petroleum ether, filtered and then 2.0 ml of extract solution was shaken with 0.1 ml dilute sodium hydroxide and a small quantity of dilute hydrochloric acid. A white precipitate indicated the presence of volatile oils[10]. The extract was also tested for free glycoside. Fehling`s solution (A and B) was added to the extract and the solution was heated on a hot plate and brick-red precipitate indicated the presence of glycosides.

Toxicity studies (LD-50)

Wistar rats of both sexes were taken for this experiment. Animals were divided in six groups (n=6) and were given different doses of plant extract (p.o.) (150, 300, 500, 1000, 2000, 3000mg/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 control group and was administered with normal saline (p.o.)[11].

Carbon tetrachloride (CCl_4) induced toxicity study in rat

Rats were divided in six groups, with six animal per group (n=6). CCl_4 in olive oil (1:1, v/v) was administered (s.c.) at a dose of 2 ml/kg body wt. Group-I (Normal control) Group received of 5% Tween-80 (5 ml/kg, b.w., p.o.) on each day for 4 days & two doses of olive oil(1 ml/kg, s.c.) on day-2 and 3. Group-II (CCl_4 control) received 5% Tween-80 like Group-I and given dose of CCl_4 suspension (2 ml/kg, b.w., s.c.) on day-2 and 3. Group-III, IV, V (Test Groups) received extract suspension (150, 300 and 500 mg/kg, p.o.) on each day & CCl_4 suspension (2ml/kg, b.w, s.c.) on day-2, & day-3 and Group-VI (Reference Group) received Silymarin suspension (100 mg kg, p.o.) in distilled water daily & CCl_4suspension (2ml/kg, b.w, s.c.) on day-2 & day-3 .

Animals were sacrificed under mild ether anesthesia on day-5, 48 hrs after CCl_4 administration. 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[12].

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.

Collection and histological study of liver

Liver 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[13]. 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.

Statistical analysis of the data

Values are expressed as Mean ± SEM & significance of inter-group differences of each parameter was analysed separately using the one way analysis of variance

(ANOVA) and $P < 0.05$ was considered to be significant. Significance within the group was analysed using Student's t test and $P < 0.01$ and $P < 0.001$ was considered to be significant[14].

Calculation of hepatoprotection (%)

Percentage of hepatoprotection for each biological parameter was calculated as follows assuming that there was no protection (100% damage) in CCl_4control group[15].

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

RESULTS AND DISCUSSION

The results of the preliminary phytochemical analysis showed the presence of volatile oil, alkaloids, tannins, flavonoids, glycosides and phenols. Methanolic extract of *Nelsonia canescens* (Lam.) Spreng did not produce any toxic symptoms or mortality up to the dose level of 3000 mg/kg body weight in rat, and hence the extract was considered to be safe and non-toxic for further pharmacological screening.

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 CCl_4 treated groups due to the blocking of hepatic triglycerides secretion into plasma. The administration of the MLE significantly lowered down the elevation ($P<0.01$) in a dose dependent manner and the MLE (500mg/kg) notably ($P<0.1$, $P<0.01$, 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 CCl_4. As shown in table-3 & 4 the concurrent treatment of MLE 500 mg/kg considerably decreased ($P<0.05$ and $P<0.01$) the elevation of enzymes by the toxin and the plant thus provides satisfactory hepatoprotection in a dose dependent manner which was comparable to the effect of the reference drug silymarin.

Histological studies also provided supportive evidence for the biochemical analysis. Normal control group illustrated a normal liver architecture; hepatocytes were very well arranged, central vein without alterations (Figure 1a). Histopathological observations on the CCl_4 induced toxicity in liver showed hepatocytes necrosis, swollen hepatocytes with excessive accumulation of fat lobules, fatty and hyaline degradation necrosis of some of the centrizonal hepatocytes along with microvesicular and macrovesicular steatosis, perilobular cloudy swelling and cell vacuolization, (Figure 1b) indicating complete damage of liver architechture. Necrosis and steatosis are the peculiar characteristic of histopathological symptoms in CCl_4 induced toxicity[16,17]. Livers of animals treated with 500 mg/kg of MLE showed satisfactory protection because there was no inflammation or necrosis and fatty degradation was seen.

Liver injuries by CCl_4 are commonly used models for the screening of hepatoprotective drugs[18]. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical[19].

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	-
	Flavonoids	+
	Glycosides	+
	Steroids	-
	Terpenoids	-
	Phenols	+

Table 2: Biochemical activity of MLE on body wt. and liver wt. (gm) in rats toxicated with CCl_4.

Group	Dose (mg/kg)	Liver wt. in gm/100 gm of body wt.
Normal control	-	2.31 ± 0.05
CCl_4 control	2 ml/kg	3.45 ± 0.03*
MLE [N.S.]	150	3.12 ± 0.07*
	300	3.02 ± 0.04*
	500	2.86 ± 0.25*
Reference	100	2.78 ± 0.11**
One way ANOVA	F	8.89
	df	5,30
	P	0.05

*P<0.1, **P<0.01, as CCl_4 control group was compared with the Normal control group and the rest other groups were compared with CCl_4 control groups. (Values are MEAN ± SEM, n=6)

Table: 3 Biochemical activity of MLE on serum enzymes (GOT, GPT, ALP, GGT) in rats toxicated with CCl_4

Group	Dose (mg/Kg)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	GGT (IU/L)
Normal control	-	23.42 ± 1.34	34.5 ± 3.68	47.9 ± 2.91	48.7 ± 4.14
CCl_4 [control]	2 ml/Kg	112.28 ± 5.07**	117.27 ± 4.9**	126.2 ± 8.4**	111.22 ± 5.42**
	150	87 ± 1.05**	87.9 ± 1.69**	95.65 ± 1.8**	93.15 ± 1.26**
MLE [N.S.]	300	51.68 ± 1.9**	57.3 ± 2.58**	82.43 ± 1.12**	74.04 ± 1.22**
	500	38.2 ± 2.08**	39.7 ± 1.2**	66 ± 2.37**	59.63 ± 2.4**
Reference	100	37.45 ± 1.23**	37.2 ± 1.66 **	65.53 ± 2.23**	51.3 ± 0.76**
	F	175.67	131.06	48.9	66.7
One way ANOVA	df	5, 30	5, 30	5, 30	5, 30
	P	0.05	0.05	0.05	0.05

** P<0.01 as CCl_4 Control group was compared with the Normal control group and the rest other groups were compared with CCl_4 control groups using Student's t-test. (Values are MEAN ± SEM, n=6)

Table 4: Biochemical activity of MLE on serum Cholesterol, Total and Direct Bilirubin in rats toxicated with CCl_4

Group	Dose (mg/Kg)	Cholesterol (Mg/dl)	Total Bilirubin (Mg/dl)	Direct Bilirubin (Mg/dl)
Normal control	-	41.2 ± 0.78	0.46 ± 0.01	0.3 ± 0.03
CCl_4 [control]	2 ml/Kg	139.57 ± 1.28**	2.4 ± 0.05**	1.36 ± 0.05**
	150	91.93 ± 1.95**	1.43 ± 0.14**	0.95 ± 0.01**
MLE [N.S.]	300	76.12 ± 1.37**	0.85 ± 0.02**	0.73 ± 0.03**
	500	57.33 ± 0.77**	0.63 ± 0.02**	0.49 ± 0.01**
Reference	100	54.57 ± 0.53**	0.53 ± 0.01**	0.41 ± 0.02**
	F	850.3	76.891	214.24
One way ANOVA	df	5, 30	5, 30	5, 30
	P	0.05	0.05	0.05

** P<0.01 as CCl_4 Control group was compared with the Normal Control group and the rest other groups were compared with CCl_4Control groups using Student's t-test.

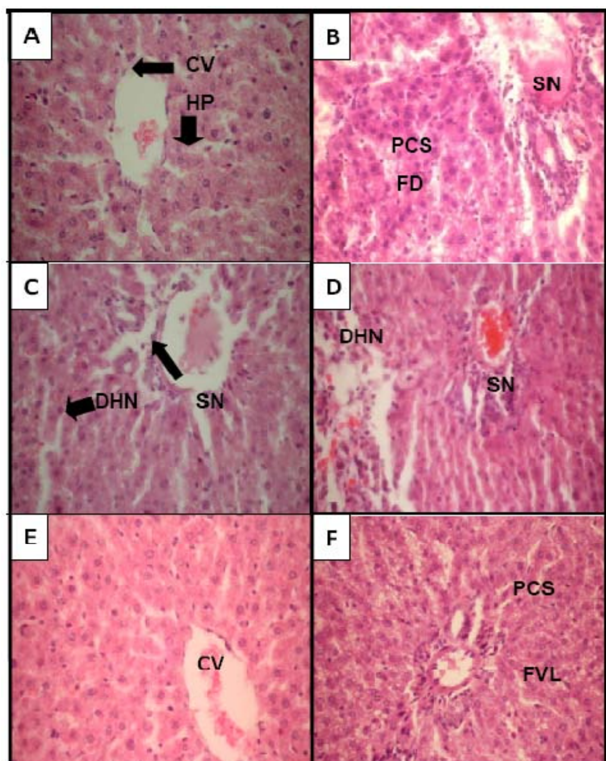

Figure 1: Photomicrograph of liver section of experimental rats (H and E; 400X). (a): Normal liver, (b): CCl_4 control rat liver, (c): CCl_4 toxicated and MLE 150 mg/kg treated liver, (d): CCl_4 toxicated and MLE 300 mg/kg treated liver, (e): CCl_4 toxicated and MLE 500 mg/kg treated liver, (f): CCl_4 toxicated and REF (silymarin) 100 mg/kg treated liver

CV ---------Central vein SN ---------Spotty necrosis
HP ----------Hepatocytes FD --------Fatty degenaration
DHN ----Diffused hyaline necrosis FVL --Fatty vacuolisation
PCS ---------Perilobular clowdy swelling

These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. These leads to the formation of lipid peroxides which in turn give products like melondialdehyde (MDA) that cause damage to the membrane. This is evidenced by an elevation of the serum marker enzymes. The increase in transaminases was the clear indication of cellular leakage and loss of functional integrity of cell. Decrease in serum GOT, GPT after treatment with the extract in liver damage induced by CCl_4 indicated the potency of the extract in normalizing the structural status of the liver and decrease in serum ALP indicated efficacy of the extract in recovering liver cholestasis whereas decrease in bilirubin indicated the effectiveness of the extract in regularizing functional status of the liver. Intoxication with CCl_4 also resulted in inhibition of synthesis of the bile acids from cholesterol which is synthesised in liver or derived from plasma lipids, leading to increase in cholesterol levels. The MLE suppressed the cholesterol level and suppression of

cholesterol levels suggests the inhibition of the synthesis of bile acids from cholesterol is reversed by the extract.

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 flavonoids moreover this plant also possess antioxidant properties. In our experiment also, it was found that this plant contains flavonoids and phenolic components. Earlier researchers also found a correlation between total phenolic content and antioxidant properties[20]. Hence it can be said that the hepatoprotective effects of *Nelsonia canescens* (Lam.) Spreng may be due to its antioxidant properties. The phenolic and flavonoid components present in this plant may play the hepatoprotection role.

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REFERENCES

1. Guyton AC, Hall JE. The liver as an organ. In, Text Book of Medical Physiology, 9th ed. W.B., Sauders Company, Bangalore, 1996, pp 883-888.
2. Handa SS, Sharma A, Chakraborty KK, Natural products and plants as liver protecting drugs, Fitoterapia, LVII (5), 1986, pp 307-351.
3. Adhikari BS, Babu MM, Saklani PL, Rawat GS, Medicinal plants diversity and their conservation status in Wildlife Institute of India (WII) campus, Dehradun. Ethnobotanical Leaflets, 14, 2010, pp 46-83.
4. Focho DA, Ndam WT, Fonge BA, Medicinal plants of Aguambu – Bamumbu in the Lebialem highlands, southwest province of Cameroon, African Journal of Pharmacy and Pharmacology, 3(1), 2009, pp 001-013.
5. Ngamo TSL, Ngassoum MB, Mapongmestsem PM, Noudjou WF, Malaisse F, Haubruge E, Logney G, Kouninki H and Hance T, Use of essential oils of aromatic plants as protectant of grains during storage, Agricultural Journal, 2(2), 2007, pp 204-209.
6. Owoyele VB, Oloriegbe YY, Balogun EA, Soladoye AO, Analgesic and anti-inflammatory properties of Nelsonia canescens leaf extract, Journal of Ethnopharmacology, 99(1), 2005, pp 153-156.
7. Sofowora A, Screening plants for bioactive agents, In, Medicinal Plant and Traditional Medicine in Africa, 2nd ed, Sptrum Books Ltd., Ibadan, 1993, pp 289.
8. Evans WC, An overview of drugs having antihepatotoxic and oral hypoglycemic activities. In, Trease and Evans, Pharmacognosy, 14th ed. U.K., Sanders Company Ltd., 1996, pp 119-159.
9. Haughton PJ, Raman A, Laboratory handbook for the fractionation of natural extracts, 1st ed., Clapman and Hall, USA, 1998, pp 22-52.
10. Dahiru D, Onubiyi JA, Umaru HA, Phytochemical screening and antiulcerogenic effect of *Moringa oleifera* aquous leaf extra, African Journal of Traditional and Complementary Alternative Medicine, 3, 2006, pp 70-75.
11. Handa SS, Sharma A, Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride, Indian Journal of Medical Resarch, 92, 1990, pp 276-283.
12. Sharma A, Singh RT, Sehgal V, Handa SS, Antihepatotoxic activity of some plants used in herbal formulations, Fitoterapia, LXII (2), 1991, pp 131-138.
13. Galigher AE and Kozoff EN, Essentials of practical microtechnique, 2nd ed., Lea and Finger, New York, 1976, pp-210-216.
14. Khan IA and Khanum A, Fundamentals of biostatistics, 1st ed. Ukaaz Publishers, India, 1994, pp-112-130.
15. Subramoniam A, Evans DA, Rajasekharan S, Pushpangadan P, Hepatoprotective activity of *Trichopus zeylanicus* extract against paracetamol - induced hepatic damage in rats. Indian Journalof Experimental Biology, 36, 1998, pp 385-389.
16. Rubin E, Hutterer F, Popper H, Cell proliferation and fiber formation in chronic carbontetrachloride intoxication – A morphologic and chemical study. American Journal of Pathology, 42, 1936, pp 715-28.
17. Rechnogel RO, Carbon-tetrachloride hepatoxicity. Status quo and future prospects. Trends in Pharmacological Sciences, 4, 1983, pp 129-131.
18. Plaa GL, Wewitt WR, Quantitative evaluation of indices of hepatotoxicity. In: Toxicology of liver, Raven Press, New York, 1982, pp 103-120.
19. Srivastava SP, Chen NO, Hotlzman JL, The *in vitro* NADPH dependent inhibition by CCl_4 of the ATP dependent Calcium uptake of hepatic microsomes from male rats. Studies on the mechanism of inactivation of the hepatic microsomal calcium pump by CCl_3. Radical, Journal of Biological Chemistry, 265, 1990, pp 8392-8399.
20. Sawadogo WR, Meda A, Lamein CE, Kiendrebeogo M, Guissou IP and Nacoulma OG, Phenolic Content and Antioxidant Activity of Six Acanthaceae from Burkina Faso. Journal of Biological Sciences, 6 (2), 2006, pp 249-252.

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