Pharmacological Profile of *Diospyros melanoxylon* Methanolic Extract

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**Keywords:** *Diospyros melanoxylon, Xestospongia muta, Alkaloids; Anti-dormant*

**Introduction**

In the recent years, many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support kidney function and treat diseases of the kidneys. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanism and mode of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Several hundred plants have been examined for use in a wide variety of disorders. Just a handful has been fairly well researched.

**Aim**

The aim of the present study is to evaluate *in-vitro* antioxidant, anti-inflammatory and nephroprotective activities of novel herbal extracts.

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**Abstract**

Present study was aimed to investigate the *in-vitro* antioxidant, anti-inflammatory and *in-vivo* nephroprotective activity novel herbal extracts. Initially the test extracts were evaluated for antioxidant potential by performing the DPPH assay. Few extracts displayed potent antioxidant activity by DPPH free radical scavenging activity. Among all the extracts Methanolic extracts of *Diospyros melanoxylon* was found to be more potent for antioxidant potential. Presence of phenols and flavonoid in the test extracts might be contributed to their potent antioxidant activity. The test extracts were also evaluated for anti-inflammatory activity by carrageenan induced paw edema model. RX- has shown moderate anti-inflammatory activity. Based on preliminary *in-vitro* antioxidant activity results *Diospyros melanoxylon* was selected for further to evaluate Nephro-protective activity against Potassium dichromate induced model. Acute oral toxicity test was performed to find out the safe dose of test extract before going to *in vivo* evaluation (Potassium Dichromate induced nephrotoxicity). Acute toxicity study of test extract was conducted in wistar rats to find the Maximum tolerated dose. The test extract did not show any toxicity and mortality symptoms during the study at the different doses studied. The Maximum Tolerated Dose (MTD) of the test extract was found to be >2000 mg/kg in rats.

*In-vivo* nephroprotective activity was conducted in wistar rats by Potassium dichromate-induced nephrotoxicity model. During the study period test extract (250 mg/kg, 500 mg/kg) were administered by oral route for the period of 7 days followed by potassium dichromate administration (15 mg/kg). At the end of the study blood samples were collected and used for estimation of kidney biochemical parameters. Results showed that significant increase was observed in biochemical parameters (BUN, CR) in PDC group compared to vehicle control. The test extract displayed significant reduction in blood urea nitrogen and serum creatinine at the dose of 500 mg/kg. Kidney tissue samples were collected on termination day of all rats and subjected for measurement of antioxidant enzymes and lipid Peroxidation to check the organ toxicity. Significant increase in lipid Peroxidation and decrease in antioxidant enzyme levels were observed in PDC control whereas test extract prevented the kidney toxicity by decreasing TBARS production and normalization of antioxidant defense enzymes at the doses studied. Gain in body weight and organ weight compared to PD control also revealed the Nephroprotective effect of *D. melanoxylon* extract at both the doses. All the data showed that both biochemical antioxidant parameters correlated together and supported the protective effect of the Herbal extract (*D. melanoxylon*) against potassium dichromate induced nephrotoxicity.

**Objectives**

To study the *in-vitro* antioxidant activity of alcoholic extracts of *Diospyros melanoxylon* Leaves.

To evaluate the anti-inflammatory activity by carrageenan induced model in wistar rats.

Acute oral toxicity studies will be conducted for active compounds as per OECD Guidelines 420.

To study the effect of methanolic extracts of *D. melanoxylon* against potassium –dichromate induced nephrotoxicity in rats. In the present study Nephrotoxicity will be assessed by measuring biochemical parameters like Blood Urea Nitrogen (BUN) Creatinine. Antioxidant parameters will also be evaluated in tissues at the end of the study (CAT, MDA, GSH, GST, NO) to determine the protective effect of the herbal extract. The plant is shown in Figure 1.

**Review of plant**

Botanical name: *Diospyros melanoxylon*

Family: Ebenaceae
**Description**

Coromandel Ebony or East Indian Ebony (*D. melanoxylon*) is a species of flowering tree in the family Ebenaceae that is native to India and Sri Lanka and that has a hard, dry bark. Its common name derives from Coromandel, the coast of southeastern India. Locally it is known as temburini or by its Hindi name tendu. In Odisha and Jharkhand it is known as kendu. The leaves can be wrapped around tobacco to create the Indian beedi, which has outsold conventional cigarettes in India [1-4].

**Taxonomy:**

Name of the: *Diospyros melanoxylon*

Synonym: Coromandel Ebony

Family: Ebenaceae

Genus: Diospyros

Species: *melanoxylon*

Vernacular names:

(Bengali): Kend, Kendu

(Hindi): Abnus, Kendu, Tendu, Timburni

(Nepali): Abnush, Tendu

(Sanskrit): Dirghapatraka

(Tamil): Karai, Karundumbi, Tumb

(Trade name): Ebony

**Diospyros melanoxylon** plant and other parts

Botanic description: *D. melanoxylon* is a medium-sized tree or shrub up to 25 m and 1.9 m girth. The bark is pelican in color, exfoliating in rectangular scales. The primary root is long, thick and fleshy at first, afterwards woody, greyish, often swollen in upper part near ground level. The roots form vertical loops in sucker-generated plants. Leaves opposite or alternate and coriaceous, up to 35 cm long, often with both petiole and leaf edge spiny, up to 3 cm long, to mentose on both sides when young, becoming glabrous above when fully grown. Male flowers are mauve in color, tetramerous to pentamerous, 1-1.5 cm [5].

Long, sessile or nearly sessile in short peduncles, mostly 3-flowered. Female flowers mauve, mostly extra-axillary or sometimes solitary, axillarily generally 2, opposite each other, larger than the male flowers.

Fruits olive green, ovoid or globose 3-4 cm across; 1-, 2-, 3-, 4-, 5-, 6-, or 8-seeded berries. Pulp yellow, soft and sweet. Seeds compressed, oblong, shiny, often banded. The generic name is derived from the Greek 'dios' (divine), and 'pyros' (fruit), referring to the excellent fruit of the genus. The specific name is Greek and means 'dark wood' [6].

Biology: The tree is deciduous or evergreen depending on its habitat. In a dry locality, it is leafless for a short time in the hot weather, regaining its leaves in May-June. In a moist locality, it is evergreen. The flowers appear from April to June on new shoots and the fruit ripens after 1 year. The edible fruits are largely eaten and disseminated by fruit bats and birds, notably hornbills. The tree produces good seed in alternate years.

**Reported pharmacological activities of Diospyros melanoxylon**

Anti-hyperglycemic effect of *D. melanoxylon* (Roxb.) bark against alloxan-induced diabetic rats: The anti-hyperglycemic activity of *D. melanoxylon* (Roxb.) bark was evaluated with scientific approach including biochemical parameters and histopathological studies of pancreas. The ethanolic extracts of the powdered bark was tested for its efficacy in alloxan-induced diabetic rats. The extracts were also evaluated for acute oral toxicity studies and its effect on different biochemical parameters. An effect of extracts was compared to that of standard glibenclamide [7]. It was revealed that ethanolic extracts has significantly (p<0.01) reversed the diabetes-induced hyperlipidemia compared to standard drug. Histopathological studies of pancreas revealed its significant effects on β-cells count. The extracts showed significant anti-hyperglycemic activity as compared to standard drug. Ethanol extract (200 mg/kg) showed beneficial effects on blood glucose and hyperlipidemia associated with diabetes, which might be due to presence of steroids, tannins, alkaloids and triterpenoids present in that extract. Thus ethanolic extract could serve as good adjuvant to other oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

This endemic plant of India and Ceylon is used in various ways. Besides being the source of Indian ebony, its wood is also utilized for making boxes, combs, ploughs and beams. The fruits are eaten and sold commercially. The bark is burnt by tribals to “cure” small-pox. The seeds are prescribed as cure for mental disorders, palpitation of heart and nervous breakdown. Above all, the leaves of this plant constitute one of the most important raw materials of the “Bidi” (Indian cheap smoke) industry.

Antimicrobial activity of *D. melanoxylon* bark from Similipal Biosphere Reserve, Orissa, India: The antimicrobial activity of five extracts of *D. melanoxylon* Roxb. bark collected from Similipal Biosphere Reserve, Orissa was evaluated against human pathogenic bacteria and fungi. The extracts including both polar and non-polar solvents; petroleum ether, chloroform, ethanol, methanol and aqueous were evaluated for their antimicrobial activity against three gram positive and five gram negative bacteria as well as three fungal strains. Although, all the five extracts exhibited promising antibacterial activities, yet maximum activity was observed in ethanol extract. In case of antifungal activity, except petroleum ether extract none of the extracts were found to be active against the fungal strains. MIC values for most of the extracts ranged from 1.5 to 6 mg/ml, while MBC values varied from 3 mg/ml to values greater than 12 mg/ml. Phytochemical analysis exhibited the presence of steroids, alkaloids, glycoside, proteins, tannins, phenolic compounds, carbohydrates, gums and...
mucilage in acetone, methanol and ethanol extracts with maximum phytochemicals in ethanol extract. Least phytochemicals was observed in case of petroleum ether. These results, so obtained, demonstrate the broad spectrum activity of *D. melanoxylon* bark extracts which may be useful in treatment of various microbial infections. However, the active components responsible for antimicrobial activity need to be evaluated [8,9].

**Material and Methods**

**Chemicals**

Chemicals used in the experiment are given in Table 1.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Chemicals</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,1-Diphenyl-2-picryl-hydrazyl (DPPH)</td>
<td>Sigma</td>
</tr>
<tr>
<td>2</td>
<td>Alcohol</td>
<td>SD fine chem. Lt</td>
</tr>
<tr>
<td>3</td>
<td>Ascorbic acid</td>
<td>Sigma</td>
</tr>
<tr>
<td>4</td>
<td>Acetic acid</td>
<td>SD fine chem. Lt</td>
</tr>
<tr>
<td>5</td>
<td>Aluminium Hexahydrate</td>
<td>SD fine chem. Lt</td>
</tr>
<tr>
<td>6</td>
<td>Dichlorofluoresceindiacetate (DCF-DA)</td>
<td>Sigma</td>
</tr>
<tr>
<td>7</td>
<td>DTNB/Ellman’s Reagent</td>
<td>Biochemika Fluka</td>
</tr>
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<td>8</td>
<td>Dimethyl Sulphoxide (DMSO)</td>
<td>Merck</td>
</tr>
<tr>
<td>9</td>
<td>Folin-Ciocalteau Reagent</td>
<td>Sigma</td>
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<tr>
<td>10</td>
<td>Formaldehyde</td>
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<tr>
<td>11</td>
<td>Gallic acid</td>
<td>Sigma</td>
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<tr>
<td>12</td>
<td>Gum Acacia</td>
<td>Loba Chemie</td>
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<tr>
<td>13</td>
<td>Hydrochloric acid</td>
<td>Rankem</td>
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<td>14</td>
<td>Hydrogen Peroxide</td>
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<tr>
<td>15</td>
<td>Naphthyl ethylene diaminedihydrochloride</td>
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<td>16</td>
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<td>19</td>
<td>Rutin hydrate</td>
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<td>20</td>
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<td>21</td>
<td>Sodium Hydroxide (NaOH)</td>
<td>SD fine chem. Lt</td>
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<td>23</td>
<td>Sodium Lauryl Sulphate (SLS)</td>
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<td>24</td>
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<tr>
<td>25</td>
<td>Sulfanilamide</td>
<td>Loba Chemie</td>
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<tr>
<td>26</td>
<td>Superoxide Dismutase (SOD) Assay kit</td>
<td>Sigma</td>
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<tr>
<td>27</td>
<td>Thio-Barbituric Acid (TBA)</td>
<td>Sigma</td>
</tr>
<tr>
<td>28</td>
<td>Tris Buffer</td>
<td>Sigma</td>
</tr>
</tbody>
</table>

**Table 1:** Chemicals used for experiment.

Apparatus used are given in Table 2.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Instruments</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Micro plate reader</td>
<td>BioTek instruments, Synergy 4, USA</td>
</tr>
<tr>
<td>2</td>
<td>UV/Visible Spectrophotometer</td>
<td>Perkin Elmer, Lambda 25, USA</td>
</tr>
<tr>
<td>3</td>
<td>Laboratory Micro Centrifuge</td>
<td>Biofuge stratus, Germany</td>
</tr>
<tr>
<td>4</td>
<td>Auto Blood Analyzer</td>
<td>Dimension X pand plus, USA</td>
</tr>
<tr>
<td>5</td>
<td>Tissue Homogenizer</td>
<td>Heidolph, Silent crusher S, Germany</td>
</tr>
<tr>
<td>6</td>
<td>PH meter</td>
<td>pH 540 GLP, WTW, Germany</td>
</tr>
<tr>
<td>7</td>
<td>Digital Balance</td>
<td>Precisa</td>
</tr>
</tbody>
</table>

**Table 2:** Apparatus used for experiment.

**Experimental animals**

Wistar Albino Rats weighing between 160-180 g were procured from NIN, Hyderabad, India. The animals were housed in a bio-safe temperature-controlled environment with a 12:12 light/dark cycle with standard conditions of temperature (25 ± 20°C) and relative humidity (30-70%) during the experimental period. The animals were fed with standard pellet diet and water ad libitum. All the animals were acclimatized under laboratory conditions for a week before the commencement of experiments. The Institutional Animal Ethics Committee (IAEC), IICT, and Hyderabad, India approved the study (Protocol No: IICT/PHARM/SRK/Feb/2013/11). The norms for Good Laboratory Practice (GCP) were followed for care of laboratory animals. The animals were maintained in accordance with the CPCSEA guidelines.

**Test compound preparation**

Required quantity of alcoholic extracts of *D. melanoxylon* Leaf were weighed and made suspension with gum acacia (2%) in de-ionized water.

**Potassium dichromate: Required quantity of potassium dichromate powder (250 mg/kg of rat body wt 500 mg/kg) weighed and made suspension with gum acacia (2%) in de-ionized water.**

**DPPH (1, 1-Diphenyl-2-picryl-hydrazil) free radical scavenging activity**

*In-vitro* studies were performed to evaluate antioxidant activity of novel herbal extracts.

Preparation of sample: Stock solution of methanolic extract (for antioxidative activity) of *D. melanoxylon* leaf was prepared by dissolving in Dimethyl Sulphoxide (DMSO) at a concentration of 5 mg/ml.

Preparation of DPPH solution: 2.8 mg of DPPH was dissolved in 13 ml of methanol.
Preparation of standard: 5 mg of ascorbic acid dissolved in 1 ml dimethyl sulphoxide (5 mg/ml). From this stock solution, further dilutions were made.

**Principle of the assay**

DPPH reduction: DPPH is one of a few stable and commercially available organic nitrogen radicals with a UV-Vis absorption maximum at 517 nm. When DPPH reacts with an antioxidant compound, which can donate hydrogen, DPPH gets reduced as shown in Figure 2 changes the color from deep violet to light yellow. The change in color (from deep violet to light yellow) is directly proportional to antioxidant potential of the compound, which was measured at 517 nm on a UV/Visible light spectrophotometer. DPPH is nitrogen centered free radical that react similarly peroxyl radicals and represents a model free radical originating in lipophilic medium. DPPH is the parameter of antioxidant activity [10].

**Figure 2: Diphenyl Picryl Hydrazine (DPPH) reduction.**

Assay procedure: DPPH free radical scavenging activity tested with 25 µl of test sample (5 mg/ml DMSO), 100 µl of 0.1M Tris HCL buffer (pH 7.2) and 125 µl of 0.5 mM DPPH (1,1-diphenyl-2-picrylhydrazyl; sigma chemicals, USA, dissolved in methanol) were mixed and shaken well. After 30 min, absorbance was recorded Spectrophotometrically (Biotek synergy, USA) at 517 nm. The free radical scavenging potential was determined as the percent discoloration of DPPH due to test samples and calculated as (1-B/A) × 100, where A is absorbance of DPPH control with solvent and B is absorbance of decolorized DPPH in the presence of test compound. All the analysis was done in triplicates; ascorbic acid was taken as reference compound. Several dilutions of primary solutions (5 mg/ml in DMSO) were made and assayed accordingly to obtain concentration of sample required for scavenging of DPPH free radical (Table 3) [11].

**Determination of Total Phenolic Content**

Preparation of samples: 5 mg of sample (alcoholic extracts of bark and leaf) dissolved in 1 ml of dimethyl sulphoxide (5 mg/ml).

Preparation of standard: 5 mg of gallic acid dissolved in 1 ml dimethyl sulphoxide (5 mg/ml). From this stock solution, further dilutions were made.

Preparation of reagents: Folin-Ciocalteau Reagent (FCR): 2.5 ml of FCR dissolved in 2.5 ml distilled water.

20% Na₂CO₃: 2 g Sodium Carbonate was dissolved in 10 ml distilled water.

**Assay procedure**

Total phenolic content in the extracts (bark and leaf of *D. melanoxylon*) was measured using Folin-Ciocalteu reagent. Briefly, 25 µl (5 mg/ml DMSO solution) of the extract was diluted with 2.5 ml of distilled deionized water followed by addition of 250 µl of Folin-Ciocalteau reagent (1M) and 250 µl of Na₂CO₃ (20% w/v). After incubation for 60 min at room temperature absorbance was measured spectrophotometrically at 765 nm (BioTeks synergy4 multi-mode microplate reader, BioTek Instruments, Inc.). Quantification was performed with respect to the standard curve of gallic acid. Results were expressed as milligrams of Gallic Acid Equivalent (GAE) per 100 g of the extract as shown in Table 4.

**Table 4: Assay procedure for experimentation.**

**Determination of Total Flavonoids Content**

Preparation of samples: 5 mg of sample (alcoholic extracts of bark and leaf) dissolved in 1 ml of dimethyl sulphoxide (5 mg/ml).

Preparation of standard: 5 mg of rutin dissolved in 1 ml dimethyl sulphoxide (5 mg/ml). From this stock solution, further dilutions were made.

Preparation of reagent: 2% AlCl₃ (Aluminum chloride hexahydrate): 0.1 g AlCl₃ dissolved in 5 ml methanol.

**Assay procedure**

Total flavonoids content in the extracts (leaf of *D. melanoxylon*) was measured using Aluminum chloride hexahydrate reagent. Briefly, 125 µl (5 mg/ml DMSO solution) of the extract followed by the addition of 125 µl of Aluminum chloride hexahydrate reagent. After incubation for 10 minutes at room temperature, Absorbance was measured at 450 nm.
nm. Quantification was performed with respect to the standard curve of Rutin. Results were expressed as milligrams of Rutin per 100 g of the extract. All determinations were performed in duplicates.

**Acute Oral Toxicity**

The acute oral toxicity study conducted as per OECD guidelines by fixed dose method adopted by OECD (420).

**Principle of the study**

The acute oral toxicity study was conducted as per OECD guidelines by fixed dose method adopted by OECD (420). The study involved a preliminary sighting study using small number of animals in order to derive the dose effect relation for toxicity and mortality and to provide information on dose selection for the main study. In the preliminary sighting study, the effect of various dosed administered to single animals of each sex was investigated in a sequential manner. The sighting study generally yields information on the dose–toxicity relationship including an estimate of the minimum lethal dose. In the main study, the test article is administered to groups of 5 males and 5 female animals at one of the fixed doses (5, 50, 300 and 2000 mg/Kg) [12-14].

**Sighting study**

In the sighting study, the effect of various doses was investigated in single animal of each sex. Dosing was sequential allowing at least 24 h. before dosing the next animal. All animals were carefully observed for signs and symptoms of toxicity continuously up to 24 h. Later up to 7 days. The sighting study was conducted with sequential doses of 5, 50, 300 and 2000 mg/kg of the test article. If the initial dose chosen did not produce severe toxicity, the next higher dose was selected. In this sighting study, the dose that produced evident toxicity but not death was identified. Dose escalation was continued up to 2000 mg/kg.

**Main study**

At least 10 animals (5 males and 5 females) for each species were used for the dose level in this study. Animals were fasted overnight and the test articles were administered as 1% gum acacia suspension by oral route. The dose used in this study is selected from one of the four levels 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg i.e. the dose that produced evident toxicity but not mortality from sighting study [15]. The animals were observed for following signs and symptoms of toxicity apart from the cage side observations which include changes in skin and fur, eyes, mucous membrane, respiratory, circulatory, and autonomic and central nervous systems. The different biochemical and hematological parameters observed on “0” day and on the termination day during the main study physical parameters like feed consumption and body weight increase were also recorded as given in Table 5.

<table>
<thead>
<tr>
<th>Observations</th>
<th>Physical examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bizarre physical position</td>
<td>Altered muscle tone</td>
</tr>
<tr>
<td>Bizarre tail position</td>
<td>Catatonia</td>
</tr>
<tr>
<td>Muscle tremors</td>
<td>Aggressive ness towards observer</td>
</tr>
<tr>
<td>Aggressiveness towards other animals</td>
<td>Cornea</td>
</tr>
<tr>
<td>Inactivity</td>
<td>Convulsions to touch</td>
</tr>
</tbody>
</table>

**Potassium-dichromate Induced Nephrotoxicity**

**Experimental Procedure**

Wistar rats weighing 160-180 g randomly selected for the present study and they were divided into four groups consisting 6 rats in each. They are as follows:

- **Group I**: Normal control rats, received distilled water for 7 days.
- **Group II**: potassium dichromate Control: Rats received distilled water and served as a negative control and on the 7th day received potassium dichromate (15 mg/kg).
- **Group III**: Treatment group: Rats received low dose (250 mg/kg) of test extract Herbal Extract (HE) p.o. from first day to seventh day followed by potassium dichromate (15 mg/kg) on day 7.
- **Group IV**: Treatment Group: Rats received high dose (500 mg/kg) of herbal extract Herbal Extract (HE) p.o. from first day to seventh day followed by potassium dichromate (15 mg/kg) on day 7.

At the end of the study blood samples were collected from all the groups of rats and used for estimation of biochemical parameters. Later animals were sacrificed by cervical dislocation and kidney tissue samples were retained for further antioxidant enzyme evaluation. During the study, body weights were recorded on day 1 and on termination day. Percentage organ weights were calculated by taking terminal body weights of all animals. All the animals were observed for toxicity signs during the study.

**Serum sample preparation**

At the end of the experimental period (9 days) blood samples were collected using heparinized capillary tubes from retro orbital plexus of the animals (rats and mice) of all groups. Blood samples were left to clot for 1 h at room temperature, and then centrifuged by using centrifuge (Biofuge stratos, Germany) at 4000 rpm for 15 min at 25°C to separate the sera, which were stored at -80°C until analysis could be completed. Serum was used for estimation of BUN and Creatinine using Auto Blood analyzer (Dimension x pand plus; USA).

**Tissue preparation and homogenization**

Animals were sacrificed by cervical dislocation with light ether anesthesia and kidney tissues were removed from rats. Tissues washed

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**Table 5: Toxicity symptoms in acute oral toxicity.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous Convulsions</td>
<td>Alterations in cardiac rhythms and rate</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>Paralysis</td>
</tr>
<tr>
<td>Sedation</td>
<td>Change in pupil size</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>Sensitivity to pain</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>Skin lesions</td>
</tr>
<tr>
<td>Abnormal Excreta</td>
<td>Corneal opacity</td>
</tr>
<tr>
<td>Salivation</td>
<td>Loss of righting reflex</td>
</tr>
<tr>
<td>Nasal Discharge</td>
<td>Grasping reflexes</td>
</tr>
<tr>
<td>Piloerection</td>
<td>Pinna reflexes</td>
</tr>
<tr>
<td>Phonation</td>
<td>Death</td>
</tr>
</tbody>
</table>
thoroughly with ice-cold normal saline and weighed. Then immediately part of the tissues were stored in formalin and used for Histopathological study. Remaining tissues were stored at -80°C and used for further estimations. Tissues were cut in to small pieces and homogenized with a Homogenizer (Heidoph, Silent crusher S, Germany) in ice-cold phosphate buffer saline (PBS) (0.05M, pH 7) to obtain 1:9 (w/v) (10%) whole homogenate. Homogenate was mixed with equal volume of 10% Trichloroacetic acid (TCA) and centrifuged at 5000 rpm for 10 min and supernatant was used for the determination of MDA, Vtc. Then remaining homogenate was centrifuged at 17,000 g for 60 min at 4°C, and supernatants were used for the measurement of antioxidant parameters, NO, CAT, GSH, GST.

**Total Lipid Peroxidation**

The concentration of MDA in homogenate as an index of lipid peroxidation and it was determined.

**Principle of the assay**

Melondialdehyde (MDA) is one of many low molecular weight end products of lipid hydro peroxides decomposition and is the most often measured as an index of lipid peroxidation. In this assay, one molecule of Melondialdehyde (MDA) reacts with two molecules of 2-Thiobarbituric Acids (TBA) at pH 3.5 to form pink chromotagen, which is measured spectrophotometrically at 532 nm with extinction coefficient of 156 M⁻¹cm⁻¹.

**Reagents**

A: 8.1% SLS (Sodium Lauryl Sulphate)
B: 20% Acetic acid (9.5 ml of Glacial acetic acid dilute up to 50 ml with DDW (pH 3.5 with 4N NaOH))
C: 0.8% aqueous solution of TBA (2-Thiobarbituric Acid)

**Method**

All the reagents were freshly prepared and to the 0.2 ml of experimental sample, 0.2 ml of 8.1% SLS, 1.5 ml of 20% Acetic acid (pH 3.5) and 1.5 ml of 0.8% aqueous solution of TBA was added and made the volume up to 4 ml with double distilled water [16]. Then heat the mixture at 95 °C for 60 min in water bath on hot plate to develop light pink Color. The mixture was allowed to cool and measure the absorbance spectrophotometrically at 532 nm using micro plate reader (BioTek instruments, synergy 4, USA) and was expressed as nmol/g wet tissue. Finally MDA content was calculated by following formula:

\[
\text{Lipid peroxides (Nmol MDA/g Tissue (Liver/Heart))} = \frac{(\text{Abs} \times 156) \times 1000}{\text{[Total Volume (4 ml)/Sample Volume (0.2 ml)]} \times \text{Dilution Factor (10)}}
\]

**Nitric Oxide Oxide (NO)**

The nitric oxide (NO) production was measured by estimating the accumulation of nitrates (NO₃⁻) in the supernatant by the method with slight modification of Griess Reagent System (Promega technical bulletin, USA).

**Principle**

Nitrite (NO₂⁻) is one of two primary, stable and nonvolatile breakdown product of Nitric Oxide. The Griess Reagent System is based on the diazotization chemical reaction which uses sulfanilamide and N-1-Napthylethylendiaminedihydrochloride (NED) under acidic (phosphoric acid) conditions according to the following reactions [17,18].

**Reagents**

A: Sulfanilamide solution (1% sulfanilamide in 5% phosphoric acid)
B: NED Solution (0.1% N-1-Naphthylethylendiaminedihydrochloride in water)
C: Nitrite Standard (0.1 M Sodium Nitrite)

**Method**

Sulfanilamide Solution, NED Solution and Nitrite Standard (0.1M Sodium Nitrite) were prepared and allowed to equilibrate to room temperature (15-30 min). A standard curve was prepared with nitrite concentration range from 0-100 µM (100, 50, 25, 12.5, 1.31, and 0.63, 0 µM), using sodium nitrite (NaNO₂) as standard. To the wells containing (duplicate) 50 µl of standard nitrite sample and experimental sample, 50 µl of the Sulfanilamide Solution was added and incubated 5-10 min at room temperature, protected from light. Then 50 µl of the NED Solution was added to all wells and incubated at room temperature for 5-10 min, protected from light. A purple/magenta color thus formed was measured by taking absorbance at 540 µm using micro plate reader (BioTek instruments, synergy 4, USA) and was expressed as µmol/g wet tissue.
ml with double distilled water. Then the mixture was incubated for 10 min at room temperature and measured the absorbance at 412 nm and calculated the GSH content from standard graph.

Catalase Assay (CAT)

Catalase activity tissues was determined by measuring the rate of decomposition of hydrogen peroxide at 240 nm, according to the method.

Principle

Catalase is a common enzyme in all-living organisms. Which catalyze the decomposition of H₂O₂ to water and oxygen and activity was measured by measuring the rate of decomposition of hydrogen peroxide at 240 nm. The difference in absorbance (ΔE 240) per unit time is a measure of the catalase activity.

Reagents

Phosphate Buffer (50 mM, pH 7)

Dissolved 6.81 g KH₂PO₄ in double distilled water to make 1000 ml.

Dissolved 7.091 g Na₂HPO₄ in double distilled water to make 1000 ml.

Mix both solutions in 1:1.55.

Hydrogen Peroxide (30 mM) (Dilute 0.34 ml of 30% H₂O₂ with phosphate buffer up to 100 ml).

Method

To The 1.95 ml phosphate buffer (50 mM, pH 7), 50 µL of experimental sample were added. Then changes in absorbance were recorded at 240 nm by addition of 1 ml hydrogen peroxide (30 mM) for 1 min at 15 sec interval and then the activity was calculated by following formula.

Catalase activity (K/min) = (1/Δt) × ln (s1/s2) = (2.3/Δt) × log (s1/s2)

Where, Δt = t₂-t₁ (time interval)

S₁ and S₂ = H₂O₂ concentrations at times t₁ and t₂.

Ascorbic Acid Assay

Principle

Ascorbic acid was assayed by the method in which Ascorbic acid is oxidized to dehydro ascorbic acid and 2,3-dioxogluconic acid with copper sulphate [20]. Further after reaction with 2,4-dinitrophenyl hydrazine it forms tris 2,4-dinitrophenyl hydrazone which on treatment with ice cold Sulfuric acid forms a complex which is quantified colorimetrically at 520 nm.

Reagents

5% Trichloroacetic acid.

DTC Reagent (3 g Dinitrophenyl hydrazine, 0.4 g thiourea, 0.05 g Urea and 0.05 g copper sulphate made upto 100 ml with 9 N sulfuric acid).

65% Sulfuric acid.

Procedure

To 0.1 ml of tissue homogenate, 1 ml of Trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min to 0.5 ml of the supernatant, 0.2 ml of DTC reagent was added and incubated at 37°C for 3 h. To the resultant, 1.5 ml of ice cold Sulfuric acid (65%) was added and again incubated at 37°C for 3 h. Absorbance of resultant solution was observed at 520 nm.

Statistical Analysis

The intergroup variation between various groups were analyzed statistically using one-way analysis of variance (ANOVA) using the Graph Pad Prism version 5.0, followed by Turkey’s Multiple Comparison Test (TMCT). Statistical significance was evaluated at P<0.05. The experimental results expressed as the Mean ± S.E.M or as percent activity compared to control animals.

Results and Discussion

DPPH free radical scavenging activity

The in-vitro antioxidant activity can be evaluated on the basis of their capability to scavenge free radical like DPPH and by estimating total phenolic content and total flavonoid content. Increased consumption of fruits, vegetables and herbal products significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and other aging-related pathologies. Photochemical, especially antioxidants of these natural products are suggested to be the major bioactive compounds for these health benefits. The details are given in Table 6.

<table>
<thead>
<tr>
<th>Test extract</th>
<th>IC-50 (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>0.6673</td>
</tr>
<tr>
<td>Herbal Extract (HE)</td>
<td>0.564</td>
</tr>
</tbody>
</table>

Table 6: IC-50 values of standard and test extracts for DPPH radical assay.

DPPH assay evaluates the ability of antioxidants to scavenge free radicals. Hydrogen-donating ability is an index of the primary antioxidants. These antioxidants donate hydrogen to free radicals, resulting in non-toxic species and therefore inhibiting the propagation phase of lipid oxidation. Antiradical antioxidants act by donating hydrogen atoms to lipid radicals. Radicals obtained from antioxidants with molecular structures such as phenols are stable species and will then stop the oxidation chain reaction. The assay has been performed with eight dilutions made serially of both the standard and the sample (Methanolic extracts of D. melanoxylon leaf). The values represent the mean and standard deviation of the triplicates done at each point. The percentage scavenging activity was calculated by comparing the absorbance of control and test. Below graph was obtained by plotting the concentration on the X-axis versus the scavenging activity on the Y-axis. The test extracts has shown significant and dose dependent antioxidant activity at all the concentrations used in the assay. Antioxidant potential of the test extracts was comparable with the standard ascorbic acid at all the concentrations used as shown in Figure 3.

ml with double distilled water. Then the mixture was incubated for 10 min at room temperature and measured the absorbance at 412 nm and calculated the GSH content from standard graph.
Anti-inflammatory effect of herbal extracts by carrageenan induced model

Carrageenan-induced paw edema is a suitable experimental animal model for evaluating the anti-inflammatory effect of new agents. Edema developed following injection of carrageenan is an index of acute inflammatory changes, and it can be determined from differences in the paw volume measured immediately after carrageenan injection. Edema induced by carrageenan is believed to be biphasic: the first phase involves the release of serotonin and histamine and the second phase is mediated by prostaglandins, cyclooxygenase products [15]. This model is the standard experimental model of acute inflammation, and it is the phlogestic agent of choice for testing anti-inflammatory drugs. Moreover, the experimental model exhibits high degree of reproducibility. In the present study the herbal extracts were evaluated for anti-inflammatory activity by carrageenan induced model in wistar rats. The test compounds were administered by oral route prior to inflammatory agent. Percentage inhibition of the test compounds was calculated using control. Among all the extracts RX-11 has shown significant (p<0.05) inhibition compared to control. Rest all extracts showed mild inhibition in paw volume which is shown in Figure 5.

Acute toxicity study acute oral toxicity

Sighting study: In the present study test compound was evaluated for different in-vitro activities and it was found to be potent. Based on these results the compounds were evaluated for maximum tolerated dose in the animals by conducting acute oral toxicity study before proceeding to in-vivo Nephroprotective activity by Potassium-dichromate-induced nephrotoxicity model.

The test extract did not show any toxic symptoms and mortality at different doses 5, 50, 300, and 2000 mg/kg throughout the study. Based on sighting study, a dose 2000 mg/kg was selected for the main study in rats for the herbal extract.

Main study: The extract was found to be safe at the dose of 2000 mg/kg and did not show any toxic symptoms. No abnormal behavior was observed in both rats and mice during the study. During the study physical parameters, body weight was measured and uniform increase in body weight was observed in all the compound groups of animals.
Based on all above observations the test extract compounds was safe up to 2000 mg/kg. Therefore, the maximum tolerated dose was found to be >2000 mg/kg in both rats shown in Table 8.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toxic signs</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>Pre-terminal deaths</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>Body weight</td>
<td>No specific change</td>
</tr>
<tr>
<td>4</td>
<td>Cage side observation</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>Motor activity</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>Tremors</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Convulsions</td>
<td>Absent</td>
</tr>
<tr>
<td>8</td>
<td>Straub reaction</td>
<td>Absent</td>
</tr>
<tr>
<td>9</td>
<td>Righting reflex</td>
<td>Present</td>
</tr>
<tr>
<td>10</td>
<td>Lacrimation and Salivation</td>
<td>Normal</td>
</tr>
<tr>
<td>11</td>
<td>Unusual vocalization</td>
<td>Absent</td>
</tr>
<tr>
<td>12</td>
<td>Sedation</td>
<td>Absent</td>
</tr>
<tr>
<td>13</td>
<td>Body temperature</td>
<td>Normal</td>
</tr>
<tr>
<td>14</td>
<td>Analgesia</td>
<td>Absent</td>
</tr>
<tr>
<td>15</td>
<td>Ptosis</td>
<td>Absent</td>
</tr>
<tr>
<td>16</td>
<td>Diarrhoea</td>
<td>Absent</td>
</tr>
<tr>
<td>17</td>
<td>Skin color</td>
<td>Normal</td>
</tr>
<tr>
<td>18</td>
<td>Respiration</td>
<td>Normal</td>
</tr>
<tr>
<td>19</td>
<td>Scratching</td>
<td>Absent</td>
</tr>
<tr>
<td>20</td>
<td>Grooming</td>
<td>Normal</td>
</tr>
<tr>
<td>21</td>
<td>Aggressiveness &amp; Restlessness</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Table 8: Acute Toxicity Study Observations.

**Effect of herbal extract on potassium dichromate induced nephrotoxicity model**

Body weight and organ weights were used to assess the toxicity effect of test compounds. During the study period, body weights were taken on every alternative day. At the end of the study, all the animals were sacrificed and liver tissues were collected. Percentage change in body weights was calculated by taking the termination day and zero day body weights. Percentage organ (kidney) weights were calculated by taking terminal body weights of rats. In the present study rats were treated with test extracts for the period of 7 days prophylactically by per oral route at the dose of 500 mg/kg. Significant (P<0.01) decrease in body weight and organ weight was observed in potassium dichromate group compared to vehicle control. Significant (P<0.05) gain was observed in test extract compared to potassium dichromate group. Significant restoration in body weights indicated the nephroprotective effect of test extracts. No evidence of toxicity symptoms were observed throughout the study. The details are shown in Figure 6 and effect of herbal extract are given in Table 9 respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in body weight (g)</th>
<th>Organ weight (Kidney) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>3.4 ± 0.32</td>
<td>1.925 ± 0.05</td>
</tr>
<tr>
<td>PD Control</td>
<td>7.8 ± 0.215</td>
<td>1.856 ± 0.08</td>
</tr>
<tr>
<td>Group 3</td>
<td>4.4 ± 0.245</td>
<td>1.93 ± 0.05</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.8 ± 0.125</td>
<td>1.892 ± 0.07</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M; n=6

Table 9: Effect of Herbal Extract (HE) on body weight and organ weight of rats in potassium dichromate induced model.

**Effect of herbal extract on biochemical parameters in potassium dichromate induced model**

It is reported that the kidney is the principal route of Cr excretion and acute exposure induces an increase in Cr kidney content on K2Cr2O7 treated rats. Exposition to Cr (VI) produced anatomical lesions at the level of the proximal tubular cells and lipid peroxidation in human kidney. Creatinine and blood urea nitrogen levels reported to be elevated in kidney dysfunction [17]. On termination animals...
were sacrificed and kidney tissue samples were collected and estimated for antioxidant enzymes. Blood samples were collected and analyzed for kidney biochemical parameters. Significant increase in creatinine and blood urea nitrogen levels was observed in PD control compared to normal control which indicates nephrotoxicity. The herbal extract at both the doses (250, 500 mg/kg) possessed significant (p<0.05) decrease in BUN levels when compared to PD. The details are shown in Figure 7a, 7b and Table 10.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Normal Control</th>
<th>Potassium control</th>
<th>Herbal Extract (250 mg/kg)</th>
<th>Herbal Extract (500 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bun</td>
<td>36.2 ± 1.356</td>
<td>45.5 ± 1.26</td>
<td>40.75 ± 4.19</td>
<td>34.5 ± 2.062</td>
</tr>
<tr>
<td>2</td>
<td>Creatinine</td>
<td>0.76 ± 0.114</td>
<td>1.12 ± 0.228</td>
<td>0.86 ± 0.152</td>
<td>0.748 ± 0.148</td>
</tr>
</tbody>
</table>

Table 10: Table represents biochemical parameters in potassium dichromate induced nephrotoxicity model.

![Figure 7a: Effect of herbal extract on blood urea nitrogen levels in potassium dichromate induced model. Blood Urea Nitrogen values of test extract Herbal Extract (HE). Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05.](image)

![Figure 7b: Effect of herbal extract on serum creatinine levels in potassium dichromate induced model. Blood Urea Nitrogen values of test extract Herbal Extract (HE). Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05.](image)

Catalase

Catalase (CAT) is an enzymatic anti-oxidant widely distributed in all animal tissue and the highest activity is found in the red cells and in liver. It decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [12]. Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the assimilation of super-oxide radicals and hydrogen peroxide. Significant decrease (Catalase) was observed in PD control compared to normal control whereas Herbal extract at the dose of 500 mg/kg showed significant increase compared to PD control as shown in Figure 8.

![Figure 8: Effect of herbal extract on antioxidant enzymes CAT level in potassium dichromate -induced nephrotoxicity model. Where Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05 as in Figure 8.](image)

MDA (TBARS)

Lipid peroxidation has been postulated as being the destructive process in kidney injury due to potassium dichromate administration.
In our study, elevations in the levels of TBARS in kidneys of rats treated with potassium dichromate were observed. The increase in TBARS levels in kidney suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. In biological systems, MDA, a degradation product of lipid hydro peroxides, is considered as an index of lipid peroxidation. Accumulating evidence suggests that ROS initiates lipid peroxidation, which not only deteriorates cellular structure and function but also produces Melondialdehyde (MDA) a potent carcinogen. In the present study, kidney tissues were collected at the end of the treatment and used for estimation of lipid Peroxidation and different antioxidant enzymes. Significant decrease (GSH) was observed in PD control compared to normal control whereas Herbal extract at the dose of 500 mg/kg showed significant increase compared to PD control as shown in Figure 9.

Figure 9: Effect of herbal extract on antioxidant enzymes MDA level in potassium dichromate- induced nephro toxicity model. Where Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05 as in Figure 9.

Effect of herbal extract on antioxidant enzymes on reduced glutathione level in potassium dichromate- induced nephrotoxicity model

Glutathione (GSH) is one of the most abundant tri-peptide, non-enzymatic biological antioxidants. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as a substrate for Glutathione Peroxidase (GPx) and GST [10]. Glutathione redox cycle is the most important intracellular antioxidant system which maintains cell integrity and participation in the cell metabolism. In our present study, the decreased level of GSH has been associated with an enhanced lipid peroxidation in Potassium dichromate treated rats compared to vehicle control group. The decrease may be because of potassium dichromate induced oxidative stress, which inactivates the enzyme Gaama-Glutamyl Cysteine Synthetase (ˠ-GCS) a rate limiting enzyme in de novo GSH synthesis. Significant decrease (GSH) was observed in PD control compared to normal control whereas Herbal extract at the dose of 500 mg/kg showed significant increase compared to PD control as shown in Figure 10.

Figure 10: Effect of herbal extract on antioxidant enzymes Reduced Glutathione level in potassium dichromate- induced nephrotoxicity model.

Where Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05 as in Figure 10.

Effect of herbal extract on antioxidant enzymes on oxidized glutathione level in potassium dichromate- induced nephrotoxicity model

The principle involves oxidation of GSH by the sulphydryl reagent 5,5’-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5’-Thio-2-Nitrobenzoic Acid (TNB), measurable at 412 nm. The Glutathione Disulfide (GSSG) formed can be recycled to GSH by glutathione reductase in the presence of NADPH. GST assay can be extended for drug discovery/pharmacology and toxicology protocols to study the effects of drugs and toxic compounds on glutathione metabolism. Significant decrease (Oxidized Glutathione) was observed in PD control compared to normal control whereas Herbal extract at both doses (250, 500 mg/kg) showed significant increase compared to PD control as shown in Figure 11.

Figure 11: Effect of herbal extract on antioxidant enzymes Oxidized Glutathione level in potassium dichromate- induced nephrotoxicity model.

Where Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05 as in Figure 11.
Effect of herbal extract on antioxidant enzymes on ascorbic acid level in potassium dichromate-induced nephrotoxicity model

Ascorbic acid was assayed by the method in which Ascorbic acid is oxidized to dehydro ascorbic acid and 2,3-dioxogluconic acid with copper sulphate. Further after reaction with 2,4-dinitrophenyl hydrazine it forms tris 2,4-dinitrophenyl hydrazone which on treatment with ice cold sulfuric acid forms a complex which is quantified colorimetrically at 520 nm. Significant decrease (Ascorbic Acid) was observed in PD control compared to normal control whereas Herbal extract at the dose of 500 mg/kg showed significant increase compared to PD control as shown in Figure 12.

Ascorbic acid assay

![Figure 12: Effect of herbal extract on antioxidant enzymes Ascorbic Acid level in potassium dichromate-induced nephrotoxicity model.](image)

Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05 as in Figure 12.

Effect of herbal extract on antioxidant enzymes on nitric oxide levels in potassium dichromate-induced nephrotoxicity model

Nitric Oxide (NO), a short-lived free radical generated endogenously, exerts influence on a number of functions including vasodilation, neurotransmission, synaptic plasticity and memory in the central nervous system. Besides mediating normal function, NO has been implicated in pathophysiological states. Overproduction of NO can mediate toxic effects, like DNA fragmentation, cell damage and neuronal cell death. Significant decrease (Nitric Oxide) was observed in PD control compared to normal control whereas Herbal extract at both doses (250, 500 mg/kg) showed significant increase compared to PD control as shown in Figure 13.

Nitric oxide assay

![Figure 13: Effect of herbal extract on antioxidant enzymes Nitric Oxide level in potassium dichromate-induced nephrotoxicity model.](image)

Values are expressed as Mean ± S.E.M; n=6. ** P<0.01, *P<0.05 as in Figure 13.

Conclusion

This study was aimed to investigate the in-vitro antioxidant, anti-inflammatory and in-vivo nephroprotective activity novel herbal extracts. Initially the test extracts were evaluated for antioxidant potential by performing the DPPH assay. Few extracts displayed potent antioxidant activity by DPPH free radical scavenging activity. Among all the extracts Methanolic extracts of D. melanoxylon was found to be more potent for antioxidant potential. Presence of phenols and flavonoid in the test extracts might be contributed to their potent antioxidant activity. The test extracts were also evaluated for anti-inflammatory activity by carrageenan induced paw edema model. RX has shown moderate anti-inflammatory activity. Based on preliminary in-vitro antioxidant activity results D. melanoxylon was selected for further to evaluate Nephro-protective activity against Potassium dichromate induced model. Acute oral toxicity test was performed to find out the safe dose of test extract before going to in-vivo evaluation (Potassium Dichromate induced nephrotoxicity). Acute toxicity study of test extract was conducted in Wister rats to find the Maximum tolerated dose. The test extract did not show any toxicity and mortality symptoms during the study at the different doses studied. The MTD of the test extract was found to be >2000 mg/kg in rats.

In-vivo nephroprotective activity was conducted in wistar rats by Potassium dichromate-induced nephrotoxicity model. During the study period test extract (250 mg/kg, 500 mg/kg) were administered by oral route for the period of 7 days followed by potassium dichromate administration (15 mg/kg). At the end of the study blood samples were collected and used for estimation of kidney biochemical parameters. Results showed that significant increase was observed in biochemical parameters (BUN, CR) in PDC group compared to vehicle control. The test extract displayed significant reduction in blood urea nitrogen and serum creatinine at the dose of 500 mg/kg. Kidney tissue samples were collected on termination day of all rats and subjected for measurement of antioxidant enzymes and lipid Peroxidation to check the organ...
toxicity. Significant increase in lipid Peroxidation and decrease in antioxidant enzyme levels were observed in PDC control whereas test extract prevented the kidney toxicity by decreasing TBARS production and normalization of antioxidant defense enzymes at the doses studied. Gain in body weight and organ weight compared to PD control also revealed the Nephroprotective effect of \textit{D. melanoxylon} extract at both the doses. All the data showed that both biochemical antioxidant parameters correlated together and supported the protective effect of the Herbal extract (\textit{D. melanoxylon}) against potassium dichromate induced nephrotoxicity.

The results of this study demonstrated that \textit{D. melanoxylon} has shown strong antioxidant potential. Significant protective effect on potassium dichromate induced nephrotoxicity in rats revealed that the herbal extract \textit{D. melanoxylon} may be find good therapeutic use in nephrotoxicity. Further studies are needed to find the exact possible mechanism of the protective effect of the plant extract.

References