International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 1(3): November-December 2011

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 21-12-2011; Accepted: 23-12-2011

EFFECT OF *NELUMBIUM SPECIOSUM* AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN ALBINO RATS

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Keywords:

Nelumbium speciosum, Carbon tetrachloride, hepatoprotective and hepatotoxicity

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ABSTRACT

The objective of the present investigation is to elucidate the hepatoprotective activity of aqueous extract of *Nelumbium speciosum* against Carbon tetrachloride induced liver damage in rats. The aqueous extract of *Nelumbium speciosum* (50mg/kg) was administered orally to the animals with hepatotoxicity induced by Carbon tetrachloride (0.7ml/kg). The plant extract was effective in protecting the liver against the injury induced by Carbon tetrachloride in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. The hepatoprotective effect of *Nelumbium speciosum* was comparable with the standard drug Silymarin. It was concluded from the result that the aqueous extract of *Nelumbium speciosum* possesses hepatoprotective activity against Carbon tetrachloride induced hepatotoxicity in rats.

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials[1]. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. It is estimated that about 7,500 plants are used in local health traditions in, mostly, rural and tribal villages of India. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1,200 plants[2].

Toxicity of a substance could be defined as the cause injury to living organisms. A highly toxic substance will damage an organism if administered in very small amounts; a substance of low toxicity will not produce an effect unless the amount is very low. Carbon tetrachloride is a clear, manmade liquid with a sweet odor. It is not flammable and does not dissolve in water very easily. It is used in the production of refrigerator coolants, propellents for aerosols, industrial degreasing agent, cleaning fluid, fire extinguishers and in laundry for spot removes. CCl4 is an injury agent for animal experiment, which induced reactive oxygen formation and depleted GSH of phase II enzyme. Liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions[3]. In absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in Ayurveda, the Indian system of medicine, are recommended for the treatment of liver disorders[4].

In the recent years, much work has appeared in the literature about the induction of liver damage by drugs and protection against it by a wide variety of herbal extracts. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical studies can lead to the development of invaluable plant drugs for many dread diseases. In India, hepatitis and other related liver disease are rampant. The hepatitis, related death rate among patients using CCl_4 is increased year by year. Hence it is interesting to report if *Nelumbium speciosum* offers protection against experimentally induced

liver damage by CCl_4 in albino rats. Therefore, the present study is focused to find out some physical (body weight and liver weight) and biochemical (SGOT, SGPT, ALP and Bilirubin) deviation in the experimental animals by induction of CCl_4 and treatment with *N. speciosum* herbal extract. And also to compare these changes between normal, CCl_4 induced animal and herbal treated animals. In addition, the effect of *N. speciosum* against liver damage by CCl_4 was also concluded.

MATERIALS AND METHODS

Nelumbium speciosum and its saline extract

The flower *N. speciosum* were collected around Tiruchirappalli district of Tamil Nadu, India in the month of January and the fresh petals of the flowers were taken and dried in room temperature, the dried petals were powdered. A suspension was prepared with 15 g of dried powder in 500 ml of saline. It is stirred magnetically for 24 h at room temperature. The residue was removed by filtration and the filtrate was dried by freeze drying and used for oral feeding. The yield of the saline extract was about 2 g from 15 g of dried flower.

Animals

Twenty four locally bred adult albino rats (80-100 g) were used. They were housed in clean poly propylene cages and fed with commercial pelleted rat chow and water *ad libitum*. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC.

Experimental procedure

A total of 24 animals were equally divided into three groups of 6 animals each. Group I- served as control, which was treated with pelted diet. Group II treated with CCl_4 at a dose of 0.7 ml per kg body weight intraperitoneally without any vehicle thrice a week for two weeks. The standard drug Silymarin (100mg/kg) was administered to Group III animals for 15 days. Group IV simultaneously treated with *N. speciosum* (50 mg/kg of body weight intraperitoneally) thrice a week for two weeks. After 15 days animals were sacrificed under mild chloroform anesthesia

condition, blood and liver sample was collected. The blood was allowed to clot for 30 min; serum was separated by centrifuging at 37°C and was used for biochemical estimations. Serum was used for the assay of hepatic marker enzymes – total bilirubin, serum aspartate transminase, serum alanine transminase, and alkaline phosphatase[5]. For histopathological study, liver tissue was quickly removed after autopsy and fixed in 10% formaldehyde.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values <0.05 were considered significant.

RESULTS

In the present study, Hepatoprotective activity of *N. speciosum* extract against carbon tetrachloride induced hepatotoxicity in rats was screened. For this screening, physical, biochemical and histological studies were made in three different groups of animals, control (group-I), CCl_4 treated (group-II) and CCl_4 + extract treated (group-III).

Table 1. Effect of aqueous extract of *N. speciosum* on CCl_4 induced hepatotoxicity in rats

Group	Control	CCl_4 treated	CCl_4 + Silymarin (100mg/kg)	CCl_4 + NS extract (50mg/kg)
Body weight (g)	87.8 ± 1.72	74.2 ± 2.4	84.2 ± 1.50*	82.2 ± 1.72*
Liver weight (g)	4.29 ± 0.23	3.6 ± 0.31	4.21 ± 0.13*	4.19 ± 0.34*
SGOT (U/ml)	16.36 ± 0.32	95.08 ± 2.62	17.5 ± 1.01*	18.8 ± 2.71*
SGPT (U/ml)	15.07 ± 1.41	59.2 ± 2.92	15.9 ± 3.2*	15.8 ± 3.7*
ALP(U/ml)	6.27 ± 0.21	16.23 ± 0.16	7.18 ± 0.10*	8.24 ± 0.18*
Bilirubin (mg %)	0.53 ± 0.03	1.1 ± 0.05	0.72 ± 0.14*	0.83 ± 0.03*

Data are expressed as Mean ± SEM, n = 6 in each group. *P<0.01 compared to control group

Effect of body weight and liver weight

Table 1 shows the body weight and liver weight of the three different groups of animals respectively. Both the liver weight and body weight were found to be significantly decreased in CCl_4 treated animals as compared to the control animals. In CCl_4 + extract treated animal's liver weight and body weight were also found to be decreased as compared to the controls but the values are not significant. But when compared to CCl_4, the extract treated animals liver weight and body weight values are significant.

Effect on serum transaminase (ALT and AST)

The activity of the serum hepatic marker enzymes namely serum glutamate oxalate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) were given in table 1 respectively. The enzyme SGOT activity was found to be significantly increased in CCl_4 treated animals in comparison with control. A very high value of SGOT was observed in CCl_4. A significant decrease in SGOT level was noted in group III animals due to the administration of *N. speciosum* extract (50 mg/kg of body weight). Like the SGOT activity, SGPT activity was found to be significantly increased in CCl_4 treated animals as compared to control. NSExt 50mg/kg administration caused significant decrease in SGPT level in group III animals.

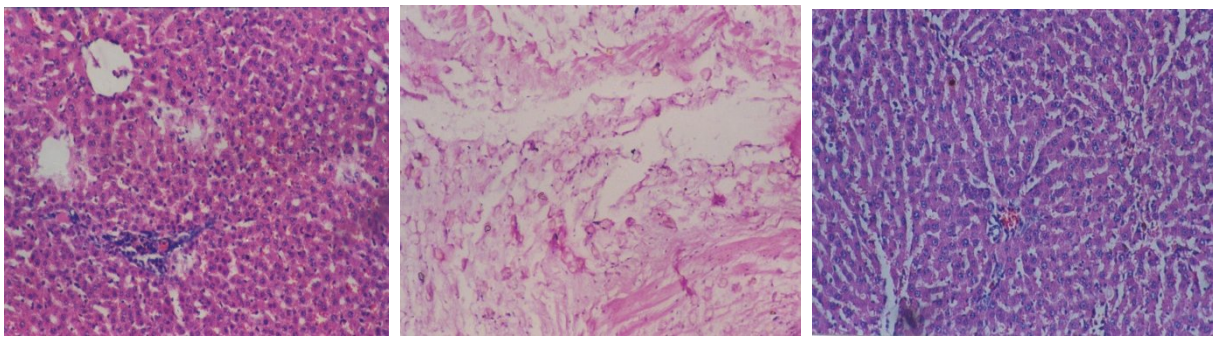
Effect of ALP activity and serum Bilirubin

Table 1 depicts the ALP activity and serum bilirubin level respectively. Hence also, ALP activity increased in CCl_4 administrated animals as compared to control animals. But simultaneous treatment of CCl_4 and extract caused decrease of these two biochemical parameters in group III animals.

Histological studies

Liver samples from the control, CCl_4 treated and extract treated animals were taken for histopathological studies. In control animals, liver sections showed normal hepatic cells with well preserved cytoplasm, prominent nucleolus and central vein (Fig.1a). In CCl_4 treated animals the section showed hydropic changes more around the central veins, cellular degenerations, fatty changes, widespread hepatocellular necrosis, kupffer cells hyperplasia, central lobular necrosis and steatosis (Fig.1b). The extract treated animals showed significant recovery from the damage (Fig.1c).

Figure 1. Slides showing histopathology of liver tissues



a. Control b. CCl_4 treated c. SNExt treated (50 mg/kg)

a) Section of the liver tissue of control rats showing normal histology **b)** Section of the liver tissue of rats treated with CCl4 showing necrosis & Fatty vacuole **c)** Section of the liver tissue of aqueous extract (50mg/kg) treated rat showing normal arrangements of hepatocytes around the central vein

DISCUSSION

Carbon tetrachloride is the one of the most commonly used hepatotoxins in the experimental study of liver diseases[6]. It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration[7]. The hepatotoxic effect of carbon tetrachloride is mainly due to its active metabolite, trichloromethyl radical[8]. This activated radical bind covalently to the macromolecules and induce lipid peroxidation and forms lipid peroxides which produce damage to the membrane[9]. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase which are cytoplasmic in location and released into circulation after cellular damages was the clear indication for the loss of functional integrity of the cell membrane[10, 11]. Amino transferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine amino transferase and aspartate amino transferase are well known diagnostic indicators of liver disease. In cases of liver damage with hepatocellular lesions and parenchymal cell necrosis, these marker enzymes are released from the damaged tissues into the blood stream[12]. Our results indicate that *N. speciosum* extract provides its protective effect against CCl_4 induced hepatotoxicity in rats.

The enzymes leak out in circulating and denote the damage to the membranes of hepatic cells. Present studies have shown that all CCl_4 treated rats (group-II) displayed, liver dysfunction as shown by their elevated levels of SGOT, SGPT, ALP, bilirubin. These data were confirmed earlier reports on hepatotoxicity of CCl_4 treatment (Shenoy, *et al.*, 2001; Buwa *et al.*, 2001). Simultaneous treatment of NSExt(50mg/kg body weight) significant recovery of the damage induced by CCl_4. This effect is evident especially with the dose of *N. speciosum* (50mg/kg body weight) because it caused drastic fall in transaminase (SOGT and SGPT) and ALP activity associated with fall in bilirubin level.

Histologically, treatment of *N. speciosum* together with CCl_4 also showed effects of hepatocytes from their damage induced by CCl_4. Many compounds exhibit liver protection against CCl_4 either by decreasing the production of CCl_3- free radicals or by impairment CCl_4 induced lipid peroxidation. The improved histology of liver after treatment with *N. speciosum* extract was compared to that seen in animals administered with CCl_4 indicates the possibility that *N. speciosum* in the dose level (50mg/kg body weight) being able to induce accelerated regeneration of the liver cells, by reducing the leakage of GPT, GOT, ALP into the blood and their by lowering their values in the serum. Serum transaminases return to normal with the healing of liver parenchyma and regeneration of liver cells.

CONCLUSION

The results of the present investigation indicate that the aqueous extract of *N. speciosum* possess good hepatoprotective activity. Further investigations has to be carried out ascertain the exact pathway.

REFERENCES

1. WHO, Regional Office For The Western Pacific, Research Guidelines For Evaluating The Safety And Efficacy Of Herbal Medicines, Manila, WHO, 1993.
2. Pushpangadan P. Role of Traditional Medicine in Primary Health Care. In: Iyengar PK, Damodaran VK, Pushpangadan P, Editors. Science for Health. Published By State Committee On Science, Technology And Environment, Govt. Of Kerala, 1995.
3. Wolf PL(1999).Biochemical diagnosis of liver diseases, Indian. J. Clin. Biochem., 14: 59–90.
4. Chatterjee TK (2000). Herbal Options (1st ed), Books and Allied (P) Ltd, Kolkata.

5. Mankani K L, Jagadeesh Singh S D, Manohara Y N et al. Evaluation of hepatoprotective activity of stembark of *Pterocarpus marsupium* Roxb. I J Pharmacol 2005; 37:165-168.
6. Johnson D E, Kroening C; Mechanism of early Carbontetrachloride toxicity in cultured rat hepatocytes, Pharmacol Toxicol 1998;83:231-9 17.
7. Shi G F, Q Li; Effects of oxymetrine on experimental hepatic fibrosis and its mechanism *in-vivo.* World J Gastroenterol 2005;11:268-271.
8. Srivastava S P, Chen N O, Holtzman J L; The *in-vitro* NADPH dependent inhibition by ccl4 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 the ccl3. radical. J biol chem 1990;265:8392-9
9. Mujeep M, Aeri V, Bagri P et al. Hepatoprotective activity of methanolic extract of *Tylophora indica* leaves. I J Green Pharmacy 2009; 125-127.
10. Saraswat B, Visen P K, Patnaik G K et al. Anticholestic effect of picroliv, active hepatoprotective principle of *Picrorhiza kurrooa* against carbon tetrachloride induced cholestatis. Indian J Exp Biol 1993; 31:316-8.
11. Sallie R, Tredger J M, William R; Drugs and the liver. Part I. Testing liver function. Biopharm Drug Disp 1991; 12:251-259.
12. Chaudhari B P, Chaware V J, Joshi Y R et al. Hepatoprotective activity of Hydroalcoholic extract of *Momordica charantia* Linn. leaves against Carbon tetrachloride induced Hepatopathy in Rats. I J ChemTech Res 2009; 1(2):355-358.