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HEPATOPROTECTIVE EFFECT OF METHANOLIC EXTRACT OF *COLDENIA PROCUMBENS* LINN AGAINST D-GALACTOSAMINE INDUCED ACUTE LIVER DAMAGE IN RATS

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ABSTRACT

Shade dried and coarsely powdered plant (1 kg) was extracted successively with Chloroform and methanol in a Saxhlet apparatus and tested for antihepatotoxic activity on rats with 200 mg/kg of D-Galactosamine (D-GalN) orally. The parameters assessed were serum levels of ASAT, ALAT, ALP, total protein, albumin, globulin, total cholesterol, total bilirubin and blood sugar changes in liver. There was significant reversal of biochemical changes induced by D-Galactosamine treatment in rats by Alcohol extract treatment, indicating promising hepatoprotective activity.

KEYWORDS: *Coldenia procumbens* Linn, Hepatoprotective activity, D-Galactosamine (D-GalN)-induced hepatic damage, Serum Glutamic Oxaloacetate Trasaminase (SGOT), Serum Glutamic Pyruvate (SGPT), Trasaminase (SGOT), Alkaline Phosphatase (ALP).

INTRODUCTION

More than 80% of the world populations rely on conventional, therapeutic practices for primary health care needs as per the statistics of world Health Organization (Kirthikar and Basu, 1975). Liver disorders are considered one of the most life-threatening circumstances in developing countries, including Egypt. The liver is concerned in wide range of functions and hence it can be exposed to toxins and drugs, as well as viral hepatitis (Nunez, 2006).

Liver disease is one of the dangerous health problems. In the absence of reliable liver protective drugs in allopathic medical practice, traditional medicines play a vital role in the administration of various liver disorders (Anonymous, 1996). Various Medicinal Plants and their formulations are use for liver disorders in ethno medical Practice as well as in traditional system of medicine in India. The majority of the herbal drugs speed up the natural healing process of liver. So the explore for effective hepatoprotective drug continues (Subramanian *et al*., 1998).

Hepatic problems due to ingestion or inhalation of hepatotoxins acetaminophen, carbon tetrachloride, ethanol, cadmium chloride and ally alcohol are interesting world wide (Turner, 1965).Anti-Inflammatory activity of the ethanolic extract of the aerial parts of *Coldenia procumbens* Linn. Has been reported (Arul et al., 2005).In traditional medicine fresh leaves of *Coldenia procumbens* are ground and applied for rheumatic swelling while dried plant is powdered with fenugreek seed and applied warm for causing suppuration of boil. (Chopra et al., 1956).The *Coldenia procumbens* is used to relieve fever, piles, leucorrhoea and menorrhagia (Bhat et.at; 1985).

The extracts of *Coldenia procumbens of* Aqueous, methanolic and acetone have been reported for their angiotensin converting enzyme (ACE) inhibitory activity, they were selected on the basis of their usage as cardiotonic, diuretic and other uses related to symptoms of hypertensions. (somanathan et al; 1999). *Coldenia procumbens* (Family. Boraginaceae) is a procumbent, Deep-rooted, hairy herb found throughout India as a weed in moist place (The wealth of India cl-cy 2001). Coldenia procumbens Linn has been widely used for a number of medicinal purposes especially in Siddha medicine (Beena et al., 2011).

The purified extract form of Silybum marianum Gaertn is Silymarin, composed mainly of flavonolignans like silybin, silibinin and its diastereoisomers isosilybin, silydianin and silychristin (Franschini F *et al., 2002*).Silymarin is commonly used in the healing of liver diseases where it is capable of protecting liver cells directly by stabilizing the membrane permeability through inhibiting lipid peroxidation (LPO) and preventing liver glutathione depletion (Gazak R *et al., 2004*).

Considering the indigenous uses of the plant, the present study was aimed at evaluating the hepatoprotective of chloroform extract of *Coldenia procumbens* Linn on rat liver damage induced by D-GalN.

MATERIALS AND METHODS

Chemicals

The solvents used for extraction of the plant material were of analytical grade.

D-Galactosamine (D-GalN) was purchased from Merck India Ltd., Mumbai, India. Autopak Siemens assay kits for estimation of serum Aspartate aminotransferase (ASAT), Alanine amino-trasaminase (ALAT), alkaline Phosphatase (ALP), total protein (TP),Albumin, Globulin, total cholesterol (TC), total bilirubin (TB), and Blood sugar were obtained from Healthcare Diagnostics Ltd. Gujarat, India and Silymarin from sigma, USA. All the other chemicals used were of analytical grade.

Collection of the plant material.

The whole plant of *Coldenia procumbens* Linn was collected during in March 2010 from Pattukkottai district, Tamilnadu. It was authenticated by Dr. Sasikala Ethirajulu, Siddha central Research Institute. A voucher specimen (ACC.No. 7311) has been deposited in the Institute.

Extraction:

Shade dried and coarsely powdered plant (1 kg) was extracted successively with chloroform and alcohol in a Saxhlet apparatus. The extracts were filtered through Whatman No.1 filter paper and distilled on a water bath to get a syrupy mass. The extracts were then dried in vacuum (yield 23 and 20 gram respectively).

The Alcohol extract was investigated for hepatoprotective activity in rats, hepatic damage induced by D.Galactosamine.

Animals

Both sex Wistar rats (120-180 g) were selected for the study and maintained at a controlled temperature of 19-25 °C with 12 h light/dark cycle and fed with a standard diet and water *ad libitum.* The experiments were conducted according to the Institutional Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (43 /PHARMA/SCRI-2007).

Preparation of suspensions

Alcohol extract of *Coldenia procumbens (CpEt)* and standard Silymarin were suspended in distilled water using sodium carboxymethylcellulose (sodium CMC, 0.3%) and administered orally to the animals with the help of an intragastric catheter.

Hepatoprotective activity

The rats were randomly divided into five groups of six animals each. Group I served as normal and received the vehicle (Sod. CMC 0.3%, 5 ml/kg body weight). Group II was served as D-GalN treated control. Groups III and IV were treated with Alcohol extract of *Coldenia procumbens* Linn at the dose levels of 200 and 400 mg/kg body weight. Group V was treated with standard drug Silymarin at 100 mg/kg body weight. All these treatments were given orally for 11 days. On the 9th day of the treatment, the animals of groups III-V received a single dose of D-GalN in distilled water at 200 mg/kg body weight intraperi-toneally. Alcohols extract of *Coldenia procumbens* Linn or standard Silymarin treatments. On the 11th day, the animals were anesthetized by anaesthetic ether and blood was collected from the retro orbital puncture and kept for 30min at 4°C. Serum was separated by centrifugation at 3000 rpm for 5min at 4°C and used for the biochemical estimations. ASAT(Expert panel of the IFCC ,1976) , ALAT(Expert

panel of the IFCC ,1976) , ALP (Z.Klin 1970)total cholesterol(Allain C.A, *et.al.,* 1974), total bilirubin(Jendrassik,L. *et.al.,*1938),totalprotein(Hentry,R.J.D.C.et.al.,1974),albumin(Doumas,B.T.et.al.,1971),globulin and blood sugar(Trinder *et.al.,*1969)were measured in an semi-auto analyzer (RA-50, manufactured by Bayer, India) using Autopak Kits.

After the collection of blood samples, the liver and kidney were excised, rinsed in ice-cold normal saline. A portion of the liver and kidney tissues were fixed in 10% formalin, cut into 5 um thick sections and stained using haematoxylin-eosin and histopathological observations were made.

Statistical analysis

The significance of the data was analyzed by student's' test and followed by one-way ANOVA and $P < 0.05$ was considered as statistically significant.

RESULTS

In the acute toxicity studies, *Coldenia procumbens* Linn did not show any toxicity and mortality up to 2000 mg/kg dose.

The elevated levels of ASAT, ALAT, ALP, total protein, albumin, globulin, total cholesterol, total bilirubin and blood sugar in D-GalN intoxication were significantly reduced in the animals pre-treated with *Coldenia procumbens* Linn as depicted in Table 1.

Treatment with Alcohol extract of *Coldenia procumbens* Linn (200 mg/kg) and (400 mg/kg) showed significant hepatoprotective activity and it was comparable with the standard Silymarin (100mg/kg).

DISCUSSION

D-GalN hepatotoxicity is considered as an experimental model of acute hepatitis and it does not affect other organs (Rasenack et al., 1980). The pre-treatment with alcohol extract of *Coldenia procumbens* Linn at 200 and 400 mg/kg body weight doses significantly reversed the levels of serum enzymes, produced by D-GalN and caused a subsequent recovery towards normalization. Hence, the possibility of the mechanism of hepatoprotection of alcohol extract of *Coldenia procumbens* Linn may be due to its antioxidant action. *Coldenia procumbens* Linn alone treatment at 400 mg/kg body weight given orally for 11 days to normal animals also showed its hepatoprotective activity.

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Table 1

Effect of Alcohol extract of *Coldenia procumbens* on biochemical parameters in D-GalN induced hepatotoxicity.

S. No	Groups	SGOT	SGPT	ALP	Bilirubin	Cholesterol	Total protein	Albumin	Globulin	Glucose
1.	Control	127.66 ± 8.84	68.66 ± 7.91	282.83 ± 56.49	0.62 ± 3.27	98.83 ± 7.86	6.5 ± 0.48	3.93 ± o.47	3.03 ± 0.13	71.16 ± 4.3
2.	D-galactosamine 400mg/Kg	148 ± 5.75	97 ± 5.19	426.33 ± 31.25	1.93± 4.02	81 ± 6.92	8.48 ± 0.10	3.25 ± 0.11	5.23 ± 0.10	94.83 ± 0.60
3.	Methanolic extract 200mg/Kg	129.16 ± 23.52*	61.66 ± 4.77*	333 ± 43.75*	0.98 ± 3.24*	74.33 ± 7.44	7. 21± 0.14**	3.21 ± 0.16*	5.73 ± 4.77*	83.16 ± 5.23*
4.	Methanolic extract 400mg/Kg	124.16 ± 11.01**	42.66 ± 2.70**	304.66 ± 38.56*	0.78 ± 5.62**	78.16 ± 5.62*	6.75± 0.25**	3.28 ± 0.12*	5.7 ± 0.24*	77.33 ± 2.30**
5.	Silymarin (100mg/Kg	117.83 ± 27.89**	63.33 ± 9.93**	289.33 ± 57.27**	0.51 ± 4.06**	59.33 ± 9.71*	7.68 ± 0.33*	4.76 ± 0.14*	2.91 ± 0.28**	86.5 ± 4.85*

Values are given as mean ± S.E.M. for groups of six animals each; values are statistically significant at P <0.05*,
P <0.01**d-GalN control vs. treated groups

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