

# Antihepatotoxic Potential of *Citrullus colocynthis* Root Extract, Fractions and Isolated Compounds

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**Abstract:** Medicinal plants are considered to be effective and safe alternative treatment for liver toxicity. The article reveals the hepatoprotective activity of the ethanolic extract of the roots of the *Citrullus colocynthis* commonly known as *INDRYAN* using carbon tetrachloride (CCl<sub>4</sub>) experimental model in albino rats. After receiving significant protection of Ethanolic extract on liver the extract further undergone fractionation into three fractions & the activity was localized in the toluene fraction. These on purification led to the isolation of two pure compounds which were identified as - Cucurbitacin B[1] and Colocynthin [2]. The pure compound shows reduction in enzymatic level viz. (SGOT 68.09%, SGPT 63.64%, ALP 76.81%, BL 68.22%) and (SGOT 71.28%, SGPT 65.24%, ALP 80.68%, BL 54.92%) at 50 mg/ kg dose respectively whereas drug Silymarin showed reduction as (SGOT 79.73%, SGPT 74.26%, ALP 87.88%, BL 82.75% ) at 25 mg/kg dose level. On comparing the obtained data it was observed that the roots of *C. colocynthis* Sch. exhibited significantly better hepatoprotective activity, thus justifying the traditional claims.

**Keywords:** *Citrullus colocynthis*, hepatoprotective, toluene fraction, isolation, ethanolic extract.

## 1. INTRODUCTION

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver diseases such as cirrhosis, fatty liver and chronic hepatitis are important world health issues.

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Conventional and synthetic drugs used in liver disease are inadequate and sometimes have serious side effects. (Luper, 1998) The use of herbal remedies for the treatment of liver diseases has long history starting with Ayurvedic treatment and extending to the Chinese, European and other systems of traditional medicine. A large number of plants and formulations have been claimed to have hepatoprotective effect. Some plants which have shown genuine utility in liver disorders are *Silybium marimum*, (Ram, 2001); *Picrorrhiza kurroa*, (Thyagarajan, 2002); *Andographis paniculata*, *Phyllanthus niruri* and *Eclipta alba*. (Bisset, 1994).

*Citrullus colocynthis* L. sch. (Cucurbitaceae) commonly known as Indrayan is widely cultivated throughout the India and Saudi Arabia (Kirtikar and Basu, 1975). The plant is very precious for Indian system of medicine particularly Ayurveda and Siddha. The leaves are diuretic and used in treatment of Jaundice and asthma. (Chadha, 1950). The fruit is purgative anthelmintic, antiepileptic, molluscicide and insecticide, and is used against gonorrhoea. Yahya-AL *et al.* (2000). Plant contains cucurbitacins A, B, C, D and  $\alpha$ -elaterin which is the important constituents used for the treatment of hepatic ailments (Wallis, 2005). The drug has exhibited anti-inflammatory, antidiabetic, and antispermatogenic activities. Abdel Hassan *et al.* (2000), Moli (2001). The plant is also useful in the treatment of liver disorder with others herbs in different traditional medicine. The aim of the present investigation is to justify the traditional claim by investigation its use in hepatic disorders.

## **2. MATERIAL AND METHODS**

### **2.1 Collection and Identification of Plant**

The plant material was purchased from the local market Lucknow and authenticated by a Taxonomist in National Botanical Research Institute (NBRI) Lucknow. The voucher specimen No. LWG-224812 was deposited in the departmental herbarium of NBRI Lucknow, India for future reference.

### **2.2 Extraction/fractionation/isolation procedure**

The roots of the plant (2.0 kg) were air shade dried and powdered after collection. The powdered plant material was extracted with petroleum ether (60°-80°C) for defatting. The defatted powdered drug was then extracted with 95% ethanol (3 x 3 L) at room temperature. The combined extracts were evaporated in a rotary evaporator below 50°C to a brown mass, which was further dried under high vacuum (24.54% w/w). A part of the ethanol

extract thus obtained was fractionated into three fractions toluene soluble fraction (CC-I), chloroform soluble fraction (CC-II) and ethyl acetate soluble fraction (CC-III). In the separate study different fractions of the plant were then subjected to hepatoprotective activity to identify the most active fraction against  $\text{CCl}_4$  induced toxicity. The hepatoprotective activity was localized significantly in toluene fraction (CC-I). This fraction was therefore purified by chromatography over silica gel separately and three compounds were isolated. Out of the three compounds, only two, i.e. Comp.-I and Comp.-II, were separated with dark absorbing spot and blue fluorescent spot and identified as Cucurbitacin-B [1] and Colocynthin [2]. These compounds were also subjected for the hepatoprotective evaluation.

### 2.3 Experimental animals

Albino rats (150-200g) of either sex were selected for study. Animals were kept in polypropylene cages for 3-4 days under standard experimental conditions before the experiment. Animals were given standard pellet diet and fresh drinking water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee of Central Drug Research Institute (CDRI), Lucknow.

### 2.4 Hepatoprotective activity of plant extract, fraction and compounds against $\text{CCl}_4$ model

The effect of extract, fractions and isolated compounds of the plant on carbon tetra chloride ( $\text{CCl}_4$ ) induced toxicity in rats were studied. Rats were divided into different groups including control, toxin, tests groups and standard with 6 animals in each group. The rats of control group (I) received 5% w/w acacia suspension. The toxin group (II) was treated with one dose of toxin ( $\text{CCl}_4$  2.5ml/kg p.o.) in olive oil mixture (1:1). (Kurma and Mishra,1997). Test groups treated with doses (100 mg/kg b.w., p.o.) ethanolic extract, toluene soluble fraction, chloroform soluble fraction, ethyl acetate soluble fraction in 5% w/w acacia suspension at 12 hrs interval and toxin ( $\text{CCl}_4$ ) one hour after the last dose (Janbaz and Gilani, 2000) and both isolated compounds with doses of 50 mg/kg b.w. p.o. Silymarin were used as standard with a dose of 25 mg/kg p.o. (Bhattacharyya *et al.* 2003). Animals were anaesthetized by light ether anesthesia and the blood was withdrawn by reterorbital plexus puncture. It was allowed to coagulate for 30 minutes in tubes and serum was separated by centrifugation at 2500 rpm.

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**Table 1:** Effect of extracts and different fractions on biochemical parameters

Extracts/ fractions/ compounds	Dose mg/kg	(% Reduction)			
		SGOT (U/ml)	SGPT (U/ml)	ALP BL (U/L) (mg/dL)	
Control group	1ml	43.95±3.98 <sup>a</sup>	33.99±2.73 <sup>a</sup>	4.45±0.42 <sup>a</sup>	0.39±0.04 <sup>a</sup>
Toxin group	2.5ml	176.22±2.91	97.45±6.05	49.85±18.25	4.06±0.14
Ethanol extract	100	90.76±4.31 (48.49)	47.45±5.96 (51.30)	11.54±0.45 (76.85)	1.32±0.15 (67.48)
Toluene soluble fraction	100	85.42±2.13 (51.52)	39.61±0.59 (59.35)	10.63±0.57 (78.67)	0.84±0.15 (79.31)
Chloroform soluble fraction	100	110.24±3.87 (37.44)	48.40±4.24 (50.33)	13.52±2.45 (72.87)	1.19±0.07 (70.69)
Ethyl acetate soluble fraction	100	104.20±4.87 (40.86)	44.90±3.05 (53.92)	12.41±2.43 (75.10)	1.12±0.03 (72.41)
Compound (1)	50	56.23±6.31 (68.09)	35.43±0.21 (63.64)	11.56±3.21 (76.81)	1.29±0.22 (68.22)
Compound (2)	50	50.61±2.51 (71.28)	33.87±5.27 (65.24)	9.63±1.21 (80.68)	1.83±0.45 (54.92)
Silymarin (Standard)	25	35.72±5.63 (79.73)	25.08±2.61 (74.26)	6.04±0.23 (87.88)	0.70±0.05 (82.75)

The value represents the mean ± S.D. for 6 rats per group. Values in parenthesis indicate percentage recovery. All groups were compared to toxic control by students-Newman-Keuls-test (a P<0.001)

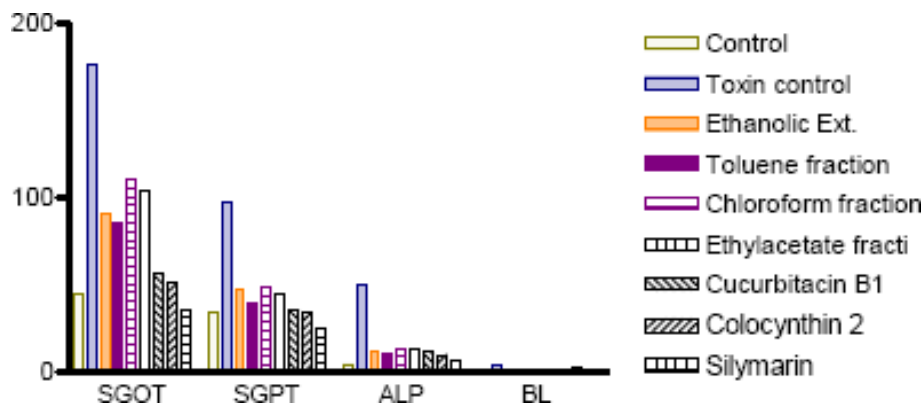
The serum was used to estimate Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total Bilirubin (BL). After recording the effect of biochemical parameters, all the extracts were tested to confirm the hepatoprotective activity by histopathological studies.

### 3. RESULTS AND DISCUSSION

#### 3.1 Antihepatotoxic activity

The ethanol extract of the roots of the *C. colocynthis* showed promising antihepatotoxic activity (reduction in biochemical parameters) at 100 mg/

kg dose level (SGOT 48.49%, SGPT 51.30%, ALP 76.85%, BL %) in experimental rats. On further fractionation the toluene fraction and chloroform fraction showed activity (SGOT 51.52%, 37.44%, SGPT 59.35%, 50.33%, ALP 78.67%, 72.87%, BL 79.31%, 70.69%) respectively at 100 mg/kg dose, while ethyl acetate fraction showed activity (SGOT 40.86%, SGPT 53.92%, ALP 75.10%, BL 72.41%) at 100 mg/kg dose level. The pure compounds isolated from toluene fractions were cucurbitacin B [1] (SGOT 68.09%, SGPT 63.64%, ALP 76.81%, BL 68.22%) and colocynthin [2] (SGOT 71.28%, SGPT 65.24%, ALP 80.68%, BL 54.92%) were found active in  $\text{CCl}_4$  model at 50 mg/kg dose whereas drug silymarin showed reduction as (SGOT 79.73%, SGPT 74.26%, ALP 87.88%, BL 82.75%) at 25 mg/kg dose level. The protective effect can be confirmed by enzymatic and non-enzymatic comparative performance which resulted in a marked reduction of serum GOT, GPT, ALP and BL levels. To sum up the above discussion altogether, it is proved that *C. colocynthis* extract, fractions and isolated compound could inhibit  $\text{CCl}_4$  induced hepatitis by regulating various biochemical parameters such SGPT, SGOT, ALP and BL and liver metabolites. These data are required not only for identification procedures that guarantee the utilization of the appropriate raw material, but also for quality-control standards demanded by health legislation.

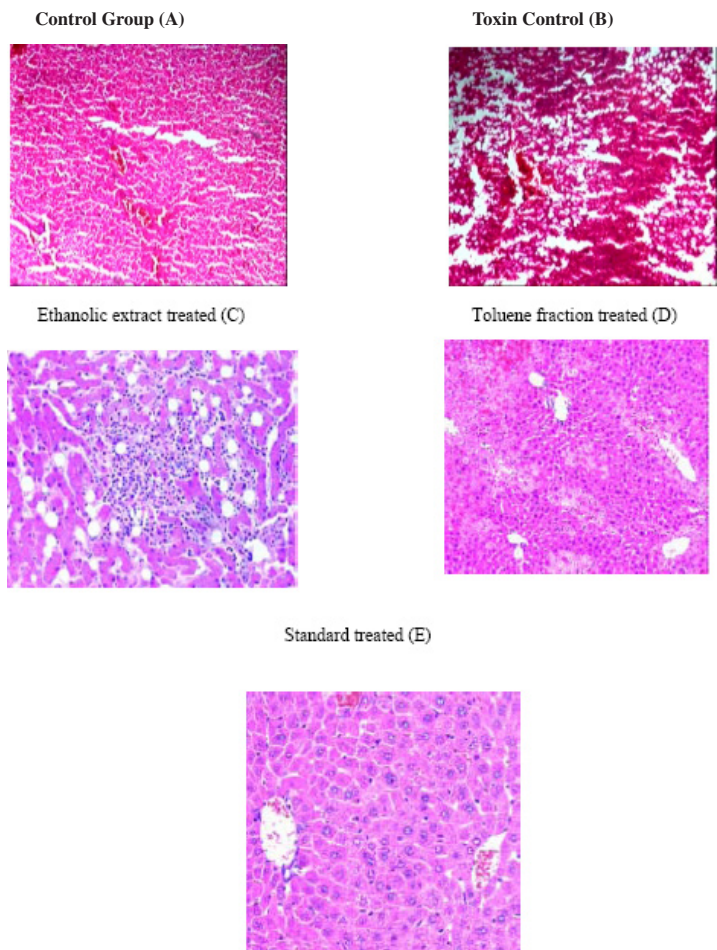


**Figure 1:** Hepatoprotective activity of extract/fractions/pure compounds from *C. colocynthis*.

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**Figure 2:** Histopathological study.

**Pathological findings:** (A) Section of control rat liver showing normal central vein and distinct nucleus. (B) The liver sections of  $\text{CCl}_4$  intoxicated group showed disarrangement of normal hepatic cells with centrilobular necrosis and vacuolization. (C) Section of rat liver treated with ethanolic extract indicated the normal hepatocytes cells without any vacuoles and necrosis. (D) Toluene fraction of ethanolic extract showed compact integrated cellular structure without necrosis and fibrosis, indicating effective hepatoprotective action. (E) The normal architecture was restored to the same as that of the standard drug Silymarin treated group.

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## 4. CONCLUSION

The result of above investigations carried out on the plants *Citrullus colocynthis* has clearly indicated that this plant possess hepatoprotective against carbon tetra chloride induced toxicity in experimental animals. On the whole, the findings offer justification for their incorporation in different polyherbal formulation and as folk medicine used for the treatment of various liver disorders.

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