INTRODUCTION

Cisplatin is a simple, water soluble, low molecular weight, platinum complex, comprising a central platinum atom, a chloride and an ammonia molecule in cis position, and is effective in the treatment of wide variety of neoplastic diseases like testicular, ovarian, bladder, lung, prostate, head and neck cancer, etc. Its potential nephrotoxicity coupled with other adverse effects limits its tolerance and dosage. The renal injury due to Cisplatin is mainly due to generation various free radicals like hydroxyl radical, super oxide radical, etc.

The Cyanotis fasciculata Var., fasciculata (Commelinaceae) is an under exploited plant of medicinal value. The hydro-alcoholic extract of this plant is reported to be useful in lymphatic leukemia, possess diuretic and antiviral properties. We found many antioxidant-phytoconstituents during preliminary phytochemical investigation of 70% hydro-alcoholic extract (CFEE) and the same showed appreciable quantities of polyphenolics during quantitative estimations. Further, the CFEE also showed commendable free radical scavenging ability in various in-vitro models. These facts drove us to anticipate that the CFEE could be useful in countering the free radical mediated renal injury induced by Cisplatin. Thus the present study was designed to assess the nephroprotective ability of CFEE. In addition, GC-MS analysis of the test extract was also performed to establish possible correlation for the results of the study.

MATERIALS AND METHODS

Plant Material

The plants of Cyanotis fasciculata were collected from Fort-hill top of Bellary, Karnataka in the month of September and were authenticated by Dr. Kotresh, Department of Botony, Karnataka University, Dharwad, Karnataka (Ref- UGC/MRP/2008-09/83). The voucher specimens of these plants were preserved in the herbarium of the pharmacognosy department of T.V.M.college of pharmacy, Bellary.

Extraction

The plants were air-dried in shade and were pulverized in a mechanical grinder to cottylny lumps. The powder was defatted with petroleum ether and exhaustively extracted with 70% hydroalcohol(CFEE) by soxhlation; extract was dried in rotary vacuum evaporation and relevant yields were calculated and stored in airtight containers at 4°C.

ABSTRACT

Cisplatin is quite effective against various neoplastic diseases, but its potential nephrotoxicity leads to increased morbidity, complications and hospitalization costs. Prevention of nephrotoxicity may allow clinicians to administer higher doses for added therapeutic benefits. So, the present investigation was designed to evaluate the ability of the 70% hydro-alcoholic extract of Cyanotis fasciculata Var., fasciculata (CFEE) to combat Cisplatin induced nephrotoxicity. Where, CFEE demonstrated dose dependent decrement of elevated biochemical markers of nephrotoxicity along with significant restoration of protective-GSH levels and suppression of LPO levels in tissues. Further, the remarkable renal-cellular rejuvenation found in histopathological studies also enunciated the organ protective activity of CFEE. It was concluded that, in addition to polyphenolics, some of the phyto-fragments found during GC-MS analysis might also contributed to the protection offered by CFEE.

KEYWORDS: CFEE, Cisplatin, Nephroprotection, BUN, Creatinine, Antioxidants, GSH, LPO.

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Later, animals were sacrificed under anesthesia and the kidneys were dissected out, washed with saline, blotted off and their weight, GSH and LPO levels were found out and some portions of kidney were stored in 10% formalin to evaluate the details of nephrotic architecture in each group microscopically.

Estimation of GSH in renal tissue

Tissue samples of kidney were homogenized in ice cold Trichloroacetic acid (1 g tissue plus 10 ml 10% TCA) in an ultra turrax tissue homogenizer. Glutathione measurements were performed using the modification of the Ellman procedure13. Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. Percentage increase in OD is directly proportional to the increase in the levels of Glutathione. Hence, Percentage increase in OD is calculated by the formula given;

\[
\text{Percentage increase} = \frac{(\text{Test OD} - \text{Control OD})}{\text{Control OD}} \times 100
\]

Estimation of LPO in renal tissue

The degree of lipid peroxide formation in the renal tissue was assayed by monitoring thiobarbituric reactive substance formation by the method of Ohkawa et al.14. Combined 1.0 ml of tissue samples of kidney (0.1-2.0 mg of membrane protein or 0.10.2μ mol of lipid phosphate) with 2.0 ml of TCA-TBA-HCl (15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25N hydrochloric acid) and mix thoroughly. The solution is heated for 1 hr in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 mins. The absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid. The malondialdehyde concentration of the sample can be calculated by using an extinction coefficient of 1.56 × 10^5 M⁻¹ cm⁻¹.

\[
\text{Percentage inhibition} = \frac{(\text{Control OD} - \text{Test OD})}{\text{Control OD}} \times 100
\]

Histopathological studies of kidney tissue

Small pieces of kidney tissues of two animals from each group were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5 - 6 microns in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissues were examined under microscope for the analyzing the altered architecture of the kidney tissue due to Cisplatin administration and improved kidney architecture due to treatment with CFEE.

GC-MS analysis

The GC-MS analysis of hydroalcoholic extract and its Ethyl acetate fraction was carried out in a GC MS Model: GCMS-QP2010 Plus. Make: SHIMADZU gas chromatograph fitted with ZB-624 30 m X 0.25mm id column. Carrier gas was helium with a flow rate of 2.5 ml/min; column temperature initially was at 120°C for 2min. Then rose to 250°C at the rate of 10°C per minute maintained at 250°C for 20minutes; injector temperature was 240°C, detector temperature 260°C, volume injected 1μL with liquid injector of 70% ethanol extract in ethanol (1g in 5ml ethanol); The mass spectra operating parameters were as follows: ion source temperature; 250°C, ionization potential, 70eV; solvent delay 3.0 min, program run time: 31 minutes and scan range 30-350 amu, EV voltage 3000 volts. Finally the structural fragments were identified on the basis of retention time by using ‘Wiley 07'-commercial library software.

RESULTS

Effect of CFEE on tissue GSH levels and LPO levels

There was a marked depletion of GSH levels in Cisplatin treated group. The CFEE successfully retarded depletion of GSH levels in renal tissue in a dose dependant manner. As a consequence of increased oxidative stress induced by Cisplatin the LPO levels were escalated. Whereas, groups treated with 400 mg/kg and 200 mg/kg of CFEE exhibited 53.95% and 47.21% of inhibition of LPO formation compared to positive control group.

Effect of CFEE on some parameters of Nephrotoxicity

There was a marked depletion of GSH levels in Cisplatin treated group. CFEE showed dose dependent sparing effect on tissue GSH. Various free radicals generated by Cisplatin also accelerated renal membrane lipid peroxidation (LPO), thus the animals of positive control (group II) exhibited increased LPO in renal tissue. But, was also a dose dependent inhibition of lipid peroxidation by both the doses of CFEE. 400 mg/kg of CFEE showed 53.95% of inhibition, whereas that of 200 mg/kg showed 47.21% of inhibition. The results are summarized in Table No. 1.

Due to renal malfunction, there was a marked decrease in the body weight of Cisplatin treated unprotected animals (-12.97%). However, there was a dose dependent sustenance of weight fall in animals treated with CFEE. Especially, 400 mg/kg dose exhibited approximately 3gms% less than the group’s initial average body weight. Increased kidney weight of animals treated with Cisplatin (1.079 gms%) clearly indicated renal damage followed by inflammation, congestion etc. But, both doses successfully reduced the kidney weight, indicating good recovery from damage. Especially, 400mg dose could reverse kidney weight to almost normal.

As expected, blood urea nitrogen and serum creatinine levels were elevated in Cisplatin treated group i.e. 71.33mg/dl & 2.11mg/dl respectively. But, our test extract significantly reduced these markers of renal damage in a dose dependant manner i.e., 40.66% & 35.04% of BUN; 46.30% & 55.51% of creatinine, for 200 and 400mg/kg treated groups respectively, when compared to the readings of positive control group. (As shown in Table No. 2) Similarly, in histopathological studies also Cisplatin treated group showed severe glomerular congestion, infiltration of inflammatory cells into interstitium, etc. But, treatment with CFEE resulted in near complete regeneration of renal cellular architecture as shown in Fig No.1-4.

During GC-MS analysis, 15and 6 compounds were identified ( ≥ 97% similarity) from CFEE and its ethyl acetate fraction, respectively(Fig No.4 & 5). Of which, many compound are already been used in food, cosmetic and pharmaceutical industries, and some of them reported to possess antioxidant property viz., 2-methyl-1-Pentene, which reacts strongly with oxidizers25; N-Methyl-2-pyrrolidine (NMP) is used as an antioxidant in cosmetics and veterinary preparations (LD₅₀=7gm/kg)16 ; Propandionic acid is a precursor for synthesis of vitamin B₃,B₆,B₁₂ and also used to prevent free radical mediated resorption of bones in broiler chicks (LD₅₀= 4gm/kg)17 ; 3-Ethoxy-2-Butanonone (Banana oil) is used as rust preventer, anti-freezing, antioxidant (LD₅₀=16.6gm/kg)8,etc.

DISCUSSION

Cisplatin inhibits antioxidant enzymes like superoxide dismutase, catalase and peroxidase in kidneys. Its direct interaction with kidney generates superoxide anion in cell free system. Further, reports are indicating that Cisplatin increases the activity of Ca⁺⁺ independent nitric oxide synthase and increases the production of nitric oxide through arginine metabolism19. All these findings confirms that Cisplatin induced renal damage is due to generation of oxidative stress (ROS) and increased NO20. As a result of raised oxidative stress the fatty acids component of cell membrane gets oxidized leading to escalation of LPO levels. While defending this free radical mediated assault on kidney, the renal tissue stocks of GSH gets depleted/ exhausted. Thus, oxidative stress worsens leading to renal injury, inflammation, congestion, increased kidney weight and...
loss of its functional integrity. Subsequently, body weight decreases along with an associated rise in serum markers of renal damage like blood urea and serum creatinine. The CFEE exhibited dose dependant normalization of the raised kidney weight, blood urea, serum creatinine, tissue LPO level. The tissue GSH levels were sustained and fall in body weight was retarded along with remarkable recovery in renal cellular histology. During in-vitro studies for antioxidant activity the CFEE exhibited an appreciable ‘NO’ scavenging ability, which is an important contributing factor in Cisplatin induced nephrotoxicity. In addition, flavonoids are also reported to inhibit the release of NO\textsuperscript{11}. So, in addition to NO scavenge, inhibition of its release by flavonoids of CFEE might have contributed to the nephro-protection. Further, other antioxidants of CFEE might have restored the downtrodden endogenous antioxidants to re-establish the healthy balance between free radicals and antioxidants. The commendable nephroprotection offered by CFEE is not only due to its phyto- antioxidants such as phenolics and flavonoids, but may also be by the virtue of some of the antioxidant, reported in GC-MS analysis viz., Propanidionic acid, banana oil, NMP, etc.

CONCLUSION

It was clear from the results of the investigation that the 70% hydroalcoholic extract of \textit{Cyanotis fasciculata} var., fasciculata offered commendable protection against Cisplatin induced nephrotoxicity at both doses by virtue of its antioxidant-phytocomstituents. So, isolation and identification of a particular phytoconstituent with nephro protective activity is under progress.

ACKNOWLEDGMENTS

The authors are grateful to principal and management, T.V.M. Pharmacy, Harapanahalli for providing necessary facilities. We wish to extend our thanks to Dr. Kotresh also for authentication of Plant.

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15. Malonic acid uses available from: en.wikipedia.org/wiki/Diethyl_malonate - Cached similar

Table No. 1: Effect of CFEE on tissue GSH & LPO levels in Cisplatin induced nephrotoxicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH levels</th>
<th></th>
<th>LPO levels</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>% increase</td>
<td>Mean ± SEM</td>
<td>% decrease</td>
</tr>
<tr>
<td>Negative Control (1ml vehicle)</td>
<td>0.812 ± 0.005</td>
<td>--</td>
<td>0.176 ± 0.005</td>
<td>--</td>
</tr>
<tr>
<td>Positive control</td>
<td>0.417 ± 0.004</td>
<td>--</td>
<td>0.430 ± 0.003</td>
<td>--</td>
</tr>
<tr>
<td>Cisplatin + CFEE (200 mg/kg p.o.)</td>
<td>0.638 ± 0.015***</td>
<td>53.00%</td>
<td>0.227 ± 0.017 ***</td>
<td>47.21%</td>
</tr>
<tr>
<td>Cisplatin + CFEE (400 mg/kg p.o.)</td>
<td>0.732± 0.024***</td>
<td>75.54%</td>
<td>0.198 ± 0.008 ***</td>
<td>53.95%</td>
</tr>
</tbody>
</table>
### Table No. 2: Effect of CFEE on some markers of Cisplatin induced nephrotoxicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kidney weight (g/100g)</th>
<th>Changes in b.wt. (g/100g)</th>
<th>BUN (mg/dl)</th>
<th>Serum Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control (1ml vehicle)</td>
<td>0.653±0.013</td>
<td>10.217±1.772</td>
<td>21.17±0.601</td>
<td>0.641±0.009</td>
</tr>
<tr>
<td>Positive control *</td>
<td>1.079±0.044</td>
<td>-12.97±2.017</td>
<td>71.33±1.145</td>
<td>2.11±0.029</td>
</tr>
<tr>
<td>Cisplatin* + CFEE (200 mg/kg p.o.)</td>
<td>0.753±0.015***</td>
<td>-5.803±1.735***</td>
<td>42.33±2.620***</td>
<td>1.133±0.154***</td>
</tr>
<tr>
<td>Cisplatin* + CFEE (400 mg/kg p.o.)</td>
<td>0.697±0.043***</td>
<td>-3.219±1.052***</td>
<td>33.5±2.975***</td>
<td>0.94±0.122***</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. of six rats/treatment. Significance *** P<0.001, **P<0.01 compared to Cisplatin treatment. b.wt. – Body weight. Cisplatin 6 mg/kg i.v. on 2nd day + CFEE 200 mg/kg p.o. for 6 days.

**Renal histopathology of Cisplatin induced model**

**Fig. 1**
Kidney architecture of Normal Control

**Fig. 2**
Kidney architecture of Cisplatin Treatment

**Fig. 3**
Kidney architecture of Cisplatin + 200 mg/kg of CFEE

**Fig. 4**
Kidney architecture of Cisplatin + 400mg/kg of CFEE
Fig. No. 5: GC-MS Report of CFEE
STARTECH LABS PVT LTD

Fig. No. 6: GC-MS report of Ethyl acetate fraction of CFEE