MECHANISM BASED PROTECTIVE EFFECT OF DELPHINIUM DENUDATUM ON CISPLATIN INDUCED NEPHROTOXICITY

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ABSTRACT

Introduction and Aim: In present investigation in-vitro and In-vivo antioxidant and nephroprotective activities of aqueous root extract of Delphenium denudatum were evaluated. Materials and Methods: Free radical-scavenging activity of aqueous roots extract was carried out by DPPH HPLC method. The in-vitro nephroprotective study was investigated by GGT assay (γ-glutamyl transpeptidase) method. In-vivo antioxidant and nephroprotective potential of aqueous root extract of Delphenium denudatum (300,600 and 800 mg kg⁻¹) were screened in animal rats model. Oxidative stress and nephrotoxicity was induced by cisplatin (5mg kg⁻¹ bw). Results: Aqueous root extract of Delphenium denudatum showed 83.38% inhibition whereas 92.67% inhibition was produced by standard reference (ascorbic acid). The IC₅₀ of root extract was found to be 189.3 where as IC₅₀ 61.29 was produced by standard drug mesna (Sodium 2-mercaptoethanesulfonate) in GGT assay. In this study the aqueous root extract of Delphenium denudatum at a dose of (300,600 and 800 mg kg⁻¹) showed significant dose-dependent reduction in the elevated blood urea, uric acid, serum creatinine and normalized the histopathological changes. However, oxidative stress was noticed in renal tissue as evidenced by a significant decrease in glutathione level, superoxide dismutase and catalase activities, also a significant increase in malondialdehyde levels when compared to control group. The animal in the curative regimen showed good response to the aqueous extract at 800 mg kg⁻¹ bw. Conclusion: It is concluded that the aqueous root extract of Delphenium denudatum possesses nephroprotective and antioxidant activity and could offer a promising role in the treatment of renal injury caused by nephrotoxins like cisplatin.
Key words: Delphinium denudatum, Nephroprotective activity, GGT, Cisplatin, Phenolics compounds, Flavonoid compounds.

INTRODUCTION

Delphinium denudatum Wall (Jadwar; Ranunculaceae) is one of the important medicinal drugs used as indigenous medicine in India, the entire plant is reported to be useful in a variety of ailments [1]. The root is used in various medical formulations in Unani and Ayurveda to reduce the withdrawal symptoms in people on de-addiction therapy [2]. Herbalists recommend the roots in the treatment of fungal infections, dysurea, calculi, asthma, cough, jaundice and nervous problems [3]. Its claims many bioactive constituent, some of which are flavanoids, triterpenoid, alkaloids including delphocurarine, staphisagrine, delphine, condelphine, denudatin and a diterpenoid alkaloid identical to condelphine [4]. The root of this plant is reputed to be in Unani medicine for its beneficial effects but the claim of its efficacy has not been scientifically explored [5]. Therefore, the present experimental study evaluates the potentiality of D. denudatum root, as a nephroprotective agent against cisplatin induced nephroprotoxicity. Cisplatin (cis-diamminedichloroplatinum II; CDDP) is an important platinum containing chemotherapeutic agent demonstrated a broad spectrum anticancer activity [6]. However, nephrotoxicity is the major side effect of this compound, correlates with the total platinum concentration present in the kidney [7]. Along with the causes of kidney failure, tubular necrosis and fibrotic lesions which occur due to ischemia or nephrotoxins like CP is the most wide spread incidence [8]. Dietary protein restriction, blood pressure control, angiotensin converting enzyme inhibitors, and angiotensin receptor blockers are the recent day therapy of renal disease [9]. However, little is known about the nephroprotective effects of herbal medicine. In fact, herbal medicines have a long history of use in the treatment of various renal diseases and evidence suggests that some herbs possess a range of important pharmacological properties in retarding kidney disease such as Astragalus membranaceus, Tripterygium wilfordii, Rheum officinale [10]. GGT (γ-glutamyl transpeptidase) initiates extracellular glutathione (GSH) breakdown, provides cells with a local cysteine supply and contributes to maintain intracellular GSH level [11]. It is part of the cell antioxidant defense mechanism [12]. The significance presence of GGT is responsible for the evidence of liver disease, obstructive jaundice, infectious hepatitis, cholecystitis and kidney diseases [13]. There is a continuous search for agents which grant nephroprotection against the renal impairment caused by drugs like cisplatin. Hence, the present study is an attempt to screen the effect of aqueous root extract of D. denudatum (DDRE) for its
nephroprotective activity. There are not many reports stating the detailed analysis of the in-vitro and In-vivo nephroprotective properties of *D. denudatum*, consequently in the present study is focusing on these aspects.

**MATERIALS AND METHODS**

Cisplatin, available commercially as Cisteen (1 mg mL⁻¹), was provided by the Miracalas Pharma Pvt. Ltd. (Mumbai, India). HPLC grade methanol and water were supplied by Merck (Darmstadt, Germany). 2, 2-Diphenyl-1-picrylhydrazyl hydrate (DPPH), gallic acid, quercetine and Folin Ciocalteu reagent were procured from Sigma Aldrich (St. Louis, MO). GGT (20×1.1) was procured from Reckon Dignostic P.Ltd. Urea estimation kit (Agappe Diagnostics, Maharashtra, India), Creatinine estimation kit (Dr. Reddy’s Laboratories, Hyderabad, India). All other inorganic chemicals and organic solvents were of reagent grade.

**Sample collection**

The sample species was collected from a local market of New Delhi, India and were authenticated by Dr. H.B. Singh (Chief Scientist & Head, Raw materials Herbarium & Museum, NISCAIR, New Delhi). A voucher specimen (RHMD/1704/04) has been deposited in the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi.

**Sample preparation**

The roots gently but thoroughly rinsed in tap water for three times and completely air dried at room temperature to a constant weight and ground to powder. The dried aqueous roots extract (DDRE) was obtained as follows: 300 g of the powdered dried roots of *D. denudatum* was boiled in 500 ml of distilled water for 30 minutes, after which it was rapidly filtered through a piece of clean cotton gauze. The filtrate was allowed to cool at room temperature for 4 hours after which it was completely lyophilized using a freeze-drying system, producing a fine and chocolate colour solid residue. The procedure was repeated 4 times with this same quantity of solvent. The dry residues obtained were weighed, pooled and stored in air and water-proof container kept in a refrigerator at 4 °C [yield: 32.43% (w/w)]. From this stock, fresh preparation was made whenever required.

**Quantification of total phenolic compounds**

Take 3ml of each standard and sample solution in a 10 ml test tube and to this 3.0 ml of FC (Folin Ciocalteu) reagent and 3.0 ml of sodium carbonate (4.0 ml, 1 M) solution were added.
The solution was kept in dark for 30 min for colour development. Absorbance was taken at 765nm against blank solution, plotted the standard calibration curve and calculated the amount of phenolics in the samples. Total phenol values expressed in terms of standard equivalent (mg g$^{-1}$ of dry mass)$^{[14]}$.

**Quantification of total flavonoid compounds**

Aluminum chloride colorimetric method was used for flavonoids determination. The DDRE (0.5 ml of 1:10 g mL$^{-1}$) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M sodium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415nm with a double beam UV/Visible spectrophotometer$^{[14]}$.

**HPLC analysis for in-vitro antioxidant activity of DDRE**

The sample was filtered through 0.2µm nylon membrane filter and an aliquot (20 µl) of the sample was injected for HPLC analysis. The reversed-phase HPLC system consisted of a Shimadzu HPLC system (LC-10 Ai, Japan) consisting of pump (LC-10 Ai), a system controller (SCL-10AVP), and a diode array detector (SPD-M10 AVP). Analyses were carried out using a 100 RP-18c column (250 × 4 mm, 5µM) (Merck, Darmstadt, Germany). Isocratic elution was carried out with methanol/water (80:20, v/v) at a flow rate of 1 mL min$^{-1}$. DPPH (100 µM) was dissolved in pure ethanol (96%). The radical stock solution of DPPH was prepared fresh daily and was used as control. The DPPH solution (1.0 ml) was added to 1 ml of extract and standard (ascorbic acid) with 3 ml of double distilled water. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in area of the resulting solution was monitored at 517 nm at 10 min. The difference in the reduction of peak area between the control and the sample was used for determining the percent radical scavenging activity of the sample. All determinations were performed in triplicate$^{[15]}$.

**In-vitro nephroprotective activity of DDRE**

The activity was determined using a modified GGT assay method described previously$^{[16-18]}$. Inhibition studies were conducted on GGT to determine the effect of mesna–disulfide heteroconjugates on the enzyme’s activity in vitro. These studies utilized a spectrophotometric assay at 405 nm that monitored the percentage inhibition of gamma-glutamyl transpeptidase (GGT) activity, mediated by mesna and aqueous extract of plant.
material. Different concentrations of mesna (3.9–500 ug mL\(^{-1}\)) and roots extract (3.9–500 ug mL\(^{-1}\)) were generated in situ and incubated with GGT.

**In-vivo nephroprotective activity of DDRE**

**Dose determination and acute toxicity study**

The rats was fed with plant extract of proposed plants suspended in carboxy methyl cellulose (CMC-1% w/v) in increasing dose levels of 10, 30, 100, 300, 600, 1000 and 3000 mg kg\(^{-1}\) body weight respectively\(^{[19]}\). The animals observed continuously for 2 h for the gross behavioral changes and then intermittently every 2 h for a period of 24 h and finally at the end of 72 h. It was found that the tested extracts were not mortal even at a dose of 3,000 mg kg\(^{-1}\) and consequently the dose 300-800 mg kg\(^{-1}\) was selected for the study.

**Experimental design**

Six groups of six rats each were used in this model. The animals were housed in a temperature (25 ± 1°C), humidity controlled room and a 12 h light-dark cycle. Rats were allowed free access to tap water and standard pellet diet. The institutional Animal Ethics Committee approved all experimental protocols (173 CPCSEA -2000). Group I administered with normal saline (1.0 ml) for 15 days. Group II treated with CP (5mg kg\(^{-1}\) i.p.) and normal saline (1.0 ml) for 15days by oral route. The rats of groups III, IV and V after initial administered of a single dose of CP 5 mg kg\(^{-1}\) body weight i.p treated with different dose of plant extract from the 6\(^{th}\) day onwards for 15 days (Curative group). Group VI was administered with 800 mg kg\(^{-1}\) p.o followed by single dose of CP (5mg kg\(^{-1}\) i.p) for 15 days (Prophylactic group). On the 16\(^{th}\) day blood withdrawn through tail vein method from all groups assessed for renal function tests (Table 4).

**Sampling and biochemical analyses**

Blood samples were collected and centrifuged for 10 min at 2500 rpm. The obtained clear sera stored at -20 °c for subsequent measurement of blood urea, serum creatinine (CR), and uric acid (UA) level using colorimetric assay kits according to the method\(^{[20]}\).

**Preparation of renal homogenate and biochemical estimation of markers of oxidative stress:**

The kidneys was removed and dissected, free from the surrounding fat and connective tissue. Subsequently, renal cortex homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates was centrifuged at 2500 rpm for 10 min at 4 °c. The resulting supernatant used for the determination of malondialdehyde (MDA) content and
reduced GSH levels and antioxidant enzyme catalase (CAT) activity using colorimetric assay. MDA content was measured according to an earlier method \cite{21}. Superoxide dismutase (SOD) activity was determined according to reported method \cite{22}. CAT activity was determined from the rate of decomposition of H2O2 according earlier method \cite{23}. GSH reductase activity was assayed according to previously reported \cite{24}. Protein content in the tissue determined by Lowry method of using bovine serum albumin (BSA) as the standard method \cite{25}.

**Histopathological examination**

10 % neutral formalin was used to fixed the pieces of kidney from each group immediately for a period of at least 24 h, embedded in paraffin, cut into 4–5 μm thick sections and stained with hematoxylin–eosin. The sections were evaluated for the pathological symptoms of nephrotoxicity.

**Statistical analysis**

Statistical analysis of differences between means was carried out using ANOVA, followed by the Dunnett Multiple Comparisons Test; p<0.01 was considered statistically significant for all tests.

**RESULTS**

**Quantification of phenolic and flavonoids compounds**

Total phenolic contents of DDRE was found in concentration of 45.583 ± 0.160 μg mL\(^{-1}\) with respect to gallic acid while the total flavonoidal contents of the same plant extract was found in the concentration of 19.45 ± 0.262 μg mL\(^{-1}\) of quercetin equivalent (Table 1).

**DPPH radical scavenging activity**

The radical scavenging activity of standard ascorbic acid and DDRE were determined by HPLC method at 517nm. A reduction in peak area revealed comparable radical scavenging activity of ascorbic acid (92.67 %) and DDRE (83.38 %) (Table 2 and Fig 1).

**Effects of mesna and DDRE on GGT activity**

Townsend *et al* (2003) demonstrated a dose-dependent inhibitory effect where the GGT activity was monitored by the release of 1.0 μmol of \(p\)-nitroaniline per min \cite{26}. In our studies, a 50% inhibition in GGT activity was observed when 62.5 ug mL\(^{-1}\) mesna (IC\(_{50}\) 61.29) and plant extract 250 ug mL\(^{-1}\) (IC\(_{50}\) 160.5) was incubated with the crude human GGT for 15 min at 25°C. The finding suggesting that mesna and plant extract had inhibitory effects on GGT.
The results of the effects of mesna and plant extract on GGT are shown in (Table 3 and Fig 2).

**Effect of DDRE on serum urea, uric acid and creatinine concentrations**

Serum urea and creatinine concentrations were significantly increased (p <0.05) in the toxic treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with the DDRE showed significant (p<0.01) decrease in (Group IV, V and VI) concentrations of serum urea and creatinine compared to the toxic (Group II) treated group. However the levels of uric acid (UA) significantly decreased (p<0.01) in the cisplatin treated groups (Group IV, V and VI), when compared to the toxic (Group II) (Table 5).

**Effect of DDRE and/or cisplatin treatment on GSH, SOD, catalase and TBARS level**

CP intoxication caused a significant decrease in renal GSH, SOD and catalase level as compared with normal controls, p<0.01. Treatment with DDRE before cisplatin (protective group) or after cisplatin (curative group) revealed a significant increase in catalase, SOD activities and GSH level as compared to cisplatin intoxicated rats, p<0.05, (Table 5). Cisplatin intoxication caused a significant increase in TBARS level as compared with normal controls, p<0.05. Treatment with DDRE revealed a significant (p<0.01) decrease in MDA levels to cisplatin intoxicated one at a dose of 800mg kg⁻¹ (Table 6).

**Effect of DDRE on CP induced renal dysfunction**

Intraparitontial injection (5mg kg⁻¹ i.p) of normal rats with CP caused a significant increase in blood urea, creatinine, uric acid levels p<0.05. Whereas in both protective and curative groups the oral administration of DDRE to CP-intoxicated rats significantly normalized renal dysfunction, the effect was prominent in the protective groups (Table 7).

**DISCUSSION**

Table 1. Total phenolic and flavanoid content (µg/mL) of aqueous roots extract of *D. denudatum*

<table>
<thead>
<tr>
<th>Sample used</th>
<th>Standard used</th>
<th>Absorbance</th>
<th>Total Phenolic Content (µg/mL)</th>
<th>Total flavanoid Content (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. denudatum</em></td>
<td>Gallic acid</td>
<td>765 nm</td>
<td>45.583 ± 0.160</td>
<td>19.45±0.262</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>415 nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value in the table was obtained by calculating the average of three experiments ± SEM.
Table 2. DPPH free radical scavenging activity of ascorbic acid and DDRE at 517nm by HPLC method

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Concentration</th>
<th>Retention Time</th>
<th>Peak Area</th>
<th>Radical Scavenging (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH Control</td>
<td>100µM/ml</td>
<td>8.292</td>
<td>1816041</td>
<td></td>
</tr>
<tr>
<td>DPPH + Ascorbic Acid (100µL each)</td>
<td>100µM ml⁻¹ (DPPH) + 100µg ml⁻¹ (Ascorbic Acid)</td>
<td>8.167</td>
<td>132943</td>
<td>92.67</td>
</tr>
<tr>
<td>DPPH + <em>D</em>denudetum (100µL each)</td>
<td>100µM ml⁻¹ (DPPH) + 100µg ml⁻¹ (Ascorbic Acid)</td>
<td>8.183</td>
<td>301849</td>
<td>83.38</td>
</tr>
</tbody>
</table>

Table 3. Effects of mesna and DDRE on GGT activity at different concentrations

<table>
<thead>
<tr>
<th>Conc ug/ml</th>
<th>% Inhibition of mesna</th>
<th>IC₅₀ of mesna</th>
<th>% Inhibition of DDRE</th>
<th>IC₅₀ of DDRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.9</td>
<td>5.68±0.57</td>
<td>62.29</td>
<td>1.71±0.27</td>
<td>189.3</td>
</tr>
<tr>
<td>7.8</td>
<td>9.32±0.41</td>
<td></td>
<td>2.99±0.33</td>
<td></td>
</tr>
<tr>
<td>15.6</td>
<td>12.12±0.47</td>
<td></td>
<td>9.72±0.82</td>
<td></td>
</tr>
<tr>
<td>31.2</td>
<td>24.71±0.06</td>
<td></td>
<td>13.83±0.33</td>
<td></td>
</tr>
<tr>
<td>62.5</td>
<td>51.82±0.90</td>
<td></td>
<td>29.91±0.87</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>61.68±0.47</td>
<td></td>
<td>41.45±0.80</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>87.44±0.15</td>
<td></td>
<td>65.90±0.88</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>90.18±0.10</td>
<td></td>
<td>80.93±0.06</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Experimental protocol and treatment schedule for different groups

<table>
<thead>
<tr>
<th>Group number (n=6)</th>
<th>Drug treatment</th>
<th>Route and dose (in mg/kg body weight)</th>
<th>Duration (in days)</th>
<th>Days of withdrawal of blood and kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline</td>
<td>1ml p.o.</td>
<td>1st-15th</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Cisplatin + Normal saline</td>
<td>5mg kg⁻¹ i.p. 1ml p.o.</td>
<td>1st-15th</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Cisplatin + Plant extract 200 mg kg⁻¹ p.o.</td>
<td>5mg kg⁻¹ i.p. (single dose) 200 mg kg⁻¹ p.o.</td>
<td>1st 6th-15th</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>Cisplatin + Plant extract 600 mg kg⁻¹ p.o.</td>
<td>5mg kg⁻¹ i.p. (single dose) 600 dose mg kg⁻¹ p.o.</td>
<td>1st 6th-15th</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Cisplatin</td>
<td>5mg kg⁻¹ i.p.</td>
<td>1st</td>
<td>16</td>
</tr>
</tbody>
</table>
Table 5. Effect of different treatment groups on blood urea, uric acid and serum creatinine

<table>
<thead>
<tr>
<th>Group number</th>
<th>Blood urea (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>33.70±1.38</td>
<td>1.98±0.011</td>
<td>0.26±0.031</td>
</tr>
<tr>
<td>Group II</td>
<td>99.57±1.62#</td>
<td>4.13±0.058#</td>
<td>3.45±0.041#</td>
</tr>
<tr>
<td>Group III</td>
<td>95.74±1.80ns</td>
<td>4.08±0.071ns</td>
<td>3.41±0.087ns#</td>
</tr>
<tr>
<td>Group IV</td>
<td>66.17±1.10**</td>
<td>3.87±0.049**</td>
<td>1.27±0.029**</td>
</tr>
<tr>
<td>Group V</td>
<td>42.89±1.51**</td>
<td>3.35±0.075**</td>
<td>0.54±0.043**</td>
</tr>
<tr>
<td>Group VI</td>
<td>41.97±1.92**</td>
<td>3.73±0.090**</td>
<td>0.52±0.027**</td>
</tr>
</tbody>
</table>

Values are mean±S.E; ns-non significant
One-way Analysis of Variance (ANOVA) followed by Dunnett test.
# P<0.01 vs group I (Normal control)
ns P>0.05 vs group II (Toxic control)
**P<0.01 vs group II (Toxic control)

Table 6: Effect of different treatment groups on TBARS, GSH, SOD and catalase

<table>
<thead>
<tr>
<th>Group number</th>
<th>TBARS (nmoles/mg protein)</th>
<th>GHS (nmoles/mg protein)</th>
<th>SOD (nmoles/mg protein)</th>
<th>CATALASE (nmoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0.14±0.038</td>
<td>2.20±0.05</td>
<td>9.45±0.11</td>
<td>15.17±0.33</td>
</tr>
<tr>
<td>Group II</td>
<td>2.88±0.102#</td>
<td>0.46±0.04#</td>
<td>1.29±0.112#</td>
<td>2.52±0.150#</td>
</tr>
<tr>
<td>Group III</td>
<td>2.77±0.046ns</td>
<td>0.49±0.024ns</td>
<td>1.38±0.023ns</td>
<td>2.65±0.248ns</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.93±0.078**</td>
<td>0.97±0.031**</td>
<td>3.69±0.015**</td>
<td>4.11±0.019**</td>
</tr>
<tr>
<td>Group V</td>
<td>0.68±0.084**</td>
<td>1.38±0.075**</td>
<td>6.80±0.095**</td>
<td>6.36±0.018**</td>
</tr>
<tr>
<td>Group VI</td>
<td>0.61±0.033**</td>
<td>0.98±0.05**</td>
<td>5.81±0.205**</td>
<td>5.57±0.116**</td>
</tr>
</tbody>
</table>

Values are mean±S.E; ns-non significant
One-way Analysis of Variance (ANOVA) followed by Dunnett test.
# P<0.01 vs group I (Normal control)
sns P>0.05 vs group II (Toxic control)
**P<0.01 vs group II (Toxic control)
Table 7. Histopathological features seen in different groups in cisplatin induced renal damage

<table>
<thead>
<tr>
<th>Groups (n=6)</th>
<th>Glomerulus</th>
<th>Proximal tubular epithelium loss</th>
<th>Interstitial Infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>WNL</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Group II</td>
<td>WNL</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Group III</td>
<td>WNL</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Group IV</td>
<td>WNL</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Group V</td>
<td>WNL</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

WNL = Within normal limit; – = absent; + = little effect, +++= appreciable effect, +++= severe effect

Fig 1. HPLC Chromatogram of DPPH at RT 8.292 and at wavelength of 517nm

Fig 2. Comparative GGT activity of mesna and DDRE at 405nm
The present study demonstrated protective and curative effects of DDRE, on CP-induced nephrotoxicity, in line with the consideration that oxygen-free radicals are important mediators of CP-induced acute renal failure. In the present study, CP administration caused marked renal dysfunction as evidenced by the significant increase in blood urea, creatinine and uric acid levels. It is well documented that CP nephrotoxicity causes acute renal failure. The destructive lipid peroxidation leads to breakdown of membrane structure and function. Further decomposition of peroxidized lipids yields a wide variety of end products, including MDA. The CP induced oxidative stress is the central pathway responsible for nephrotoxicity. In our work, oral administration of DDRE to CP-intoxicated rats normalized blood urea, creatinine and uric acid suggesting that the extract (800 mg/kg) under investigation may introduce protection against CP-induced nephrotoxicity, possibly by attenuating the oxidative stress induced by administration of CP. GSH depletion is a common outcome of increased creation of ROS. Increased consumption of GSH in non-enzymatic removal of oxygen radicals is an explanation to cellular GSH depletion after kidney toxicity. SOD activity diminished in renal tissue and most of the antioxidant enzymes become inactive in response to oxidative stress. It has demonstrated that CP generates ROS that mediate biomolecules oxidation in the kidney. The excessive ROS can damage the protein sensitive thiols. Therefore, CP inhibits the activities of antioxidant enzymes, SOD and depletes thiol cellular content. In our work, DDRE consumption improves GSH content and SOD as well as catalase activities. Significant free radical scavenging activity show by DDRE in DPPH scavenging model (Table 2). Hence, the probable mechanism of nephroprotection by D. denudatum may be recognized to its antioxidant and free radical scavenging property. It was reported that mesna bypass GGT metabolism pathway and not be the substrate for GGT xenobiotic pathway. In contrast to this mesna have inhibitory effect on GGT. This is an observation thought to be effective mechanism through which D. denudatum may have a nephroprotective effect against CP-induced nephrotoxicity. Shirwaikar et al., 2004 reported the protective activity of ethanolic extract of Aerva lanata in CP and GM-induced nephrotoxicity in male Wistar albino rats. The study revealed that the flavonoids are well known potent antioxidant and free radical scavengers and bring improvement in kidneys. Natural antioxidants such as flavonoids and polyphenols are believed to possess antioxidant properties due to their reducing and chelating capabilities. Flavonoids and polyphenols are secondary plant metabolites that are widely distributed in plants parts with free radical scavenging abilities. The antioxidant activity of DDRE could be credited to its polyphenolic contents. Flavonoids act as scavengers.
and quenchers of singlet oxygen of various oxidizing species or peroxyl radicals. Phenols are very important plant constituents. However there is a highly significant relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups and effective hydrogens donors \[37\].

**CONCLUSION**

In conclusion, it is proposed that the nephroprotective activities of DDRE in CP-induced nephrotoxicity may be due to presence of phytochemicals like phenolics and flavonoids. Thus DDRE has a promising role in the treatment of renal injury induced by nephrotoxins, especially cisplatin. Further DDRE could constitute novel drug which will be helpful for treatment of drug-induced nephrotoxicity.

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**REFERENCE**

5. Baytar Ibn:Jame al Mufradat al advia wal aghzia. Cairo:Bulak Press;1874.159
8. Greggi ALM, Darin JD, Bianchi MD. Protective effects of Vitamin C against cisplatin


