Evaluation of Hepatoprotective Effect of *Cryptocoryne Spiralis* against Thioacetamide Induced Acute Liver Failure

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Abstract

Aim of the study was to investigate the hepatoprotective effect of Cryptocoryne spiralis against thioacetamide induced acute liver failure. Male Wistar rats were used in the study. Thioacetamide induced acute liver failure model was developed by three day treatment protocol with 350 mg/kg dose i.p. *Alcohol extract of C. spiralis root and rhizome (ECS) was administered p.o. at 300 mg/kg dose seven days prior to and three days post induction. Silymarin 50 mg/kg served as standard. Serum parameters ALT, AST, GGT, ALP, total proteins, total bilirubin, albumin, and ammonia were estimated prior to administration of thioacetamide followed by 24 and 48 h post administration. ECS and silymarin did not demonstrate any hepatoprotective effect rather aggravated the hepatotoxicity induced by thioacetamide as evidenced by the results of biochemical estimations and histopathology studies. Thus this study could not substantiate the claim regarding hepatoprotective effect of C.spiralis made in traditional systems of medicine.*

Key Words: Acute Liver Failure, Thioacetamide, *Cryptocoryne Spiralis*

1. INTRODUCTION

Tagara is a drug in Ayurveda is used for the treatment of anaemia (*pandu*), jaundice (*kamala*), mental disorders (*unmadha*), epilepsy (*apasmara*), fevers (*jwara)*, cough, and asthmatic conditions (*swas-kas*) and it is also a general and brain tonic[1]. The botanical source of Tagara is *Valeriana jatamansi* Jones, (Valerianaceae) and *Cryptcoryne spiralis* (Retz.) Fisch. ex. Wydl family Araceae, is one of the alternate sources [1]. *C. spiralis* is also known as Nattu atividayam and is used traditionally for the treatment of fever, jaundice, vomiting, cough, abdominal complaints [2]. Chemical analysis revealed that root and rhizome of *C. spiralis* contain alkaloid, glycosides and reducing sugars [3], carbohydrate, tannins, protein, fixed oil and saponins [4]. Active compounds of corms of *C. spiralis* are oxo fatty acid esters, ethyl 14 oxo-tetracosanoate and 15-oxoeicosanyl 14-oxoheptadecanoate, two fatty acid 22-oxononacosanoic acid and 26-oxohentriacontanoic acid [5]. *C. spiralis* is used traditionally for the treatment of jaundice but no scientific work has been reported yet. Hence this study was undertaken to evaluate the protective effect of alcohol extract of *C. spiralis* root and rhizome in thioacetamide induced acute liver failure model.

2. METHODOLOGY

2.1 Plant material

Root and rhizome of *C. spiralis* [6] were collected from vicinity of Gopalasamudran tank, Tirunelveli district, Tamil Nadu during March 2010. The plant was identified and authenticated by Dr. S N Yoganarasimhan, Plant Taxonomist, of Faculty of Pharmacy, Bangalore and herbarium specimen (037) has been deposited in the herbarium of Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bangalore. Combined alcohol extract of *C. spiralis* root and rhizome (ECS) was prepared by Soxhlation with 95% ethanol (yield 4% w/w).

2.2 Animals:

Wistar rats of either sex were used in the study. Animal maintenance was in accordance with CPCSEA guidelines.

2.3 Acute toxicity study:

Acute toxicity study was performed following OECD guideline 423 [6].

2.4 Standardization and development of rodent model of thioacetamide induce acute liver failure:

On the day 0, blood was collected from the rats from retro orbital sinuses and plasma was separated to check liver function. Animals were randomized based on the body weight, bilirubin and AST levels. Freshly prepared thioacetamide TAA (350, 525, 750 mg/kg) was injected (i.p.) on day 0, day 1, day 2 and day 3. To prevent TAA induced hypoglycaemia and dehydration animals were supplemented with 40 ml/kg of 5% glucose and 20 nm potassium chloride in 0.45% saline orally [7]. All experimental groups were observed for mortality at different time intervals of 24, 48, 72 and 96 h. Every 24 h blood was collected and plasma was used for liver function test after TAA administration. The experimental group are describing as follow. (n=6)

1. Group 1 – Normal control
2. Group2 – Path control TAA (350 mg/kg)
3. Group3 – Path control TAA (525 mg/kg)
4. Group4 – Path control TAA (750 mg/kg)

2.5 Hepatoprotective activity

Animals were randomized based on the body weight, bilirubin level and AST level. Six animals were pre-treated with alcohol extract of *C. spiralis* and another 6 animals treated with silymarin for 10 days (day-7 to day 3). On the day 0, day 1, day 2, day 3 freshly prepared TAA (350, 525, 750 mg/kg) was administered (i.p.). To prevent TAA induced hypoglycaemia and dehydration animals were supplemented with 40 ml/kg of 5% glucose and 20 nm potassium chloride in 0.45% saline orally [8]. All experimental groups were observed for mortality at different time interval of 24, 48, 72 and 96 h. Every 24 h blood was collected and plasma was analyzed for liver function test after TAA administration. The experimental groups were as follows (n=6).

1. Group1- Normal control
2. Group2- Path control TAA (350mg/kg)
3. Group3 – TAA (350 mg/kg) + *C. spiralis* extract (300 mg/kg)
4. Group4 – TAA (350 mg/kg) + Silymarin (50 mg/kg)

2.6 Histopathology

Histopathology studies were performed following standard procedures [9].

2.7 Statistical analysis

Data are expressed as Mean ± SEM. Statistical evaluation was done using Two Way Analysis of Variance (ANOVA) followed by Tukey Kramer multiple comparison test.

3. RESULTS

Preliminary phytochemical study of alcohol extract of *C. spiralis* revealed the presence of carbohydrates, glycosides, fixed oil, phenolic compounds, tannins and saponins. In the acute toxicity study, ECS was found to be safe up to 2000 mg/kg. Change in body weight of the animals is a characteristic of acute liver failure. On day 1 and day 2 there was no significant difference in body weight of animals of path control groups when compared with the animals of normal control. While on day 3, a significant ($p<0.001$) decrease in the body weight of animals treated with 350 mg/kg of TAA was observed. ECS 300 mg/kg and silymarin 50 mg/kg attenuated the TAA induced decreased in body weight. Different enzyme marker including AST, ALT, ALP, GGT, total protein, total bilirubin, albumin and ammonia etc are associated with the liver diseases. Any alteration in enzyme markers, indicate liver injury [10]. In this study, TAA injection caused liver damage as demonstrated by elevation in the markers ALT, AST, GGT, ALP, total bilirubin, ammonia, and decrease in total proteins and albumin. On day 1, animals treated with 350 and 525 mg/kg of TAA did not show alterations in the biochemical parameters (Table 1). It was found that ECS 300 mg/kg and silymarin 50 mg/kg significantly elevated the hepatic marker levels, indicating a possible increase in hepatic damage (Table 2). However *C. spiralis* extract treatment prior to thioacetamide administration, did not manifest any kind of toxicity but when ECS was administered along with thioacetamide, it aggravated the liver damage.

Histopathological examination of liver in normal control group (Fig 1) revealed normal hepatocytes, sinusoidal spaces, central vein and portal triad was observed. Pathological control (Fig 2) group (350 mg/kg) revealed mild degree of multifocal fatty changes and dilatation of the sinusoidal spaces. TAA and ECS treated (Fig 3) group revealed moderate degree of infiltration of inflammatory cells predominantly lymphocytes around the central vein and between the hepatocytes, mild degree of fatty changes, apoptotic hepatocytes and dilatation of sinusoidal spaces. TAA and silymarin treated (Fig 4) group revealed severe degree of infiltration of inflammatory cells predominantly lymphocytes around the central vein and between the hepatocytes, mild degree of fatty changes, apoptotic hepatocytes and dilatation of sinusoidal spaces.

4. CONCLUSION

From this study it is concluded that thioacetamide (350 mg/kg) is the optimal dose for induction of acute liver failure in Wistar rats. Extract of *C. spiralis* root and rhizome did not reveal any protective effects against thioacetamide induced acute liver failure for the treatment period adopted in this study.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Table 1. Change in enzyme level after induction of acute liver failure

S.N	Parameters	Groups (day 1)				Group (Day 2)			
		Normal control	TAA (350mg/kg)	TAA (525mg/kg)	TAA (750mg/kg)	Normal control	TAA (350mg/kg)	TAA (525mg/kg)	TAA (750mg/kg)
1	ALT (IU/L)	53.9±1.4	3474.1±212***	2179.3±518.4***	220.3±39.7	48.8±5.2	1176.4±274.5***	114.4±12.3	80.5±8.1
2	AST (IU/L)	110.9±5.5	9737.6±755.4***	4293.8±1111.4***	356.7±10.5	112.7±5.3	1706.3±90.6	303.5±39.7	245.5±31.6
3	ALP (IU/L)	135.8±0.6	287.1±27.9***	215.5±32.4*	121.7±21.9	125.5±11.5	233.3±17.4***	143.9±19.8	147.7±12.7
4	GGT (U/L)	5.6±0.4	9.5±0.6***	7.7±0.2**	6.8±0.3	7.5±0.5	7.8±0.6	6.2±0.4	6.3±0.1
5	Total proteins (mg/dl)	6.4±0.1	5.5±0.2**	5.7±0.3*	5.4±0.1	6.4±0.1	6.6±0.1	6.4±0.1	6.4±0.1
6	Total bilirubin (mg/dL)	0±0	1.7±0.4***	1±0.2**	0.3±0.1	0.1±0	0.3±0	0.1±0	0.1±0
7	Albumin (mg/dL)	3.7±0	3.4±0.1	3.6±0.2	3.1±0.2*	3.7±0.1	3.9±0.1	3.8±0	3.9±0.1
8	Ammonia (mcg/dL)	60.3±0	168.8±0***	136.3±0***	102.5±0	64.3±0	101.3±0	83.1±0	61.7±0

Value Expressed as Mean± SEM

p<0.05 in comparison with normal control

p<0.01 in comparison with normal control

p<0.001 in comparison with normal control

Table 2. Evaluation of hepatoprotective effect of *C. spiralis* extract against thioacetamide induced acute liver failure

Sl. No	Parameters	Groups (day 1)				Groups (day 2)			
		Normal control	TAA (350 mg/kg)	ECS (300 mg/kg)	Silymarin (50 mg/kg)	Normal control	TAA (350 mg/kg)	ECS (300 mg/kg)	Silymarin (50 mg/kg)
1	ALT (IU/ml)	44.3±1.3	1176.4±274.5	4806.2±504.5	5921.2±670.8**	50.3±2.2	1176.4±274.5	3399.1±1074.6**	4710.3±911.2***
2	AST (IU/ml)	107.5±10.2	9737.6±755.4	11752.4±1806.2	10617.8±807.8	91.5±1.9	1706.3±90.6	5219±213.3**	7833.2±1130.5***
3	ALP (IU/ml)	109.4±11.8	287.1±27.9	241.3±28.8	249.6±11.8	125.6±7	233.3±17.4	275.5±47.6	255.2±40
4	GGT (U/L)	6.3±0.2	9.5±0.6	10.4±0.7	11.3±0.1	5.4±0.3	7.8±0.6	9.9±1*	9.6±1*
5	Total proteins (mg/dL)	6.3±0.3	5.5±0.2	5.2±0.1	5.2±0	6.8±0.2	6.6±0.1	6.9±0.1	6.9±0.1
6	Total bilirubin (mg/dL)	0.1±0	1.7±0.4	3.3±0.7*	3.3±0.4	0.1±0	0.3±0	1.9±0.9*	1.6±0.6*
7	Albumin (mg/dL)	3.8±0.3	3.4±0.1	3.2±0	3.7±0.5	3.9±0.1	3.9±0.1	4.2±0.2	4±0.1
8	Ammonia (mcg/dL)	78.4±3.5	168.8±0	186.5±36.8	177.9±19.4	78.4±3.5	101.3±0	150.5±39.3	248.4±31***

Value Expressed as Mean± SEM

p<0.05 in comparison with path control

p<0.01 in comparison with path control

p<0.001 in comparison with path control

Histopathology Study

Fig 1.Normal control

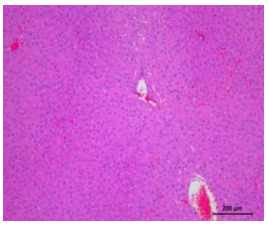

Fig 2. Path control

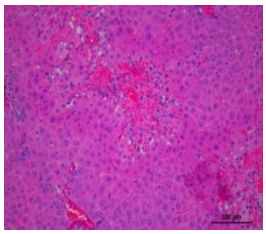

Fig 3. ECS

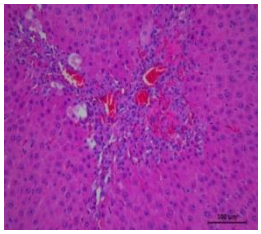

Fig 4. Silymarin

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