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Original Research Article

HEPATOPROTECTIVE ACTIVITY OF *ZIZYPHUS NUMMULARIA* (BURM.F.)WIGHT & ARN. AGAINST CCL_4 INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Effect of ethanol and aqueous extracts of leaves of Zizyphus nummularia (ZPN) was investigated against carbon tetra chloride-induced hepatic damage. carbon tetra chloride at the rate of 1 ml/kg produced liver damage in rats as manifested by the significant (P<0.001) rise in serum levels of Serum oxaloacetate transaminase(SGOT), Serum glutamate pyruvate transaminase(SGPT), alkaline phosphatase (ALP), Totalprotein Totalbilirubin and Directbilirubin compared to respective control values. Histopathological observation also revealed that pretreatment with ZPN protected the animals from carbon tetra chloride induced liver damage. The results indicate that the leaves of Zizyphus nummularia possess the Hepatoprotective activity. This property may be attributed to the flavonoids present in the leaves of Zizyphus nummularia.

Key words: *Zizyphus nummularia*, carbontetra chloride, hepatotoxins, transaminases, Histopathological studies.

INTRODUCTION

Uncontrolled environmental pollution, poor sanitary conditions, alcohol intoxication and indiscriminate use of potent drugs predispose liver to a vast array of disorders[1]. However, infections by viruses still remain as the major cause of liver diseases[2]. According to global estimates, there are about 796,000 deaths every year due to liver cirrhosis caused by hepatitis[3]. Many indigenous drugs are claimed to possess hepatoprotective activity. One such plant used by the tribals of South India, Zizyphus nummularia *(Burm.f.)Wight&Arn.* (Rhamnaceae), is claimed to have control over hepatic dysfunction[4]. Preliminary studies have given positive results regarding the same. Hence, this work was undertaken to have a systematic study on the exact mechanism of the drug on male albino rats. Even though it is quite safe in human beings, massive hepatic necrosis is becoming a major problem.. Hence, carbon tetra chloride -induced liver toxicity was studied, with special emphasis given to the ability of liver to recover spontaneously after carbon tetra chloride challenge.

MATERIALS AND METHODS

Plant material:

Fresh leaves of Zizyphus nummularia *(Burm.f.)Wight&Arn.*Were collected from kaaripatti, Salem (Dt) Tamilnadu. The plant was then authenticated by the Botanist A. Balasubramanium, consultant-central Siddha Research, salem-Tamilnadu.

PREPARATION OF THE EXTRACT/ DRUG:

The fresh leaves of Zizyphus nummularia collected and dried under shade, sliced into small pieces with mechanical grinder. The powder was passed through sieve no.30 and stored in a container.Then the marc was defatted with Ethanol 95% (75-78^{o}C) by using hot percolation method (soxhlet apparatus). The marc was then subjected to cold maceration using distilled water for 72hrs. The extracted was concentrated using a rotary vacuum evaporator and then dried under reduced pressure and kept in the dessicator. The extracts were suspended in Tween80 for presented study. The extract obtained was subjected to various chemical tests as per the procedure mentioned in the standard reference books[5,6]. The extract was used for pharmacological evaluation.

Experimental animals:

Swiss albino mice (20-25 g) and rats of Wistar strain (150-200 g) of either sex were fed with a standard diet and water ad libitum. In addition to pellet diet guinea pigs were supplemented with Lucerne. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27^{o}C and 12 hours light / dark cycle) throughout the experimental period[7,8]. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute no (P.COL/57/2010/IEAC/VMCP).

Acute toxicity test[9]:

The ethanol and aqueous extract of Zizyphus nummularia was screened for acute toxicity, following the standard method (OECD/OCDE No: 423). Albino mice of female sex weighing 20-25 gm were used in this study. Animals were maintained on normal diet and water prior to and during the course of experiment. The dose of ethanol and aqueous extract was prepared with saline and was administered by intubations. The acute toxicity was tested at the doses of 5, 50 300 and 2000mg/kg.

CHEMICAL AND INSTRUMENTS

All chemicals used were analytical grade. Silymarin was obtained from micro lab Bangalore and other chemicals were obtained from Rankam India pvt ltd. UV spectra were recorded in Shimadzu 1601 UV-Visible spectrophotometer.

HEPATOPROTECTIVE STUDY

Induction of Liver Damage:

The rats were divided into 7 groups of six animals each. Group I served as a control, Group II rats were administered with carbon tetrachloride (1 mg/kg administered by intra peritonieal route.). Group III rats were pretreated with standard drug of silymarin (100mg/kg) orally for a period of 7 days. Groups IV, V animals were pretreated with Ethanol extract of Zizyphus nummularia (200mg/kg, 400mg/kg respectively) for a period of 7 days. Groups VI, VII animals were pretreated with aquoues extract of Zizyphus nummularia (200mg/kg, 400mg/kg respectively) for a period of 7 days and carbon tetrachloride (1 mg/kg)intra peritonially an interval of every72 hours for a period of 7 days. After the experimental period, Blood was collected by retro orbital method and the serum was separated and used for the assay of Serum oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase(SGPT), alkaline phosphatase (ALP), , total protein, total &direct bilirubin were estimated respectively. The rats were sacrificed by cervical dislocation method. The liver are removed and weighted for histological examination.

STATISTICAL ANALYSIS

The results were expressed as mean±SEM. The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's "t" test. The values of $P<0.01$ and $P<0.001$ were considered to indicate a significant difference between the groups.

RESULTS AND DISCUSSION

Phytochemical analysis:

Ethanol and aqueous extract of Zizyphus nummularia *(Burm.f.)Wight&Arn.* leaves was concentrated under vacuum using rotary vacuum evaporator to a dry residue and kept in a desiccator. The percentage yield was 11.4%w/w and 16.6 %w/w. The Phyto chemical screening of the percentage crude yields of extracts studied has shown that the leaves of Zizyphus nummularia *(Burm.f.)Wight&Arn.* were rich in lignands,

flavonoids, glycosides, sterols, sugars, amino acids and triterpenoids.

Acute toxicity:

The mice treated with oral administration of Zizyphus nummularia*(Burm.f.)Wight&Arn.* Aqueous and ethanol extract upto 2000mg/kg did not produce any toxic effects in rats. No mortality was observed and Zizyphus nummularia*(Burm.f.)Wight & Arn.* Ethanol and aqueous extract was found to be safe at given doses. Dose selected for pharmacological evaluation were 200 mg/kg and 400 mg/kg.

Hepatoprotective Study:

Liver plays a significant role in the regulation of physiological process[10]. It is involved in several functions such as metabolism, secretion and storage[11]. Furthermore detoxification of a variety of drugs and xenobiotics occurs in liver[12]. The bile secreted by liver has, among other things, an important role in digestion. Liver disease is among the most series aliment[13].

Carbon tetrachloride (CCl4) is potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to CCl3 by liver Cytochrome P450-dependent monooxygenase its enzymatic transformation by CYP2E1 into a highly reactive free radical (CCl3•) and subsequent derivatives (Cl3COO) Those free radicals initiate and promote the propagations of lipid peroxidation[14,15]. Hepatic cells appear to participate in a variety of enzymatic metabolic activities and both carbon tetrachloride and produced marked liver damage at the given doses as expected. Administration of carbon tetrachloride showed significantly elevated levels of, SGOT, SGPT, ALP,TP and TB, DB,due to its enzymatic activation of CCl3 free radical, which in turn alters the structure and function of liver cells. Treatment with ethanol and aqueous extract of Zizyphus nummularia*(Burm.f.)Wight&Arn.*showed a dose-dependent protection against the injurious effects of CCl4. **(Table no-1)**

Table.1.Effect of Ethanolic and Aqueous extracts of leaves of Zizyphus nummularia*(Burm.f.)Wight&Arn*.extracts on bio chemical parameters in serum by ccl_4 induced hepatotoxicity

Treatment	SGOT U/L	SGPT U/L	ALP IU/L	T.Protein g/dl	T.Billirubin	D.Billirubin
Control	173.83± 0.16	165.66 ± 1.35	267.83 ± 3.37	5.41±0 .08	0.41 ± 0.06	0.24 ± 0.01
CCl_4 1 ml/kg i.p	365.16± 0.94###	348.00 ± 1.46###	395.33 ± 1.28###	4.66±0.07###	0.82±0.01###	0.84± 0.01###
Silymarin(100mg/kg.)	187.50± 0.99***	175.00 ± 0.57***	253.00 ± 1.35***	5.55±0.01***	0.54±0.01***	0.43±0.01***
Ethanolic extract of Zizyphus nummularia. (200mg/kg)	272.50± 0.95***	233.00 ± 0.96***	333.66 ± 1.14***	5.91±0.01***	0.62±0.02***	0.60±0.01***
Ethanolic extract of Zizyphus nummularia (400mg/kg)	224.83± 1.49***	212.83 ± 1.24***	262.5 ± 1.25***	5.72±0.01***	0.61±0.03***	0.56±0.01***
Aqueous extract of Zizyphus nummularia.(200mg/kg)	253.83± 0.94***	233.83 ± 0.98***	325.33 ± 1.35***	5.93±0.01***	0.66±0.03***	0.62±0.01***
Aqueous extract of Zizyphus nummularia.(400mg/kg)	212.66± 1.14***	192.85 ±1.24***	243.33 ±1.35***	5.63±0.01***	0.55±0.02***	0.52±0.03***

#, normal control group compare with ccl4 treated group
*, compare with ccl4 treated group

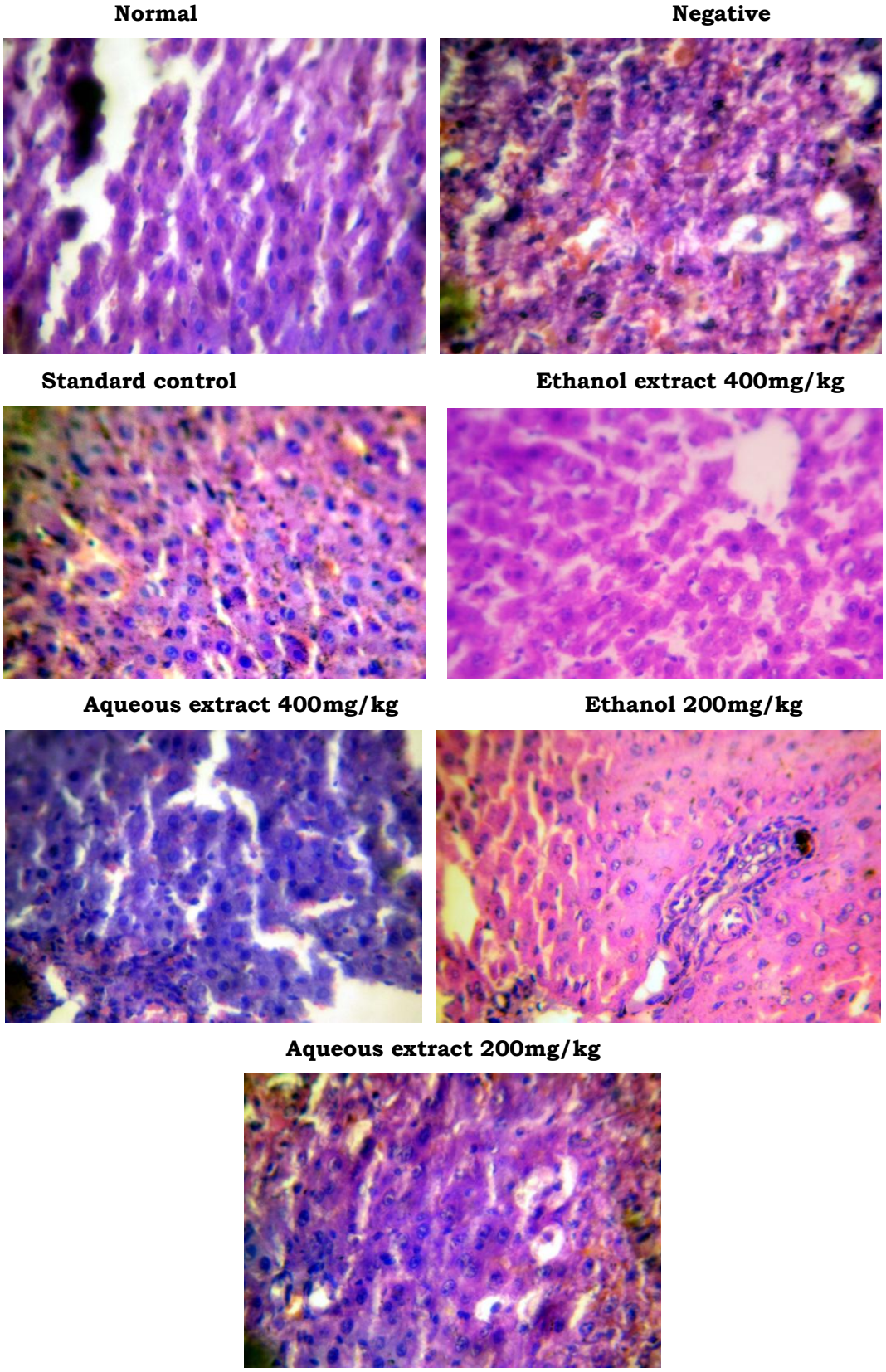

Fig.1.Histopathology of Rats Liver (CCL_4 induced)

An obvious sign of hepatic injury is the SGOT,SGPT,ALP,TP,TB,and DB levels are elevated in CCL_4 - induced hepatotoxicity. In the present study, elevation in the levels of biochemical parameters were found in CCL_4 -treated animals. But Z.nummularia has significantly reduced these enzyme levels. The observed reversal produced by the drug in serum biochemical parameters may be a manifestation of the reduction in the cell membrane disturbances.

CONCLUSION

The present study concludes that the ethanol and aqueous extracts of the leaves of Z.nummularia possesses significant hepatoprotective activity in Carbon tetrachloride (CCl4) induced male rats at a dose of 1 mg/kg/i.p Thus, it has been scientifically proved that the traditional knowledge obtained from the tribal people of South India is true and the extracts has enough potential as a hepatoprotective agent and hence worth investigative.

REFERENCES

1. Wolf P L, Biochemical diagnosis of liver disease, Indian J Clin Biochem, 14(1999) 59.
2. Hikino h & Kiso Y (1988) Natural Products for liver disease, pp. 39-42. Academic Press London.
3. The wealth of India: A Dictionary of indian Raw materials and Industriel Products—Raw Materials Series, Publications& Information Directorate, CSIR, New Delhi, Revised ser, 1992, Vol,3 (Ca-Ci), pp.213-214.
4. Mander M (1998) Marketing of indogenous medicinal plants in South Africa. A case study in KwaZulu-Natal, FAO Rome.
5.J.B. Harborne. Phytochemical methods: A guide to modern technique of plant analysis (3rd edition). Chapman and Hall co. New York, pp. 1–302 (1998).
6.C.K. Kokate, C.K., Pharmacogonosy. 16th Edn, Nirali Prakashan, Mumbai, India (2001).
7. Kevin Robinson for BioPharm International, Aug 1, 2003. GLPs and the Importance of Standard Operating Procedures.
8. "OECD Principles of Good Laboratory Practice (as revised in 1997)". *OECD Environmental Health and Safety Publications* (OECD) 1. 1998.
9. OECD guidelines 423, 2001.
10. Brodie B B (1956) J Pharmacol 8. 1-17.
11. Karandikar G V.Chitle G K & Balwani J H (1963) Acta Pharmacol 20, 274-280.
12. Weber LW, Boll M, Stampfl A Institute of Toxicology, GSF-National Research Center for Environment and Health.
13. Arthur C. Guyton, text book of medical physiology, 1998, 8th edition.
14. Pandi S. Sur TK,Jana Udebnath P K. Sen S& Bhattacharya D, Prevention of carbon tetra chloride- induced hepatotoxicity in rats by Adhatoda vasica leaves, Indian J Pharmol, 36 (2004) 313.
15. Clwson G A, Mechanism of carbon tetrachloride hepatotoxicity, Pathol Immunol Res, 8 (1989) 104.