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Research Article

Hepatoprotective Potential of Total Anthraquinone Fraction of *Rheum emodi*: *In vitro* and *In vivo* Studies

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ABSTRACT

Aim: The present study aimed to evaluate the hepatoprotective potential of total Anthraquinone glycoside fraction of *Rheum emodi*. *In vitro* in freshly isolated rat hepatocytes and *in vivo* in wistar rats intoxicated by carbon tetrachloride (CCl_4).

Methods: Dried rhizomes of rheum emodi were subjected to extraction with 70% ethanol and separation of total Anthraquinine glycoside fraction. In *in-vitro* study freshly isolated rat hepatocytes were treated with CCl_4 and various concentrations of the total anthraquinone glycoside fractions (200-400 µg/ml). In *in-vivo* study, the hepatoprotective activity of total anthraquinone glycoside fraction of *Rheum emodi* was analyzed in liver injured CCl_4 treated rats. The hepatoprotective activity was assessed using various biochemical parameters like serum bilirubin, protein, Alanine transaminase, Aspartate transaminase and alkaline phosphatase along with the Histopathological alterations.

Results: Anthraquinone glycoside fraction pre-treatment improving the removal rate of bromsulphalein from hepatic cells and also increases the cell viability this has been as a clinical evidence of hepatoprotective potential of rheum emodi. Anthraquinone glycoside administration shows significant dose dependent restoration of serum enzymes and antioxidants levels. In addition histomorphological pattern of all the experimental groups treated with Anthraquinone glycoside were significant restoration of the normal morphological pattern of hepatic cells.

Conclusion: The result obtained from the study of SOD, MDA, SGOT, SGPT and ALP clearly directing antioxidant (or) free radical scavenging property and hepatoprotective property of *Rheum emodi*.

Keywords: *Rheum emodi*, Total Anthraquinone glycoside content and Hepatoprotective.

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INTRODUCTION

Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. [1]However, reactive oxygen species also have useful cellular functions, such as redox signaling. Thus, the function of antioxidant system is not to remove oxidants entirely, but instead to keep at an optimum level. [2] Oxidative stress plays an important role in the

pathogenesis of many liver diseases. It has a fundamental factor in the pathological changes observed in various liver diseases. Oxidative stress plays an important role in the pathophysiological changes that progress to liver cirrhosis and finally to hepatocellular carcinoma.

Carbon tetrachloride (CCl_4) is acute hepatotoxic agent, in liver CCl_4 is biotransformed into Trichloromethyl free radical CCl_3 by the action of Cytochrome p450.[3] which induces per oxidative degeneration of membrane lipids this leads to formation of lipid peroxide which in turn into toxic aldehyde that causes liver damage. This involvement of toxic intermediate radicals such as CCl_3 (Trichloromethyl), $OOCCl_3$ (Trichloromethylperoxy) and Cl (Chlorine) free radicals as well as aldehyde products of lipid per oxidation. This radicals can react with O_2 to form high reactive species CCl_3OO (Trichloromethylperoxy) radical this will be initiates chain reactions of lipid per oxidation which causing hypo perfusion of the membrane. This affects the permeability of cell resulting in homeostasis. Carbon tetrachloride (CCl_4) increases Cytosolic enzymes like SGPT, SGOT and ALP in the blood where as decreasing hepatic glutathione, protein, albumin and SOD. Accordingly, effects of antioxidants or free radical scavengers have been widely tested for the prevention, treatment of acute and chronic liver injuries.

The use of herbal medicine for liver disease is continuing since centuries in the world. A number of medicinal plants are used in traditional system of medicinal for the management of liver disorders. In recent years many researchers have examined the therapeutic effectiveness of certain plants or extracts of the plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. Plant anthraquinones are widely distributed amongst plants and have a wide range of biological properties. Anthraquinone has been shown in certain studies to help aid in digestion as a laxative, reduce inflammation in arthritis patients, and to inhibit the growth of cancer cells. Anthraquinones that are used for medical purposes found to be naturally occurring implants and are not made through chemical reactions. In view of these reports Rhizomes of Rheum emodi plant was selected to screen the possible hepatoprotective activity.

Rheum emodi is an important medicinal plant which is extensively using in the ayureda and unani systems of medicines. *Rheum emodi* is commonly known as Indian rhubarb/ Revanchini belongs to family Polygonaceae.[4]It has the property of purgative, hemostatic, antipyretic, antihelmintic, laxative, atonic indigestion, constipation , diarrhea, dysentery,antibacterial, antitumor, antifungal, diuretic, hemostatic, cholagogue, antihypertensive, lowers serum cholesterol, anti-inflammatory and antioxidant activity. [5–7] The present study was designed to assess the protective potential of anthraquinone glycoside fraction of *Rheum emodi* rhizomes against ccl4 toxicity on liver.

MATERIAL AND METHODS

Chemicals: Chemicals and solvents required for study were obtained from S.D chemicals, Mumbai, Loba chemie Indo Austranel Co, Mumbai, Ranbaxy Laboratories Ltd, Punjab, Sigma fine chemicals, Mumbai and Hi media laboratories, Mumbai, India. For various biochemical estimations, kits were procured from Ecoline, E.Merck Ltd., M.I.D.C., Tolaja.

Extraction, isolation and standardization of the Total Anthraquinone glycosides

Rheum emodi rhizome powder 500 g was extracted with 3x 3 L of 70% ethanol using a soxhlet apparatus. The extract was evaporated to dryness to yield crude ethanol extract. A portion of the crude ethanol extract (75 g) was further extracted by adding 170 ml of water and 30 ml of 70% ethanol. After 10.5% w/v ferric chloride hexahydrate solution (50 ml) was added, the mixture was refluxed for 30 min before adding 20 ml concentrated hydrochloric acid and refluxed for another 30 min and allow the mixture to cool. When the mixture was cooled, it was filtered and the filtrate was extracted with 5 x 200 ml of chloroform. The collected extracts from chloroform layer were evaporated after aqueous layer was added with 0.1 g of sodium bicarbonate to adjust to neutral pH. The solution was then centrifuged at 4000 rpm for 20 min. The supernatant which contained Anthraquinone glycoside was evaporated finally brown dried power with 2.25% of yield represented total Anthraquinone glycoside mixture and it was designated as total Anthraquinone glycoside fraction of *Rheum emodi* (TAGF of *Rheum emodi*). It showed positive result for Borntragers test, indicating the presence of Anthraquinone glycosides.

TAGF of *Rheum emodi* was standardized by TLC profile using precoated silica gel plates (60 F254) the stationary phase, Petroleum ether: Ethyl acetate: Formic acid (75:25: 1), and 10% methnolic KOH as the spray reagent. TLC revealed the presence of four spots on the plate

with R_f values of 0.52, 0.38, 0.27 and 0.84 confirmed the presence of Anthraquinone glycosides The TLC profile of this investigation was similar to that reported in the literature.

Acute Toxicity Studies

Acute oral toxicity study was carried out on total Anthraquinone glycoside fraction of *Rheum emodi* as per guidelines of the Organization for Economizing Co- operation and Development, following the up-and-down method (Guideline 425).Based on the method, a limit test was performed to categorize the toxicity class of the compound and then main test was performed to estimate the exact LD_{50}. The animals (Wister rats weighing 150-200 g) were selected by random sampling technique for the study and were divided into 5 groups of 5 animals each. The animals were fasted overnight prior to administration of test sample, provided only water, a single oral dose of the extract was administered at the dose level of 5 mg/kg body weight and the group was observed for any toxic symptoms, behavioral changes, locomotion, convulsions and mortality for 72 hrs. The Anthraquinone glycoside fraction of *Rheum emodi* in the study had no signs of toxicity in the first group tested and hence higher doses of 50, 300, 2000 and 4000 mg/kg were administered to the groups by single oral administration and the animals were observed initially for any behavioral changes during 72 hrs and subsequently for toxic symptoms and mortality for a period of 14 days. The dose of 4000 mg/kg was studied in the present study to know the LD50 between 2000- 4000 mg/kg as the drug is popular and extensively used, the information will help in safeguarding the human health. As there is no mortality even at the highest dose tested the LD50 was estimated to be more than 4000 mg/kg and accordingly 1/10 of the LD50 value 400mg/kg body weight was selected as high dose and 200 mg/kg body weight was selected as low dose for the study.

Hepatoprotective effect of Anthraquinone glycoside fraction of Rheum emodi in freshly isolated rat hepatocytes

Isolation and culture of hepatocytes: Hepatocytes were isolated using modified method of Seglen (1976). [8] The animals was anesthetized with inter peritoneal injection of pentobarbital sodium (i.p. 35 mg/kg body weight). Cut the skin above the formal vain and inject heparin into the femoral vein (200IE/100gBW) followed by perfusion of liver immediately with ca^{2+} free HEPES (Hydroxyl ethyl piperazineethane sulfonic acid Solution). The liver was swelling and slowly changes its color dark red to grayish white. After the perfusion liver was transferred to a sterile Petridis containing ca^{2+} free HEPES and cut into small pieces, which were transferred into a sterile conical flask containing 10ml of 0.075% of collagenase in HEPES and the crud cell suspension was stirred with the help of a magnetic stirrer for 5min to release hepatocytes into the solution. The cell suspension thus obtained was filtered through a nylon mesh (250µ) and filtrate was centrifuged at 1000 rpm for 15 min. The cells were washed with ca^{2+} free HEPES this washing procedure was repeat 2 to 3 times and suspended in complete medium and incubated at room temperature for 1.30hrs.The viability of the isolated cells was determined by trypan blue exclusion assay by counting stained and unstained cells.[9] The cell viability showing is more than 95%. The isolated rat hepatocytes were culture in Hams F12 Medium supplemented with calf serum, antibiotics, dexamethasone and bovine insulin at density of 1×10^6 cells/ml was incubated at 37^{oc} for 30 min in a humidified incubator under 5% co_2.

Carbon tetrachloride induced in vitro hepatocytes injury

The primary rat hepatocytes thus obtained were used for studying the hepatoprotective potential of total Anthraquinone glycoside fraction of *Rheum emodi*. Isolated hepatocytes were incubated with different concentrations of *Rheum emodi* (200-400 µg/ml) and toxicant CCl_4 (1%). In the CCl_4 control tubes media was added instead of Anthraquinone glycoside fraction while in normal tubes contains no CCl_4. Silymarin (250µg/ml) was used as standard reference hepatoprotective. The above cell suspensions were incubated for 1 hour. After 60 min of incubation cell viability was determined and suspension was centrifuged at 1500 rpm for 20 min. The sample obtained after centrifugation was used for estimation of hepatic marker enzymes like aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (ALP), triglycerides (TGL), total proteins, albumin, total bilirubin and lactate dehydogenase (LDH) in the medium were measured as an indication of hepatocytes necrosis using Ecoline diagnostic kits.[10]

In Vivo Hepatoprotective Effect

Animals were divided into five groups with six animals in each group. Group I received single oral dose of CMC (Sodium CMC 0.3%) daily and served as control and was not treated with the toxicant. Group II received CCl_4 (1 ml/kg

body weight, i.p, 30% CCl_4 suspended in olive oil1:1 v/v mixture of CCl4and olive oil) once in every 24 h served as CCl_4 -treated control. Acute toxicity studies were performed and the dose was fixed at low dose of 200 mg/kg body weight, high dose 400mg/kg body and 250 mg/kg body weight for anthraquinone glycoside fraction and standard Silymarin, respectively. Group III received the standard Silymarin (250mg/kg b.w.) and group IV and V received a suspension of the total anthraquinone glycoside fraction of *Rheum emodi* (200 mg/kg b.w., 400 mg/kg b.w.). The animals received these treatments by the oral route for a period of 7 days. On the seventh day except group I, all other groups received 30% CCl_4 suspended in olive oil (1 ml/kg b.w.) i.p. After 24 h of intoxication, on the 8 th day, blood was collected from the all groups of animals by cardiac puncture. The collected blood was allowed to clot and centrifuged at 3000 rpm for 10 min to obtain the serum. The biochemical parameters like serum enzymes ASAT, ALAT, ALP, TGL, total proteins, albumin, total bilirubin and LDH using Ecoline diagnostic kits.[10-11] The liver tissue was dissected out half of the liver was used for estimation of antioxidant enzymes and remaining half was subjected for histppathological study.

Bromsulphalein uptake test

Bromsulphalein clearance test is the most sensitive and dependable method to assess the physiological status of liver function and also for measuring the detoxification capacity of liver it indicates the excretory function of the liver. The abnormal functional effects produced by CCl_4 are easily demonstrated by the retention of BSP. On 8th day Livers were purfussued and part of liver was subjected to BSP uptake test. Liver slices kept in ice cold phosphate buffer (0.2 M) at pH 7.4 were incubated in media (KCl: 10 mM, $MgSO_4$: 1 mM, NaCl: 1 mM in phosphate buffer) containing 30mg BSP/ml at 38°C. An aliquot of reaction mixture was analyzed after 10, 20 and 30 min to determine the concentration of BSP in the media at 580 nm. [12]

Thiopentone induced sleeping time

Thiopentone induced sleeping time is one of the functional parameter for assessing the hepatoprotective activity of the herbal plants.[13]Sleeping induced by short acting thiopental sodium is significantly prolonged in the event of any liver damage and this can be used as a measure of the function of the drug metabolizing enzymes. On the 8th day, a single dose of thiopentone (4 mg/kg, i.p.) was given to the animals and the time between loss of the righting reflex and its recovery was taken as duration of thiopentone induced sleeping time. [14]

Estimation of free radical scavenging ability of liver

Free radical mediated cell injury plays an important role in chemical induced hepatotoxicity. The dissected Livers were treated with 0.9% saline and homogenated in ice cold phosphate buffer. Centrifuge the homogenate at1000 rpm for 15 min followed by centrifugation of the supernatant at 1200 rpm for 20 min to get mitochondrial fractions. These mitochondrial fractions were used for estimation of following enzymes. GSH (Reduced Glutathione) reduces H_2O_2 directly to water or react directly with the free radicals such as O_2^{2-}, OH^-, and O^- by a radical transfer process, which yields thiol radicals. This thiolradical presents in glutathione forms a colored complex with DTNB, which is measured calorimetrically at 412 nm. [15], LPO (Lipid peroxidation) Lipid peroxidative degradation of bio membranes is one of the principal causes of hepatotoxicity of CCl_4, [16] Thiobarbituric acid reactive substance of (malondialdehyde) lipid per oxidation was determined following the method of Okhawa et al. [17] SOD (Superoxide dismutase) activity was assayed by the inhibition of nicotinamide adenine dinucleotide (reduced) phenazine methosulphate nitrobluetetrazolium reaction system as adapted method of Misra and Fridovich. [18]

Histopathological Examination

The liver was per fused and excised from the all group animals for histopathological investigation. Part of liver was fixed in Aqua Bouin's fluid and processed for Histopathological assessment of liver damage by modified method of Nanji et al. [19]

Statistical Analysis

The values are represented as mean ± S.E.M. Statistical analysis was carried out by one way analysis of variance (ANOVA) and comparison of mean values of different groups treated with different dose levels of total anthraquinone glycoside fraction and positive control with normal was performed by Turkey's Multiple Comparison Test. With help of Graph Pad prism 5.0 software value $P < 0.05$ was considered significant.

RESULTS

Hepatoprotective Effect of TAGF of Rheum emodi in Freshly Isolated Rat Hepatocytes

A significant increase in the levels of ASAT, ALAT, ALP, LDH, total bilirubin and a

significant reduction in the TGL, albumin and total protein levels as compare to control was observed in CCl_4 exposed hepatocytes in Table.1.These cells, when treated along with different concentrations of TAGF of *Rheum emodi* (200-400 µg/ml) showed a significant restoration in the altered biochemical parameters toward the normal and is dose dependent manner. A similar result was obtained when CCl_4 -intoxicated hepatocytes were treated with the standard Silymarin. When compared to standard Silymarin *Rheum emodi* at 400µg/ml was significant and more than that produced by the standard Silymarin at 250µg/ml.

In Vivo Hepatoprotective Effect of TAGF of Rheum emodi on CCl_4 intoxicated rats

A significant increase was observed in ASAT, ALAT, ALP, LDH, total bilirubin and a significant reduction in the TGL, albumin and total protein levels after exposed to CCl_4 when compared with normal control rats ($P < 0.001$) (Table:2). However administration of TAGF of *Rheum emodi* at dose levels 200 mg/kg b.w, 400 mg/kg b.w and Standard Silymarin 250 mg/kg b.w, showed a significant restoration in the altered biochemical parameters toward the normal. All biochemical findings were positively supported by the histopathological results.

Thiopentone induced sleeping time

Treatment with TAGF of *Rheum emodi* at doses 200mg/kg, 400 mg/kg rats have been showing statistical significant ($p < 0.001$) hepatoprotective activity (96.28%) by reducing the sleeping time (65.72 ± 2.43 min to24.62 ± 2.90, 18.72 ± 1.12 min) compared to the toxicant control (CCl4) group in Table 3.

Estimation of liver free radical scavenging ability

TAGF of *Rheum emodi* at the dose levels of 200 mg/ kg, 400 mg/kg showed significant increase in SOD and Glutathione levels compare to the CCl_4 intoxicated rats group. Treatment with TAGF of *Rheum emodi* 200 mg/kg, 400 mg/kg shows significant reduction in lipid peroxide levels compare to elevated levels in CCl_4 intoxicated rats group Table.4.

Viability of liver cells

CCl_4 treated with hepatic cells showing 61.56% viability Compare to the normal cells (92.99%). When CCl_4 intoxicated cells treated with different concentrations of TAGF of *Rheum emodi* (200 mg/kg, 400 mg/kg) and Silymarin (250 mg/kg) shows significant increase in the percentage of cell Viability as 75.25%, 88.78% and 87.89% shown in Table.5.

Histopathological parameters

CCl_4 intoxicated rats showed significantly increasing the liver weight 6.28 ±1.48g Compare to the normal 4.39 ±1.29g.When CCl_4 intoxicated rats treated with TAGF of *Rheum emodi* (400 mg/kg) and Silymarin (250 mg/kg) showed hepatoprotection by reducing the liver weight 4.92 ±1.52 and 4.86 ±1.74g, respectively shown in Figure :1.

CCl_4 intoxicated animals liver section examination showed vacuolated hepatocytes with focal necrosis, degenerated nuclei and vesicular fatty changes which indicate completely damaged cyto architecture of liver. Liver sections of rats treated with TAGF of *Rheum emodi* (400 mg/kg) and Silymarin (250 mg/kg) showed central vein with mild degree of fatty changes and necrosis like near to normal hepatic cell compare to control group liver sections. Histopathological observations and photomicrographs of liver slices are shown in Figure: 2

Bromsulphalein uptake test

Liver slices of TAGF of *Rheum emodi* treated animals showed extremely statistical significant ($P < 0.001$) Hepatoprotection (74.33%) at 400mg/kg dose. It showed 91.44 ±3.42µg of BSP uptake per gm of liver tissue compared to 42.18 ±3.28µg of toxicant control (CCl_4) group (Figure: 3).

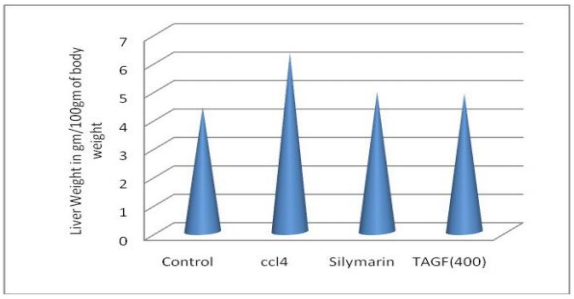

Figure1: Effect of Total anthraquinone glycoside fraction of *Rheum emodi* on relative liver weight of CCl_4 treated rats. n = 6.P< 0.001 when compared to negative control (CCl_4 treated) group

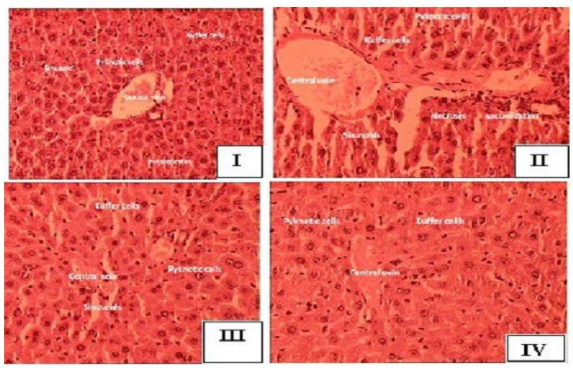

Figure 2: Assessment of CCl_4 induced hepatotoxicity by histopathology in hematoxylin eosin stained liver section. [I]: Section from normal liver tissue. [II]: CCl4 intoxicant,

liver tissue [III]: Silymarin treated liver tissue. [IV]: *R.emodi* TAGF (400mg/kg) pre treatment, liver tissue.

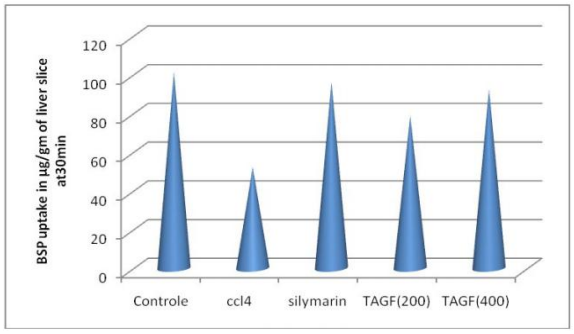

Figure 3: Effect of Total anthraquinone glycoside fraction of *Rheum emodi* on BSP uptake of CCl_4 treated rat liver slice. n = 6. P< 0.001 and ns = not significant when compared to negative control (CCl4 treated) group.

DISCUSSION

The present study was to explore the hepatoprotective effect of TAGF of *Rheum emodi* in CCl_4 induced hepatic injury. CCl_4 induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effect of medicinal plants. Hepato toxicant CCl_4 get convert into (CCl_30^-) Trichloromethyl radical in liver by action of Cytochrome p-450. Trichloromethyl radical that binds to lipoprotein and leads to peroxidation in turn gives malonaldihyde that cause damage to cell membrane and endoplasmic reticulum [20].The per oxidative products induce hypo perfusion of the membrane and finally cytosolic enzymes appear in the blood [21]. As result levels of liver marker enzymes SGOT, SGPT and ALP are found to be elevated levels in cytoplasm as well as in blood. significant elevation in SGOT, SGPT, ALP and serum bilirubin levels favor liver cell necrosis and those conditions enhance permeability of hepatic cell witch lead severe damage in the hepatic tissue membrane after exposing with CCl_4. Treatment with TAG of *Rheum emodi* at dose levels 200 mg/kg, 400mg/kg prevent their rise in levels of ALT, AST, ALP and bilirubin. It indicates possible stabilization of Cytosolic membrane as well as repair of hepatic tissue damage caused by CCl_4. The hepatotoxic effect of CCl_4 prolongs duration of sleeping time for hexobarbitone, thiopentone and Pentobarbitone, etc in animals due to damage of drug metabolizing enzymes in liver. Thiopentone induced sleeping time in animals with hepatic intoxication is increased as the enzymes responsible for metabolism of thiopentone is destroyed or reduced [22] .Treatment with TAG of *Rheum emodi* at dose levels 200 mg/kg, 400mg/kg showed decrease in thiopentone induced sleeping in treated animals may be due to protection of hepatic drug metabolizing enzymes as evidenced for protective effect of TAGF of *Rheum emodi*.

Hepatoprotective activity is associated with antioxidant activity; highly reactive CCl_3^- radical induced lipid peroxidation disturbs Ca^{2+} homeostasis in hepatic cell and finally results in hepatic cell death. The antioxidant enzymatic systems are natural protector against lipid per oxidation. Antioxidant enzymes (SOD, CAT) protect the cellular constituents from oxidative damage by preventing the generation of hydroxyl radicals.[23] TAGF of *Rheum emodi* and Silymarin treatments showed significant elevation in glutathione and SOD levels indicates the restoration of vital molecules such as NAD, Cytochrome, SOD and glutathione. SOD can help in cellular defense mechanism by preventing cell membrane oxidation. It indicates antioxidant activity of *Rheum emodi*.

The rate of reduction in serum concentration of BSP is a sensitive measure of liver function. [24] TAGF of *Rheum emodi* at higher dose has significantly improved the capacity of the damaged liver to take up BSP. This increased uptake of BSP by the hepatic cells showed that it enhanced capacity to excrete the dye from the blood. Simultaneous treatment of total anthraquinone glycoside fraction of *Rheum emodi* with CCl_4 exhibited less damage to the hepatic cells as compared to the rats treated with CCl_4 alone.

The histopathological observations, showed disarrangement of hepatic cells with centrilobular necrosis and vacuolated hepatocytes with degenerative nuclei were observed in CCl_4 treated animals. Treatment with TAGF of *Rheum emodi* and Silymarin produced mild degenerative changes and moderate centrilobular necrosis compared with control. *Rheum emodi* drug treatments showing reducing the cell injury to preserve the normal hepatic physiological mechanisms against CCl_4, all these results indicate a hepatoprotective potential of the *Rheum emodi*.

The result obtained from this study conform the hepatoprotective activity of *Rheum emodi* on CCl_4 induced hepatic damage. It can be concluded that hepatoprotective potential of rhizomes of *Rheum emodi* due its antioxidant, free radical scavenging property and synergistic effect of anthraquinone glycosides present in the rhizome. The data of this finding could lead to the use of *Rheum emodi* as an herbal remedy for jaundice and other liver disorders, suggesting the feasibility of developing herbal formulation and clinical studies.

Treatment/ Concentration	ASAT U/L	ALAT U/L	ALP U/L	Albumin g/L	Total bilirubin mg/dL	Total Protein g/dL	TGL mg/dL	LDL U/L
Vehicle control	18±0.58	14 ± 0.51	37 ± 0.13	3.66 ± 0.18	0.23±0.014	2.2±0.16	165±9.20	121±0.018
CCl_4 (1%)	87±2.98 [a]	61±0.60 [a]	101±0.65 [a]	1.9±0.12 [a]	0.72± 0.15 [a]	1.4±0.18 [a]	74±3.18 [a]	301±0.14 [a]
CCl_4 (1%) + Silymerin (250 µg)	21±0.92 [b]	17±0. 95 [b]	39±1.36 [b]	3.18±0.15 [b]	0.31± 0.08 [b]	2.14±0.08 [b]	159±12.59[b]	130±0.16 [b]
CCl_4 (1%) + *R.emodi* TAGF (200 µg)	27±1.46 [b]	23±0.63 [b]	41±1.14 [b]	3.28± 0.17	0.33± 0.013 [b]	2.10±0.15 [b]	154±9.92 [b]	133±0.18 [b]
CCl_4 (1%) + *R.emodi* TAGF (400 µg)	25±0.96 [b]	16±0.98 [b]	39±1.34 [b]	3.14±0.16	0.30±0.014 [b]	2.16±0.14 [b]	160±10.20[b]	128± 0.16 [b]

Table 1: Hepatoprotective effect of Total anthraquinone glycoside fraction of *Rheum emodi* on freshly isolated rat hepatocytes.
Note: Values expressed as mean ± SEM (n=6); a= P < 0.001, when compared to the normal group, b=P < 0.001, when compared to the CCl_4 -treated group.

Treatment /Concentration	ASAT U/L	ALAT U/L	ALP U/L	Albumin g/L	Total bilirubin mg/dL	Total Protein g/dL	TGL mg/dL	LDL U/L
Vehicle control	89.48±0.537	60.65±13.54	326.42±2.106	3.283±0.295	0.46±0.54	7.385±3.127	78.57±0.22	258.49±0.051
CCl_4	170.04±2.04[a]	116.28±12.29[a]	596.14±3.604[a]	1.470±0.104 [a]	1.98±0.02 [a]	4.402 ± 1.42 [a]	28.24±0.68 [a]	520.38±0.014 [a]
CCl_4 + Silymerin (250 mg/kg b.w.)	80.87± 1.47[b]	62.15± 12.16[b]	358.06±2.43 [b]	3.374±0.324[c]	0.44±0.01[b]	6.459±2.47[c]	68.02±0.09 [c]	305.72±0.012[b]
CCl_4 + *R.emodi* TAGF (200 mg/kg b.w.)	96.64± 2.36[b]	79.46 ± 10.89 [b]	398.12± 2.65[b]	3.126±0.109[c]	0.64±0.015[b]	6.546±1.38[c]	82.12±1.28[c]	312.24±0.016 [b]
CCl_4 + *R.emodi* TAGF (400 mg/kg b.w.)	86.92± 1.29[b]	64.52± 12.08[b]	370.09± 2.38[b]	3.368± 0.28[c]	0.48±0.013[b]	6.982±2.71[c]	67.08±0.17[c]	286.46±0.028 [b]

Table 2: Hepatoprotective effect of Total anthraquinone glycoside fraction of *Rheum emodi* on CCl_4 intoxicated rats
Note: Values expressed as mean ± SEM (n=6); a= P < 0.001, when compared to the normal group, b=P < 0.001, c=P < 0.001 when compared to the CCl 4 -treated group

Treatment (mg/kg, p.o)	Sleeping time (min)	Hepatoprotection (%)
Vehicle control	16.26 ± 1.54	---
CCl_4	65.72 ± 2.43[c]	---
CCl_4 + Silymerin (250 mg)	18.56 ± 1.08[c]	96.86
CCl_4 + *R.emodi* TAGF (200)	24.62 ± 2.90[c]	78.42
CCl_4 + *R.emodi* TAGF (400)	18.72 ± 1.12[C]	96.28

Table 3: Hepatoprotective effect of Total anthraquinone glycoside fraction of *Rheum emodi* on thiopentone induced sleeping time against CCl_4 induced liver damage on rats
Note: p < 0.001 When compared to negative control (ccl4) group Sleeping time was expressed in mean ± standard error of mean; n = 6. Percentage of Hepato protection was calculated using the equation: H = [1 - (T – V / C – V)] × 100. Where T is mean value of group treated with test drug, C is the mean value of control animal.

Treatment (mg/kg, p.o)	Superoxide dismutase (unit/mg of protein)	Lipid peroxidase (n mol/g of protein)	Glutathione (mg/g of liver)
Vehicle control	5.96 ± 0.54	4.62± 1.38	28.12 ± 049
CCl_4	1.52± 0.64	33.12 ±0.52	6.98± 1.26
CCl_4 + Silymerin (250 mg/kg b.w.)	5.16±0.49[a]	4.92 ±1.26[b]	23.65± 1.62[b]
CCl_4 + *R.emodi* TAGF (200 mg/kg b.w.)	3.68 ±1.72[ns]	16.24 ±1.26[b]	19.62 ±1.42[a]
CCl_4 + *R.emodi* TAGF (400 mg/kg b.w.)	4.98 ±1.82[a]	8.56±1.42[b]	22.92 ±1.54[b]

Table 4: Effect of total anthraquinone glycoside fraction of *Rheum emodi* on free radical scavenging ability of CCl_4 treated rat liver.
Values are expressed in mean ± standard error of mean: n = 6. a=P< 0.05, b=P< 0.01 and ns not significant when compared to negative control (CCl_4 treated) group.

Treatment (mg/kg, p.o)	No of cell counted	No of viable cell (% of viable cells)
Vehicle control	232.33 ± 4.45	217.45 ± 2.53 (92.99)
CCl_4 (1%)	205.54 ± 4.78	146.55 ± 2.99 (61.56)
CCl_4 (1%) + Silymerin (250 mg/kg b.w.)	227.55 ± 4.42	202.43 ± 3.63 (87.89)[c]
CCl_4 (1%) + *R.emodi* TAGF (200 mg/kg b.w.)	227.65 ± 6.02	176.25 ± 3.52 (75.25)[c]
CCl_4 (1%) + *R.emodi* TAGF (400 mg/kg b.w.)	229.25 ± 5.15	203.55 ± 2.11 (88.78)[c]

Table 5: Effect of total anthraquinone glycoside fraction of *Rheum emodi* on hepatic cell viability
Values are expressed in mean ±standard error of mean: n = 6. The viability was calculated as percentage from the following equation: % of viable cells = no. of cells excluding dye/total no. of cells counted× 100. C=P<0.001, Significant reduction compared to hepatotoxin (cP < 0.05).

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