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Hepatoprotective activity of *Spillanthes acmella* Extracts against CCl_4-induced liver toxicity in rats

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ABSTRACT

Objective: Hepato protective activity of Spillanthes acmella Extracts (70% ethanol, methanol, ethyl acetate and hexane) were investigated. **Methods:** Hepatoprotective activity was assessed against CCl4 induced liver intoxication. **Results:** The extracts were produced concentration dependent percentage protection in the reduction of enzymes (Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase, Alkaline phasphatase and Total bilirubin) levels against CCl4 induced liver intoxication in rats. Among all extracts methanol extract showed better activity compare to other extracts with percentage protection of SGOT (84.39%), SGPT (79.04%), ALP (78.15%) and Total bilirubin (80.00%) levels at a dose of 500mg/kg. **Conclusions:** From the results obtained during the present study it could be concluded that Spillanthes acmella extracts has components that have hepato protective effects.

1. Introduction

Liver diseases are one of the most serious health problems in the world, but their prevention and treatment options still remain limited. Recently, the most common in vivo model used in the investigation of new hepato protective agents was liver injury induced by carbon tetrachloride (CCl_4), a chemical hepato toxin that causes a free radical -mediated hepato cellular damage [1,2]. Hepatic damage induced by CCl4 resulted in an increase in Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline phasphatase (ALP) and Total bilirubin (TB) concentrations [3-5]. Hepatoprotective studies by many researchers on medicinal plants reported that plants have active ingredients that are capable of free radical scavenging in living systems (Hepato protective) [6-8].

Spilanthes acmella L. (tooth- ache plant) is an annual herb belonging to the family Compositae. The genus is widely distributed throughout the tropics and subtro- pics and can be found in damp pastures, at swamp margins, on rocks near the sea and as a weed of road- sides and cultivations. The flower heads were chewed to relieve the toothache and other mouth related troubles. Leaves are used externally in treatment of skin diseases. Root decoction is used as purgative. Leaf decoction is used as diuretic and lithotriptic. Whole plant is used in treatment of dysentery [9].

The present study was aimed to evaluate the Hepato protective activity for different extracts of Spillanthes acmella against CCl4 induced rats.

2. Materials and Methods

2.1. Drug and Chemicals

Silymarin and Carbon tetrachloride (CCl4) were purchased from Sigma chemicals, USA. Serum Glutamate Oxaloacetate

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Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), Serum Total bilirubin (TB) kits were purchased from Span diagnostics Ltd, Gujarat, India. All other chemicals used were analytical grade.

2.2. Plant Material and Preparation of extracts

Freshly collected aerial parts of Spillanthes acmella plant was dried under shade and powdered. The coarse powder was extracted with 70% v/v ethanol, methanol, ethyl acetate and hexane separately in a Soxhlet apparatus. The liquid extracts were filtered and evaporated under reduced pressure by using rotary evaporator (Buchi R-210) until a soft mass obtained and then four extracts were used for further investigation.

2.3. Animals

Adult Wistar rats (National Institute of Nutrition, Hyderabad, India) of either sex weighing 200-250 g were used in the studies. The animals were maintained under standard laboratory conditions at an ambient temperature of 23±2℃ having 50± 5% relative humidity with 12-h light and dark cycle. The use and care of the animals in the experimental protocol has been approved by the local Institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA) following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4. Acute toxicity studies

Acute toxicity study was conducted according OECD Guide lines No.423. After fasting overnight, mice were administered with extracts of Spillanthes acmella in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed continuously for 1 h and then hourly for 6 h and finally after every 24 h up to 15 days for any toxicological symptoms or mortality.

2.5. Assessment of Hepatoprotective activity against CCL4 induced liver intoxication

Carbon tetrachloride intoxication in rats is an experimental model widely used to study necrosis and statues of liver [10-11]. The animals were divided in to XV groups, each consisting of 6 animals. The vehicle, standard and test group animals were treated with 5% gum acacia, 50 mg/kg dose of Silymarin and 125mg/kg, 250mg/kg and 500 mg/kg doses of 70% ethanol, methanol, ethyl acetate and hexane extracts of *S. acmella* for 5 days. On 6th day, 1hr after treatment with standard and test doses, the animals were intoxicated with CCl_4: liquid paraffin (1:1) (1ml/kg,p.o). On 7th day the blood samples were collected and analyzed for biochemical parameters like serum enzymes, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) were estimated by Reitman and Frankel, 1957 method, Serum Alkaline Phosphatase (ALP) by King and Armstrong, 1980 method and Serum Total bilirubin (TB) by Jendrassik and Grof, 1938 method by using commercial reagent kits in Autoanalyzer (RM4000, Biochemical systems International, Italy) [12-14].

2.6. Statistical Analysis

Data was analyzed by using One-Way ANOVA followed by post hoc Dunnett's test using Graph pad Prism-5 software. The results are expressed as Mean ∫ S.E.M. $P<0.05$ was considered as significant.

3. Results

The CCl4-induced hepatotoxicity model was used to evaluate the hepato protective effects of drugs and plant extracts. The hepato protective effect of Ethanol70%, Methanol, Ethyl Acetate and hexane extracts of Spillanthes acmella at doses of 125mg/kg, 250mg/kg and 500mg/kg assessed by measuring liver related biochemical parameters (SGOT, SGPT, ALP and TB) following CCl_4-induced hepatotoxicity. The percentage protection produced by the standard drug and extracts were calculated based on reduction of SGOT, SGPT, ALP and TB levels on 7th day of experiment in each case and the results were given in Table No. 1. In our studies, CCl_4-damaged rats that were previously treated with extracts showed a significant reduction in serum GOT, GPT, ALP and T. bilirubin levels. This is evidence that both stabilization of the plasma membrane and repair of CCl_4-induced hepatic tissue damage by the extracts of Spillanthes acmella. The standard drug silymarin and higher dosages of extracts showed a strong hepato protective effect against CCl_4-induced liver injury.

Group I was treated with vehicle showed no significant change in the biomarkers of enzymes (SGOT, SGPT, ALP and TB). Group II was treated with CCl_4, there is increase in SGOT, SGPTT, ALP and TB levels. Group III was treated with Silymarin, at a dose of 50mg/kg and after one hour followed by CCl4 intoxication, the reduced enzymes (SGOT, SGPT, ALP and TB) levels and percentage protection offered by the silymarin was 93.55%, 94.32%, 89.04% and 80% respectively.

Groups IV, V and VI treated with hydro-alcoholic extract of Spillanthes acmella orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl4 intoxication. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and TB levels were 43.10%, 30.92%, 57.23% and 60.00%, 55.80%, 42.87%, 66.94%, and 70.00%, 74.53%, 70.51%, 72.25% and 80.00% respectively.

Groups VII, VIII and IX were treated with Spillanthes acmella Methanolic extract orally at doses of 125mg/kg, 250mg/kg and

Table 1

Enzymes levels and Percentage (%) protection due to the effect of Vehicle, CCl4, Silymarin and Spillanthes acmella extracts at different doses

Name of the Extract	Amount of the extract											
	125mg				250mg				500mg			
	SGOT (U/L) 7th day	SGPT(U/L) 7th day	ALP(U/L) 7th day	TB(mg/dl) 7th day	SGOT (U/L) 7th day	SGPT(U/L) 7th day	ALP(U/L) 7th day	TB(mg/dl) 7th day	SGOT (U/L) 7th day	SGPT(U/L) 7th day	ALP(U/L) 7th day	TB(mg/dl) 7th day
Vehicle (5% um acacia)	96.17 ±2.85	56.00 ±1.46	217.5 ±1.06	0.17±0.01	96.17 ±2.85	56.00 ±1.46	217.50 ±.06	0.17±0.01	96.17 ±2.85	56.00 ±1.46	217.50 ±.06	0.17±0.01
CCl_4	194.3 ±2.73	141.0 ±1.88	471.5 ±12.6	0.27±0.0	194.3 ±2.73	141.0 ±1.88	471.5 ±2.16	0.27±0.0	194.33±2.73	141.00 ±.88	471.50±2.16	0.27±0.0
Silymarin (50mg/kg)	102.50±1.61	60.83±1.08	245.33±2.70	0.17±0.01	102.50±1.61	60.83±1.08	245.33±2.70	0.17±0.01	102.50±1.61	60.83±1.08	245.33±2.70	0.17±0.01
Percentage (%) protection	93.55	94.32	89.04	80.00	93.55	94.32	89.04	80.00	93.55	94.32	89.04	80.00
Ethanol (70%)	152.02±0.5	114.72±0.65	57.48±0.68	0.21±0.0	139.56±0.4	104.56±0.4	301.46±1.28	0.20 ±0.01	121.17±1.19	81.07±0.55	287.98±1.10	0.19±0.00
Percentage (%) protection	43.10	30.92	57.23	60.00	55.80	42.87	66.94	70.00	74.53	70.51	72.25	80.00
Methanol	147.4±0.46	109.96±0.36	316.34±0.63	0.21±0.00	140.18±0.57	94.06±0.82	293.86±0.79	0.20±0.00	111.49±0.75	73.82±0.69	273.0±0.93	0.19±0.00
Percentage (%) protection	47.81	36.52	61.10	60.00	55.17	55.22	69.94	70.00	84.39	79.04	78.15	80.00
Ethyl Acetate	165.64±0.75	122.28±1.12	327.57±0.33	0.21±0.00	145.36±0.58	102.19±0.73	314.46±0.81	0.20±0.01	131.54±0.51	85.94±0.52	299.84±0.82	0.19±0.00
Percentage (%) protection	29.33	22.02	56.67	60.00	49.89	45.66	61.83	70.00	64.01	64.82	64.58	80.00
Hexane	17.99±0.67	126.17±0.38	344.47±0.51	0.23±0.01	140.18±0.42	110.37±0.54	329.32±0.50	0.22±0.00	142.03±0.45	99.56±0.59	309.02±0.74	0.20±0.01
Percentage (%) protection	18.68	17.45	50.01	40.00	40.27	35.29	58.18	60.00	53.28	48.75	63.97	70.00

All groups were compared with CCl_4 group. Values are mean ±S.E.M., $n = 6$ animals per group.

Values in the parenthesis indicate percent protection in individual biochemical parameters from their elevated values caused by the hepatoprotection. The percentage of the protection is calculated as 100 × (values of CCl_4 −values of sample)/ (values of CCl_4 control − values of vehicle). The results are expressed as Mean ±S.E.M. All values are $P<0.05$ was considered as significant

500 mg/kg and after one hour followed by CCl4 intoxication. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and TB levels were 47.81%, 36.52%, 61.10% and 60.00%, 55.17%, 55.22%, 69.94% and 70.00%, 84.39%, 79.04%, 78.15% and 80.00% respectively.

Groups X, XI and XII were treated with Spillanthes acmella ethyl acetate extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl4 intoxication. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and TB levels were 29.33%, 22.02%, 56.67% and 60.00%, 49.89%, 45.66%, 61.83% and 70.00%, 64.01%, 64.82%, 64.58% and 80.00% respectively.

Groups XIII, XIV and XV were treated with Spillanthes acmella Hexane extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl4 intoxication. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 18.68%, 17.45%, 50.01% and 40.00%, 40.27%, 35.29%, 58.18% and 60.00%, 53.28%, 48.75%, 63.97% and 70.00% respectively. The results were given in Table No. 1.

The order of hepatoprotective activity of Spillanthes acmella extracts based on SGPT (ALT) levels is as follows:

Silymarin (50mg/kg)(93.55%) > Methanolic extract (500mg/kg) (79.04%) > Hydro-alcoholic extract (500mg/kg)(70.51%) > Ethyl acetate extract (500mg/kg)(64.82%) > Hexane extract (500mg/kg)(48.75%).

4. Discussion:

The liver plays an astonishing array of vital functions in the body. It is involved with almost all the biochemical pathways including detoxification, protein synthesis, and production of biochemical necessary for digestion and synthesis as well as breakdown of small and complex molecules, many of which are necessary for normal vital functions. Therefore, the maintenance of a healthy liver is vital to overall health and well being. But it is continuously and variedly exposed to environmental toxins, and abused by poor drug habits, and alcohol and prescribed & over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease. Growth factors and cytokines, such as HGF and IL-6, promote hepatic survival by stimulating liver regeneration and providing hepatoprotection in a variety of liver-injury models, including Fas-mediated injury, toxic damage caused by hepatotoxins (such as CCL4), and ischemic liver injury. These growth factors provide protection against chronic liver injury that ultimately leads to cirrhosis. Part of this protection is mediated by induction of anti apoptotic proteins that regulate the caspase cascade.

There are no specific allopathic medicines used as hepatoprotective, although different research works are going on some drug. Herbal drugs are more widely used than allopathic drugs as hepatoprotectives because they are inexpensive, have better cultural acceptability, better compatibility with the human body and minimal side effects. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. At the same time, surprisingly, we do not have readily available satisfactory plant drugs/formulations to treat severe liver disease. Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model.

Normally, SGOT and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells

and their levels in the blood increases. SGPT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. SGPT is more selectively a paranchymal liver enzyme than SGOT. Assessment of liver function can be made by estimating the activities of serum SGPT, SGOT, ALP and TBL which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage. Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with the extract indicates the effectiveness of it in normal functional status of the liver [15-17].

The results of biochemical parameters revealed the elevation of enzyme level in CCl_4 treated group, indicating that CCl_4 induces damage to the liver. A significant reduction was observed in SGOT, SGPT, ALP and T.BIL levels in the groups treated with silymarin and Spillanthes acmella extracts at different doses (table no 1). The results obtained for biochemical parameters are comparable with silymarin, the standard hepatoprotective drug. The groups which received methanol extract at the doses showed a significant decrease in the elevated levels of enzymes compared to other extracts (Ethanol 70%, Ethyl Acetate and Hexane). Therefore, as like silymarin the extracts have restored the altered level of enzymes significantly. It was found that the extracts decreased the CCl4 induced elevated levels of the enzymes levels, indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. The isolation and characterization of the common constituents of all extracts of Spillanthes acmella and screening of pharmacological action for isolated compounds against the liver damage has to be carried out to identify an efficient hepato protective drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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