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Full Length Research Paper

Hepatoprotective activity of *Nilgirianthus ciliatus* (Nees) bremek in paracetamol induced toxicity in Wistar albino rats

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***Nilgirianthus ciliatus* (Nees) Bremek is used in traditional system of India to treat various diseases. In the present study, the hepatoprotective effect of the methanolic extract of the bark of *N. ciliatus* against paracetamol toxicity (2.5 mg/kg b.wt, p.o) liver damage in Wistar albino rats was evaluated. The test drug was used at two different concentrations as 250mg/kg b.wt p.o, and 500mg/kg b.wt p.o.,and Silymarin (100mg/kg b.wt. p.o) as positive control. The biochemical parameters comprising of liver marker enzymes SGOT, SGPT, ALP levels were measured in all the groups. The study showed the methanolic extract of *N. ciliatus* having significant hepatoprotective effect as evidenced by the decreased liver marker enzyme levels when compared with the control. The histological study on liver, kidney and heart tissues of treated groups showed the supporting evidence for the hepatoprotective activity of plant extract.**

Key words: *Nilgirianthus ciliatus*, hepatoprotection, liver marker enzymes, paracetamol intoxication, Indian medicinal plants.

INTRODUCTION

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. Paracetamol is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Boyd and Bereczky, 1966). Overdose of paracetamol in rats is reported to decrease their sensitivity to its hepatotoxic effects, and associated with oxidative stress (Thamizh Selvam *et al.,* 2011). Paracetamol toxicity is due to the formation of toxic metabolites by ctytochrome P-450 activity (Dahlin *et al.,*1984*)*. Introduction of cytochrome P-450 or depletion of glutathione is a prerequisite for paracetamol induced hepatotoxicity (Moron *et al.,1979;*Gupta *et al. 2006).* Though the modern medicinal system has grown phenomenally, the identification of a hepatoprotective plant-based component against toxicity still remains a dream. Indian Medicinal plants and many herbal formulations belonging to the traditional systems of medicine like Ayurveda, have been investigated as liver protective drugs (Jose and Kuttan, 2007). *Nilgirianthus ciliatus* (Nees) Bremek. (Synonym *Strobilianthes ciliatus* Nees) belongs to the family Acanthaceae. *N.ciliatus* is found to be distributed throughout the evergreen forests of Western ghats of India up to 1200 meters. It is a slender shrub with sub-quadrangular white dotted dark green or purple stems and branches. The roots are reported as bitter, thermogenic and emollient, depurative, expectorant in ancient science literature (Warrier *et al.,* 2002). But still no scientific and methodical investigation has so far been reported in literature regarding its action on the liver. Therefore, the present study has been designed to evaluate the hepatoprotective activity of *N. ciliatus* in paracetamol intoxicated Wistar albino rats.

MATERIALS AND METHODS

Plant Material

The whole plant of *N. ciliatus* was collected from the medicinal plant garden of Oushadi, Thrissur (Government Organization, Kerala). The plant was authenticated by the Kerala Forest Research Organization (KFRI), Peechi,

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Table 1. Effect of methanolic extract of *Nilgirianthus ciliatus* on liver marker enzymes in paracetamol induced liver damage in Wistar rats.

Experimental Group	Biochemical Parameters			
	SGOT (IU/L)	**SGPT (IU/L)**	**ALP (IU/L)**	**Total Protein (g/dl)**
Normal Saline (Control)	133.56 ± 21.61	69.75 ± 8.98	91.82 ± 13.67	8.73 ± 0.75
Normal Saline +Paracetamol (Negative Control)	358.0 ± 22.14*	293.86 ± 10.19*	266.77 ± 9.75**	7.79 ± 1.83
Silymarin + Paracetamol (Standard drug)	164.91 ± 23.85*	74.58 ± 11.77*	117.36 ± 16.47**	7.95 ± 1.16
Extract *N.ciliatus* (250mg/kg)	198.48 ± 32.50**	113.47 ± 21.30*	106.50 ± 11.82*	8.30 ± 0.56
Extract *N.ciliatus* (500mg/kg)	192.62 ± 37.87**	92.76 ± 19.16*	141.13 ± 12.70*	7.84 ± 0.76

Values are Mean ± SEM, n=6 animals in each group. .*p< 0.05, **p<0.01 when Disease control was compared with Healthy control, and Treatments groups were compared with Disease control.

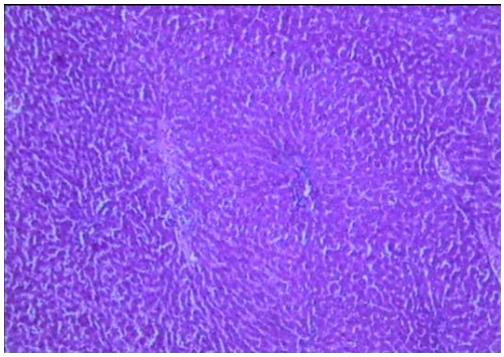

Figure 1.a.Section of Liver of Control group (Normal Saline) showing normal nuclei and cytoplasm. Central venous system and sinusoidal spaces are normal. (40 X)

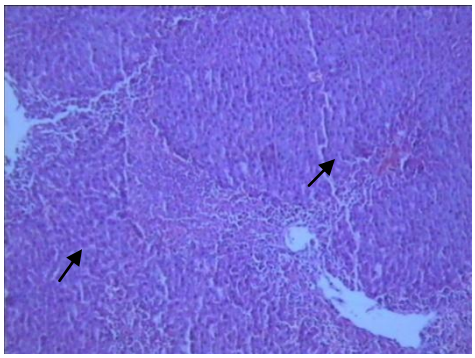

Figure 1.b. Section of paracetamol treated rat liver showing extensive areas of hemorrhage and necrosis in the liver parenchyma, hepatocytes show vacuolated cytoplasm. Collection of inflammatory cells and siderophages are observed. (40 X).

Kerala. The bark material was used for the present study.

Preparation of extract

The bark of *N. ciliatus* were washed thoroughly in tap water and then shade dried and powdered coarsely. The powder material weighing 200 gm was taken for extraction by soxhlet method using 2 lit of methanol, as methanol is highly polar solvent and dissolves most of the phytoconstituents. The hot solvent extraction was carried out for 12 hours (6 hours per day). Methanol was removed by condensation and the extract was collected and stored in refrigerator for the experimental purpose. The extract was suspended in normal saline to the required concentration for the experimental purpose.

Experimental Animals

Thirty numbers of adult Wistar albino rats were purchased from the Laboratory Animal Breeding Station,

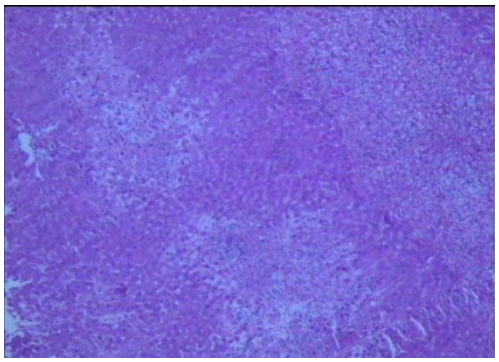

Figure 1.c. *N.ciliatus* treated group showing normal architecture of livers. Portal areas normal. Hepatocytes show vacuolated cytoplasm, some of them mostly towards the central veins. (40 X).

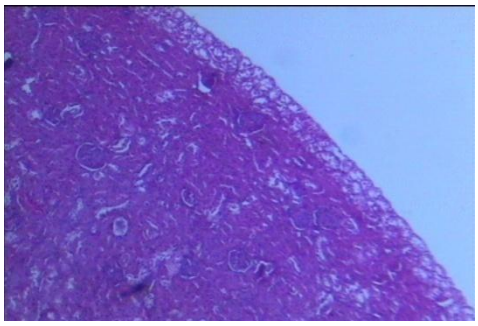

Figure 2.a. Section of kidney of helathy control. Glomeruli are normal with normal cellularity. Renal tubules and interstitial tissue also appear normal. (40 X).

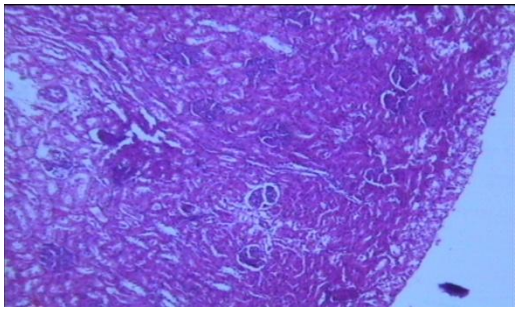

Figure 2.b. Paracetamol group. Glomeruli show edema. Renal tubules show vacuolation of the lining epithelial cells. (40 X).

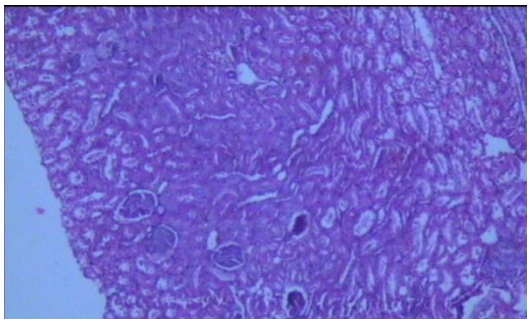

Figure 2.c. *N. ciliatus* group showing normal glomeruli. Interstitial tissues appear normal. (40 X).

Veterinary College, Thrissur, Kerala. Animals of weighing 150-200gm were used for the present study. The rats were divided in to five groups of six animals each comprising of 3 male and 3 female in each group. Animals were housed in polypropylene cages as three animals per cage and maintained under standard laboratory conditions with dark and light cycle. The rats were allowed free access to standard dry pellet (Amrut, Bangalore) and water *ad libitum*. The rats were acclimatized to laboratory conditions for 15 days before commencement of experiment. The study protocol was approved by the Institutional Animal Ethical Committee as per the requirement of CPCSEA guidelines (No.615/03/A/CPCSEA).

METHODOLOGY

Paracetamol (Acetaminophen) (Glaxo) was suspended in normal saline and administered orally at a dose of 2.5g/kg. This dosage is known to cause liver damage in rats (Mitchell *et al.,*1973) [8]. Rats were divided into five groups of six animals in each group. Group I, the normal control was given a singly daily dose of normal saline for 8 days. Group II, the paracetamol control group received a daily dose of normal saline for 8 days and 2 ml of paracetamol (2.5 g/kg b.wt, p.o) suspension on day 8. Group III (Standard/positive control) animals received Silymarin at a dose of 100mg/kg b.wt. p.o on all the days and paracetamol suspension (2.5g/kg) on eighth day, 30 minutes after administration of Silymarin. Group IV and Group V received a daily dose of test extract p.o, for 8 days (250mg/kg and 500mg/kg. b.wt respectively) and paracetamol suspension (2.5g/kg) on day 8, 30 minutes after test extract administration. The animals were sacrificed 48h after paracetamol administration by mild ether anesthesia. The blood samples and organs, liver, kidney, heart were collected for biochemical and histological studies. Liver marker enzymes like Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Serum alkaline phosphatase (ALP) were assayed according to standard methods (Reitman and Frankel *et al.,*1957; Kind and King *et al.,*1954).

Statistical analysis

The data were expressed as mean ± SEM and statistically assessed by one-way analysis of variance.

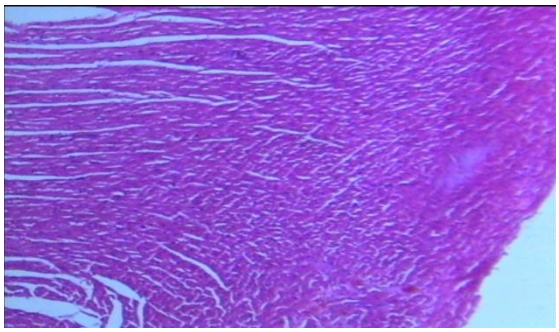

Figure 3.a. Section of heart (Normal saline group) showing normal endocardium and myocardium. (40 X).

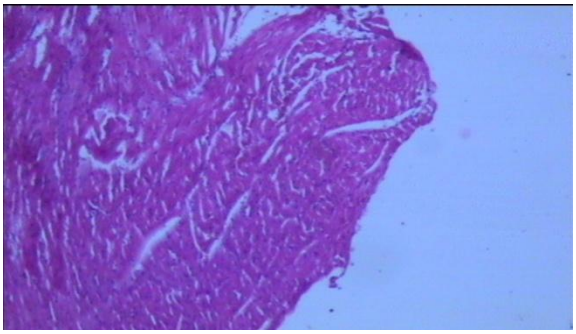

Figure 3.b. Section of heart (paracetamol group) showing normal endocardium and myocardium. (40 X).

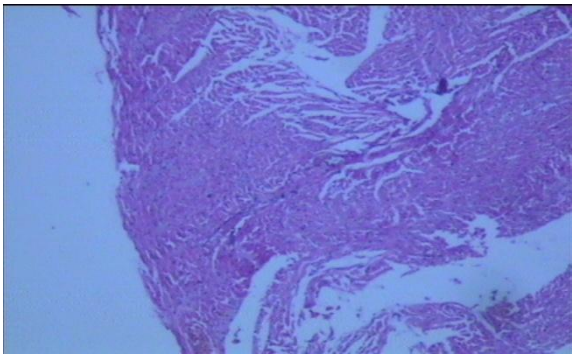

Figure 3.c. Section of heart (*N. ciliatus* treated group) showing normal endocardium and myocardium. (40 X).

RESULTS

Administration of paracetamol (2.5g/kg, p.o) induced a marked increase in the serum hepatic enzyme levels including SGOT, SGPT, ALP as compared to normal control. Pre-treatment of the rats with *N. ciliatus* extract prior to paracetamol administration caused a significant reduction ($P < 0.05$) in the values of SGOT, SGPT and ALP in a dose dependent manner almost comparable with Silymarin treated group (Table 1). The total serum protein level was found to be decreased in the paracetamol group when compared to the normal control, and there was remarkable improvement in treatment groups. The hepatoprotective effect of *N. ciliatus* was confirmed by the histopathological examinations of the liver tissue of control and treated groups. The histological architecture of paracetamol treated group showed extensive areas of hemarrohage and necrosis in the liver parenchyma; hepatocytes showed vacuolated cytoplasm and collection of inflammatory cells and siderophages. The test extracts administered groups showed almost normal architecture of liver and it is comparably with the level of Silymarin treated group (Figure 1a-c). The histological architecture of paracetamol treated kidney section showed edema in glomeruli; renal tubules showed vacuolation of the lining epithelial cells, interstitial tissues showed focal collections of inflammatory cells and areas of hemorrhage (Figure 2a-c). The extract treated group showed normal glomeruli and interstitial tissue comparable with Silymarin. There were not much change observed in the histology of heart tissues in the control groups and treated groups (3a-c). The kidney and heart histology also proved the safety of the test extract as the prescribed dosage.

DISCUSSION

Paracetamol is a common antipyretic agent, which is safein therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses(Kumar and Rex, 1991; Eriksson *et al.,* 1992). Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several several investigations (Ahmed and Khater, 2001;Asha *et al.,*2004; Kumar G *et al.,*2004; Sing and Hand, 1995; Visen *et al.*, 1993)). Liver is highly affected primarily by toxic agents and so that the liver marker enzymes parameters have been found to be of great importance in the assess-

ment of liver damage. Paracetamol is metabolized in the liver to excrete glucuronide and sulphide conjugates (Torrielli, 1978; Jollow *et al*., 1974). An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disturbance caused in the transport functions of hepatocytes (Schmidt et al., 1975). So the present study was designed to evaluate hepatoprotective activity of plant extract by analyzing the liver marker enzymes in the paracetamol induced hepato toxicity in Wistar albino rats. In the present study, overdose of paracetamol caused significant hepatic damage, which was observed through a substantial increase in the concentration of serum parameters. Pre-treatment of the rats with *N. ciliatus* test extract at 250 mg/kg b.wt and 500 mg/kg b.wt concentration for 8 days followed by paracetamol administration resulted in a significant protection of paracetamol induced elevation of serum marker enzymes. The test extract appears to be effective in reducing the injurious effect of paracetamol, observed in the study. This was an indication of stabilization of plasma membrane, as well as repair of hepatic tissue damage caused by paracetamol. The results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Shukla *et al.,* 1992;Thabrew *et al*., 1987). A reduction in total serum protein observed in the paracetamol treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein (Lesch *et al.,*1970). Further, the stimulation of hepatic regeneration was known to make the liver more resistant to damage by toxins. The histological observation basically supported the results from the serum assays as test extract administration reversed to a large extent, hepatic lesions produced by paracetamol. The histology of kidney and heart also showed high possibility for safety of the test extract as there were not any remarkable changes. So, the present study concludes that *N. ciliatus* extract has potent hepatoprotective action upon paracetamol induced hepatic damage in rats.

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