Effect of chrysin isolated from *Oroxylum indicum* against cisplatin-induced acute renal failure.

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Abstract: Present study is designed to evaluate the effect of chrysin isolated from *Oroxylum indicum* against on cisplatin-induced nephrotoxicity. chrysin was isolated from dried roots of *Oroxylum indicum*. Nephroprotector activity was evaluated in male Albino rats. Nephrotoxicity induced by intraperitoneal administration (6 mg/kg, i.p., single dose) of cisplatin. Chrysin was administered by gastric intubation at two dose levels. Animals were divided into 5 groups. Group I animals received vehicle (1% CMC). Group II animals received cisplatin on day 1. Group III received 40mg/kg Bd.Wt chrysin for ten days, Group IV and V received 20 mg/kg and 40mg/kg Bd.Wt. chrysin respectively from day 1 to day 10 and On day 5 these groups received cisplatin injection. On day 9, urine was collected and estimated urinary functional parameters. Blood was collected on day 10 and estimated Blood urine nitrogen (BUN), Serum creatinine (SC). Malondialdehyde levels were also estimated in kidney tissue. Histological studies were also conducted. Cisplatin caused acute renal damage characterized by elevation of Blood Urine Nitrogen, Serum creatinine, Malondialdehyde level, marked drop in creatinine clearance Animals which received chrysin reversed all the effects induced by cisplatin in dose dependent manner. Histological studies also substantiated above results. Present study reveals that chrysin attenuated the nephrotoxicity of cisplatin in rats.

Key-words: *Oroxylum indicum*, Cisplatin, Chrysin, Nephroprotector activity

1. Introduction

Flavonoids are broad class of poly phenolic compounds found in fruits, vegetables, nuts and seeds as well as most types of tea and red wine [1]. Flavonoids have been reported to exhibit a wide range of biological effect including anti oxidant and anti proliferative properties [2]. Recent reports evidenced that number of flavonoids such as quercetin, luteolin, rutin [3,4] showed good nephroprotective activity. chrysin is one such flavonoid which possess broad spectrum of biological activities [5,6,7]. Over the past several years the number of persons suffering from kidney problems increased and estimated that 26 million peoples are suffering with kidney problems, but till now there is no allopathic drug to treat kidney problems, but through search on previous papers it was revealed that substances with anti oxidant potential such as flavonoids, showed good protection against cisplatin-induced nephrotoxicity. Hence present study was planned to evaluate nephroprotector activity of chrysin against on cisplatin-induced nephrotoxicity.

2. Materials And Methods

2.1. Isolation of chrysin

The roots were powdered and extracted with light petroleum to remove oily matter and then with ether. The ether extracts were concentrated to a small volume and shaken successfully with 20% aqueous sodium carbonate, 0.2% aqueous sodium hydroxide and 4% aqueous NaOH. The sodium carbonate extract on acidification yielded a brown solid, which on fractionation from ether yielded crude chrysin which was further purified by recrystallisation from alcohol.

2.2. Screening of nephroprotector activity of chrysin

2.2.1. Animals

Healthy Wistar adult male albino male rats between 2 and 3 months of age and weighing about 150-200g were used in the present study. Housed in polypropylene cages and fed with standard rat pellet diet, water *ad libitum*. Animals
were acclimatized to our lab environment for about a week. Animals were handled according to the rules and regulations of Institutional Animal Ethics Committee (IAEC) (Reg. No. IAEC/930/a/06/CPCSEA).

Cisplatin was prepared in distilled water to give 1mg/ml solution. The chrysin was suspended in 1% CMC to give 6mg/ml solution and used for in vivo studies.

2.2.2. Treatment Protocol for Chrysin

Animals were divided into 5 groups each of 5 rats.

(1) Vehicle (1% CMC)
(2) Cisplatin (6mg/kg body wt)
(3) Chrysin (40mg/kg)
(4) Chrysin (20mg/kg) + Cisplatin (6mg/kg)
(5) Chrysin (40mg/kg) + Cisplatin (6mg/kg)

Group I animals received only 1% CMC for 10 days. Group II animals received vehicle, 5 days prior to the intraperitonial (i.p.) administration of Cisplatin (6mg/kg) and thereafter throughout the study i.e., up to day 10. Group-III animals received only chrysin with a dose of 40mg/kg body wt. for 10 days.

Group-IV and group-V animals were treated with chrysin (20mg/kg and 40mg/kg, p.o.) by gastric intubation respectively, 5 days prior to intraperitonial administration of cisplatin (6mg/kg) and there after throughout the study i.e., up to day 10.

2.3. Assessment of nephroprotector activity

On day 9 animals were housed in metabolic cages for 12hrs for urine collection and urinary functional parameters like urinary total proteins [8] and urinary creatinine [8] were determined. Five days after cisplatin treatment i.e. on day 10 animals were sacrificed and blood samples were taken by heart puncture to measure blood urea nitrogen [8] and serum creatinine [8] using commercially available kits. Kidneys were removed and washed thoroughly with ice-cold normal saline and homogenates (10% W/V) were prepared in phosphate buffer solution (50mmol/l, P\text{H} 7.4). Homogenates were centrifuged at 10,000g for 10min in a cooling centrifuge at 4\textdegree C, after removing the cell debris; supernatants were used for the assay of lipid peroxides (LPO) in kidney [9].

Creatinine clearance (Cl_{cr}): Creatinine clearance was estimated by alkaline picrate method (Godkar, 1994).

Creatinine clearance = \frac{\text{Urinary creatinine}}{\text{Serum creatinine}} \times \frac{\text{Urinary volume/ hr}}{\text{Cl_{cr}}}

2.4. Histological Studies

Two animals from each group were sacrificed on day ten and kidneys were isolated. The kidney sections were stained with hematoxylin and eosin and observed under light microscope.

3. Statistical Analysis

The results are expressed as mean±SEM and the data was analysed by one way analysis of variance followed by post hoc Student-Keuls test using SPSS computer software for in vivo studies. Statistical significance was set at P≤ 0.05.

4. Results

Animals which received Chrysin (group III) for ten days exhibited no change in serum markers level and urinary functional parameters. Hence, Chrysin did not show any deteriorative effects on kidney. To assess the neproprotector activity of extract the data obtained from Chrysin treated Groups (IV, V) was compared with Group II (animals which received only cisplatin injection).

4.1. Effect of chrysin on Cisplatin induced nephrotoxicity:

Table-1 lists the effect of chrysin on cisplatin-induced nephrotoxicity in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum markers</th>
<th>Urinary functional parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>II</td>
<td>Cisplatin</td>
<td>Elevated</td>
<td>Elevated</td>
</tr>
<tr>
<td>III</td>
<td>Chrysin</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>IV</td>
<td>Chrysin + Cisplatin</td>
<td>Dose related protection</td>
<td>reversed the effect caused by cisplatin</td>
</tr>
<tr>
<td>V</td>
<td>Chrysin + Cisplatin</td>
<td>Dose related protection</td>
<td>reversed the effect caused by cisplatin</td>
</tr>
</tbody>
</table>

4.1.1. Effect on Serum markers

Animals which received cisplatin alone (group-II, single dose, 6mg/kg) showed significant elevated levels of BUN, when compared to group-I animals. Administration with chrysin in group-IV, group-V animals exhibited a dose related protection against cisplatin-induced effect.

The SC levels were significantly increased in Group-II animals when compared to group-I animals. Group-IV and Group-V animals which received chrysin at doses of 20mg/kg and 40mg/kg respectively showed significant protection against cisplatin-induced nephrotoxicity.

4.1.2. Effect on Urinary functional parameters

Animals received cisplatin alone excreted high amount of U_{TP} in Group-II when compared to normal control animals. Administration of chrysin with doses of 20mg/kg and 40mg/kg in Group-IV and group-V animals respectively, reversed the effect caused by cisplatin. Animals which received cisplatin alone exhibited decreased levels of Cl_{cr}, when compared to
normal control Group-I animals. On oral administration of chrysin 20mg/Kg body weight in Group-IV and 40mg/kg in Group-V animals respectively, showed significant increase in Clcr.

4.1.3. Effect on Lipid peroxidation

Animals which received cisplatin alone exhibited increased levels of LPO when compared to normal control animals. Administration of chrysin with doses of 20mg/kg in group-IV and 40mg/kg in group-V exhibited decrease in LPO levels.

4.2. Histological studies

The section of kidney isolated from rats treated with cisplatin showed degenerative glomeruli and degenerative tubular epithelial cells, indicating cisplatin-induced renal injury.

There was a reduction in the congestion of glomeruli and congestion of tubule in the section of kidneys isolated from rats which are treated with extract (400mg/Kg) and chrysin (40mg/Kg), indicating the regenerative changes.

Table-1: Effect of chrysin on cisplatin-induced nephrotoxicity in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>BUN (mg/dl)</th>
<th>SC (mg/dl)</th>
<th>Uric Acid (mg/24 hrs)</th>
<th>Clcr (ml/hr/1.73 bd wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal (1% CMC)</td>
<td>26.2±1.0</td>
<td>0.7±0.06</td>
<td>15.9±0.3</td>
<td>16±1.6</td>
</tr>
<tr>
<td>II</td>
<td>Cisplatin (6mg/kg)</td>
<td>62.1±2.4</td>
<td>1.8±0.3</td>
<td>32.4±2.3</td>
<td>8.5±0.2*</td>
</tr>
<tr>
<td>IV</td>
<td>Chrysin (20mg/kg)</td>
<td>39.5±1.6*</td>
<td>1.1±0.2*</td>
<td>24.1±1.4</td>
<td>10.4±0.4*</td>
</tr>
<tr>
<td>V</td>
<td>Chrysin (40mg/kg)</td>
<td>28.8±1.4*</td>
<td>0.9±0.1*</td>
<td>18.3±0.6</td>
<td>15.1±1.0</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E.M from 6 animals in each group.

*p<0.01 when compared to normal group.

a: p<0.01 when compared to control group.
b: p<0.01 when compared to group-II

c: p<0.05 when compared to normal control animals.

* : p<0.01 when compared to group II

![Light microscopy of renal tissue of chrysin treated rats](image1)

**Plate 1: Section of rat kidney treated with cisplatin (6mg/kg).**

**DG** = Degenerative Glomeruli; **DT** = Degenerative Tubule

**Plate 2: Section of kidney showing normal organization; DFT= Dense fatty tissue**

**Plate 3: Section of kidney treated with chrysin RT= Renal tubule; E = Epithelial cells; BC= Bowmann’s capsule**

![Graph](image2)
Light microscopy of renal tissue of cisplatin-induced nephrotoxicity

Plate 4: Section of kidney treated with cisplatin (6mg/kg)  
RT= Renal tubule; ERPC=enlarged renal tubule E = Epithelial cells

Plate 5: Section of kidney treated with chrysin (20mg/kg + cisplatin)  
VRT= Vacuolization of renal tubule; AGMH= Acute glomeruli with mild haemorrhages

Plate 6: Section of kidney treated with chrysin (40mg/kg + cisplatin)  
BC= Bowmann’s capsule RT= normal renal tubule (indicating regenerative changes)

5. Discussion

Cisplatin is one of the most effective anticancer drugs administered to treat a variety of cancers such as ovarian cancer, testicular, bladder, head and neck and uterine cervix carcinomas [10]. A high dose of cisplatin is more effective than low doses in ovarian and colorectal cancer [11], however high dosage treatment induces nephro- and neuro toxicity. Nephrotoxicity is a dose limiting factor in clinical uses of cisplatin. The mechanism of cisplatin nephrotoxicity is not fully understood several mechanisms were proposed such as cisplatin induces apoptosis in sensitive non-renal cells [12,13], however the generation of free oxygen radicals in tubular cells has been proposed as an important pathogenic process [14]. Cisplatin has been shown to cause nephrotoxicity in all species including mice, rats [15,16], dogs [17], and humans [18]. The rat model of cisplatin-induced nephrotoxicity is considered to be sensitive and reproducible system [19, 20, 21].

Number of people suffering from kidney disorders increasing day to day but till today there is no allopathic drug which is effectively treats the renal toxicity. The search for nephroprotector agents results in exploitation of natural products.

Urea is the major nitrogen containing metabolic product of protein catabolism in human. It is accounts for more than 75% of the non protein nitrogen eventually excreted. More than 90% of urea is excreted through the kidneys. Creatinine formed as the end product of creatine metabolism is a waste product. It is filtered at the glomeruli and secreted by the tubules and its excretion in urine per 24hrs is 1.5-3.0gm. During renal damage, the kidneys unable to excrete the urea and creatinine hence these serum markers levels are increased in blood. Cisplatin-induced nephrotoxicity in rats was established by elevated blood urea nitrogen (BUN), serum creatinine (SC) levels and marked drop in creatinine clearance levels. Animals which received chrysin at doses 20mg and 40mg/kg body.wt showed significant decrease in BUN, SC and significant raise in creatinine clearance levels.

The protein most commonly found in the urine is those derived from the blood plasma and consists of a mixture of plasma albumin and a globulin. These are not normally filtered through the glomeruli, but in kidney diseases, due to the alteration in glomerular permeability, these proteins appear in urine. Animals received cisplatin alone exhibited high amount of protein excretion in urine, which indicate that cisplatin-induced renal damage. chrysin significantly reduced the urinary protein excretion in dose dependent manner, which is indicative of chrysin is inducing regenerative changes in kidney.

Number of studies suggested that cisplatin induces nephrotoxicity through LPO
Lipid peroxidation is a degenerative pathway of membrane components mediated through free radicals produced in the cells. In present study on administration of cisplatin a significant increase in the malondialdehyde (MDA) content was observed when compared to the normal group. Upon treatment with chrysin (20mg/kg and 40mg/kg) there was significant decrease in MDA content indicating decrease in lipid peroxidation and the effect was dose dependent manner.

Previous reports suggested that plants containing antioxidant principles [23,24,25] and antioxidants such as Ascorbic acid, flavonoids like Quercetin (Devi Priya et al., 1999), Naringeninn [26] were exhibited good nephroprotector activity. Present study also evidenced that chrysin is also showed significant protection against cisplatin-induced nephrotoxicity. However further investigations are essential to elucidate the interference of chrysin in the anti-tumour efficacy of Cisplatin for its clinical application.

6. Acknowledgements

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References:


