8-DEHYDROXY CHRYSOPHENOL ISOLATED FROM EXTRACT OF RHEUM EMODI ENHANCE GENTAMICIN INDUCED NEPHROTOXICITY IN RATS MODEL

Mohd Aslam¹*, Rameshwar Dayal², Kalim Javed³, Mohd Samim³, Deepak Yadav¹, SM Arif Zaidi⁴, Surener Singh⁵

¹Department of Ilmul-Advia, Faculty of Medicine, Jamia Hamdard, New Delhi-110062 India
²Chemistry Division, Forest Research Institute, Dehradun, India
³Department of Chemistry, Faculty of Science, Jamia Hamdard, New Delhi-110062 India
⁴Department of Surgery, Faculty of Medicine, Jamia Hamdard, New Delhi-110062 India
⁵Department of Pharmacology, AIIMS, New Delhi, India

ABSTRACT

Revand Hindi (Rheum emodi) is considered as a substitute for Revand Chini. In traditional system of medicine, R emodi used as diuretic, liver stimulant, purgative, cathartic, and stomach disease etc. It is useful in kidney disorders and has been reported in different other treatments. The effects of different extracts (petroleum and chloroform) of R. emodi in gentamicin induced kidney damage were observed, Blood urea nitrogen (BUN), serum creatinine were significantly reduced in petroleum extract while chloroform extract increased the blood urea nitrogen (BUN) and serum creatinine and body weight. Chloroform extract was further fractionated with liquid–liquid extraction with sodium bicarbonate (SB), sodium carbonate (SC) and sodium hydroxide (SH) to find out a fraction which is responsible for toxicity increase by R emodi. When chloroform extracts (CH) shaking with aqueous solution of sodium bicarbonate, a brown ppt was obtained (RE). The chloroform layer after liquid-liquid extraction was coded as CHL. The effects of SB, SC, SH, CHL and RE fractions of chloroform extract of R. emodi in gentamicin induced kidney damaged was observed. Among fractions RE enhance gentamicin toxicity. TLC examination of RE showed a single spot. It was crystallized with methanol, its MASS, IR, ¹HNMR were recorded and characterized as 8-dehydroxy chrysophenol.

Keywords: Rheum emodi, Gentamicin, nephrotoxicity, Rat model, NMR, MASS.
INTRODUCTION

The definition of nephrotoxicity was chosen to be a change in creatinine clearance greater than 20% (Kirkpatrick et al. 2003). Any adverse functional or structural change in the kidney due to the effect of chemical or biological product, that is inhaled, ingested or absorbed or which yields metabolite with an identifiable toxic effect on the kidney is characterized as nephrotoxicity. By extension, the concept of nephrotoxins is occasionally applied clinically to the renal effects of physiological substances circulating in abnormal concentration such as may occur in hypercalcemia, hyperuricaemia or hypokalemic nephrotoxicity (Sheriner Maher, 1965). The usage of certain drugs in long run may lead to nephrotoxicity. The drugs tend to get accumulated in the form of by products which lead to renal failure or nephropathy. Aminoglycosides are the most widely used class of antibiotics for G–ve infections. A major complication of the use of these drugs especially gentamicin is nephrotoxicity. Investigators who have used well defined measure of nephrotoxicity indicate an incidence rate of 7 to 36%. In a review of various studies Kahlmeter and Dahlanger, (1984) have indicated that the average incidence of gentamicin nephrotoxicity is around 15%.

The plant of Rheum emodi (Revand chini) is a perennial stout herb; belong to a large genus `Rheum’ (Anonymous, 1972). The Rhubarb is distributed in the temperate and sub tropical region of the world, chiefly in Asia. About ten species occur in India. The majority of authors considered that purgative action of Rheum emodi is due to the presence of Chrysarobin and after that it produces constipation due to rheotannic acid (Anonymous, 1972; Bunney and Shah, 1992; Clark, 1995). Some authors narrated that the Rheum emodi produced constipation in low dose, and purgation in high doses (Morton, 1977; Khory and Katrak, 1985; Wren, 1968). Chopra et al quoted that Revand Chini should not use in constipation and a continuous aperient although it’s seldom act as an irritant (Chopra et al., 1958; Chatterjee and Pakrashi, 1991; Clark, 1995). In a case of sluggish bowl it is used with ginger (Anonymous, 1972; Chatterjee and Pakrashi, 1991) and in children diarrhoea due to indigestion is used with NaHCO3 and Magnesia. Cooked stalks act as a powerful purgation (Clark, 1995). Decoction of root bark is bitter tonic in wines (Chatterjee and Pakrashi, 1991; Kirtikar and Basu, 1987; Bunney and Shah, 1992).

Chinese Rhubarb used as a flavour component in alcoholic and non-alcoholic beverages, Backed goods, Candy, frozen dairy, desserts, Gelatin and puddings (Duke, 1985; Uphof, 1968). After taking to Rheum emodi the colour of urine changed to bloody red colour.
(Chatterjee and Pakrashi, 1991). Strong water solution burns to Aluminium and form a hole due to high acid contents. A pack of *R. officinale* in hot water applied on hair for 30-minute change the colour golden to light brown or fair hair (Bunney and Shah, 1992). Stem of *R. officinale* and *R. rhaponicum* are used as a food (Anonymous, 1972; Clark, 1995; Wren, 1968), and flowers and leaf stalks also (Agarwal, 1986).

The anthraquinones aloe emodin, emodin and rhein were found to inhibit in vitro, the growth of *Bacillus subtilis* and *Staphylococcus aureus*, with rhein being the most effective compound (Fuzellier et al. 1981). Four anthrone C, O-glycosides named rheinosides A, B, C and D were isolated from rhubarb root. Rheinosides A and B are stereoisomeric 5-O-β-D-glucopyranosyl 19-C- β-D-glucopyranosyl 9-hydroxy rhein anthrones. Rheinosides C and D differ from rheinosides A and B by the absence of the 9-hydroxy group. The anticancer effect of aloe-emodin has been established in two human cancer cell lines, Hep G2 and Hep 3B. Aloe-emodin inhibited cell proliferation and induced apoptosis in both examined cell lines by different antiproliferative mechanisms (Kuo et al. 2002). Plant aqueous extract caused significant prolongation (p<0.001) in pentobarbital-induced sleeping time in aqueous extract treated group. The increased effects of pentobarbital on sleeping time and strychnine on the lethality of rats conclude that crude aqueous extract of ‘Rheum emodi wall’ inhibits hepatic microsomal P450 enzyme (Tahir et al. 2009).

In addition to essential oil, roots contain an unidentified terpenic alcohol and a product believed to be methyl-n-heptyle ketone. In a study of the anthraderivatives in roots of *Rheum emodi*, three new anthrone C-glucosides, namely 10 hydroxycascaroside C, hydroxycascaroside D and 10R- chrysaloin1-O-beta-D glucopyranoside were isolated, beside the rare compounds cascaroside C, cascaroside D and cassialoin (Krenn et al., 2004). In this study, we investigated the *R. emodi* activity with Gentamicin.

**MATERIALS AND METHODS**

**Chemicals**

Acetic acid, Benzoic acid, Paraffin wax, Petroleum ether (60-80°C), Potassium chloride purchased from E. Merck (India) Pvt. Ltd. Mumbai, India. Copper Sulphate (CuSO4), Formalin, Glycerin, Hematoxylin & Eosin (H & E), Hydrochloric acid, Orthophosphoric acid, Sodium potassium tartarate, Sulphuric acid purchased from S.D. Fine Chem. Ltd. Mumbai, India. Gentamicin was bought from Cadila Healthcare Ltd Ahmedabad, India. Double distilled water was used whenever required.
Plant material

The plant material was purchased from Ajmal and Brothers, Khari Baoli, Delhi, India. Their botanical identity was established as *Rehum emodi* (Revand Chini) rhizomeat NISCAIR (National Institute of Science Communication and Information Resources), Dr. K.S. Krishnan Marg, Pusa Gate, New Delhi, India, 110012 under Ref. NISCAIR/RHM/F-3/2004Consult/-486/62.

Extraction

The air-dried rhizomes (1 kg) of *Rheum emodi* were coarsely powdered and sequentially extracted with petroleum ether (3 Liter) (60-80°C) and chloroform (3 Liter) in soxhlet apparatus by refluxing on a heating mantle. The removal of the solvent yielded two extractives on water water bath. The solvents were removed by distillation method to give respective extracts which were coded as petroleum ether (PET) and chloroform (CH) respectively. The CH was dissolved in chloroform and transferred to a separating funnel. It was shaken successively with aqueous sodium bicarbonate (SB) solution (5 %), sodium carbonate (SC) solution (5 %) and sodium hydroxide (SH) (2 %). These fractions were acidified separately with dilute HCl to give respective brown precipitates which were filtered, dried and coded as SB, SC and SH respectively. The yield was 33 %, 3 % and 18 % respectively. The chloroform layer left after liquid-liquid extraction was coded as CHL.

When CH dissolved in CHCl₃ was shaken with sodium bicarbonate, a brown precipitate was obtained which was removed by filtration and washed with dilute HCl and then with plenty of distilled water. It was dried and coded as RE. All extract was dried in oven at 50°C for 3 days and further used for the identical and biological activities.

Compound isolation and characterization

The chloroform extract of rhizomes of *Rheum emodi* on shaking with aqueous sodium bicarbonate gave a brown precipitate which was filtered, washed with dilute HCl followed by distilled water to make it acid free. The solid obtained was crystallized with methanol to give a yellow needles (m.p: 199-200°C) and designate as RE. This compound (RE) was further characterized using Infra-Red (IR, PerkinElmer, Thane, MH), proton Nuclear magentic resonance ($^1$H NMR, spectrophotometer (Bruker, 300MHz)) and Mass spectrophotometry (Bruker, MS-632) at IIT, New Delhi, India.
Experimental animals
All the experiments were carried out on either sex of albino rats of Wistar strain (180-250 g), supplied by the Central Animal House Facility of Jamia Hamdard, New Delhi (Registration no. 173/CPSEA). All animals were housed in groups in polypropylene cage and maintained on a standard pellet diet (Amrit Laboratory rat and mice feed, New Maharashtra Chakan Oil Mills Ltd., Mumbai, India) and water ad libitum. The animals were kept under standard laboratory conditions at a temperature of 25 ± 1°C. The experiments were performed in accordance with the guidelines for the care and use of laboratory animals, laid down by the committee for the purpose of control and supervision of experiments in animals (CPCSEA), Ministry of Social Justice and Empowerment, Govt. of India, Jan. 2000. In all experiments 1% CMC in water was used as vehicle and given in volume of 10 ml/kg. All the treatments were given in the form of suspension in the vehicle and given in volume of 10 ml/kg. The animals were anaesthetized with anesthetic ether and sacrificed.

Effect of petrol and chloroform extract of Rheum emodi on BUN and serum creatinine in normal rats
Study was done in two phase in first phase, the rats were divided into four groups of six animals in each. Group first served as control and given 1 % CMC in distilled water (10 ml/kg per day, p.o), while group second was given gentamicin (100 mg/kg bw) and group third and fourth was administered petrol and chloroform extract of Rheum emodi (100 mg/kg bw) after gentamicin administration once (100 mg/kg bw), respectively for 15 days. Dose was given orally. On the next day blood sample were collected from retro-orbital plexus. The serum was separated to measure the concentration of BUN and serum creatinine followed by Kanter, 1975 and Hare, 1950 respectively and kidney were isolated after sacrificing the animals.

In Second phase, the rats were divided into seven groups of six animals each groups I was given vehicle (1% CMC) in distilled water (10 ml/kg/d, p.o.), group II was administered orally gentamicine (100 mg/kg bw) while groups III, IV, V, VI and VII were given SB, SC, SH, RE and CHL (50 mg/kg bw) orally suspended in the vehicle (10 mg/kg) respectively after gentamicin administration once (100 mg/kg bw), for 15 consecutive days (Walker, 1987). On the next day, blood samples were collected from retro-orbital venous plexus. The serum was separated to measure concentration of urea nitrogen and creatinine. Animals were
sacrificed and their kidneys were isolated for histopathological studies and post mitochondrial supernatant (PMS).

**Biochemical estimation**
After 8 days of oral administration of extract, the animals were fasted overnight and anesthetized with chloroform; blood was collected by cardiac puncture with help of syringe, and the serum was separated by centrifugation at 3,000×g for 7 min for the assessment of enzymatic activity from serum samples. Urea nitrogen (BUN) was estimated at 340 nm and creatinine (CRE) at 490 nm using a UV–vis spectrophotometer 3600-Shimadzu by standard protocol using analytical kits, obtained from Span Diagnostic Private Ltd., Udhana, Surat, Gujrat, India.

**Estimation of serum creatinine (SCr)**
SCr was estimated by the alkaline picrate method (Hare, 1950). Protein free filtrate was prepared to 1.0 ml of plasma/serum, 1.0 ml of sulphuric acid (0.6 N), 1.0 ml of sodium tungstate (5 % w/v), and 1.0 ml of distilled water were added and mixed thoroughly. The mixture was then centrifuged at 800 g for 5 min. The supernatant was added to a mixture containing 1.0 ml of picric acid (1.05 % w/v) and 1.0 ml of sodium hydroxide (0.75 N). The absorbance was recorded at 520 nm after 20 min of incubation.

**Estimation of blood urea nitrogen (BUN)**
BUN was estimated by diacetyl monoxime method (Kanter, 1975). Protein free filtrate was prepared and to a 0.5 ml of it, 3.5 ml of distilled water, 0.8 ml diacetylmonoxime (2 %) and 3.2 ml of sulphuric acid, phosphoric acid reagent (prepared by mixing 150 ml of 85 % w/v phosphoric acid with 140 ml of water and 50 ml of concentrated H₂SO₄) were added. The reaction mixture was placed in a boiling water bath for 25 min and then cooled immediately. Absorbance was recorded at 520 nm.

**Post-mitochondrial supernatant (PMS) preparation**
Kidneys were perfused immediately after removing with ice-cold saline (0.85 % w/v NaCl) and homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17 % w/v), using a Potter Elvehjem homogenizer. The homogenate was centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The supernatant was then centrifuged at 10,500 g for 20 min to obtain PMS.
Lipid Peroxidation (LPO)

LPO in various kidney regions (FC) was measured by estimating malondialdehyde (MDA) levels by thiobarbituric acid reactive substances (TBARS) following the method of (Utley, et al. 1967). In brief, homogenate was prepared in 0.15 M KCl (5 % w/v homogenate) and aliquots of 0.6 ml were incubated for 0 and 1 h at 37°C. The reaction was stopped by addition of 1.2 ml of 28 % w/v TCA and the volume was made up to 3 ml by adding 1.8 ml of water. Following centrifugation at 3,000 rpm for 10 min, 2.5 ml of the supernatant was removed and colour was developed by addition of 0.5 ml of 1 % w/v TBA dissolved in 0.05 N NaOH and kept in boiling water bath for 15 min until the appearance of pink color. The absorbance was read at 532 nm. The result was expressed as nmol (MDA) formed/min/mg protein.

Estimation of reduced GSH

Reduced GSH in kidney was assayed by the method of Jollow et al. (1974). One millilitre aliquot of renal PMS (10% w/v) was precipitated with 1.0 ml of sulphosalicylic acid (4 % w/v). The samples were kept at 4°C for 1 h and then centrifuged at 1200 g for 15 min at 4°C. The supernatant was used for GSH determination. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml DTNB (40 mg/ 10 ml of phosphate buffer 0.1 M, pH 7.4) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm.

Assay for superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was measured by the method of Beauchamp and Fridovich, (1971). The reaction mixture of total volume 1.0 ml consisted of 0.5 M phosphate buffer pH 7.4, 0.1 ml PMS, 1.0 mM xanthine, and 57 µM NBT. It was incubated for 15 min at room temperature and reaction was initiated by the addition of 50-mU xanthine oxidase. The rate of reaction was measured by recording change in the absorbance at 550 nm due to formation of formazan, a reduction product of NBT.

Assay for Catalases (CAT)

Catalase activity in kidney regions was assayed by the method of Sinha (1972) using H₂O₂ as substrate. The reaction mixture of 2.0 ml contains 1.0 ml phosphate buffer (0.01 M, pH 7.0), 0.4 ml distilled water and 0.1 ml 10 % w/v homogenate (prepared in 0.1 M phosphate buffer). Reaction was started by adding 0.5 ml H₂O₂ followed by, incubation at 37°C for 1 min and was stopped by the addition of 2.0 ml of dichromate acetic acid reagent. The tubes were immediately placed in a boiling water bath for 15 min and centrifuged for 10 min (3000 rpm).
The green colour developed during the reaction was read at 570 nm spectrophotochemically. Control tubes, devoid of enzyme, were also processed in parallel. The enzyme activity was calculated using molar extinction coefficient of H₂O₂ and expressed as µmol H₂O₂ consumed/minute/mg protein.

**Histopathological study of kidney**

Formulation-treated groups were compared with the normal groups, gentamicin group for the observations of the tissues. Kidney tissues were stained with hematoxylin and eosin (H and E) stain to visualize and differentiate. Last day of the experiment, the rats were anesthetized with ether and perfused transcardially with saline. Kidneys were removed quickly and postfixed in buffered formalin (10 %) for 24 h and further 3-4 mm pieces of the tissues dehydrated and embedded in paraffin (Yadav et al. 2013). Sections were taken from each tissue in 5-µm thickness, washed with xylene and mounted with DPX. The slides were observed for histopathological changes, and microphotographs were taken using an Olympus BX50 microscope system (Olympus, Japan) at Jamia Hamdard, New Delhi, India.

**Statistical analysis**

The results are expressed as mean ± SE. Statistical evaluation was carried out using ANOVA followed by Dunnnett's test between the groups. Differences were considered significant at p<0.05 and highly significant at p<0.01.

**RESULTS**

**Compound RE (8-Dehydroxychrysophenol) analysis**

The solid on crystallization with methanol yielded yellow needles (111 mg), m.p: 117°C. The compound RE (8-dehydroxychrysophenol) infrared spectrum showed characteristic absorption bands for hydroxyl group (3385cm⁻¹), carbonyl function (1701 cm⁻¹) and aromatic rings (1625, 1545, 867 cm⁻¹). Its mass spectrum exhibited a molecular ion peak at m/z 238 consistent with the molecular formula C₁₅H₁₀O₃. The ¹H NMR spectrum of compound RE displayed three double doublets at δ 7.84 (J =1.2, 7.8 Hz,), 7.69 (J =8.4, 2.8 Hz,), 7.30 (J =1.2, 7.2 Hz) integrating for one proton each were assigned to ortho-, meta- coupled H-2, H-4 and H-7 protons, respectively. Two one-proton doublets at δ 7.28 (J =7.2 Hz) and 7.11 (J =1.6 Hz) were ascribed to ortho - coupled H-8 and meta- coupled H-5 proton. A one proton multiplet at δ 7.66 was due to H-3 proton. A three-proton broad singlet at δ 2.47 was due to C-9 methyl protons located to the aromatic ring. On the basis of foregoing evidences the
Mohd Aslam et al.

World Journal of Pharmacy and Pharmaceutical Sciences

structure of compound RE was elucidated as 8-dehydroxychrysophenol, a known compound (Fig. 1).

\textbf{IR} \nu_{\text{max}} \text{(KBr)}: 3385, 2925, 2850, 1701, 1625, 1365, 1135, 930, 867 \text{ cm}^{-1}

\textbf{\textsuperscript{1}H NMR (CDCl}_3, \text{\delta}): 7.84 \text{ (1H, dd, } J = 1.2, 7.8 \text{ Hz, H-2)}, 7.69 \text{ (1H, dd, } J = 8.4, 2.8 \text{ Hz, H-4)}, 7.66 \text{ (1H, m, H-3)}, 7.30 \text{ (1H, dd, } J = 1.2, 7.2 \text{ Hz, H-7)}, 7.28 \text{ (1H, d, } J = 7.2 \text{ Hz, H-8)}, 7.11 \text{ (1H, d, } J = 1.6 \text{ Hz, H-5)}, 2.47 \text{ (3H, brs, CH}_3\text{-9)}.

\textbf{FAB-MS (m/z, rel. int.):} 239 \text{ [M}^+ + \text{H]} \text{ (5.2), (C}_{15}\text{H}_{10}\text{O}_3).}

\textbf{Biological Evaluation of Rheum emodi

The Gentamicin induced nephrotoxicity were revealed after experimental protocol of 15 days. Animals were treated with gentamicin and showed a significant nephrotoxicity and oxidative stress. When the stress and damage to kidney for this group were compared to the normal group biochemically, the increased levels of BUN, SCr and lipid peroxide (LPO) levels were found (P<0.01); whereas the levels of CAT, SOD, and GSH were found to be decreased (P<0.01).

The nephroprotective effect of petroleum ether and chloroform, and SB, SC, SH, CHL and RE were evaluated. Petroleum ether showed nephroprotective effect in comparison to gentamicin group while chloroform extract enhanced the nephrotoxicity (Table 1). Further fractions of chloroform extract SB, SC, SH, CHL and RE were showed that only RE fraction enhanced the nephrotoxicity while all other fractions showed nephroprotective effect (Table 2).

The effects of SB, SC, SH, CHL and RE fractions of \textit{R. emodi} extract on MDA levels were measured to demonstrate the rate of LPO in the kidney of gentamicin-induced nephrotoxic group. There were significant changes in MDA levels in the gentamicin treated group compared to control group. RE enhanced the MDA level significantly (P<0.01) while as SB, SC, SH, and CHL normalized the MDA level of gentamicin-induced nephrotoxicity (Table 3). Effects of SB, SC, SH, CHL and RE fractions of \textit{R. emodi} extract on the activity of CAT, SOD, and GSH in the gentamicin-induced nephrotoxic group. The activity of CAT, SOD, and GSH in RE group decreased significantly with gentamicin-induced nephrotoxic group (P<0.01). On the other hand, the activities of SB, SC, SH, and CHL were normalized the activities significantly (P<0.05) compared to the control group.
Table 1: Effect of petrol and chloroform extracts of *R. emodi* in gentamicin-induced kidney damage.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>9.42 ± 1.74</td>
<td>0.146 ± 0.02</td>
</tr>
<tr>
<td>II (Toxicant)</td>
<td>Gentamicin</td>
<td>100 mg/kg</td>
<td>27.68 ± 3.18*</td>
<td>0.365 ± 0.03*</td>
</tr>
<tr>
<td>III</td>
<td><em>R. emodi</em> Petroleum ether extract</td>
<td>100 mg/kg</td>
<td>22.45 ± 2.69*</td>
<td>0.319 ± 0.02</td>
</tr>
<tr>
<td>IV</td>
<td><em>R. emodi</em> Chloroform extract</td>
<td>100 mg/kg</td>
<td>49.43 ± 4.61*</td>
<td>0.521 ± 0.04*</td>
</tr>
</tbody>
</table>

n=6, values are expressed as Mean± S.E.M.

*p<0.05 indicate control Vs toxicant and #p<0.05 indicates toxicant Vs drug treated group.

Table 2: Effect of SB, SC, SH, CHL and RE fractions of chloroform extract of *R. emodi* in gentamicin-induced kidney damage on BUN and Creatinine.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>BUN (mg/dl) Mean ± SE</th>
<th>Creatinine (mg/dl) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>10.01 ± 3.03</td>
<td>0.156 ± 0.02</td>
</tr>
<tr>
<td>II</td>
<td>Gentamicin</td>
<td>100 mg/kg</td>
<td>26.45 ± 1.58*</td>
<td>0.326 ± 0.048*</td>
</tr>
<tr>
<td>III</td>
<td>SC</td>
<td>50 mg/kg</td>
<td>38.86 ± 3.87*</td>
<td>0.246 ± 0.068</td>
</tr>
<tr>
<td>IV</td>
<td>SB</td>
<td>50 mg/kg</td>
<td>24.95 ± 4.22</td>
<td>0.284 ± 0.091</td>
</tr>
<tr>
<td>V</td>
<td>SH</td>
<td>50 mg/kg</td>
<td>26.45 ± 2.85</td>
<td>0.251 ± 0.048</td>
</tr>
<tr>
<td>VI</td>
<td>RE</td>
<td>50 mg/kg</td>
<td>44.45 ± 3.93*</td>
<td>0.308 ± 0.053*</td>
</tr>
<tr>
<td>VII</td>
<td>CHL</td>
<td>50 mg/kg</td>
<td>20.89 ± 3.15</td>
<td>0.233 ± 0.067</td>
</tr>
</tbody>
</table>

n=6, values are expressed as Mean± S.E.M.

*p<0.05 indicate control Vs toxicant and #p<0.05 indicates toxicant Vs drug treated group.
Table 3: Effect of SB, SC, SH, CHL and RE fractions of chloroform extract of *R. emodi* in gentamicin-induced kidney damage on LPO, SOD, Catalase and GSH.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>LPO (n moles TBARS formed /mg of protein)</th>
<th>SOD (Unit/mg of protein)</th>
<th>Catalase (µM of H$_2$O$_2$ consumed /min/mg of protein)</th>
<th>GSH (µg/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>2.8 ± 0.057</td>
<td>6.69 ± 0.44</td>
<td>180.0 ± 3.64</td>
<td>0.316 ± 0.01</td>
</tr>
<tr>
<td>II (Toxicant)</td>
<td>Gentamicin</td>
<td>100 mg/kg</td>
<td>26.6 ± 0.127***</td>
<td>3.32 ± 0.13***</td>
<td>126.4 ± 4.4**</td>
<td>0.169 ± 0.01*</td>
</tr>
<tr>
<td>III</td>
<td>SC</td>
<td>50 mg/kg</td>
<td>7.38 ± 0.135#</td>
<td>4.48 ± 0.13##</td>
<td>173.0 ± 2.25#</td>
<td>0.294 ± 0.002#</td>
</tr>
<tr>
<td>IV</td>
<td>SB</td>
<td>50 mg/kg</td>
<td>5.36 ± 0.09 # #</td>
<td>5.36 ± 0.12 #</td>
<td>158.2 ± 4.13#</td>
<td>0.275 ± 0.012#</td>
</tr>
<tr>
<td>V</td>
<td>SH</td>
<td>50 mg/kg</td>
<td>6.45 ± 0.29 # #</td>
<td>6.45 ± 0.13#</td>
<td>166.12 ± 1.43#</td>
<td>0.275 ± 0.023#</td>
</tr>
<tr>
<td>VI</td>
<td>RE</td>
<td>50 mg/kg</td>
<td>30.21 ± 0.43## # #</td>
<td>1.43 ± 0.43## # #</td>
<td>103.4 ± 5.1##</td>
<td>0.109 ± 0.04#</td>
</tr>
<tr>
<td>VII</td>
<td>CHL</td>
<td>50 mg/kg</td>
<td>8.02 ± 0.123##</td>
<td>5.12 ± 0.33##</td>
<td>156.67 ± 2.73#</td>
<td>0.298 ± 0.005##</td>
</tr>
</tbody>
</table>

n=6, values are expressed as Mean± S.E.M.; *p<0.05, **P<0.01, ***P<0.001 indicate control Vs toxicant; #p<0.05, ##p<0.01, ###p<0.001, indicates toxicant Vs drug treated group.

**Histopathological observation**

H and E staining is used to visualize and differentiate between tissue components in normal and pathological conditions. The histological examination of the H and E-stained control kidney tissues showed normal architecture of normal glomerular and tubular histology. The tubules were largely intact without the presence of any mononuclear infiltrates in the interstitium and blood vessels were also unremarkable (Fig. 2a & 3a). Kidney section of gentamicin-induced nephrotoxic group showed glomerular, peritubular and blood vessel congestion along with interstitial edema, desquamation and necrosis of the tubular epithelial lining cells in the tubular lumen in kidneys of rats exposed to toxicant (Fig. 2b & 3b). Group III of I phase study of (PET extract) showed the tubules were some intact and no necrosis, while group IV showed more necrosis as compared to group II and infiltrated glomeruli (Fig. 2c & 2d). In II phase study RE fraction showed more cell necrosis and minimal cell swelling and cytoplasmic vacuolations were present in group II (Fig. 3f). While other fractions SB, SC, SH and CHL showed minimal necrosis involving only a few tubular lining cells of the proximal convoluted tubule, loss of brush border and cytoplasmic vacuolations in the figure 3c, 3d, 3e and 3g, respectively (Fig. 3).
Figure 1: Structure elucidated by analytical characterization (8-dehydroxychrysophenol).

Figure 2: Morphological changes of kidney tissue stained with haematoxylin and eosin (H & E). a), b), c) and d): sections of kidney tissues from group I, II, III, and IV of phase I study, respectively (×400).
DISCUSSION

Revand Hindi (*Rheum emodi*) is considered as a substitute for Revand Chini (*Rheum officinalis*) in Unani system of medicine as both have similar medicinal properties. It has been described having diuretic (Baitar, 1987), liver stimulant, purgative / cathartic, stomachic...
In this study, the renal effects of various fractions of Revand Hindi (Rheum emodi) were investigated on gentamicin induced nephrotoxicity in rats and normal rats by monitoring the level of blood urea nitrogen and serum creatinine (Azhar et al. 2005). These investigators reported that water soluble fraction has nephroprotective effect on all the proximal tubule segments. Water insoluble fraction also improved the renal function by protecting S₂ segment of proximal tubule nephrotoxicity by metals. However, this fraction has been found to enhance gentamicin nephrotoxicity in rats. In the present study attempts were made to find out constituent(s) responsible for enhancing gentamicin nephrotoxicity.

Gentamicin induced nephrotoxicity is a model of acute renal failure caused by oxidative stress (Maldonadu et al, 2003) generated through the induction of superoxide. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubules. It is a complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal tubular necrosis, followed by (Silan et al, 2007) deterioration and renal failure. The toxicity of gentamicin is believed to relate to generation of reactive oxygen species (ROS) in kidney. Several reports have documented the pathogenesis of aminoglycoside induced renal tubular cell injury, such as derangement of lysosomal, mitochondrial and plasma membrane structure. Furthermore, results of many studies have been shown that the altered concentrations of various biochemical indicators of oxidative stress in kidney tissue are due to gentamicin. Because of the obvious mediation of ROS in gentamicin induced renal damage, several antioxidant agents have been (Yaman et al, 2010; Reiter et al, 2002) used to block gentamicin induced nephrotoxicity.

The coarse powder of rhizomes of R. emodi was extracted successively with hot petroleum ether (PET) and chloroform (CH) in a soxhlet apparatus. Removal of the solvents on a water bath yielded the petroleum and chloroform extracts respectively. The effect of different extracts of R. emodi in gentamicin induced kidney damage was observed as blood urea nitrogen 23.86 % and serum creatinine 0.359 % in PET and in CH blood urea nitrogen (48.82 %) and serum creatinine (0.481 %) respectively. The results clearly indicate that CH fraction enhance the Gentamicin induced toxicity.
The effect of SB, SC, SH, CHL and RE fractions of CH extract of *R. emodi* in Gentamicin induced kidney damage observed as blood urea nitrogen 26 % and serum creatinine 0.276 % in SB, in SC blood urea nitrogen 39 % and serum creatinine 0.254 %, in SH blood urea nitrogen 25 % and serum creatinine 0.24 %, in CHL blood urea nitrogen 21 % and serum creatinine 0.223 % and in RF blood urea nitrogen 41 % and serum creatinine 0.298 %.

Among these fractions only RE enhanced the Gentamicin toxicity. It was crystallized with methanol to give a yellow crystalline solid (m.p 199°C). The IR, $^1$H NMR and Mass spectra of this compound were recorded and it was characterized as 8-dehydroxychrysophenol. On the basis of these findings, it may be concluded that 8-dehydroxychrysophenol may be responsible for enhancing gentamicin toxicity.

**CONCLUSION**

Coumarins comprise a vast array of biologically active compounds ubiquitous in plants. A large number of coumarins derivatives have been reported to show substantial antioxidant activity in vitro and in vivo. Based on the evidences available, it may be concluded that extract of *R. emodi* showed enhanced gentamicin nephrotoxicity possibly through 8-dehydroxy chrysophenol present in this plant.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

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