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## Original Research Article

Antioxidant and hepatoprotective effects of *Ougeinia dalbergioides* benth against paracetamol and  $\text{CCl}_4$  induced liver damage in ratsSarita Karole<sup>1</sup>, Deepak Kumar Jain<sup>2\*</sup>, Sahil Abbas<sup>3</sup>, Nisha Abbas<sup>3</sup>, Pradeep Kare<sup>3</sup><sup>1</sup>Dept. of Pharmacy, Oriental College of Pharmacy, Bhopal, Madhya Pradesh, India<sup>2</sup>Dept. of Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Central University, Bilaspur, Chhattisgarh, India<sup>3</sup>Dept. of Pharmacy, College of Pharmacy, Agra, Uttar Pradesh, India

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## ABSTRACT

Hepatic diseases are flatter ordinary day by day and pose grave health threats to the life of humans. In sort to treat these diseases, the awareness of man is diverting towards herbal drugs, which are much safer and cost effectual than synthetic drugs. The aspire of this work was to explore antioxidant and hepatoprotective activity of ethanolic and aqueous extract of *Ougeinia dalbergioides* (*O. dalbergioides*) bark for treatment of liver damage induced by  $\text{CCl}_4$  and paracetamol (PCM) in rats. In vitro antioxidant activities aqueous and ethanolic extracts were also checked using 1-1-diphenyl-2-picryl hydrazine (DPPH), nitric oxide (NO), ABTS scavenging method. *O. dalbergioides* at chosen oral doses of 250 mg/kg and 500 mg/kg levels of serum marker enzymes such as ALP, SGPT, SGOT, total bilirubin and direct bilirubin was reduced which indicate major hepatoprotective effects, when contrasted with silymarin and negative control. All the parameters were observed at oral dose of 500 mg/kg with extreme effects. The significant anti-oxidant activity was also demonstrated by the extracts. The preliminary screening of phytochemical *O. dalbergioides* indicates the presence of phenolics, tannins, flavonoids, carbohydrate and phytosterols. It is conceivable that extract of *O. dalbergioides* has activity of hepatoprotective against  $\text{CCl}_4$  and paracetamol induced hepatotoxicity, due to the scavenging of free radical mechanisms demonstrated by phenolics and flavonoids, thus asserting its medicinal therapeutic role in liver diseases.

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## 1. Introduction

The sequence of liver disorders influenced by the oxidative stress.<sup>1</sup> Liver disease prevents by the cellular antioxidant systems by diminishing oxidative stress.<sup>2</sup> Cell membranes, DNA and proteins injured because of high oxidative stress. Thus, it imparts toxicity, like muscular dystrophy and liver cancers. Some adverse effects are related with presently accessible synthetic antioxidants. Consequently, natural antioxidants are being recognized because their improved effectiveness and safety.<sup>3-6</sup> Liver is the major

internal organ of body having hepatocytes which are its functional entities. Liver independently performs up to 500 functions, typically by interacting with other systems and organs. The chief job of liver is to detoxify toxic substances from body and to metabolize ingested substances (nutritional supplements, food, alcohol or medicines). Along with it, liver controls glycogen storage and breakdown of red blood cells; produce plasma proteins and synthesize hormones.<sup>7</sup> The damage produced in liver is hepatotoxicity and it is resulting by chemicals.<sup>8</sup> Hepatotoxicity is also defined as obliteration or malfunction to liver because of xenobiotic or drugs known as hepatotoxins when they are given in overeat.<sup>9</sup> Liver injury

\* Corresponding author.

E-mail address: [jaindeepak2022@gmail.com](mailto:jaindeepak2022@gmail.com) (D. Kumar Jain).

can also be induced as a consequence of oxidative stress which is persuaded by stimulation of enzymes in CYP 450 system like CYP<sub>2E</sub>.<sup>10</sup> Hepatotoxicity injures liver in both gentle and severe forms. The capability of any compound, formulation or product to stop damage to the liver is known as hepato protection or anti-hepatotoxicity. CCl<sub>4</sub> induced hepatotoxicity accrues in parenchymatic cells of liver and cytochrome P450- dependent monooxygenases in liver metabolized it to CCl<sub>3</sub> \* radicals, thus rapidly injured the hepatocytes.<sup>11</sup> Paracetamol is an extensively used analgesic drug but over dosage of its causes hepatotoxicity and nephrotoxicity as it is detoxified in the liver in the form of sulphate or glucuronide which escorts to creation of reactive N-acetyl p-benzoquinonimine metabolite which binds to cellular macromolecules covalently and begin cell damage.<sup>12</sup> Numerous drugs which are synthetic being administered in the executive of liver may have grave side effects, because of which there is an increasing interest to trail universal study method and to evaluate technical basis for conventional natural drugs that are conserved to retain hepatoprotective activity.<sup>13</sup> There is an integer of herbal preparations in Ayurveda for management of hepatic diseases in the deficiency of a dependable liver defensive drug in contemporary remedy.<sup>14</sup> Numerous studies have defensible that phytotherapeutic agents who possess antioxidant activity are effectual in producing hepatoprotection against PCM and CCl<sub>4</sub> induced hepatotoxicity.<sup>15</sup>

## 2. Materials and Methods

### 2.1. Plant material

The barks of *O. dalbergioides* were obtained from the Medicinal Garden of Oriental College of Pharmacy Raisen Rd, Patel Nagar, Bhopal, MP 462022 in March 2019. The barks were identified by Dr. Padma Shrivastava, Professor (Botany), Govt. P.G College, Mathma Gandhi Road, Pipalani, Berkhedha, P.O. Bhel, Huzur (T), Bhopal (Dist.), MP 462022. A receipt sample (Accession no.650) of the genuine *O. dalbergioides* barks has been placed in the herbarium of the College.

### 2.2. Drugs and chemicals

liquid paraffin, CCl<sub>4</sub> and sucrose was procured from Qualigens Fine Chemicals, Mumbai, India. Silymarin, 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), sodium nitroprusside, potassium ferricyanide, ascorbic acid was procured from Sigma Aldrich Company (St. Louis, MO, USA). Paracetamol acquired as gift sample by Ranbaxy Lab Pvt. Ltd. Dewas. Assessment of marker enzymes for liver functions such as SGOT, SGPT, total bilirubin, ALP were performed using kits supplied by Span Diagnostic Ltd., Surat, India. Other required chemicals and reagents

used during experimentations were of analytical grade and accessible commercially via renowned sellers.

### 2.3. Extraction

Parts of herbal plant (barks) of *O. dalbergioides* were dried in gloom at room temperature. The dehydrated barks in shade were coarsely grounded and extracted using soxhlet apparatus with petroleum ether (60-75°C). The process of extraction was stopped after defatting of the material. Initially 250gm of plant powered was packed into the thimble and 2 liters of solvent (Water, Ethanol) used for extraction was poured into flask (Round Bottom) separately. The soxhlet extraction process was executed for 18-24hrs till the obtained liquid in siphon tube shows clear nature. Afterward under low pressure extracted liquid was evaporated to obtain dried powder of extract. At the end % yields of the dried extracts were determined.<sup>16</sup>

### 2.4. Qualitative phytochemical analysis of extract

The *O. dalbergioides* plant extract obtained was subjected to the preliminary phytochemical analysis following standard methods.<sup>17,18</sup> The collected extract was monitored for identification of different active principles such as flavonoids, phenolic compounds, saponins, tannins, alkaloids, glycosides, carbohydrates, fixed oils or fats, amino acids and protein.

### 2.5. Experimental animals

Wistar rats weighing 180-240gm of either sex procured from the central animal house, Pinnacle Biomedical Research Institute (PBRI) Bhopal MP which was used in the experiment. The procured rats were housed in clean, big cages of polypropylene in a specific temperature-controlled room 22±2°C having RH (44-55%) where 12-hr light and dark cycles are maintained. All the rats were adapted to environment of laboratory for at least one week prior to study. In a noise-free room all the experiments were carried out between 08.00 to 15.00 hr. Rats were provided with a clean drinking water ad libitum and standard rodent pellet diet purchased from Lipton India, Bangalore, India. The use and care of experimental animals were severely according to the guidelines given by the Institutional Animal Ethical Committee constituted as per the norms of CPCSEA, India.

#### 2.5.1. Acute oral toxicity

Wistar rats which are healthy, fasted overnight were screened for acute toxicity identification studies for the determination of non-observable adverse effect dose level by using of acute toxic class method of oral toxicity as per guidelines of Organization for Economic Co-operation and Development (OECD) 423. The experimental rats (n=3) were consumed *O. dalbergioides* extract in the

maximum test dose of 2000 mg/kg and detected continually for autonomic profiles, neurological, and behavioral for the period of 2hr and after for 24, 72hr and after that up to 14 days for any moribund state, lethality or expiry of animal. In another group of rats (n=3) the limit test was repeated for estimated LD50 determination and corroboration.<sup>19</sup>

## 2.6. In-vitro antioxidant activity

By using the standard methods, the ethanolic and aqueous extract of *O. dalbergioides* was screened for its in vitro antioxidant activity. The particular conc. Of the obtained extract was used in all these methods. In this, after addition of all reagents it gives a final conc. Of 50-1000 µg/ml. The absorbance (Abs) of the extract or standards, but without the reagents was calculated against a blank solution. Without the extract or standards the control test was executed. IC50 value and % scavenging which is the conc. of the sample obliged for scavenging of 50% of the free radicals was determined.

### 2.6.1. DPPH radical scavenging method

This test was conceded out as given by.<sup>20</sup> In 96-well plate having 2.5ml of various concentrations (50-300 µg/ml) of the extract or standards, an aliquot of 0.5ml of 0.1mM DPPH in methanol was added. At room temperature mixing of reaction mixture is carried out and detained for 20min. The Abs was checked at 517nm against a blank. By comparing Abs with that of a blank solution the scavenging activity of each fraction of free radical was determined. By using following equation the capability of DPPH radical scavenging was calculated which is expressed as % inhibition:

$$2.7. \text{ DPPH scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where  $A_1$  is the Abs of the sample and  $A_0$  is the Abs of the control.

### 2.7.1. NO radical inhibition assay

NO was measured by using Griess reaction which is created from sodium nitroprusside (SNP). At physiological pH in aqueous solution the SNP impulsively creates NO which interrelates with oxygen to yield nitric ions which is determined by Griess reagent. NO scavengers compete with the oxygen leading to declined production of nitric oxide. The mixture of reaction (6ml) containing phosphate buffer saline (PBS, pH 7.4, 1ml), SNP (10mM, 4ml) and standard solution or extract (1ml) at different concentrations which was incubated at 25°C for 150min. 0.5ml of the reaction mixture containing nitrite ion was removed after incubation process, 1ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and kept aside for 5min. After completion of diazotization reaction, 1ml of NEDD was added, stirred and kept aside for 30min in diffused

light. The Abs of sample was measured at 540nm against the blank solutions.<sup>21</sup>

### 2.7.2. ABTS radical scavenging activity

To 0.2ml of different concentrations of standard or extract, 0.16 ml of ABTS solution and 1.0ml of distilled DMSO were added. The reaction mixture was mixed homogeneously and incubated for at least 20 min. Abs of these samples were measured at 734nm spectrophotometrically.<sup>22</sup>

## 2.8. In vivo hepatoprotective activity

### 2.8.1. CCl<sub>4</sub> induced hepatotoxicity in rats

The dilution of CCl<sub>4</sub> was carried out using liquid paraffin (1:1) prior to consumption. The experimental rats were alienated into 7 groups, every group containing 6 rats. The rats were then screened to either one of the following treatments for next 9 days

1. **Group 1:** Control; distilled water (1ml/kg, po)
2. **Group 2:** Toxicant; distilled water for 9 days + CCl<sub>4</sub> (1ml/kg, po) on ninth day.
3. **Group 3:** Standard; silymarin (100mg/kg/day, po) for 9 days + CCl<sub>4</sub> (1ml/kg po) on ninth day.
4. **Group 4:** Test; aqueous extract (250mg/kg/day, po) for 9 days + CCl<sub>4</sub> (1ml/kg po) on ninth day.
5. **Group 5:** Test; aqueous extract (500mg/kg/day, po) for 9 days + CCl<sub>4</sub> (1ml/kg po) on ninth day.
6. **Group 6:** Test; ethanolic extract (250mg/kg/day, po) for 9 days + CCl<sub>4</sub> (1ml/kg po) on ninth day.
7. **Group 7:** Test; ethanolic extract (500mg/kg/day, po) for 9 days + CCl<sub>4</sub> (1ml/kg po) on ninth day.

Rats were fastened 12 hr before CCl<sub>4</sub> administration to increase the acute liver damage in rats of groups 2-7. The rats were sacrificed after the 24hr of administrations of CCl<sub>4</sub>. Blood samples from sacrificed rats were collected and the serum was used for assay of various marker enzymes such as SGOT, SGPT, ALP, and serum bilirubin.<sup>23</sup>

### 2.8.2. PCM induced liver damage in rats

The similar procedure was followed which is mentioned above other than the liver was damage using PCM (1g/kg po) which is diluted with sucrose solution (40% w/v). PCM was consumed in 3 equal doses on day 9 and animals were sacrificed after the 48hr of consumption of PCM. Blood sample from animals were collected and serum was used for assay of marker enzymes such as SGOT, SGPT, ALP, and serum bilirubin.<sup>23</sup>

### 2.8.3. Biochemical studies

At the 9<sup>th</sup> day of the experimental studies, blood sample was withdrawn by using micro-capillary method from the retro-orbital plexus of rat by giving light ether anesthesia. A glass capillary is inserted by using a slight thumb pressure

into the medial canthus of the eye (30 degree angle to the nose) to enter the plexus/sinus by puncturing tissues. When the plexus is punctured, blood will withdraw through the glass capillary tube which was collected in 1.5ml Eppendorff tubes. The glass capillary tube is then softly removed and wiped with sterile cotton. Bleeding can be blocked by applying light finger pressure on the eye. The collected blood samples were allowed to clot for 45min at RT. Serum was separated from blood by centrifugation at 2500 rpm for 15 min at 30°C and used for the determination of different biochemical parameters namely SGOT, SGPT, ALP and serum bilirubin using semi-autoanalyser.<sup>24-28</sup>

### 2.9. Statistical analysis

The multiple comparisons with the Dunnett-t-test were followed by one-way analysis of variance (ANOVA) to compare various parameters between the groups. A p value  $P < 0.01$  was considered significant.

## 3. Results

The outcomes of qualitative analysis of phytochemicals of the raw powder barks of *O. dalbergioides* are exposed in Table 1. Ethanolic and water extracts of barks of *O. dalbergioides* indicates the occurrence of phenolics, flavonoids, carbohydrate, tannins and Phytosterols. ABTS and scavenging activity of DPPH radical of aqueous and ethanolic extract of *O. dalbergioides* was given in Table 2. The extract showed potent activity against radical scavenging in the both methods in conc. dependent way (50-300 µg/ml). The value of IC<sub>50</sub> of extract was equivalent to the standards (ascorbic acid) used. The aqueous extract showed lower IC<sub>50</sub> value than ethanolic extract in both models. Ethanolic extract of *O. dalbergioides* demonstrated a strong activity of nitric oxide scavenging which was equivalent to aqueous extract of *O. dalbergioides*. The IC<sub>50</sub> value of ethanolic extract of *O. dalbergioides* was less than aqueous extract of *O. dalbergioides* but close to ascorbic acid. IC<sub>50</sub> values of standards and extract were shown in Table 2. Acute oral toxicity studies illustrated that the extract of *O. dalbergioides* was secure up to a dose of 2,000 mg/kg of body weight and NOAEL dose is greater than 2,000 mg/kg. No death or moribund state or any toxic reactions were seen up to the end of the observation time period of 14 days. Alterations in the activities of serum enzymes ALP, SGOT and SGPT and total bilirubin in the serum of PCM (1gm/kg po) and CCl<sub>4</sub>-induced (1ml/kg po) liver injuries in experimental rats as confirmation from Table 3, 4. The serum marker enzyme levels ALP, SGOT, SGPT and bilirubin were shows considerable increase in CCl<sub>4</sub> and PCM — induced liver injuries in rats when contrasted with control group ( $P < 0.01$ ). Although, management with aqueous and ethanolic extract at oral dose 250 and 500 mg/kg for 9 days showed

noteworthy hepatoprotective activity in terms of serum ALP, SGOT, SGPT and level of total bilirubin in CCl<sub>4</sub> and PCM-induced liver injuries in experimental rats as contrasted to control groups. Silymarin (100mg/kg) also major decline in the serum enzymes level and bilirubin level in PCM and CCl<sub>4</sub>-treated groups as contrasted with respective control group.

**Table 1:** Phytochemical evaluation of *O. dalbergioides* bark extracts

| S. No. | Constituents             | Ethanol extract | Aqueous extract |
|--------|--------------------------|-----------------|-----------------|
| 1.     | Alkaloids                | -               | -               |
| 2.     | Glycosides               | -               | -               |
| 3.     | Flavonoids               | +               | +               |
| 4.     | Saponins                 | -               | -               |
| 5.     | Phenolics                | +               | -               |
| 6.     | Proteins and amino acids | -               | -               |
| 7.     | Carbohydrate             | +               | +               |
| 8.     | Tannins                  | +               | -               |
| 9.     | Phytosterols             | +               | -               |

## 4. Discussion and Conclusion

Free radicals are defined as the chemical species which holds one or more unpaired electrons. They are extremely unstable and cause destruction of other molecules by removing electrons from them to conquer stability. They are formed into the system and are potentially damaging, highly reactive and fleeting chemical species. These radicals are necessary for detoxification, chemical signaling, energy supply and immune function therefore they are incessantly generated in the body of human. Endogenous antioxidant enzyme system regulates free radicals, but because of more generation of free radicals by contact to ecological oxidant elements such as UV radiation, smoking of cigarette etc or a malfunction in mechanism of antioxidant defense or impairment to cellular structures, the risk enhance for numerous disorders such as liver diseases, cardiovascular disorders, ulcerative colitis, mild congestive impairment, parkinson's disease, alzheimer's disease, cancer and inflammation.<sup>29</sup> Fascinatingly, the human body has defense mechanisms counter to oxidative stress induced by free radical, which includes repair and defensive mechanisms, i.e. physical and antioxidant defense. Enzymatic antioxidants like catalase, glutathione peroxidase, superoxide dismutase, etc as well as non-enzymatic antioxidants like ascorbic acid, phenolic compounds, flavonoids, carotenoids, etc act by one or more than one mechanisms like scavenging of free radicals, reducing activity, quenching of singlet oxygen and potential complexing of pro-oxidant metals. It is potential to decrease the threats of chronic disorders and prevention of disease progression by either increasing the

**Table 2:** DPPH, ABTS, NO radical scavenging activity of *O. dalbergioides* extract (mean±SEM)

| IC50 (µg/ml)*<br>Drug/Std. | DPPH radical scavenging activity |              | ABTS radical scavenging assay |              | Nitric oxide radical scavenging assay |              |
|----------------------------|----------------------------------|--------------|-------------------------------|--------------|---------------------------------------|--------------|
|                            | Aq. Extract                      | Eth. Extract | Aq. Extract                   | Eth. Extract | Aq. Extract                           | Eth. Extract |
| <i>O. dalbergioides</i>    | 230.02±4.23                      | 249.98±5.88  | 175.66±2.55                   | 225.15±3.64  | 263.72±2.42                           | 215.03±5.86  |
| Ascorbic acid              | 198.08±1.02                      | 198.08±1.02  | 159.97±3.38                   | 159.97±3.38  | 200.42±5.90                           | 200.42±5.90  |

\*Mean of three determinations

**Table 3:** Effect of aqueous and ethanol extract of *O. dalbergioides* on enzyme SGOT, SGPT, ALP and total bilirubin levels in serum of CCl<sub>4</sub> induced hepatotoxicity (Each values represent the Mean ± SEM)

| Treatment                          | Dose (po, mg/kg) | SGOT(U/L)     | SGPT(U/L)     | ALP(U/L)      | Direct Bilirubin(mg/dl) | Total Bilirubin(mg/dl) |
|------------------------------------|------------------|---------------|---------------|---------------|-------------------------|------------------------|
| Vehicle control                    | —                | 96.02±1.79**  | 81.53±3.30**  | 224.85±1.90** | 0.11±0.03**             | 1.5±0.13**             |
| CCl <sub>4</sub> Control           | —                | 309.69±1.96   | 250.75±4.72   | 479.54±4.75   | 2.27±0.06               | 3.47±0.16              |
| CCl <sub>4</sub> + Silymarin       | 100              | 124.32±3.10** | 100.58±0.73** | 258.43±5.90** | 0.64±0.19**             | 2.18±0.09**            |
| CCl <sub>4</sub> + Aqueous extract | 250              | 198.05±4.14** | 180.06±2.45** | 387.66±3.03** | 1.9±0.03**              | 3.02±0.11**            |
| CCl <sub>4</sub> + Aqueous extract | 500              | 158.05±5.15** | 121.08±3.49** | 295.96±4.00** | 1.3±0.04**              | 2.96±0.04**            |
| CCl <sub>4</sub> + Ethanol extract | 250              | 187.34±3.27** | 175.3±1.68**  | 389.21±1.88** | 1.85±0.20**             | 3.86±0.01**            |
| CCl <sub>4</sub> + Ethanol extract | 500              | 147.94±3.28** | 111.3±1.79**  | 289.41±2.89** | 0.85±0.10**             | 2.86±0.02**            |

(n=6). \*\* P&lt; 0.01)

**Table 4:** Effect of aqueous and ethanol extract of *O. dalbergioides* on enzyme SGOT, SGPT and total bilirubin levels in serum of paracetamol induced hepatotoxicity (Each values represent the Mean ± SEM)

| Treatment             | Dose (po, mg/kg) | SGOT(U/L)  | SGPT(U/L)     | Total Bilirubin(mg/dl) | Direct Bilirubin(mg/dl) |
|-----------------------|------------------|------------|---------------|------------------------|-------------------------|
| Vehicle control       | —                | 130±2.08** | 52.39±0.41**  | 1.14±0.02**            | 0.62±0.03**             |
| PCMControl            | —                | 275.1±1.82 | 163.12±1.57   | 3.35±0.13              | 2.57±0.02               |
| PCM+Silymarin         | 100              | 145±1.26** | 65.04±0.52**  | 1.17±0.01**            | 1.75±0.03**             |
| PCM + Aqueous extract | 250              | 250±1.26** | 153.25±1.54** | 3.02±0.01**            | 1.79±0.03**             |
| PCM + Aqueous extract | 500              | 212±1.29** | 81.29±1.57**  | 2.74±0.02**            | 1.32±0.02**             |
| PCM+ Ethanol extract  | 250              | 258±2.41** | 125.12±0.33** | 2.98±0.01**            | 1.64±0.01**             |
| PCM+ Ethanol extract  | 500              | 209±2.91** | 75.19±0.73**  | 2.22±0.01**            | 1.29±0.03**             |

(n=6). \*\* P&lt; 0.01)

body's natural antioxidant protection or complementing with established antioxidants. Therefore detection of herbal antioxidants is a major thrust area.<sup>30</sup> Different in vitro models were used to study antioxidant activity of the aqueous and ethanolic extract of *O. dalbergioides*. Subsequently, free radicals are of various chemical bodies, to corroborate its antioxidant activity it is important to test extract against several free radicals. IC<sub>50</sub> values found were compared with the standard samples. ABTS radical scavenging activity is reasonably new method, which includes a severe radical, chemically generated and is regularly used to screen versatile mixtures of antioxidant like plant beverages, extracts and biological fluids. The capability in aqueous and organic media and the stability in a large pH range increased concentration in the use of ABTS<sup>+</sup> for judgment of antioxidant activity.<sup>31</sup> In ABTS method, the extract disclosed efficient antioxidant activity which is equal to the standard. Here, direct role of phenolic compounds in free radical scavenging was shown by the extract's radical scavenging activity. The DPPH has been widely accepted, stable free radical, which is used as tool for determining free radical scavenging activity of antioxidants. DPPH accepts hydrogen radical or an electron to become a stable diamagnetic fragment.<sup>32</sup> The reduction ability of DPPH radical is estimated by the reduction in absorbance at 517 nm persuaded by antioxidants. The investigational informations of the extract exposed that the extract have the free radicals scavenging activity. From the outcomes we observe a relationship in the DPPH radical scavenging activity which is dose dependent. The contribution of free radicals, mainly their enhanced generation, seems to be a characteristic of most of the human disorders including cancer and heart diseases. It has been established that flavonoids, tannins, tocopherols, ascorbic acid, cysteine, aromatic amines and glutathione decrease and by their hydrogen donating ability it decolorize the DPPH. Phenolic compounds and Flavonoids of *O. dalbergioides* extracts are perhaps intricate in its activity of antiradical.<sup>33</sup> Nitric oxide produced during its reduction in presence of oxygen or with various superoxides, such as N<sub>2</sub>O<sub>4</sub>, NO<sub>2</sub>, N<sub>3</sub>O<sub>4</sub> is highly reactive. These radicals are accountable for changing the functional performance and structure of numerous components of cell. The *O. dalbergioides* extracts showed improved activity with oxygen to react with nitric oxide and thus blocks the production of anions and the activity is comparable to the standards. The secondary metabolites of the plant may have properties to offset the outcome of nitric oxide generation and in turn may be significantly interested in avoiding the adverse effects of extreme nitric oxide production in the human body. Additionally scavenging activity also helps to arrest the reactions or chains initiate by surplus formation of nitric oxide that is hazardous to the health of human. Nitric oxide is also concerned for cancer, inflammation

and other diseases. In the appraisal of liver injury by CCl<sub>4</sub> and PCM hepatotoxin, the estimation of levels of different enzymes such as SGOT and SGPT is mainly used. Membrane damage or necrosis discharges the enzyme into blood circulation; therefore, it can be determined in serum. Increased levels of SGOT shows liver injuries like viral hepatitis as well as cardiac infarction and muscle damages. SGPT catalyses the alteration of alanine to glutamate and pyruvate which is released in a similar way. So, SGPT is highly specific to the liver, thus better parameter for identifying liver damages.<sup>34,35</sup> Our outcomes using the model of CCl<sub>4</sub> and PCM — induced liver toxicity in the rats proved that *O. dalbergioides* extracts at oral dose 250 and 500 mg/kg caused remarkable reduction of SGOT and SGPT levels. Bilirubin and ALP levels in serum are related to the function of liver cell. Increase in serum level of ALP is due to increased synthesis in presence of increasing biliary pressure.<sup>36</sup> Our results confirmed that *O. dalbergioides* extracts at 250 and 500 mg/kg caused noteworthy inhibition of ALP and bilirubin levels. Efficient manage of bilirubin level and alkaline phosphatase activity points towards an early development in the secretory mechanism of the hepatic cell. In present years, interest has been focused on the role of biotransformation of chemicals to highly reactive metabolites that begin cellular toxicity. Many compounds, including clinically useful drugs can cause cellular damage through metabolic activation of the chemical to extremely reactive compounds such as free radicals, carbenes and nitrenes. CCl<sub>4</sub> has perhaps been studied more expansively both biochemically and pathologically than any extra hepatotoxin. CCl<sub>4</sub> hepatotoxicity depends on the reductive dehalogenation of CCl<sub>4</sub> catalysed by Cyt 450 in the liver cell endoplasmic reticulum foremost to the generation of an unstable complex CCl<sub>3</sub> radical. This trichloromethyl radical reacts quickly which is reported as an extremely reactive species. These free radicals assault microsomal lipids leading to its peroxidation and also covalently attach to microsomal lipids and proteins eventually initiating a site of secondary biochemical processes which is the final cause for the unfolding of the panorama of pathological consequences of CCl<sub>4</sub> metabolism.<sup>37</sup> Phytochemical investigated on *O. dalbergioides* have reported the presence of hydroxylupeol, betulin, lupeol and isoflavanones such as homoferreirin, dalbergioidin and ougenin.<sup>38,39</sup> A number of scientific reports indicated certain flavonoids, triterpenoids and steroids have defensive effect on liver due to its antioxidant properties.<sup>40–42</sup> Presence of those compounds in *O. dalbergioides* may be accountable for the defensive effect on PCM and CCl<sub>4</sub>-induced liver damage in rats. In conclusion, the results of this study reveal that *O. dalbergioides* has a potent hepatoprotective action upon PCM and CCl<sub>4</sub>-induced hepatic damage in rats. Our results demonstrate that the hepatoprotective effects of *O. dalbergioides* may be due to its antioxidant and free

radical scavenging properties. Additional, investigation is in progress to determine the precise phytoconstituents that is accountable for its hepatoprotective effect.

## 5. Source of Funding

None.

## 6. Conflict of interest

None.

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**Deepak Kumar Jain**, Associate Professor

**Sahil Abbas**, Associate Professor

**Nisha Abbas**, Assistant Professor

**Pradeep Kare**, Research Scholar

## Author biography

**Sarita Karole**, Professor

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