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Research Article

INVESTIGATION OF PHYTOCHEMICAL SCREENING AND HEPATOPROTECTIVE ACTIVITY OF *RHEUM EMODI* IN CCL_4 INTOXICATED CHICKS

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Abstract

The liver is the key organ of metabolism and detoxification. The body depends on liver to regulate, synthesize, store and secrete many important proteins and nutrients and to purify, transform and clear toxic substances. Continuous exposure to a variety of environmental toxic agents may cause hepatic injury. Natural antioxidants have been proposed to prevent and treat hepatopathies induced by oxidative stress. The aim of this research project was to investigate the hepatoprotective activity of *Rheum emodi* in CCl_4 intoxicated chicks. Curative and preventive effect of aqueous extract and powdered plant material of *Rheum emodi* was evaluated. The results showed that curative effect of solid plant material was greater than that of aqueous extract as well as from preventive effect of both powdered plant material and aqueous extract.

Keywords: Hepatoprotective, *Rheum emodi*, phytochemicals, liver.

Introduction

The liver is an organ of vital importance. Due to its distinctive and significant regenerative capacity, even a moderate cell injury is not reflected by measurable change in its metabolic functions. However, some of its functions are so sensitive that abnormalities start appearing depending upon the nature and the degree of initial damage. The etiology of the liver disorders depends on various factors as nutritional, biochemical, bacteriological, viral, or environmental aberration. The liver plays a considerable role not only in the metabolism and disposition of the chemicals to which it is exposed directly or indirectly, but also in the metabolism of fats, carbohydrates, proteins, and immunomodulation.

Rheum emodi Linn (Polygonaceae) is a leafy perennial herb distributed in altitudes ranging from 2800 to 3800 m in the temperate and subtropical regions of Himalayas from Kashmir to Sikkim in India (Nazir et al., 2013). The herb has been traditionally used for treating pathological ailments like fevers, ulcers, bacterial infections, fungal infections, jaundice and liver disorders (Agarwal et al., 2000 ; Babu et al., 2003). Rhubarbs, the rhizomes of *Rheum* species are used in remedies of blood stagnation syndrome, which includes diabetes, atherosclerosis, ischemia, and inflammation in Japanese and Chinese traditional medicine. *R. emodi* constitutes an important food source in various different forms for the people of Kashmir predominantly in the rural and high altitude locations of the valley. *R. emodi* is also used for making pies that are used as antipyretic, antihelminthic, laxative, atonic indigestion, constipation, jaundice and liver disorder (Alam et al., 2005). Rhubarb contains a variety of compounds like flavonoids, anthraquinone glycosides, tannins, volatile oils and saponins (Ye et al., 2007; Aslam et al., 2012) and has long been used as an ingredient of purgative, laxative and stomachic.

Herbal drugs are playing an important role in health care programs worldwide, and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. Hepatoprotective effect of some plants like *Spirulina maxima*, *Eclipta alba*, *Boehmeria nivea*, *Cichorium intybus*, and *Picrorhiza*

kurroa has been well established (Duran et al., 1999; Saxena et al., 1994; Lin et al., 1998; Zafar and Mujahid 1998; Saraswat et al., 1999). Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity (Doreswamy et al., 1995).

The present work was carried out to evaluate the hepato protective effect of extracts of *Rheum emodi* in CCl_4 intoxicated chicks.

Materials and Methods

Sample collection and preparation of aqueous plant extract

Samples of *Rheum emodi* were collected from rail bazaar, local market, Faisalabad. Plant material was washed with cold water to remove dirt, dried in shade and ground to powdered form. Aqueous extract of plant was prepared by heating powdered plant material (30 g) in a round bottom flask for 2 hours with 300 mL distilled water. The mixture was filtered after cooling and the filterate was concentrated to yield semisolid material (39.15 %) (Badami et al., 2005).

Phytochemical screening

Detection of alkaloids

The extract for the detection of alkaloids were prepared by adopting the following methods.0.5 g dry powder of plant material was made into slurry by grinding it with 1 g of sand and 10 mL of chloroform. The slurry was made acidic with 5 mL of 2N H_2SO_4 and then filtered. The filterate was made alkaline with 5 mL of 2N NaOH and then centrifuged for 15 minutes. The upper aqueous layer was removed with the dropper and tested for its alkaloidal contents with the method of Brain and Turner (Culvenor and Fitzgerald, 1963; Brain and Turner, 1975).

Detection of Glycosides

The glycosides were identified by Stas-Otto procedure (Brain and Turner, 1975) .5 g of dry powdered plant material was boiled with 30 mL of 70% ethyl alcohol and filtered then 5-8 mL of 4% lead sub acetate solution was added till no further precipitation took place. The chlorophyll and other pigments (sugar, phenol, organic acids etc.) were precipitated, filtered it. 0.5 to 1.0 mL lead sub acetate solution was added into the 2 mL of the filterate. Excess of lead sub acetate was removed by adding 15 mL of distilled water saturated with H_2S gas. The black ppt. of PbS thus formed were removed by filteration. 0.5-1 mL of distilled water saturated with H_2S gas was added to 1-2 mL of filterate. This lead sub acetate free filterate was concentrated to 5 mL by heating it on an electric hot plate. To this (5 mL) concentrated filterate was added Benedict (Adam et al., 1970) and Fehling's solution separately. Red ppt. indicated the presence of glycosides.

Detection of cardiac glycosides

For detection of cardiac glycosides the methods described by Brain and Turner was used.

Powdered plant material (10 g) was boiled in 70% alcohol (100 mL) for 5 minutes and filtered. The filterate was diluted with 2 volumes of distilled water. It was followed by the addition of concentrated lead sub acetate solution (10 mL). The precipitate of chlorophyll and other pigments were removed by filteration. The excess lead sub acetate from the filterate was removed by passing hydrogen sulphide gas and subsequent filteration. The filterate was then extracted with chloroform (50 mL). The chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3.5 % ferric chloride in glacial acetic acid (3 mL), and concentrated sulphuric acid (1.5 mL) was added to it in such a way that it ran along the wall of test tube. Bluish black colour indicated the presence of cardiac glycosides.

Detection of flavonoids

For the detection of flavonoids, following methods were used.

Shinoda test

Powdered plant material (10 g) was boiled with 80% alcohol (50 mL) for 10 min. and filtered. After cooling, small pieces of magnesium ribbon were added, followed by the addition of concentrated HCl (3 mL) drop wise.

1) Ammonia test

A filter paper dipped in an alcoholic solution of flavonoid was exposed to vapours of ammonia solution (Siddiqui and Ali, 1997).

Detection of steroids and triterpenoids

The methods adopted were those developed by Segal and Harbone and later modified by Siddiqui and Ali.

1) Salkovaski test

To the chloroform solution of plant material, 84% sulphuric acid (2 mL) was added. Brownish colour was appeared.

2) Libermann-Burchard test

A few drops of acetic anhydride and 1 mL of concentrated sulphuric acid was added to the chloroform

solution of plant material. Yellowish colour was appeared (Segal, 1960; Harbone, 1972).

3) Antimony trichloride test

A saturated solution of antimony trichloride containing 20% acetic anhydride was added to the chloroform solution of plant material and then heated for 10 minutes.

Detection of Tannic acids

The methods adopted by Siddiqui and Ali were described as,

i) To an alcoholic solution of tannic acids, added slowly a solution of ferric chloride. After it, dilute sulphuric acid was added in it drop wise.
ii) The alcoholic solution of tannic acid was added to a solution of gelatin, albumin and some alkaloids.
iii)

Detection of Anthraquinones

10 g of powdered plant material was boiled with 100 mL of hot water for 30 minutes. Filtered hot, cooled the filterate and extracted it with 100 mL CCl_4. Took off CCl_4 layer, washed with 50 mL of water and was shaken with 50 mL diluted ammonia solution. A pink to cheery red colour was appeared in ammonical layer which indicated free anthraquinones.

Hepatoprotective studies

Animals

For this study chicks were selected which were kept under standard conditions of food and water.

Lethal dose determination

The chicks were subjected to a series of different concentrations of aqueous extract and powdered plant material. The concentration (4-10 g/kg, body weight) were administered orally to two chicks for each concentration. The LD_{50} of the aqueous extract and powdered plant material was found to be 9 and 10 g/kg body weight respectively. Aqueous extract and powdered plant material administration did not produce any abnormalities in the animals throughout the experimental period. The dose level selected for the present study was non-toxic and safe.

Experimental protocol

Study protocol consisted of two modes.
1) Preventive mode
2) Curative mode

Chicks were randomly divided into six groups with two animals in each as follows:

Group 1
It included two untreated chicks.

Group 2 (Experimental control)
Chicks were treated orally with 50 % CCl_4 in a dose of 0.4 mL/100g body weight for days 1, 2 and 3.

Group 3 (Treated group)
Curative effect of aqueous extract of *Rheum emodi*
Chicks were treated with 0.4 mL of 50 % CCl_4/100 g body weight for days 1 and 2. On 6, 8 and 10 day, aqueous extract was administered intraperitoneally.

Group 4
Preventive effect of aqueous extract of *Rheum emodi*
Animals were provided 0.2 g/30 g body weight of aqueous plant extract for day 1, 2 and 3. A dose of 50 % CCl4 (0.4 mL/ 100 g) was administered on 6th and 8th day.

Group 5
Curative effect of powdered (solid) plant material of *Rheum emodi*
Chicks were treated with CCl_4 as described in group 3 for 1 and 2 days. On 6th, 7th and 8th day, powdered plant material was administered orally.

Group 6
Preventive effect of powdered (solid) plant material of *Rheum emodi*
Chicks were orally administered 0.2 g/kg body weight powdered plant material on day 1, 2 and 3. On day 6 and 8 they were treated with CCl_4 50 % in olive oil 0.4 mL / 100 g body weight.

After 14 days all chicks were slaughtered and blood samples were collected for further studies. The blood samples were allowed to clot for 45 min at room temperature. Serum was prepared by centrifugation at 3000 rpm for 15 min. The clear supernatant was used for the estimation of biochemical parameters namely Serum glutamyl pyruvate transaminase (SGPT), Serum glutamyl oxaloacetic acid transaminase (SGOT).

Biochemical assessment

Biochemical assessment was performed with serum enzymes SGPT and SGOT by using ELISA reader.

Biochemical analysis

Serum samples were analysed by following the kinetic method using the Microlab 200 (Merck, Germany) for the

estimation of SGOT/AST and SGPT/ALT according to the recommendations of the expert panel of the IFCC (International federation of clinic chemistry).

Estimation of SGOT

50 µL of sample / serum were pipette into cuvet to which 500 µL of the working reagent was added and incubated at 37 ºC for one minute. The absorbance, taken against air was read at wavelength of 340 nm after one minute.

IU / L = Abs./ min x -1745

Estimation of SGPT

50 µL of sample / serum were pipette into cuvet to which 500 µL of the working reagent was added and incubated at 37 ºC for one minute. The absorbance, taken against air was read at wavelength of 340 nm after one minute.

IU / L = Abs./ min x -1745

Results and Discussion

The results of phytochemical screening and hepatoprotective potential of *Rheum emodi* were as follows.

Phytochemical screening

The roots of *Rheum emodi* belonging to family Polygoneceae were analyzed for alkaloids, flavonoids, glycosides, tannic acid, steroids and triterpenoids. The results obtained by qualitative and quantitative analysis of various phytoconstituents in powdered roots of *Rheum emodi* are summarized in table 1.

Table 1: Results obtained by qualitative and quantitative analysis of various phytoconstituents in *Rheum emodi*

Sr. no.	Phytoconstituents	+/-	% yield
1	Alkaloids	+	3
2	Anthraquinones	+	2
3	Flavonoids	+	13
4	Crude glycosides	+	6.8
5	Saponins	-	-
6	Steroids and triterpenoids	+	3
7	Tannic acids	+	17

Where, + = Present; = Absent

Alkaloids

Chloroform and alcoholic extract of plant showed precipitation on addition of various alkaloid detecting reagents such as brownish precipitates with Dragendroff's and Wagner's reagent. These observations suggested the presence of alkaloids in *Rheum emodi*. The semi quantitative estimation with the method of Brain and Turner revealed 3 % alkaloid in *Rheum emodi* (Brain and Turner, 1975).

Flavonoids

As an orange red crimson colour was obtained on treatment of the alcoholi**c** extract of plant with magnesium ribbon and concentrated HCl. Also, paper strip dipped in alcoholic extract of plant turned pink in ammonia test. These observations indicated that flavonoids were present in *Rheum emodi*. The semi quantitative estimation with the method of Brain and Turner revealed 13 % flavonoids in *Rheum emodi* (Brain and Turner, 1975).

Glycosides

For the detection of glycosides, an alcoholic extract of plant was prepared by adopting stas-otto method. This extract gave blue precipitates with Fehling's solution and green ppt. with Benedict's solution. These observations indicated the presence of glycosides. The semi quantitative estimation with the method of Brain and Turner revealed 6.8 % glycosides in *Rheum emodi* (Brain and Turner, 1975).

Steroids and triterpenoids

Chloroform extract gave brownish colour with acetic anhydride followed by addition of concentrated sulphuric acid by Salkovaski and Liberman-Burchard test. Also, the formation of pink ring at the junction in Salkovaski test indicated the presence of steroids and triterpenoids in *Rheum emodi.* The semi quantitative estimation with the method of Brain and Turner revealed 3 % steroids and triterpenoids in *Rheum emodi* (Brain and Turner, 1975).

Tannic acids

Alcoholic extract of plant gave bluish black colour with ferric chloride, which disappeared later with the formation of yellow brown precipitate by the addition of dilute sulphuric acid. Also, the appearance of thick precipitation in albumin test indicated the presence of tannic acid in *Rheum emodi*. The semi quantitative estimation with the method of Brain and Turner revealed 1.7 % tannic acids in *Rheum emodi* (Brain and Turner, 1975).

Anthraquinones

Cherry colouration was seen after the addition of ammonium hydroxide in the washed CCl_4 extract of *Rheum emodi*. This indicated the presence of anthraquinones in plant roots. The semi quantitative estimation with the method of Brain and Turner revealed 2 % anthraquinones in *Rheum emodi* (Brain and Turner, 1975).

Hepatoprotective studies

Curative effect of an aqueous plant extract and powdered plant material against CCl_4 induced rise of SGOT

The (Mean ± SEM) SGOT level of the control group was 233 ± 3.0 IU / L where as in CCl_4 treated group SGOT increased significantly upto 242 ± 20 IU / L which showed the hepatotoxic role of CCl_4. The post treatment with aqueous extract and powdered plant material of *Rheum emodi* inhibited the rise of SGOT by CCl_4 upto 225 ± 18.00 IU / L and 206 ± 9.00 IU / L, respectively (Fig 1).

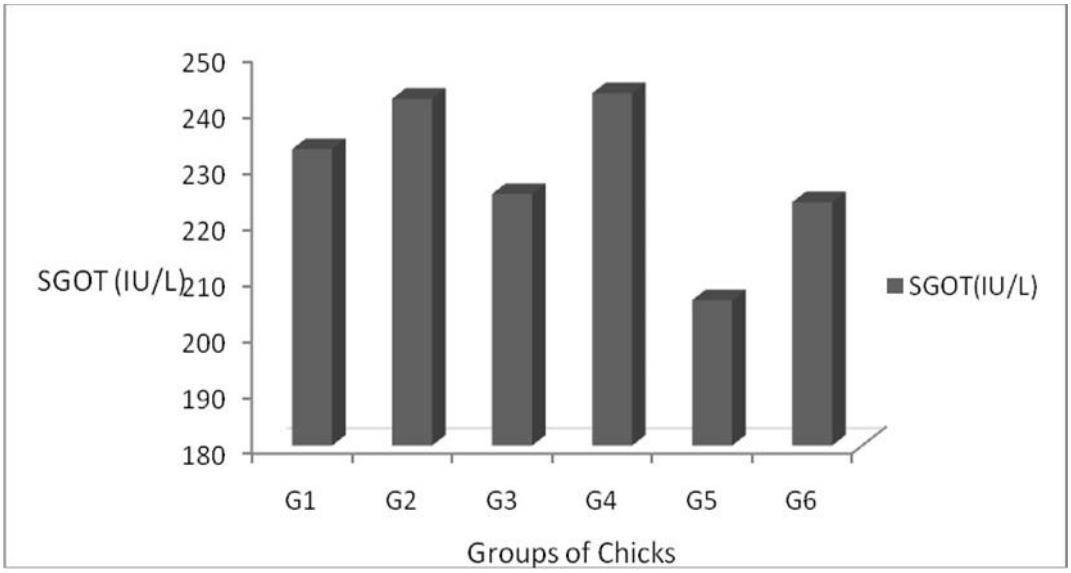

Fig. 1: Effect of *Rheum emodi* on activity of SGOT in CCl_4 induced hepatotoxicity

The hepatoprotective efficiency of solid plant material was greater than the aqueous extract of *Rheum emodi*. Solid plant material had greater tendency to decrease the increased level of SGOT by CCl_4. Aqueous extract slightly decreased the SGOT level as compared to CCl_4 treated group but efficiency was not greater than the solid plant material treated group.

Aqueous extract of plant lacked tannic acids which were present in solid plant material. Tannic acids are effective as hepatoprotective agent against CCl4 intoxication because these are free radical scavengers (Bruneton, 1999).

Curative effect of an aqueous plant extract and powdered plant material against CCl_4 induced rise of SGPT

The (Mean ± SEM) SGPT level of the control group was 8.5 ± 0.5 IU / L where as in CCl_4 treated group SGPT increased significantly upto 16 ± 1.0 IU / L which showed the hepatotoxic role of CCl_4. The post treatment with aqueous extract and powdered plant material of *Rheum emodi* inhibited the rise of SGPT by CCl_4 upto 7.2 ± 2.00 IU / L and 4.5 ± 0.5 IU / L, respectively (Fig 2).

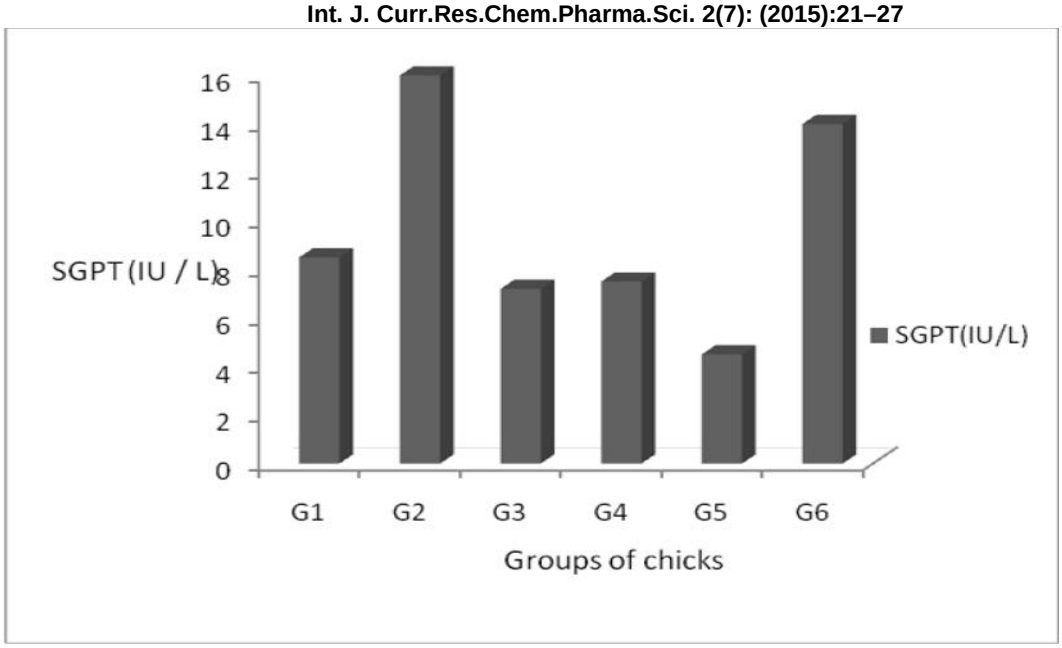

Fig. 2: Effect of *Rheum emodi* on activity of SGPT in CCl_4 induced hepatotoxicity

The hepatoprotective efficiency of solid plant material was greater than the aqueous extract of *Rheum emodi*.

Preventive effect of an aqueous plant extract and powdered plant material against CCl_4 induced rise of SGOT

The (Mean ± SEM) SGOT level of the control group was 233 ± 3.0 IU / L where as in CCl_4 treated group SGOT increased significantly upto 242 ± 2.0 IU / L. The pre treatment with aqueous extract of *Rheum emodi* caused increase in the SGOT value by CCl_4 upto 243 ± 24.00 and pretreatment with powdered plant material inhibited the rise of SGOT by CCl_4 upto 223 ± 25.5 (Fig. 1).

The hepatoprotective efficiency of solid plant material was greater than the aqueous extract of *Rheum emodi*. Pretreatment with aqueous extract showed no protective effect against CCl_4 increased level of SGOT. Solid plant material showed significant hepatoprotective potential.

Preventive effect of an aqueous plant extract and powdered plant material against CCl_4 induced rise of SGPT

The (Mean ± SEM) SGPT level of the control group was 8.5 ± 0.5 IU / L where as in CCl_4 treated group SGPT increased significantly upto 16 ± 1.0 IU / L. The pre treatment with aqueous extract and powdered plant material of *Rheum emodi* inhibited the rise of SGPT by CCl_4 upto 7.5 ± 0.5 and 14 ± 5.00, respectively (Fig.2).

The hepatoprotective efficiency of aqueous plant extract was greater than the solid plant material. The phytochemicals present in solid plant material possesses antioxidant properties and found to be useful in treatment of liver diseases.

Conclusion

In conclusion the data obtained in the present studies clearly suggested that *Rheum emodi* posseses hepatoprotective properties and support the empirical use of plant drug in the traditional system of medicine, so commonly practiced in the world.

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