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## **Research Article**

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

# R.GANESAN<sup>1\*</sup>, MATHURAM VENKATANARASIMHAN<sup>2</sup>, SHARAD PAWAR<sup>1</sup>, G.PRAMOD REDDY<sup>1</sup>, T. ANANDAN<sup>1</sup> AND G. MASILAMANI<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Siddha Central Research Institute, Anna Hospital Campus, Arumbakkam, Chennai 600106.Tamilnadu, India

<sup>2</sup>Department of Chemistry, CSMDRIA &S, Anna Hospital Campus, Arumbakkam, Chennai-600106.Tamilnadu, India \*For correspondence email: <u>dayanand01@gmail.com</u>

#### ABSTRACT

Hepatoprotective activity of Coldenia procumbens Linn whole plant chloroform Extract - 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 Serum Glutamic Oxaloacetate Trasaminase (SGOT), Serum Glutamic Pyruvate (SGPT), Trasaminase (SGOT), Alkaline Phosphatase (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 chloroform extract treatment, indicating promising hepatoprotective activity.

**KEYWORDS**: Coldenia procumbens Linn, Hepatoprotective activity, D-Galactosamine (D-GalN)-induced hepatic damage.

#### **INTRODUCTION**

Liver diseases remain one of the serious health problems<sup>1</sup>. Modern medicines have little role to alleviation of hepatic diseases and the plant-based preparations which are chiefly available medicines employed for the treatment of liver disorders<sup>2</sup>. Liver performs several diversified functions. It is the central organ of body's metabolism<sup>3</sup>. The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory and neoplastic insults<sup>4</sup>. The metabolic activities of the liver are essential for providing fuel to the brain, muscle, and other peripheral organs<sup>5</sup>.

Coldenia procumbens (Family: Boraginaceae) is a procumbent, Deep-rooted, hairy herb found throughout India as a weed in moist place<sup>6</sup>. Coldenia procumbens Linn has been widely used for a number of medicinal purposes especially in Siddha medicine<sup>7</sup>. Fresh leaves-ground and applied to rheumatoid swellings<sup>8</sup>.

It is used in external application of boils. In folklore medicine it is used to treat rheumatic swelling, immature abscesses, leucorrhoea, menorrhagia, ant diabetic, anti-arthritic and hypertensive<sup>9</sup>. In vitro Antibacterial activity of different extracts of leaves of Coldenia procumbens has been reported<sup>10</sup>. The powdered roots enter into a compound formulation given in leucorrhoea and menorrhagia<sup>11</sup>. Anti-Inflamatory activity of the ethanolic extract of the aerial parts of Coldenia procumbens Linn. was reported<sup>12</sup>.

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 serum aspartate aminotransferase (ASAT), alanine amino- transaminase (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

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Shade dried and coarsely powdered plant (1 kg) was extracted successively with chloroform and methanol 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 chloroform 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**

Chloroform 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 Chloroform 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 9<sup>th</sup> 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. Chloroform extract of Coldenia procumbens Linn or standard silymarin treatments. On the 11<sup>th</sup> 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, ALAT, ALP, total cholesterol, total bilirubin, total protein albumin, globulin and blood sugar were measured in an semiautoanalyzer (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 heamatoxylin-eosin and histopathological observations were made.

#### Statistical analysis

The significance of the data was analyzed by students 't' 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 chloroform 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<sup>13</sup>. The pretreatment with chloroform 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 chloroform extract of 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 Chloroform extract of Coldenia procumbens on biochemical parameters in d-GalN induced hepatotoxicity

S. NO	GROUPS	SGOT	SGPT	ALP	BILIR UBIN	CHO LEST EROL	TOTA L PROT EIN	ALB UMI N	GL OB ULI N	GLUC OSE	GLUC OSE
1.	Control	207.66 ± 8.84	68.66 ± 7.91	282.83 ± 56.49	0.62 ± 3.27	98.83 ± 7.86	6.5 ± 0.48	3.93 ± 0.47	3.03 ± 0.13	71.16 ± 4.3	71.16 ± 4.3
2.	D- galactosamine 400mg/Kg	841 ± 5.75	95 ± 5.19	624.33 ± 31.25	1.43± 4.02	81 ± 6.92	8.48 ± 0.10	2.95 ± 0.11	5.23 ±0.1 0	94.83 ± 0.60	94.83 ± 0.60
3.	Chloroform extract 200mg/Kg	134.33 ± 11.66*	52.66 ± 3.74* *	513.5 ± 88.47*	0.48 ± 8.39*	70.33 ± 5.22**	8.53 ± 0.42**	3.03 ± 0.14*	5.5 ± 0.46 *	87 ± 4.2*	87 ± 4.2*
4.	Chloroform extract 400mg/Kg	130.66 $\pm$ 10.05* *	62.5 ± 3.41*	433.16 ± 35.24*	0.35±3. 59**	85.5±5 .85*	8.36±0 .21*	5.31± 0.10* *	5.2± 0.12 **	93 ±3.8	93±3.8
5.	Silymarin (100mg/Kg	171.83 ±27.89 **	63.33 ± 9.93*	389.33 ± 115.27**	0.515±4 .06	59.33± 9.71*	7.68±0 .33*	4.76± 0.14*	2.9± 0.28 **	86.5 ± 4.85*	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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