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Hepato protective activity of Hydroalcoholic extract of *Nardostachys jatamansi in* Carbon tetra chloride induced Hepatotoxicity in Rats.

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ABSTRACT

Nardostachys jatamansi (*NJ*) belongs to the family Valirenaceae possess different pharmacological activites. In Ayurveda & unani treatment roots and rhizomes of *Nardostachys jatamansi* are used to treat hysteria, epilepsy, fungal infection, and neurological disorders. The hepatoprotective activity of the Hydroalcoholic rhizomes extracts HAE1& HAE2 was carried out against CCl_4 induced liver damage in rats. Treatment of the animals with Hydroalcoholic extracts caused a significant reduction in the values of sGOT, sGPT, sALP and sB nearly similar to standard silymarin. The Hepatoprtective activity of the rhizomes was confirmed further by histopathological studies of liver tissue of control and extract treated animals.

The Hydroalcoholic extract HAE1 at a dose (500 mg/kg) exhibited significant Hepatoprotective activity than extract HAE2 at a dose (500 mg/kg) in rats.

Key words - *Nardostachys jatamansi*, Hepatprotective, CCl_4, silymarin, hydroalcoholic extract.

1. INTRODUCTION

The *Nardostachys jatamansi* (*N J)* a flowering plant belongs to the family Valirenaceae that grows in the Nepal, Himalayas of India and China. The plant grows to one meter in height with pink, bell-shaped flowers. It is found in the altitude of about 3000–5000 meters. In unani & Ayurveda treatment roots and rhizomes of *N. Jatamansi* are used to treat hysteria, epilepsy, and convulsions. The decoction of the *N. Jatamansi* is also used in neurological disorders, problems related to cardiovascular system and insomnia. Rhizomes are reported to contain a terpenoid ester, Nardostachysin. The sesquiterpenes (Jatamansone, Jatamansic acid), lignans and neolignans are reported to be present in the roots of this plant. To date much research has been undertaken to evaluate the drug to treat various neurological and cardiovascular disorders in different animal models and is widely used in ayurvedic & unani formulations. It is reported to possess many

activities like anti-depressant activity, anticonvulsant activity, antiarrhythmic activity, and possess hepatoprotective activity, usefull in Alzheimer & cereberal ischemia, have antifungal property, anxiolytic & hypolipidimic activity [(1).]

The liver is an important organ as it regulates many imperative metabolic functions. Hepatic damage is associated with alteration of these metabolic functions [(2)]. Liver is the first organ in the body which expose to toxins absorbed from the GIT resulting in many liver diseases, this is key organ of metabolism and excretion. Thus liver diseases remain one of the serious health concerns. Allopathic medicines have failed to treat hepatic diseases completely without adverse effects, chiefly the plant based preparations are in use for their treatment of liver diseases with minimum adverse effects. Unfortunately very few drugs are available for the treatment of liver diseases [(3, 4)].

Therefore, many folk medicines from plant origin are brought under study to find out its possible antioxidant and hepatoprotective properties against different toxicant-induced liver ailments in animals. Hepatotoxicity induced by CCl_4 model is often used for the study of hepatoprotective properties of drugs and plant extracts [(5, 6).]

2. MATERIAL AND METHODS

2.1 Chemicals:

The CCl_4 used as toxicant in this study was obtained from Sigma chemicals, other chemicals used were of analytical grade. Silymarin obtained from Micro lab Bangalore, for estimation of biochemical parameter, biochemical kits like ALP, serum bilirubin, total bilirubin, $_S$GOT,$_S$GPT were obtained from Span Diagnostics Ltd.

2.2 Plant Material

The rhizome of plant *Nardostachys jatamansi* used for the investigation was obtained from M/s Munnalal Dawasaz and company, Hyderabad. The plant identification was done by experts in the Dept of Botany, Bhavan's New Science College, Narayanaguda, and Hyderabad. AP, India.

2.3 Hydro alcoholic Extraction.

The rhizome were collected and washed thoroughly with water to remove any type of contamination, and air dried and subjected to pulverization to get coarse powder. The coarse powder about 1 kg of *Nardostachys jatamansi* was used for hydroalcoholic extraction (ethanol and water in a ratio 75:25 for HAE1 and 50:50 for HAE2) in soxhlate apparatus. The extracts were dried in rotary evaporator to produce a semi solid mass and stored in refrigerator below 10^0C using air tight containers.

2.4 Experimental animals

Adult Wister rats of either sex, weighing around 200 gm were used in the study. The animals were kept under standard conditions i.e. controlled temperature (26 ± 1°C) and humidity (30%–40%) in standard polypropylene cages from 48 hours prior to the study, in order to minimize if any of non-specific stress. Rats were fed with a standard rat diet. Water was supplied to the animal's ad labium. The rats were obtained from the animal house of the Mahaveer enterprises, Hyderabad. Experimental protocols were approved by the approval of the Institutional Animal Ethics Committee (IAEC) of C.P.C.S.E.A. was taken prior to the experiment (reference no 1330/ac/10/ CPCSEA). All the protocol and the experiments were conducted in strict compliance according to ethical principles and guide lines provided by CPCSEA.

2.5 Acute oral toxicity studies

Acute oral toxicity study was carried out as per OECD-423 guidelines [(1, 7)]**.** AOT was performed on Wister rats of either sex. The animals were kept on fasting overnight providing only water ad libitium, after which the extracts were administered orally at the dose level of 5 mg, 50 mg, 500 mg, 1000 mg, 2000 mg, and 5000 mg/kg/body weight and observed the mortality of animal for 3 days.

2.6 Evaluation of Hepatoprotective potential

The hepatoprotective activity of hydro alcoholic extract of *Nardostachys jatamansi* rhizome was

evaluated by using CCl_4 induced acute hepatotoxicity model[8].

In the hepatotoxic model, toxicant CCl_4 was administered with a dose of 0.5ml/kg i.p daily for seven days to the animals of group II, III, IV & V. The standard drug silymarin was administered with dose of 50 mg/kg p.o. Wistar rats of either sex were divided into 5 groups consisting of 6 animals in each group. Group I received 1% tween 80 for 7days & served as normal control. Group II served as negative control received 1% tween 80 p.o for seven days. Group III and Group IV received HAE1 and HAE2 respectively a dose of 500 mg/kg, p.o for seven days. Group V received standard drug silymarin with a dose of 100mg/kg ,p.o for seven days.

The Animals were sacrificed 24 hr after last treatment. The animals were anesthetized using ether and blood sample were collected by retro orbital plexux for biochemical estimation i.e. sALP, serum bilirubin, total bilirubin, sGOT, sGPT. After coagulation of blood at room temp serum was islolated by centrifugation at 2500 rpm for 20 minute. One liver from each group were separated. The livers after washing by normal saline were preserved in 10% formaldehyde for histopathalogical evaluation.

2.7 Statistical Analysis:

All the values of biochemical estimations expressed as mean ± Standard error mean (SEM). The comparison of difference was calculated by using ANOVA (one-way analysis of variance) followed by Dunnet's t test. *P* values < 0.01 were considered as significant.

3. RESULTS:-

Phytochemical evaluation of hydroalcoholic extract of *NJ* revealed the presence of chemical constituent like Alkaloids, Carbohydrates, Triterpenoids, Tannins,

Tannins, Lignins, Volatile oils, Coumarin & Steroids.

3.1 Acute Toxicity Study

NO mortality was reported up to the dose of 5000 mg/kg body weight. Hence the extract was found to be safe up to the dose level of 5000 mg/kg. $1/10^{th}$ of this dose i.e. 500 mg/kg of the dose selected for pharmacological evaluation[1]

3.2 Hepatoprotective activity

Toxicant CCL_4 on administration results in distinctive elevation in serum hepatic levels, $_S$GPT, $_S$ GOT, s ALP, Serum bilirubin & Total bilirubin when compared with the normal controls indicating liver damage (necrosis). Pretreatment of the animals with hydroalcoholic extracts (HAE1, HAE2) earlier to c administration caused a marked reduction in the values of $_S$GPT, $_S$ GOT, s ALP, Serum bilirubin & Total bilirubin ($p<0.01$) almost compareable to the silymarin (table 1).

Table 1:- Effect of the *Nardostachys jatamansi* rhizome's extracts on serum enzyme in ccl_4 induced liver injury in rats

Groups	SGPT (IU/L)	SGOT (IU/L)	Serum ALP (IU/L)	Serum bilirubin(mg/dl)	Total bilirubin (mg/dl)
GroupI Normal	51.5±1.56	80.5±2.14	97±1.71	0.49±0.0071	1.49±0.0220
Group II, CCL_4 treated	268.5±9.74	290.33±3.04	318.33±1.76	1.05±0.0331	4.29±0.1592
Group III, CCL_4 & HAE1 treated	84.5±2.59**	118. 33±3.11**	135.67±1.61**	0.61±0.0096**	1.91±0.02801**
Group IV, CCL_4 & HAE2 treated	96.2±1.47**	132.16±2.32**	145.83±1.01**	0.68±0.0133**	2.71±0.1833**
Group V, CCL_4 & Silymarin treated	72.8±1.25**	104.5±3.03**	119.33±0.99**	0.56±0.0162**	1.65±0.0183**

Data are presented as mean ± SEM, (n=6), **p<0.01, when compared with CCl_4 control group. Using repeated measure ANOVA followed by Dunnets t test.

3.3 Histopathological Results:-

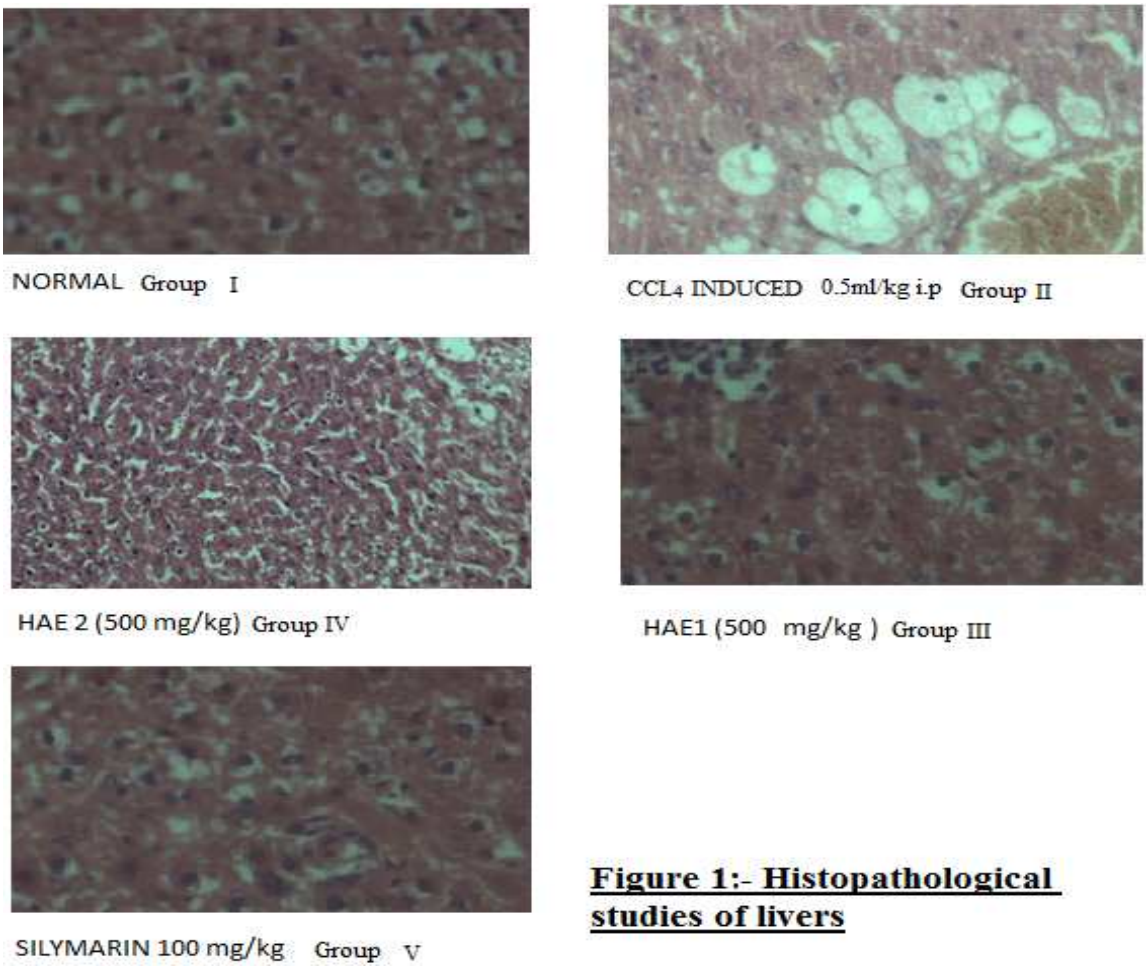

Figure 1:- Histopathological studies of livers

The results of histopathological studies reveal that the histological architecture of liver sections of control (group 1) has normal hepatic cells with prominent nucleus, well preserved cytoplasm, nucleolus and clearly noticeable central veins. The hepatoprotective effect of hydroalcoholic extracts of *Nardostachys jatamansi* rhizome was established by histopathological results of the liver tissue of control (group 2) and treated (group 3,4) animals. The (group 2) CCl_4 treated liver section showed severe central vein congestion, massive fatty changes, centrilobular necrosis, entrilobular degeneration, ballooning of hepatocytes, inflammation and loss of cellular boundaries. The histological architecture of liver section of the animals treated with hydroalcoholic extracts showed more or less normal lobular pattern with a mild degree of fatty change, centrilobular necrosis, central vein congestion and mild inflammation almost similar to the silymarin treated and control groups. Silymarin treated group had maintained the normal histology with negligible damage (Fig 1).

4. DISCUSSION:-

In the recent time, many studies have been undertaken on conventional medicines, in an effort to develop new drugs without any harmful side effects, for the treatment of liver ailments [10]. Trichloro methyl (CCl_3) free radical is a metabolite of carbon tetra chloride (CCl_4) which biotransformed by cytochrome P-450. This free radical initially formed as relatively un reactive, reacts very fast with oxygen to form a highly

reactive trimethyl peroxy radical (CCl_3O_2). These two radicals are capable of binding to proteins or lipids and initiate lipid peroxidation[10,11]. Lipid peroxidation process may responsible for peroxidative tissue damage in inflammation. Therefore, inhibition of the cytochrome P-450 dependent oxygenase activity could cause a reduction in the level of toxic reactive metabolites and a decrease in tissue damage. An increase in plasma $_S$GPT, $_S$ GOTs, ALP & bilirubin indicating considerable hepatocllular injury. Further the extent of toxicity was estimated by histopathalogical studies of liver.

Many liver protective agents which are beneficial against carbon tetrachloride mediated liver damage exert their protective action by either via a decreased production of CCl_4 derived free radicals or by antioxidant activity of the protective compound themselves [12].

The results of this study indicating that *Nardostachys jatamansi* (500mg/kg), significantly reduced the increased serum enzyme activity induced by toxicant carbon tetra chloride. Among the two hydroalcoholic extracts HAE1 showed more significant results than HAE2. Results indicate that tannins [13], steroids [14] are may be responsible for hepatoprotective activity of *NJ*.

5. CONCLUSION

In conclusion the hydroalcoholic extracts of *Nardostachys jatamansi* rhizome possess a protective activity against hepatotoxicity induced by CCl_4 in rats, as witnessed by biochemical & histological parameter. The hepatoprotective activity of *Nardostachys jatamansi* might be due to the presence of tannins and steroids. Further studies require to isolate active component responsible for the hepatoprotective activity and to understand the exact mechanism.

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