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Evaluation of Hepatoprotectivte Activity of Citrullus Colocynthis Roots Against CCl_4 induced Toxicity in Albino Rats

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Abstract – Hepatoprotective activity of different extracts of *Citrullus colocynthis L. sch.* (roots) (cucurbitaceae) was investigated in albino rats by inducing hepatotoxicity with carbon tetrachloride. The alcoholic extract of *Citrullus colocynthis sch.* 100 mg/kg b.w. has been shown to posses significant hepatoprotective effect by lowering the serum level of transaminases (GPT & GOT), alkaline phosphate (ALP) and bilirubin ($P < 0.05$ to $P < 0.001$).

Keywords – *Citrullus colocynthis*, carbon tetrachloride, hepatoprotective, SGOT, SGPT, alkaline phosphtase

Introduction

Citrullus colocynthis L. sch. (Cucurbitaceae) commonly known as "indrayan" widely cultivated through out the India and Saudi Arabia (Kirtikar and Basu, 1975). The plant is very precious for the Indian system of medicine particularly *Ayurveda and Siddha.* The leaves are diuretic and used in treatment of jaundice and asthma (Chadha, Y.R., 1950). The fruit is purgative, anthelmentic, antiepileptic, molluscicide and insecticide, and is used against gonorrhoea (Yahya-AL, *et al.*, 2000). Plant contains cucurbitacins A, B, C, D and α-elaterin which is the important constituents used for the treatment of hepatic ailments (Wallis, T.E., 2005). The drug exhibited anti-inflammatory, antidiabetic and antispermatogenic activities (Abdel Hassan, I.A., *et al.*, 2000; Moli, 2001). The plant is also useful in the treatment of liver disorder with other herbs in different traditional medicine. The aim of the present paper is to justify the traditional claims by investigation its use in hepatic disorders with the help of various validated models. The drug was studied using CCl_4 as a hepatoxin.

Experimental

Plant materials – The plant materials were purchased from the local market, Lucknow U.P. India. The plant material was identified and authenticated by taxonomically by National Botanical Research Institute (NBRI), Lucknow.

A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference (Voucher specimen number is LWG-224812).

Preparation of extracts – The shade dried plant materials was crushed, powdered and exhaustively defatted by Petroleum ether (60 - 80 °C) and then successively extracted with benzene, chloroform, 70% alcohol and water. All the extract were filtered, pooled and concentrated under reduced pressure using rotavapour (Buchi, USA.)

Preliminary phytochemical analysis – The Preliminary phytochemical screening of extract of *C. colocynthis* roots gave positive test for resins, carbohydrates, saponin, anthraquinone and Steroids (Brain and Turner 1975). Fruits and roots of plant reported to contain cucurbitacins A, B, C, D and α-elaterin.

Test animals

Animals : Ducrane albino rats (150 - 200 g) of either sex were used for the study. The animals were kept under standard conditions temp 23 ± 2 °C, relative humidity 55 ± 10% and 12 hrs. light and dark cycle. The animals were maintained under standard pellet diet and water ad libitum in animal house of Central Drug Research Institute (CDRI), Luckhnow, India. Initial body weight of each animal was recorded and they were given seven days time to get acclimatized with the laboratory condition. All experiments were performed in the morning according to current guidelines for the laboratories animals and ethical guidelines for investigation of experimental of conscious

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Table 1. Distribution of groups with dose of drugs : -

S.No.	name of the group	amount of the drug
1.	group I-control	5% w/w acacia suspension
2.	group II-toxin-CCl_4	2.5 ml/kg p.o in olive oil (1 : 1)
3.	group III--*C. colocynthis* aqueous extract	100 mg/kg b.w. p.o.
4.	group IV--*C. colocynthis* alcoholic extract	100 mg/kg b.w. p.o.
5.	group V-silymarin--standard	25 mg/kg b.w. p.o

Table 2. Effect of *C. colocynthis* on carbon tetrachloride-induced hepatotoxicity in rat serum

S. No.	name of the group	bilirubin total (mg %)	alkaline phosphate	SGOT (Unit/ml)	SGPT (Unit/ml)
group I	control	$0.24 \pm 0.02^{a,B}$	$4.45 \pm 0.42^{a,B}$	29.52 ± 1.78^{a}	$15.03 \pm 2.30^{a,B}$
group II	toxin-CCl_4	4.06 ± 0.14	18.52 ± 0.64	114.01 ± 8.82	77.52 ± 9.26
group III	aqueous extract	1.70 ± 0.06	15.43 ± 0.68	32.61 ± 2.95	35.86 ± 3.48
group IV	alcoholic extract	1.32 ± 0.15	11.54 ± 0.45	38.19 ± 2.50	47.45 ± 5.96
group V	silymarin	0.27 ± 0.04	11.65 ± 0.36	27.16 ± 3.41	11.67 ± 0.57

Each group contains 6 animals.
Normal control (group I) was compared to toxic control by students-Newman-Keuls-test.
($^{a}P < 0.001$, $^{b}P < 0.01$,$^{c}P < 0.05$)
Normal control (group I) was compared to group III and IV by students-Newman-Keuls-test.
($^{A}P < 0.001$, $^{B}P < 0.01$,$^{C}P < 0.05$).

animals (Zimmerman, 1983).

Experimental induction of hepatic damage – Rats were divided into five groups control, toxin, tests and standard groups with 6 animals in each groups. According to the Table 1, the rats of control group (I) received 5% w/w acacia suspension. The toxin group (II) was treated with one dose of toxin (CCl_4 2.5 ml/kg p.o.) (Kumar and Mishra, 1996) in olive oil mixture (1 : 1). All the test groups (III and IV) treated with four dose (100 mg/kg b.w. p.o.) of extracts in 5% w/w acacia suspension at 12 hrs interval and toxin (CCl_4) one hr after the last dose (Janbaz and Gilani, 2000). The std. group (V) also treated with silymarin 25 mg/kg p.o. (Saraswat, *et al.*, 1996) Animal were anesthetized with ether 36 hrs of CCl_4 administration and blood was collected by puncturing the retro orbital plexus (Thakur, *et al.*, 1991).

Blood samples were transferred to the centrifugation tubes and Serum was separated by centrifugation at 2500 rpm for 30 minutes. Serum was collected and analyzed for biochemical estimations.

Biochemical estimation – Serum was analyzed to biochemical estimations of different parameters like Aspartate aminotransferase (AST) Alanine aminotransferase (ALT), (Reitman and Frankel, 1957) Alkalinephosphatase (ALP) (Kind and Kings, 1954) and Bilirubin (T. Bill and D. Bill), (Malloy and Evelyn, 1937).

Histopathology – Small pieces of liver tissue were removed after autopsy to Bouin's fluid. The tissues wereprocessed for paraffin embedding after fixing for 48 hr in 10% formasaline. The 5 μm - 10 μm section of liver stained with hematoxyline and eosin were observed for histopathological changes under optical microscope (Handa and Sharma, 1990); (Pathak, *et al.*, 1991).

Statistical analysis – All the data were presented as mean ± S.E.M. and analysed by One way ANOVA followed by Students Newmans keul's multiple comparison test for the possible significant identification between the various groups (Osol, *et al.*, 1975). $P < 0.05$ was considered statistically significant. Statistical analysis was carried out using Graph pad prism 3.0 (Graph pad software, San Diago, CA).

Results

A significant ($p < 0.001$) increase in the total serum bilirubin level was reported in the toxic control as compared to the normal control.

Significantly lower levels of bilirubin were observed after the treatment with aqueous and alcoholic extracts of C. colocynthis.

There was significant lowering in the levels of the alkaline phosphatase after the treatment of aqueous and alcoholic extract comparable to the normal control group.

Serum transminases levels (SGOT and SGPT) were also significant in groups (group III and group IV). Group V was treated with the standard drug silymarin (25 mg/kg) and the level of all the enzymes were significantly reduced (Table 2).

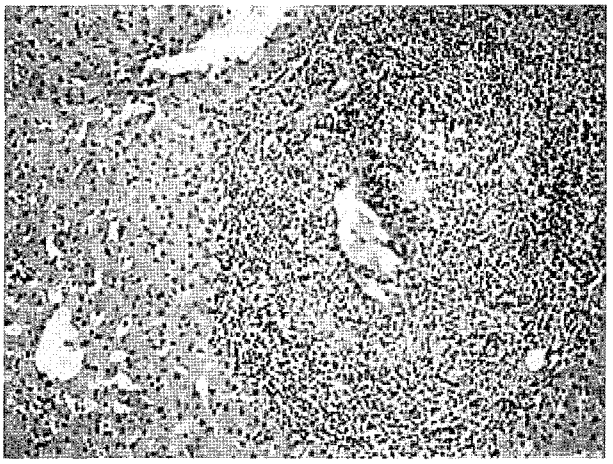

Diagram 1. CCl_4 damaged rat liver.

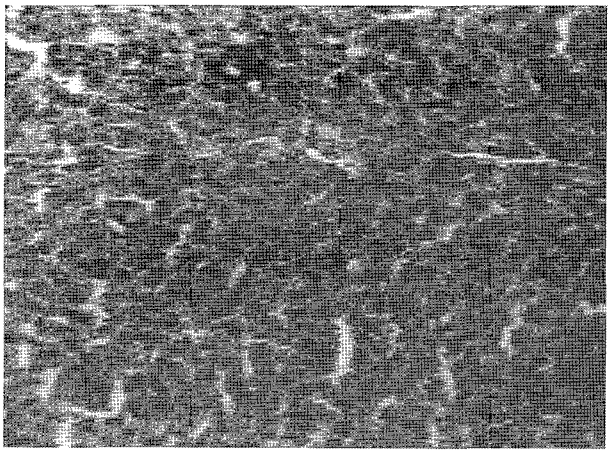

Diagram 2. After treatment with alcoholic extract (*Citrullus colocynthis*).

Histopathological profile of liver from carbon tetrachloride treated rats revealed cellular degeneration steotosis and hydrophic changes more around the central veins. (Diagram 1). The section of liver of rat treated with alcoholic extract of C. colocynthis showed significant improvement of hepatocellular architecture by the presence of normal hepatic cord, absence of necrosis and steotosis (Diagram 2).

Discussion

Extracts of Citrullus colocynthis administered prophylactically, exhibited preventive against CCl_4 induced liver damage. The preventive action in liver damage induced by CCl_4 is mainly used as an indicator of the liver protective activity of drugs & their extracts.

The hepatotoxicity produced by carbon tetrachloride is due to its enzymatic activation to CCl_3 halogenated free radical (HFR) by hepatic cytochrome P_{450} which in turn disrupt the structure & function of lipid & protein macromolecules in the membrane (Packer, J.E., *et al.*, 1978; Drotman, R,B., and Lawharn, G.T., 1978).

The rise in serum level of GOT, GPT and ALP has been attributed to the damaged structural integrity of the liver.

Hence alcoholic and aqueous extracts of drug mediated reduction in level of SGOT, SGPT, towards the respective normal values which indicate the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4 intoxication. The alkaline phosphatase (ALP) is the prototype of these enzymes that reflect the pathological alteration in biliary flow. CCl_4 induced elevation of this enzymatic activity in serum is in line with increased level of serum bilirubin content. The suppression of increased ALP activity with concurrent depletion of raised bilirubin level suggests the possibility of the drug being able to stabilize biliary dysfunction in rat liver during chronic hepatic injury with toxin. (Ploa, G.L., and Hewitt, W.R., 1989). Histopathological changes in the liver section also reveal that alcoholic extract of C. colocynthis significantly enhances the process of regeneration and reduces the necrosis.

Thus experiment clearly divulges that the protective action of drug extract against experimentally induced liver damage in rats and so its use in traditional folk medicine for liver affection was justified.

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