Journal of Experimental Sciences 2012, 3(9): 43-45
ISSN: 2218-1768
Available Online: http://jexpsciences.com/

journal of
experimental sciences

Antihepatotoxic potential of *Citrullus colocynthis* root extract, fractions and isolated compounds

Alok Mukerjee[1]*, Shanti Bhushan Mishra[1] and Shubhini Saraf[2]

[1]United Institute of Pharmacy, UCER, Allahabad, India.
[2]Department of Pharmacy, BBDNIT&M, Lucknow, India.

Abstract

Natural remedies from 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* in rats using carbon tetrachloride (CCl_4) model. On further fractionation of the ethanolic extract into three fractions, the activity was localized in the toluene fraction. These on purification led to the isolation of 2 pure compounds – Cucurbitacin B [1] and Colocynthin [2]. These two compounds showed promising activity in CCl_4 model at 50 mg/kg dose level.

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

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. 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 marinum*, (Ram;2001) *Picrrorhiza kurroa*, (Thyagarajan; 2002) *Andographis paniculata*, *Phyllunthus niruri* and *Eclipta alba*.(Bisset;1994).

Citrullus colocynthis L. sch. (Cucurbitaceae) commonly known as indrayan 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 gonorrhea (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, 2005). The drug 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.

Received: July 10, 2012; Revised: Oct 14, 2012; Accepted: Nov 26, 2012.

*Corresponding Author

Alok Mukerjee
United Institute of Pharmacy, UPSIDC Industrial area, Naini, Allahabad-211010 (India)

Tel: +91-9415456913, Fax: 0532-2687142
Email: alokmukerjee2107@gmail.com

MATERIAL AND METHODS

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.

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 defatation. The defatted powdered drugs were 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 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.

Hepatoprotective activity of plant extract, fraction and isolated compounds against CCl4 model

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

The effect of extract, fractions and isolated compounds of the plant on carbon tetra chloride (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 (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 (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 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. The serum was used to estimate Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Total Bilirubin (BL). Percent reduction of biochemical parameters was calculated by following equation.

$$\% \text{ Reduction} = \frac{\text{AUC Toxin control} - \text{AUC treatment}}{\text{AUC control}} \times 100$$

RESULTS AND DISCUSSION

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 are 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 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 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.

Table 1. Hepatoprotective activity the extract/fractions/pure compounds of the *C. colocynthis*

Extracts/fractions/ compounds	Dose mg/kg	(%Reduction) SGOT (U/ml)	SGPT (U/ml)	ALP (U/L)	BL (mg/dL)
Control group	1ml	29.52±1.78 [a]	15.03±2.30 [a]	4.45±0.42 [a]	0.24±0.02[a]
Toxin group	2.5ml	114.01±8.82	77.52±9.26	18.52±0.64	4.06±0.14
Ethanolic extract	100	38.19±2.50 (89.73)	47.45±5.96 (48.11)	11.54±0.45 (49.60)	1.32±0.15 (71.72)
Toluene soluble fraction	100	85.42±2.13 (68.6)	39.61±0.59 (91.1)	29.37±2.36 (59.7)	0.84±0.15 (77.1)
Chloroform soluble fraction	100	112.41±4.22 (48.2)	48.40±4.24 (77.2)	41.28±2.45 (24.9)	1.19±0.07 (59.3)
Ethyl acetate soluble fraction	100	104.20±4.87 (54.4)	44.90±3.05 (82.8)	34.41±2.43 (45.0)	1.12±0.03 (62.9)
Cucurbitacin B (1)	50	35.61±2.36 (88.6)	68.47±4.05 (92.2)	28.10±1.60 (61.4)	1.29±0.22 (82.2)
Colocynthin (2)	50	38.11±3.12 (84.4)	71.32±3.16 (89.5)	26.42±2.65 (68.0)	1.96±0.45 (66.5)
Standard (Silymarin)	25	79.77±2.98 (72.9)	44.59±2.28 (83.2)	27.62±1.70 (64.8)	0.70±0.05 (84.2)

Each group contains 6 animals. Values in parenthesis indicate percentage recovery. All groups were compared to toxic control by students-Newman-Keuls-test(a P<0.001)

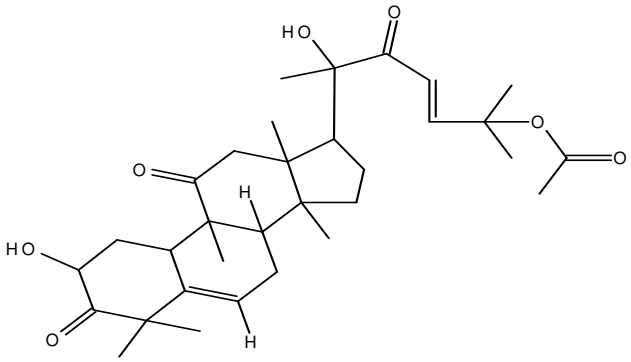

Figure 1 (a) Chemical structure of compound cucurbitacin B [1]

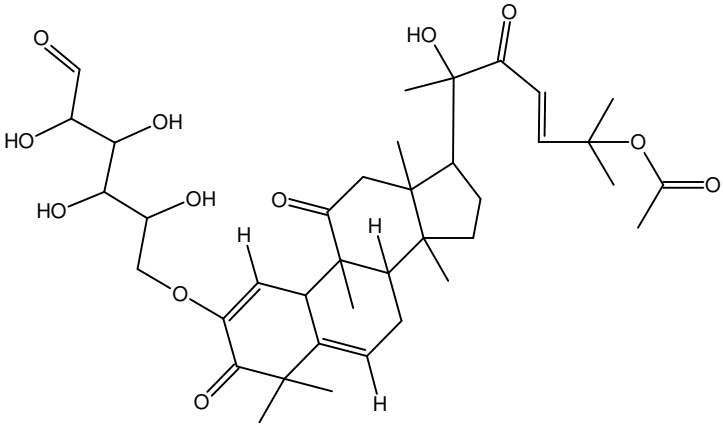

Figure 1 (b) Chemical structure of compound colocynthin [2]

ACKNOWLEDGEMENT

The authors wish to acknowledge the constant encouragement, supports of Dr. PKS Visen and facilities provided by Central Drug Research Institute, Lucknow for successful completion of this work.

REFERENCES

[1] Abdel-Hassan, I.A., Abdel-Barry, J.A. and Tariq Mohammeda, S. 2000. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits, J Ethnopharmacol. 71 (1-2): 325-30

[2] Al-Yahya, M.A., Al-farhan, A.H. and Adam, S.E.I. 2000. Preliminary toxicity study on the individual and combined effects of *C. colocynthis* and *N. oleander* in rats. Fitoterapia. 71: 385-391

[3] Bhattacharyya, D., Pandit, S., Mukherjee, R., Das, N. and Sur, T.K. 2003. Hepatoprotective effect of Himoliv: A polyherbal formulation in rats. Ind J Physiol Pharmacol. 47: 435–440

[4] Bisset, N.G. 1994. Herbal drugs and phytopharmaceuticals. A handbook for practice on a specific basis. Sttutgart: Medpharm Scientific, pp - 302-314.

[5] Chadha, Y.R. 1950. Wealth of India, Raw material Vol. II, CSIR New Delhi, pp-185-186

[6] Janbaz, K.H. and Gilani, A.H. 2000. Studies on preventive & curative effects of berberine on chemical-induced hepatotoxicity in rodents. Fitoterapia. 71: 25-33

[7] Kirtikar, K.R. and Basu, B.D. 1975. Indian medicinal plant Vol II, 2nd edition, Periodical experts New Delhi. pp. 1146-1147

[8] Kurma, S.R. and Mishra, S.H. 1997. Isolation and assessment of hepatoprotective activity of fumaric acid obtained for the first time from *Sida cordifolia* Linn. Indian Drugs. 34: 702-706.

[9] Luper, S.N.D. 1998. A review of plants used in the treatment of liver disease: Part 1. Alt Med Rev. 3: 410-21.

[10] Moli, P.C. 2001. Antispermatogenic effect of alcoholic extract of *C. colocynthis* roots. Pharm Biol. 39 (2): 113-114

[11] Ram, V.J. 2001. Herbal preparations as a source of hepatotoprotective agents. Drug News Perspec. 14: 353-63.

[12] Thyagarajan, S., Jayaram, S., Gopalkrishnan, V., Hari, R., Jeya, K.P. and Sripathi, M. 2002. Herbal medicines for liver diseases in India. J Gastroenterol Hepatol. 3: 370-376.

[13] Wallis, T.E. 2005. Textbook of Pharmacognosy, 5th edition, CBS publications and distributor, New Delhi.