**Introduction**

*Rheum emodi* Wall. ex Meissn (family Polygonaceae) is a stout perennial herb that grows predominantly in sub-tropical and temperate regions of Asian countries including India, Nepal, China and Bhutan. In India, it is distributed in altitudes ranging from 2800 to 3800 m in the temperate and subtropical regions of Himalayas [1]. It is commonly known as Rhubarb in English and revand-chini in Hindi. It is also known by some other names such as amla-vetasas, Indian rhubarb, Himalayan rhubarb, archu, Chinese Rhubarb, Bangla Revanchini, Reval-chini, Rhabarber, Rheuchini, Tursak and Varyattu. Roots of *R. emodi* are widely used in Ayurvedic and Asian folk medicine as a stomachic, purgative, astringent, and tonic. It is also used by traditional healers in certain skin diseases, fevers, ulcers, bacterial infections, fungal infections, jaundice and liver disorders [2-5]. Compounds isolated from *R. emodi* reported to have, anti-viral, anti-bacterial, anti-fungal, tumor cell-growth inhibitory and cytostatic activities. Anthraquinones, a type of polyphenolic compounds with much pharmaceutical importance are the major active constituents of this plant showing significant biological activities [3-7]

**Taxonomic Classification**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
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</tr>
<tr>
<td>Superivision</td>
<td>Spermatophyta</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
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<tr>
<td>Order</td>
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<tr>
<td>Family</td>
<td>Polygonaceae</td>
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<tr>
<td>Genus</td>
<td><em>Rheum</em></td>
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<tr>
<td>Species</td>
<td><em>emodi</em></td>
</tr>
<tr>
<td>Binomial name</td>
<td><em>Rheum emodi</em> Wall. ex Meissn</td>
</tr>
</tbody>
</table>

**Traditional uses**

It is an important medicinal plant, which is reported to be extensively used in Ayurvedic, Unani and Chinese systems of medicine [8]. It is reported to be used as antipyretic, laxative, stomachic, purgative, hemostatic, anthelmintic and cathartic in different systems of traditional medicines. Roots of this plant exhibit a purgative action to be used in the treatment of constipation. *Rheum emodi* is extensively used in Chinese medicine. Moreover, different hydroxyanthraquinone
derivatives are also used as preservatives these are also used in textile industries as ecofriendly natural dyes.

**Phytochemistry**

Phytochemical investigations have showed the presence of wide range of chemical compounds in different parts of this plant. Anthraquinones are the major active constituents of this plant. Rhizomes of *Rheum emodi* contain several hydroxyanthraquinone derivatives including emodin, chrysophanol, aloe-emodin, rhein, physcion, and their glycosides.

![Chemical structures of various compounds](image)

Emodin 8-O-β-D-glucopyranosyl-6-O-sulfate, a sulfated glucoside of emodin was isolated from roots of *R. emodi* collected from Nepal. In addition, four other compounds carpusin, maesopsin, torachrysone 8-O-β-D-glucoside and epicatechin have also been identified in the same collection
[9]. The activity guided fractionation of petroleum ether extract of the rhizomes resulted in isolation of two oxanthrone esters revandchinone-1, revandchinone-2 and β-asarone. In the similar way, chloroform extract resulted in identification of new anthraquinone ether, revandchinone-3 as well as an oxanthrone ether, revandchinone-4. In addition to these new compounds, emodin, Chrysophanol and physcion were also isolated [3].

**Pharmacology**

Several pharmacological studies on the different active fractions and compounds obtained from *Rheum emodi* have been carried out. The important biological activities are discussed here.

The chloroform and petroleum ether extracts of *Rheum emodi* rhizomes exhibited antibacterial and antifungal activities. Revandchinones 1-4 as well as other compounds isolated from ether extract of rhizomes showed significant anti bacterial and anti fungal activities [3]. Chrysophanol exhibited the antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*. [10]. Carpusin and maesopsin showed significant antioxidant activity. Different anthraquinones were shown to be able to directly affect the transcription through intercalating into DNA [11].

Emodin, as well as aloe-emodin has been shown to induce significant effect on different stages of cell cycle [12, 13]. The cell-cycle arrest is postulated to be mediated by increased expression of p53 and p21. These molecules were also found to induce G1/S cell-cycle arrest in different cancer cell lines. This effect is shown to be mediated by p16-Rb-E2F pathway. Diverse studies have shown that emodin show anticancer effect through inducing the apoptosis in cancer cells which is postulated to be mediated by ROS dependent as well as caspase dependent mechanism.

Different studies have postulated that anthraquinone derivatives isolated from different parts of *Rheum emodi* particularly aloeemodin, emodin and rhein exhibit significant antiproliferative activity against different human cancer cells. Emodin has been shown to exhibit antiproliferative activity against breast, prostate, lung, colorectal and cervical cancers cells [14–17].
Aim and design of work

The aim of our study is to isolate the chemical constituents from ethanolic extracts and fractions of *Rheum emodi* rhizomes based on biological activity. Owing to the immense therapeutic potential of emodin and other anthraquinone derivatives isolated from this plant, considerable interest has been focused on evaluation of antidyslipidemic and antiulcer activity.

Present study

The rhizomes of *Rheum emodi* were purchased from a local market and identified by Botany Department of Central Drug Research Institute, Lucknow, India. A voucher specimen (Voucher
No. 3755) has been preserved in the herbarium of the Institute for future reference. The chapter deals with the isolation of compounds from 95% ethanolic extract of rhizomes and biological evaluation of these extract, fractions and active constituents.

**Extraction, fractionation Isolation and characterization of compounds**

The air dried and ground rhizomes (4.0 kg) were extracted with ethanol (5×4.0 lit.) at room temperature and the total combined extract was filtered and concentrated under reduced pressure below 50°C to dryness as a dark brown mass (crude ethanolic extract, 300 g). 200 g. of this extract was fractionated into n-hexane soluble fraction (F1, 3.4 g), chloroform soluble fraction (F2, 5.2 g), 5% methanol in chloroform soluble fraction (F3, 89.0 g) and insoluble fraction (F4, 102.4 g) by maceration with n-hexane, chloroform and 5% methanol in chloroform successively. As F3 has shown prominent activity, 60 g. of this fraction was repeatedly chromatographed on a silica gel (100–200 mesh) column yielding four compounds RE-1 (K1, 240 mg), RE-2 (K2, 2.4 g), RE-3 (K3, 1.6 g) and RE-4 (K4, 760 mg). These compounds were finally purified by HPLC on reverse phase C18 R.P columns using solvent A (acetonitrile: methanol; 95:5, v/v) and solvent B (water: acetic acid; 99.9:0.1, v/v, pH 3.5) as the mobile phase, with a linear gradient elution at flowrate-1.0 ml/min as per method described previously [18]. These compounds were characterized as chrysophanol (K1), emodin (K2), chrysophanol 8-O-β-D- glucopyranoside (K3) and emodin 8-O-β-D-glucopyranoside (K4) using MS, $^1$H and $^{13}$C NMR spectral data and comparing with those reported in literature [19-21]. A summary of isolation procedure is given in flow sheet 4.1 and the compounds isolated are given in table 4.1.

**Table 4.1:** Compounds isolated from *Rheum emodi* rhizomes

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Characterized as</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE-1</td>
<td>C$<em>{15}$H$</em>{10}$O$_{4}$</td>
<td>254</td>
<td>Chrysophanol</td>
</tr>
<tr>
<td>RE-2</td>
<td>C$<em>{15}$H$</em>{10}$O$_{5}$</td>
<td>270</td>
<td>Emodin</td>
</tr>
<tr>
<td>RE-3</td>
<td>C$<em>{21}$H$</em>{20}$O$_{9}$</td>
<td>416</td>
<td>Chrysophanol 8-O-β-D-glucopyranoside</td>
</tr>
<tr>
<td>RE-4</td>
<td>C$<em>{21}$H$</em>{20}$O$_{10}$</td>
<td>432</td>
<td>Emodin 8-O-β-D-glucopyranoside</td>
</tr>
</tbody>
</table>
Structure of isolated compounds

Flow sheet 4.1: Summary of extraction, fractionation and isolation procedure

Rheum emodi (rhizomes) (Air dried, powdered)

- Extraction with 95% Ethanol
  - 95% Ethanol Extract
    - Fractionation with i) n-Hexane, ii) Chloroform and iii) 5% Methanol in Chloroform
      - Hexane Soluble Fraction (F1)
      - Chloroform Soluble Fraction (F2)
      - 5% Methanol Soluble Fraction (F3)
      - Insoluble Fraction (F4)
        - Isolation (Repeated Chromatography and crystallization in methanol)
          - Chrysophenol (RE-1)
          - Emodin (RE-2)
          - Chrysophenol 8-O-β-D-glucopyranoside (RE-3)
          - Emodin 8-O-β-D-glucopyranoside (RE-4)
BIOLOGICAL ACTIVITY: ANTIDYSLIPIDEMIA

Atherosclerosis is the foremost cause of heart disease, stroke and death all over the world [22]. It is known to be initiated with inflammation in the walls of blood vessels in response to retained low-density lipoprotein (LDL) molecules. LDL molecules are oxidized by reactive oxygen species which in turn triggers a cascade of immune responses which eventually can produce an atheroma [23]. Moreover, hyperlipidemia following oxidative stress and overproduction of oxygen free radicals may cause oxidative modifications in low density lipoproteins, which may play an important role in the initiation and progression of atherosclerosis and related cardiovascular diseases [24, 25]. Drug therapy and dietary changes reduce blood lipid levels and decrease the risk of atherosclerosis and cardiovascular diseases. Drug therapy includes statins, fibrates, nicotinic acid and bile acid resins [26]. Statins are the most popular and widely prescribed group of medications which act through inhibiting HMG-CoA reductase [27]. However, several adverse effects including asymptomatic creatine kinase elevation in muscles and rhabdomyolysis have been shown to be associated with statins and fibrates [28]. Furthermore, these drugs specifically target one component of the lipid profile, with smaller effects on other parameters. Hence there is an urgent need of new antidyslipidemic agents with more efficacies. Several species of *Rheum* genus were studied for their cholesterol lowering activities [29]. The present study deals with the evaluation of antidyslipidemic potential of *Rheum emodi* rhizomes and its active constituents in dyslipidemic rats emphasizing on different components of lipid profile along with the determination of possible mechanism of action.

Material and Methods

Drugs and Standards

Standard drugs gemfibrozil, pravastatin and other chemicals were obtained from Sigma Chemical Company, St. Louis, MO, USA.

Animals

Male adult rats (Charles foster strain, body weight 150 -200, age 2-4 weeks old) were kept in a room with controlled temperature 25-26 °C, humidity 60-80% and 12/12 hours light/dark cycle, (light on from 8.00 to 20.00 hrs.) under hygienic conditions. Animals were acclimatized for one week before starting the experiment. The animals had free access to the normal diet and water *ad
libitum. All experimental protocols were approved by the Ethical Committee of CDRI, Lucknow, which follows CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines and fulfils the International norms of Indian National Science Academy.

**Lipid lowering activity in triton induced hyperlipidemic rats**

Rats were divided into 12 groups with six rats in each group, group 1–control, group 2–triton treated, group 3–triton + ethanolic extract (200 mg/kg b.w.) treated, group 4 to 7– triton + fractions F1, F2, F3 and F4 (200 mg/kg b.w.) treated, group 8 to11– triton + compounds K1, K2, K3 and K4 (100 mg/kg b.w.) treated; and group 12–triton + standard drug gemfibrozil (100 mg/kg b.w.) treated groups. In the 18 h acute experiment, triton WR-1339 (Sigma Chemical Company, st. louis, M O, U.S.A) was administered intraperitoneally at a dose of 400 mg/kg b.w. to animals of all groups except the control to induce hyperlipidemia. After that extract, fractions, pure compounds and Gemfibrozil were macerated with 0.2% w/w aqueous gum acacia and given orally to their respective groups. The same amount of gum acacia suspension (vehicle) was given to animals in group– 1 and group– 2. After dosing, the rats were fasted for 18h and anaesthetized with sodium thiopentone solution (50 mg/kg i.p.) prepared in normal saline and 1.0 ml blood was withdrawn from retro orbital plexus using glass capillary in EDTA coated tubes. The blood was centrifuged at 2500g for 10 min at 4°C and plasma was separated for biochemical analysis.

**Biochemical analysis**

The levels of total cholesterol (TC), phospholipids (PL), triglycerides (TG) and High density lipoprotein (HDL) were estimated as reported earlier [30], Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were calculated using formulas: (VLDL = TG/5); (LDL = TC - HDL - TG/5) [30]. The effect of compounds K1–K4 on HMG-CoA reductase was measured using an assay kit (Sigma-Aldrich, St. Louis, MO). Lecithin-cholesterol acyl transferase (LCAT) and post heparin lipolytic activity (PHLA) in plasma and LPL activities in liver homogenate were measured according to standard methods as reported earlier.

**Antioxidant activity (in vitro)**

The free radical scavenging potential of ethanolic extract, its fraction F3 and compounds K1-K4 against formation of Superoxide anions (O$_2^-$) and hydroxyl free radicals (OH’) in enzymatic and
non-enzymatic systems were determined in absence or presence of plant samples (100μg and 200μg/ml) [31].

**Statistical analysis**

Data were analyzed using one way analysis of variance (ANOVA) and the significance of mean difference between different groups was done by Tukey’s post hoc test. A two tailed (α=2) probability p<0.05 was considered statistically significant (p < 0.001 = ***, p < 0.01 = **, p < 0.05 = *, p >0.05 (ns) = not significant). Number of independent determinants n=6 animals for *in vivo* experiments and n=3 for triplicate *in vitro* experiments.

**Results**

**Effect of extract, fractions and compounds in triton induced dyslipidemia**

The acute administration of triton WR-1339 caused a marked increase in serum levels of TC (+4.23 fold), PL (+2.71 fold), TG (+3.79 fold), VLDL (+3.79) and LDL (+8.71) while decreased the HDL level (-37%). A significant decrease was noticed in TC (-24%), PL (-23%), TG (-24%), VLDL (-24%) and LDL (-28%) followed by increase in HDL (+16%) after treatment with ethanolic extract (200 mg/kg b.w.) which indicated antidyslipidemic potential of this plant (Figure 4.1). Subsequently it was fractionated in to four fractions and evaluated for lipid lowering activity at the same dose. Fraction F3 has shown maximum activity, it decreased the serum level of TC, PL, TG, VLDL and LDL by 24%, 24%, 24%, 24% and 27% respectively while increased the level of HDL by 17%. Consequently it was chromatographed repeatedly and purified which resulted in isolation of compounds K1-K4. All the four compounds were assessed for their lipid lowering activity. The results were significant and in good agreement with preliminary screening of the ethanolic extract and fraction F3. Compounds K1, K2, K3 and K4 (100 mg/kg b.w.) significantly lowered the serum level of TC by 20%, 23%, 20% and 22%, PL by 18%, 23%, 22% and 21%, TG by 20%, 22%, 20%, and 21%, VLDL by 20%, 22%, 20% and 21% and LDL by 23%, 27%, 23% and 26% while increased the serum HDL level by 16%, 21%, 17% and 18% respectively (Figure 4.1).

Triton administration caused inhibition of plasma LCAT activity (54.54%) and PHLA (46.80%). Treatment with compounds K1 to K4 significantly reactivated LCAT (23%, 27%, 23% and 25%) and PHLA (22%, 25%, 24% and 24%) respectively (Figure 4.2).
Figure 4.1 Effect of ethanolic extract (200 mg/kg), fractions F1-F4 (200 mg/kg) and pure compounds K1-K4 (100 mg/kg) on plasma lipid levels in triton induced dyslipidemic rats. Gemfibrozil = Standard drug. Data is presented as means±SD of six rats (n=6). The triton treated group was compared with the control; triton plus drug treated groups were compared with triton treated group. ***p<0.001, **p<0.01, *p<0.05 and ns p>0.05.
Figure 4.2: Effect of compounds K1, K2, K3 and K4 (100 mg/kg each) on LCAT activity and PHLA in triton induced dyslipidemic rats. Data is presented as means±SD of six rats (n=6). Triton treated group was compared with control; Triton plus drug treated groups were compared with Triton treated group. ***P<0.001, **P<0.01.

In-vitro study was performed to evaluate the inhibition of HMG–CoA reductase enzyme by compounds K1-K4 at five different concentrations ranging from 5µM to 100 µM in triplicate. All the compounds showed a dose dependent inhibitory response which is comparable to that of pravastatin (Figure 4.3). Compounds showed maximum inhibition (81.58%, 81.89%, 82.98% and 82.09% respectively) at 100 µM.
Figure 4.3: Effect of compounds K1–K4 on 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase activity (in vitro) in a dose-dependent manner. P<0.01 for 100 µM dose, P<0.05 for 50 µM dose and p>0.05 for remaining dose groups.

Antioxidant effect of Rheum emodi rhizomes

The antioxidant activity of ethanolic extract, fraction F3 and compounds K1-K4 has been shown in Table 4.2. Ethanolic extract showed significant inhibition in generation of superoxide anions (15% and 23%) and hydroxyl radicals (14% and 26%) as well as plasma lipid peroxidation (10% and 29%) at 100 and 200µg/ml concentrations, respectively. Fractions F-3 showed significant inhibition of superoxide anions (16% and 23%), hydroxyl radicals (15% and 26%) and lipid peroxidation (18% and 21%) at 100 and 200µg/ml concentrations, respectively. Compound K2 showed potent inhibition of superoxide anions (18% and 26%), hydroxyl radicals (15% and 26%) and lipid peroxidation (18% and 24%) at 100 and 200µg/ml concentrations, respectively. Other compounds K1, K3 and K4 also showed significant antioxidant activity.
Table 4.2: Antioxidant activity of ethanolic extract, fraction F3 and compounds K1 to K4 from *Rheum emodi* rhizomes

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Dose (µg/ml)</th>
<th>Formation of O$_2^-$ <em>a</em> (% decrease)</th>
<th>Formation of OH$^-$ <em>b</em> (% decrease)</th>
<th>Plasma Lipid peroxidation <em>b</em> (% decrease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>100</td>
<td>-15%*</td>
<td>-14%*</td>
<td>-10%*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>-23%***</td>
<td>-26%***</td>
<td>-29%***</td>
</tr>
<tr>
<td>F3</td>
<td>100</td>
<td>-16%*</td>
<td>-15%*</td>
<td>-18%*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>-23%***</td>
<td>-26%***</td>
<td>-21%**</td>
</tr>
<tr>
<td>K1</td>
<td>100</td>
<td>-15%*</td>
<td>-9% NS</td>
<td>-9% NS</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>-21%**</td>
<td>-15%*</td>
<td>-22%**</td>
</tr>
<tr>
<td>K2</td>
<td>100</td>
<td>-18%*</td>
<td>-15%*</td>
<td>-18%*</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>-26%***</td>
<td>-26%***</td>
<td>-24%***</td>
</tr>
<tr>
<td>K3</td>
<td>100</td>
<td>-13%*</td>
<td>-11%*</td>
<td>-7% NS</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>-22%**</td>
<td>-24%***</td>
<td>-11%*</td>
</tr>
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<td>K4</td>
<td>100</td>
<td>-19%*</td>
<td>-8% NS</td>
<td>-8% NS</td>
</tr>
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<td></td>
<td>200</td>
<td>-22%**</td>
<td>-15%*</td>
<td>-14%*</td>
</tr>
<tr>
<td>Standards</td>
<td>200</td>
<td>68%*** (Allopurinol)</td>
<td>-48%*** (Mannitol)</td>
<td>-45%*** (α-Tocopherol)</td>
</tr>
</tbody>
</table>

Units: *a* n mole formazone formed/min, *b* n mole MDA formed/h, O$_2^-$ superoxide anions, OH$^-$ hydroxyl radicals. Values are shown mean±SD of triplicate experiments. * P<0.05, ** P<0.01 *** P<0.001; ns p>0.05 (non-significant).

**Discussion**

Recent studies have demonstrated that altered blood lipid levels followed by increased oxidative stress and generation of large quantities of reactive oxygen species increase the risk of atherosclerosis and cardiovascular disease [32, 33]. Natural agents with antioxidant and lipid lowering activities may act as a lead in drug discovery for these diseases. Present study established the antidyslipidemic and antioxidant potential of *Rheum emodi* rhizomes and its active constituents using triton and high fat diet induced dyslipidemic rat models. The results
revealed that treatment with ethanolic extract, its fraction F3 and pure compounds K1 to K4 showed significant lipid lowering and antioxidant potential. Compounds K1 to K4 significantly increases PHLA and LCAT activity. The post-heparin lipolytic activity of plasma mainly consists of two activities—triglyceride lipase and lipoprotein lipase. Activation of PHLA is responsible for decreased level of TG, PL, LDL and VLDL. Increased activity of LCAT lowers cholesterol levels in plasma. In conclusion, it can be stated that Rheum emodi rhizomes have significant lipid lowering and antioxidant potential. The isolated compounds emodin and related anthraquinone glycosides emerge as novel antihyperlipidemic agents whose mechanism of action seems to be through activation of LCAT and PHLA.

**BIOLOGICAL ACTIVITY: ANTIULCER (GASTROPROTECTIVE)**

Gastric ulcer is a very common gastrointestinal disorder affecting a large number of people worldwide. It arises due to an imbalance between aggressive (acid, pepsin and Helicobacter pylori infection) and protective (mucin secretion, prostaglandin, epidermal growth factors and bicarbonate) factors in the stomach [34]. Stress, smoking, alcohol consumption, *H. pylori* infection and excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) are considered as etiological factors for this disorder [35]. Major therapeutic approaches to treat gastric ulcer disease include regular feeds and adequate rest, drug therapy and averting ulcerogenic agents. Drug therapy involves reduction of gastric acid production as well as reinforcement of gastric mucosal protection. Antacids, proton pump inhibitors, and histamine H2 receptor antagonists are commonly used drugs [36, 37]. However, beside their therapeutic efficacies, several incidences of relapse, adverse effects and drug interactions have been shown to be associated with these drugs [38]. Hence, research interest has been focused on search for new anti-ulcer molecules from medicinal plants. As a part of anti-ulcer drug discovery program of our lab several Indian medicinal plants have been reported to possess anti-ulcer activity [39]. In present study, the antiulcer potential of indigenous medicinal plant *Rheum emodi* has been determined.

**Material and Methods**

**Experimental Animals**

Adult Sprague Dawley rats of either sex, weighing 180-200g were housed in raised bottom mesh cages to prevent coprophagy and were kept in environmentally controlled rooms (temperature 25±2°C, humidity 60-80% and 12 hours light and dark cycle).
Treatment Schedule

Graded doses of extract (50, 100 and 200 mg/kg, b.w.), fractions and pure compounds (10, 20 and 40 mg/kg p.o.) as well as reference drugs omeprazole (10 mg/kg) and sucralfate (500 mg/kg) were prepared in 1% carboxymethyl cellulose (CMC) as suspension and administered orally (1ml/200g b.w), 45 mins prior to exposure of ulcerogens. Animals were fasted for 16 h before ulcerogens exposure and were divided into three groups, (n=6). Group I (Ulcer control): Animals treated with vehicle (1% CMC), Group II (Treatment groups): Animals treated with graded doses of extract or fractions or compounds and Group III (Standard groups): Animals treated with reference anti-ulcer drugs omeprazole (Omz) (10 mg/kg, p.o.) in CRU, Aspirin and Pyloric ligation models and Sucralfate (500 mg/kg, p.o.) in Alcohol induced ulcer model.

Anti-ulcer activity

Cold restraint induced gastric ulcer (CRU)

Animals were subjected to cold restraint stress after 45 mins of treatment with extract, fractions, compounds or reference drug omeprazole (Omz). Animals in all groups were immobilized in restraint cage and kept at 4°C in an environmental chamber [40]. After two hours, animals were sacrificed and stomachs were observed and scored under Magnascope for ulcers.

Alcohol induced gastric ulcers model (AL)

Chilled absolute alcohol (1ml/200g, body weight) was given to animals for induction of gastric hemorrhage [41]. Chrysophanol, emodin and sucralfate (SUC) were administered 45 minutes before alcohol treatment. After 1 hour of alcohol administration, animals were sacrificed and stomachs were cut open along the greater curvature to observe the gastric lesions appearing as hemorrhagic bands along the mucosal ridges of the stomach. Lengths of the lesions were measured using Biovis image analyzer software and summated to give a total lesion score.

Aspirin induced gastric ulcer model (AS)

Chrysophanol, emodin and reference drug omeprazole (Omz) were administered 45 mins before the treatment of aspirin (150 mg/kg body weight). Animals were sacrificed after 5 hours and the stomachs were dissected out, incised along the lesser curvature and the lesions were scored [42].
Pyloric ligation induced gastric ulcer model (PL)

After 45 mins of administration of chrysophanol, emodin and omeprazole (Omz), ulcer was induced by pyloric ligation under chloral hydrate anesthesia (300mg/kg, i.p.). Abdomens were opened and pyloric part of stomach from each rat was ligated avoiding any damage to the adjacent blood vessels [43]. Stomachs were replaced carefully and the animals were allowed to recover with free access to water. After 4 hours, animals were sacrificed and stomachs were dissected out. Lesions were scored and gastric fluid was collected and centrifuged at 2000 rpm for 10 mins. Supernatant was collected and used for estimation of gastric secretion and mucin level.

Gastric secretion study

Free and total acidity was measured from the collected gastric juice by titrating against 0.01N NaOH, using phenolphthalein as an indicator and expressed in terms of μ equiv./ml [44]. Mucin level in gastric juice was quantified as per method reported by Crowther, et al., [45].

Ulcer Scoring

Magnascope (5X magnification) were used for ulcer scoring after induction of ulcer via different ulcerogens. Ulcer were scored According to method reported earlier [46].

In vitro assay of H⁺ K⁺-ATPase activity

Effect of compounds on proton pump or H⁺ K⁺-ATPase activity was analyzed in microsomes isolated from stomachs of normal fasted rats [47]. Microsomes were incubated with different concentrations of chrysophanol, emodin and reference drug omeprazole (Omz) for 10 min at 37°C. Then assay buffer (pH 7.2) containing 150 mM KCl, 10 mM PIPES, 1 mM MgSO4, 5 mM Mg ATP, 1 mM EGTA, 0.1 mM ouabain, 10µg/ml valinomycin and 2.5µg/ml oligomycin was added. The reaction was carried out at 37°C for 20 min and was stopped by adding 10% ice-cold trichloroacetic acid. After centrifugation (2000 g for 1 min), inorganic phosphate release was determined in supernatant at 310 nm wavelength [48] and expressed as μM/hr/mg protein.

PGE2 estimation

PGE2 was determined in gastric tissue obtained from sham, control and treatment groups. Briefly, mucosa was scrapped and rapidly rinsed with ice-cold saline. The tissue was weighed
and homogenized in 10 volumes of phosphate buffer (0.1 M, pH- 7.4) containing 1 mM EDTA and 10 μM indomethacin. The homogenate was centrifuged (10,000 rpm, 10 min, 4°C), and the supernatant was processed for PGE2 estimation using Biotrak enzyme immunoassay kit (Cayman), following the manufacturer’s instructions. Results were expressed as pg/mg protein.

**Statistical analysis**

All values shown in the figures and tables represent the means ± S.E.M. IC50 values with 95% confidence limits were estimated using Maximum Likelihood Iterative Procedure [49]. Statistical analysis was performed with Prism version 3.0 software using one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. P<0.05 was considered to be statistically significant (p<0.001 = ***, p<0.01 = **, p<0.05 = *, p >0.05 (ns) = not significant).

**Results**

**Effect of extract and fractions of R. emodi rhizome on cold restraint induced ulcer in rats**

Administration of 95% ethanolic extract at graded doses of 50, 100 and 200mg/kg, p.o. in CRU model exhibited 37.5%, 50.0% and 52.5% protection respectively which indicated antiulcer potential of this plant (Figure 4.4). Subsequently it has been fractionated into four fractions. Graded doses of these fractions (10, 20 and 40mg/kg, p.o.) were evaluated in CRU model. F2 showed potent activity while F3 showed moderate protection in CRU model. (Table 4.3).

**Table 4.3:** Graded dose analysis of various fractions and reference drug omeprazole (OMZ) on percentage protection of ulcer against cold restraint induced gastric ulcer models in rats.

<table>
<thead>
<tr>
<th>Fraction prepared from 95% ethanolic extract</th>
<th>% Protection in cold restraint induced ulcer (CRU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/kg, p.o.</td>
</tr>
<tr>
<td>Fraction F1</td>
<td>0</td>
</tr>
<tr>
<td>Fraction F2</td>
<td>25± 5.065</td>
</tr>
<tr>
<td>Fraction F3</td>
<td>0</td>
</tr>
<tr>
<td>Fraction F4</td>
<td>0</td>
</tr>
<tr>
<td>Omeprazole (10 mg/kg, p.o)</td>
<td>75.0± 6.340</td>
</tr>
</tbody>
</table>
Data expressed as mean % protection ± S.E.M..

Effect of chrysophanol and emodin on cold restraint induced ulcer in rats

The most active fraction F2 yielded chrysophanol (CP) and emodin (ED) as major constituents. Both compounds were screened at graded doses of 10, 20 and 40mg/kg, p.o. in CRU model and exhibited (25.0%, 50.0%, 52.5%) and (37.5%, 62.5%, 66.67%) protection respectively as compare to reference drug omeprazole (Omz) (75.00%) as shown in Figure 4.4. 20 mg/kg, b.w. dose was found effective and selected for further studies.

Effect of chrysophanol 8-O-β-D-Glucopyranoside and emodin 8-O-β-D-Glucopyranoside, on cold restraint induced ulcer in rats

Fraction F3 was chromatographed repeatedly and purified to yield Chrysophanol 8-O-β-D-Glucopyranoside (CPG) and Emodin 8-O-β-D-Glucopyranoside (EDG). These compounds showed moderate protection in CRU model at doses of 10, 20 and 40mg/kg, p.o. (Table-4.4).

Effect of chrysophanol and emodin on alcohol induced ulcer in rats

Chrysophanol and emodin showed significant anti-ulcer activity against ethanol induced ulcer showing 70.51% and 78.48% protection respectively whereas the reference drug, sucralfate (SUC), showed 65.00% protection as depicted in Figures 4.4 and 4.5.

Table 4.4: Graded dose study of active constituents of fraction F3 and omeprazole (OMZ) on percentage protection of ulcer against cold restraint induced gastric ulcer model in rats.

<table>
<thead>
<tr>
<th>Names of Compounds isolated from fraction F-3</th>
<th>% Protection in cold restraint induced ulcer (CRU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/kg, p.o.</td>
</tr>
<tr>
<td>Chrysophanol -8-O-β-D Glucopyranoside (CPG)</td>
<td>0</td>
</tr>
<tr>
<td>Emodin -8-O-β-D Glucopyranoside (EDG)</td>
<td>0</td>
</tr>
<tr>
<td>Omeprazole (10 mg/kg, p.o)</td>
<td>75.0± 7.024</td>
</tr>
</tbody>
</table>

Data expressed as mean % protection ± S.E.M.
**Figure 4.4:** Effect of: a) 95% ethanolic extracts (RE) at graded doses 50, 100 and 200 mg/kg, b.w. b) & c) Chrysophanol (CP) and emodin (ED) at different doses 10, 20 and 40mg/kg p.o. compared with reference drug omeprazole (Omz) (10 mg/kg) in cold restraint induced gastric ulcer model in rats. d) Chrysophanol and emodin against alcohol, aspirin and pyloric ligation induced gastric ulcer models in rats. Reference drugs sucralfate (SUC) (500 mg/kg) used for alcohol model and omeprazole (Omz) (10 mg/kg) used for aspirin and pyloric ligation models. Data expressed as mean % protection ± S.E.M. * P<0.05 and **P< 0.01, n = 6 in each group.
Effect of chrysophanol and emodin on aspirin induced ulcer in rats

Chrysophanol and emodin showed potential anti-ulcer activity (37.5% and 50.0% protection respectively) in aspirin induced ulcer model, whereas omeprazole showed 50.0% protection in comparison to control as shown in Figure 4.4.

Effect of chrysophanol and emodin on pyloric ligation induced ulcer in rats

Anti-ulcer activity of chrysophanol and emodin was also observed against pyloric ligation induced ulcer model in rats where it showed protection of 52.5% and 62.5% respectively whereas reference drug omeprazole (Omz) showed 70.0% protection (Figure 4.4).

Effect of chrysophanol and emodin on gastric secretion

As shown in Table 4.5, treatment with Chrysophanol and Emodin at a dose of 20 mg/kg body weight significantly reduced the free acidity by 10.72% and 25.61% and total acidity by 15.01% and 32.98%, respectively. On the other side, chrysophanol and emodin at a dose of 20 mg/kg body weight increased the mucin secretion by 32.64% and 46.64% respectively.

![Figure 4.5: Photographs of ulcerated stomachs obtained from rats of control and chrysophanol (CP), emodin (ED) and reference drug sucralfate (SUC) treated groups against alcohol induced gastric ulcer models in rats, n = 6 in each group.](image)

Effect of chrysophanol and emodin on H+ K+-ATPase activity

To establish the gastroprotective activity of the compounds chrysophanol and emodin, we investigated the effect of these compounds on H+ K+-ATPase inhibitory activity in gastric microsomes isolated from rat stomach. Chrysophanol and emodin inhibited the proton pump activity with an IC₅₀ 187.1319µg/ml and 110.3046µg/ml respectively comparable to reference
drug omeprazole with an IC$_{50}$ 30.24 µg/ml, signifying the anti-secretory activity of the chrysophanol and emodin (Figure 4.6a).

**Table 4.5**: Effect of chrysophanol (CP), emodin (ED) and omeprazole (OMZ) on free acidity, total acidity and mucin contents in pyloric ligation model (n= 6 in each group).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Free acid (µequiv./ml)</th>
<th>Total acid (µequiv./ml)</th>
<th>Mucin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>75.26±3.242</td>
<td>136.90±3.570</td>
<td>544.03±47.39</td>
</tr>
<tr>
<td>CP (20mg/kg)</td>
<td>67.19±8.027</td>
<td>116.35±8.310</td>
<td>807.69±35.06*</td>
</tr>
<tr>
<td>ED (20mg/kg)</td>
<td>55.98±5.634*</td>
<td>91.74±4.096*</td>
<td>1019.57±92.48**</td>
</tr>
<tr>
<td>Omz (10mg/kg)</td>
<td>49.07±6.801**</td>
<td>76.59±6.124**</td>
<td>990.41±49.50*</td>
</tr>
</tbody>
</table>

Data expressed as mean ± S.E.M. * P<0.05 and **P< 0.01.

**Effect of chrysophanol and emodin on PGE2 level**

PGE2 levels in the ulcer control group were 2814±256 pg/mg tissue protein. The PGE2 values of chrysophanol and emodin and omeprazole (OMZ) treated group were found to be 3273±355, 4214±206, 4315±305 pg/mg respectively (Figure 4.6b).

**Figure 4.6**: a) Effect of chrysophanol (CP), emodin (ED) and reference drug omeprazole (Omz) on H$^+$K$^+$-ATPase activity in the rat gastric microsomes. Dots and lines are mean ± S.E.M. of experiments performed in triplicates (n=3). b) Effect of chrysophanol, emodin and omeprazole on gastric PGE2 level in comparison to ulcer control group. *P<0.05 and **P< 0.01, n = 6 in each group.
Discussion

At present times, Natural products have gained powerful attention due to its effective roles in development of chemo-therapeutic agents. The anti-ulcer activity of ethanolic extract of *Rheum emodi* rhizomes and its active constituents chrysophanol and emodin isolated from its active chloroform fraction has been studied against various models of experimentally induced gastric ulcer in order to evaluate its mechanism of action involved in prevention of gastric ulcer.

We performed a dose dependent anti-ulcer study of chrysophanol and emodin in CRU model. CRU is a well-accepted model for the induction of gastric ulcers, in which peripheral sympathetic activation and increased acid secretion play important roles [50]. In addition, chrysophanol and emodin exerted a protective effect against ethanol-induced gastric lesions. Ethanol damages the superficial epithelial layers and inhibits the release of mucosal prostaglandins and depresses the gastric defensive mechanisms. Chrysophanol and emodin appear to augment the gastric mucosal defense indicating the cytoprotective potentials.

Furthermore, gastric acid is an important factor for the genesis of ulceration in pyloric-ligated model [43]. In this model, auto-digestion of mucosa by gastric acid results in the development of ulcers [51]. Chrysophanol and emodin significantly reduced free and total acidity in this model, which suggests its anti-secretory potency.

In an attempt to clarify the mode of action of chrysophanol and emodin, through the anti-secretory pathway, its influence on gastric secretion was studied using inhibition of H⁺ K⁺-ATPase (Proton pump). Proton pump is a membrane bound enzyme that catalyses H⁺ transport at the expense of ATP hydrolysis. Thus the inhibition or the blockade of H⁺ K⁺-ATPase may account for suppressed acid secretion observed in the *in vivo* studies. The results obtained with gastric microsomes isolated from rat stomach showed that chrysophanol and emodin potently inhibited the H⁺ K⁺-ATPase activity comparable to the positive control omeprazole, thus suggesting that both of these compounds might be imparting anti-ulcer activity through decrease in acid secretion via proton pump inhibition.

The cytoprotective ability of chrysophanol and emodin was evident with increase in mucin content in pyloric ligation model and protection against ethanol induced ulcer model in comparison with the reference drugs. To further substantiate the cytoprotective potency of chrysophanol and emodin, its effect against NSAIDs induced ulcer model was explored. Studies
suggested that NSAIDs induce ulcers through their effect on cyclooxygenase enzyme leading to reduced prostaglandin production and increase in acid secretion [51, 52]. Our result demonstrated that chrysophanol and emodin significantly increased gastric level of PGE2 and exerted significant protection in ethanol induced gastric lesions in rats.

Though different biological activities of *R. emodi* have been reported earlier, anti-ulcer mechanism of this plant and its active constituents has not been reported till date. Our study is the first of its kind to establish the anti-ulcer potential of this plant.

Emodin and some other compounds isolated from *Rheum emodi* are well known for anticancer activity with well established mechanisms. The antiproliferative and anti-neoplastic effects of emodin is shown to be mediated by its capability to induce apoptosis in various cancer cells. Recent studies have postulated that emodin-induced apoptosis is mediated by ROS generation and alteration in mitochondrial membrane potential [53, 54]. Some studies showed the role of pathways involving akt and ERK. In other studies, it is shown to be mediated by decrease in Mcl-1 level independent of ROS generation [55]. Anthraquinones are highly active molecules, with high redox potential, leading to the formation of reactive oxygen species that in turn initiate a wide range of biological effects. The ROS generation may contribute to mitochondrial damage leading to reduction of mitochondrial transmembrane potential, release of cytochrome c and Smac, and subsequently activation of caspase and apoptosis [56].

Medicinally active compound with more than one biological target can provide additional benefits to patients suffering with disease with several inter related complications including cancer and metabolic syndrome. In the similar way, targeting an intracellular molecule with central roles in different signaling pathways may provide opportunity to find a novel activity in already existing drugs [57, 58].

Several HMG-CoA reductase inhibitors are reported to possess significant anticancer potential beside their potential role in treatment of cardiovascular disorders. This class of compounds showed significant anticancer potential through inducing the apoptosis, decreasing the cell proliferation as well as inhibiting the invasion of neighboring tissues. Several research groups are involved in exploring the potentials of HMG-CoA reductase inhibitors as anticancer agents. Statins have reported to possess very significant effect when given as combination therapy with other cytotoxic agents even in advanced or recurrent metastatic stage. Moreover these HMG-
CoA reductase inhibitors are significantly effective as maintenance therapy in localized tumor after receiving cytoreductive therapy. Several natural product based HMG-CoA reductase inhibitors including lovastatin, mevastatin, simvastatin and others which are extensively used for the treatment of cardiovascular diseases have now recognized as potent agents for the treatment of cancer.

Moreover, the gastric ulcer is considered as a major side effect related to most of the anticancer drugs and thus an anticancer drug possessing gastroprotective potential may provide better option for chemotherapy.

Keeping the above facts in consideration and in order to explore the therapeutic potential of these anticancer agents against other related disorders, the antidyslipidemic and antiulcer potential have been evaluated using rat models.

The ethanolic extract of *Rheum emodi* rhizomes as well as compounds isolated through the bioactivity guided fractionation of this extract showed prominent antidyslipidemic and antiulcer potential proving the immense potential of these anticancer agents as broad spectrum and safe drug.
REFERENCES


