Evaluation of Antioxidant Activity of Ocimum canum Hydro-alcoholic Leaf Extract in the Prevention of Hepatic Ischaemia

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Author(s): Behera S, Panigrahi R, Ramani Y, Babu S, Choudhury PK

Abstract

The hydro-alcoholic leaf extract of Ocimum canum (OC) – Kala Tulsi was studied for its antioxidant and hepatoprotective activity. The in vitro antioxidant models employed were DPPH (di (phenyl)-(2, 4, 6-trinitrophenyl) iminoazanium) radical scavenging assay and hydroxyl radical scavenging activity which proved the plant to be rich in antioxidants. The study was carried out at different concentrations (250, 500, 1000, 2000 µg/ml) and was compared with the control Ascorbic Acid. Further the plant’s antioxidant potential was studied by using an in vivo method to prove its potency in preventing ischemia by incorporating hepatic ischemia-reperfusion in albino rats. Ocimum canum (OC) hydro-alcoholic leaf extract were administered in doses of 100, 200 and 300 mg/kg/day, orally for 29 days before I/R injury respectively and repeated before the reperfusion period. After the experimental period all rats were sacrificed and antioxidant defense system and oxidative stress in hepatic tissue was investigated. Liver samples were taken for histological examination and determination of hepatic malondialdehyde (MDA) and super oxide dismutase (SOD) activity. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined to assess liver functions. Lactate dehydrogenase (LDH) was assayed in serum samples for the evaluation of generalized tissue damage. Ischemia/reperfusion caused a significant decrease in hepatic SOD and significant increase in MDA levels. Serum AST and ALT levels, as well as LDH activity levels were also elevated in the I/R group. Treatment with OC hydro-alcoholic leaf extract reversed all these biochemical parameters as well as histological alterations induced by I/R. In all the in vitro testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals. Simultaneously, all in vivo tests showed a significant correlation between concentrations of the extract and alteration in the biochemical and histological parameters. These findings suggest that the hydro-alcoholic extracts are able to scavenge free radicals, and can therefore act as primary antioxidants. OC hydro-alcoholic leaf extract reduced I/R-induced organ injury through its ability to balance the oxidant–antioxidant status. The results of present study indicate that the hydro-alcoholic leaf extract of Ocimum canum has significant antioxidant activity and can prevent ischemia.

Introduction

Oxidative stress associated injury is a direct result of an imbalance between an increase in ROS production and a decrease in antioxidant reserve under various pathological processes. Ischemic injury occurs when there is reduced blood supply or complete occlusion of an artery. The causes for ischemic insults vary from organ to organ, and rupture of atherosclerotic plaques with resultant formation of thrombi represents a major cause for acute ischemic injury in the heart, brain, lung, intestinal tract and other organs. Intermittent constriction or compression from the outside of vessels also causes a reduction or cessation of blood supply. Lung, heart and liver transplantation remains the only effective therapy for end-stage lung, heart or liver diseases. Ischemic insults occur as results of a variety of conditions, leading to an accumulation of reactive oxygen species (ROS) and an imbalanced redox status in the tissues [1, 2]. The oxidant stress may activate signaling mechanisms provoking more toxic events, and eventually causes tissue damage. Reactive oxygen species (ROS) are largely generated from mitochondrial energy metabolism via oxidative phosphorylation in the respiratory chain of eukaryotes. Because of the existence of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, and antioxidants, such as the reduced form of glutathione (GSH), as well as vitamin C and E, the redox balance is well maintained. Upon injurious insults including, inflammation, drugs, alcohol intake, or environmental pollutants, there is increased production of superoxide anion (O2) or other ROS from various sources resulting in the disturbance of this delicate balance. The increase in ROS consumes endogenous antioxidant compounds, such as GSH,
and induces expression of antioxidant enzymes in order to maintain the redox balance [3, 4]. When the injury is pronounced or persistent, compensatory responses become inadequate to correct the imbalance redox state, giving rise to oxidant stress, with activation of subsequent signaling events leading to inflammatory responses and tissue damage. Cardiac, cerebral, pulmonary or intestinal ischemic attacks often take place secondary to arterial thrombosis or emboli from other sites. In these cases, enhanced oxidant stress exists along with chronic pathologic changes within the involved vascular wall and surrounding tissue. In the event of reperfusion (I/R)-induced donor organ damage, oxidant stress depends on the donor conditions (living donor or cadaveric), preservation method and duration, the match of tissue typing, as well as the complexity of surgical procedure of implantation [5-8]. More profound oxidant stress usually occurs when the blood supply is re-established for either ischémic tissue or implanted grafts. Thus, oxidant stress represents one of the major causes of ischemic injury, and antioxidant therapy may ameliorate the injury when it is properly delivered during an optimal time window and at right doses. A variety of antioxidants, scavengers, or scavenger mimetics have been evaluated in various ischemic conditions. Therefore, treatments with antioxidants, free radical scavengers and their mimetics, as well as gene transfer approaches to over express antioxidant genes represent potential therapeutic options to correct the redox imbalance.

Antioxidant enzymes play a fundamental role in maintaining the delicate redox balance in the body and are essential in keeping the physiological function and in coping with oxidant stress from endogenous or exogenous sources [9-11]. Two major approaches have been developed to treat ischemic stroke: recanalization and neuro-protection. At present, alteplase, recombinant tissue-type plasminogen activator (rt-PA) is the only approved therapy for acute ischemic stroke. Among more than 700 drugs which have been studied and found to be effective in animal stroke models, yet none has been proven efficacious on the basis of a positive phase III trial except a new free-radical tapering agent, NXY-059. Antioxidant therapy at an early stage is considered as an adjuvant regimen in a variety of ischemic disorders [12].

Natural products are important sources for biologically active drugs. There has been an increasing interest in the medicinal plants as natural products in different parts of the world [13]. Medicinal plants containing high antioxidant properties play an important role in the prevention of various degenerative diseases in the society. The medicinal value of these plants depends on bioactive phytochemical constituent’s action in the human body.

Plants of genus Ocimum belong to family Lamiaceae and are collectively called as Basil are a diverse and rich source of aromatic essential oil. The species such as Ocimum basilicum L., O canum Sims., O gratissimum L. and Ocimum sanctum L. are composed of phenyl-propenes e.g. eugenol, methyl eugenol and methyl chavicol [14]. Two types of flavonoids are also present in this Ocimum species. Lipophilic flavonoid glycosides (external flavonoids), often highly methylated, are found in glandular hairs on the surface of the leaves, stems and inflorescences. These have been the subject of two recent studies [15, 16]. The second type of flavonoids is polar flavonoid glycosides, which are stored in the vacuoles of aerial plant parts. Nevadensin and salvigenin are found in O. canum Sims [17].

Ocimum canum Sims (Hairy Basil) is a traditional medicinal plant which distributes throughout Odisha and it is commonly known as Kala Tulasi in Odiya has an unusual mint-like flavor. The plant branches out from its base, with angle stems and open foliage. The plant shows a pungent, aromatic flavor and is commonly cultivated for culinary purposes. O. canum is used specially for treating various types of diseases and lowering blood glucose and also treats cold, fever, parasitic infestations on the body and inflammation of joints and headaches [18]. Essential oil from the leaves of O. canum possesses antibacterial and insecticidal properties [19]. It is used in ritual as an incense as well to protect the home and welcome newborns into the world. It is an unusual and very useful addition to the medicinal garden. The hairy leaves and decorative flowers are very aromatic and form a lush mound about 2 feet in height. This annual plant grows well in full sun, well-drained soil and plenty of heat. The plant branches from the base and has an angled stems and oval pubescent leaves. Its leaves are tiny and fuzzy and have violet or white flowers, having a sweet scent resembling that of the clove. The leaves of the Ocimum canum are opposite and toothed. It is irregular and occurs in crowded whorls. The Ocimum canum has a small corolla. These plants have intense floral-fruity aromas. The oil of the Ocimum canum is composed of Linalool. The seeds may provide fiber or reduce constipation. Medicinal properties may be due to the presence of the external flavonoids, as some specimens produce very high levels of these compounds, especially nevadensin [16],...
which has antioxidant activity [20]. Keeping in view the above data and literature support the present work aims at exploring the possible role of plant based antioxidants in imparting protection against ischemia employing in vitro and in vivo methods. The present study, therefore, investigated the protective effect of *Ocimum canum* hydro-alcoholic leaf extract against oxidative stress during I/R injury of the liver, by measuring biochemical values and conducting histological examinations.

**Materials and methods**

**Method:** The experimental protocols were conducted with the approval of the Animal Research Committee at Royal College of Pharmacy and Health Sciences, Brahmapur. Odisha. All animals were maintained in accordance with the recommendations of the CPCSEA.

**Drugs and Chemicals:** Hydrogen peroxide, Ascorbic acid, DPPH, Potassium persulphate, H2SO4, Potassium Iodide, Mercuric Chloride, Bismuth Carbonate, Glacial acetic acid are purchased from Nobel Enterprises, Brahmapur, Odisha.

**Animals:** Adult rats of either sex (150-200gm) were obtained from the animal house of R.C.P.H.S. and were housed and divided into 5 groups containing 6 animals each. All the experimental procedures and protocols used in this study were reviewed and approved by Institutional Animal Ethical Committee.

**Preparation of Plant Extracts:** The dried leaves were coarsely powdered and extracted with a mixture of methanol:water (7:3, v/v) by a Soxhlet apparatus at 50°C. The solvent was completely removed and obtained dried crude extract which was used for investigation. Further the extracts were subjected for the phytochemical study as well as pharmacological screening.

**In vitro Antioxidant Study:** *Ocimum canum* hydro-alcoholic leaf extract was tested for its antioxidant activity using two different in vitro models as follows at concentrations of 250, 500, 1000 and 2000 µg/ml.

1. **DPPH Radical Scavenging Activity (517 nm):** To the Methanol solution of di (phenyl) - (2, 4, 6 - trinitrophenyl) iminoazanium i.e. DPPH (1 mM) an equal volume of the extract dissolved in alcohol was added at various concentrations from 250 to 2000 µg/ml in a final volume of 1.0 ml. An equal amount of alcohol was added to the control. After 20 min, absorbance was recorded at 517 nm. Experiment was performed in triplicate [21].
2. **Hydroxyl free radical scavenging method (Deoxyribose degradation assay) (532nm):** The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al.,* (1987) [22]. Stock solutions of EDTA (1mM), FeCl3 (10 mM), ascorbic acid (1mM), H2O2 (10mM) and deoxyribose (10mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 ml of EDTA 0.01 ml of FeCl3, 0.1 ml of H2O2, 0.36 ml of deoxyribose, 1.0 ml of plant extract (250-2000 µg/ml), 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1hr. About 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA to develop the pink chromogen, measured at 532 nm. The hydroxyl radicals scavenging activity was calculated using the following equation:-

\[
\% \text{Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where, \(A_0\) was the absorbance of the control (blank) and \(A_1\) was the absorbance in the presence different concentrations of the extract.

**In vivo Evaluation:** Hepatic Ischemia in the Rat (Yuxin Chen., *et al*) [23] Hepatic Ischemia (HI) / Reperfusion Injury in Rats were performed.

Under anesthesia, a midline laparotomy was made using minimal dissection. The abdomen was shaved and a transverse incision was performed. The bowel loops were covered with saline-soaked gauze. Total hepatic ischemia was induced for 45 min by clamping the hepatic artery, the portal vein and the bile duct using a vascular clamp and the rats were then allowed to reperfuse for 60 min. Abdominal incision was closed in layers with 4-0 dexon and 2-0 nylon during reperfusion stage in order to prevent the loss of body fluid and quantity of heat.

Animals were divided into seven groups consisting of six rats each. *Ocimum canum* (OC) hydro-alcoholic leaf extract was dissolved in water and administered to the animals. *Ocimum canum* (OC) hydro-alcoholic leaf extract used in this study contains external flavonoids, especially nevadensin.
Group-I: NAIVE-Normal control-rats in this group did not undergo ischemia or reperfusion and served as the control group.

Group-II: SHAM-Sham-operated (animals subjected to the identical procedure of surgery without ischemia-reperfusion injury) plus physiologic saline treatment.

Group-III: I/R-Animals subjected 45 minutes of total hepatic ischemia, followed by reperfusion for 60 mins and served as untreated experimental control.

Group-IV: OC control- Sham operated plus Ocimum canum control (400 mg/kg body wt. treatment up to 15 days).

Group-V: OC 100mg/kg + I/R- Hepatic I/R plus Ocimum canum hydro-alcoholic leaf extract 100 mg/kg body wt. treatment up to 15 days

Group-VI: OC 200mg/kg + I/R- Hepatic I/R plus Ocimum canum hydro-alcoholic leaf extract 200 mg/kg body wt. up to 15 days.

Group-VII: OC 300mg/kg + I/R- Hepatic I/R plus Ocimum canum hydro-alcoholic leaf extract 300 mg/kg body wt. up to 15 days.

None of the animals died during these procedures. At the end of the reperfusion period, animals were decapitated and trunk blood samples were collected to determine serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activity, the indicators of liver functions and generalized tissue damage, respectively. The hepatic tissue samples were stored at –20°C. Afterwards, tissue malondialdehyde (MDA) levels, an end product of lipid peroxidation, superoxide dismutase (SOD), the key endogenous antioxidant, were measured in these samples. The hepatic tissue samples were also placed in formaldehyde (10%) for histological evaluation.

Biochemical Measurements:
1. Measurement of serum index of hepatotoxicity
   Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activity was measured using commercial kits (Crest Biosystems, Bambolim Complex, Goa, India) and the results are expressed in international units per liter [24].
2. Measurement of hepatic oxidative stress markers
   a. Lipid peroxidation was used as an indirect measure of oxidative damage induced by ROS (free radical-induced injury). Lipid peroxidation was assayed as the malondialdehyde (MDA) level in liver homogenate by the thiobarbituric acid method using tetraethoxypropane as the standard [25]. A mixture of 8.1% sodium dodecylsulphate (0.2 ml, Merck), 20% acetic acid (1.5 ml), and 0.9% thiobarbituric acid (1.5 ml, Merck) was added to 0.2 ml of 10% tissue homogenate. Distilled water was added to the mixture to bring the total volume to 4 ml. This mixture was incubated (95°C, 1 hr). After incubation, the tubes were placed in cold water and 1 ml of distilled water plus 5 ml of n-butanol/pyridine (15:1, v/v) was added, followed by mixing. The samples were centrifuged (4,000 x g, 10 min). The organic phase (supernatant) was removed, and absorbances were measured with respect to a blank at 532 nm. 1, 1, 3, 3-Tetraethoxypropane was used as the standard. Lipid peroxide levels were expressed as nmol MDA/g of wet tissue.
   b. The hepatic antioxidant activity, superoxide dismutase (SOD), was assessed in the homogenized liver by the method of Sun et al [26]. [0.3 mM xanthine, 0.6 mM Na₂EDTA, 0.15 mM nitroblue tetrazolium (NBT), 0.4 M Na₂CO₃, and 1 g/L bovine serum albumin (BSA)] was added 100µl of the tissue supernatant. Xanthine oxidase (50µl, 167 U/L) was added to initiate the reaction and the reduction of NBT by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system, was determined by measuring the absorbance at 560 nm. Cu,Zn-SOD activity was expressed as units of SOD/mg of tissue protein, where 1 U is defined as that amount of enzyme causing half-maximal inhibition of NBT reduction To 2.45 ml of assay reagent In addition, the total protein content in the liver tissues was determined according to the Lowry’s method [27].

Histological Procedures:
Liver specimens from all groups were rapidly taken and fixed in Bouin’s solution and processed for light microscopic study using hematoxylin and eosin stain [28]. For light microscopic investigations, hepatic tissue specimens were fixed in 10% formaldehyde, dehydrated in alcohol series, clearing in toluene and embedding in paraffin. Paraffin sections (5 µm) were stained with hematoxylin and eosin (H&E) and examined under a photomicroscope. All tissue sections were examined microscopically for the characterization of histopathological changes by an experienced histologist in blind fashion (Procedures were carried out in NIDAN diagnostics, Berhampur).
Odisha, INDIA).

**Statistical Analyses:**
Results are presented as the mean ± SEM. All statistical analyses were performed using Graph Pad Prism Software program (version 5.04) [29]. In vitro data were statistically by One way analysis of variance (ANOVA) followed by Student’s t-test. Values are expressed in Mean ± SEM (P< 0.05). In vivo data were analyzed using analysis of variance followed by Bonferroni’s post-test. The Kruskal-Wallis 1-way analysis of variance by ranks was used to simultaneously test the pathologic score for the I/R and I/R ± Ocimum canum groups. A P value of < 0.05 was considered statistically significant.

**Results**

Previously done phytochemical screening of the extracts revealed the presence of carbohydrates, flavonoids and tannins.

The DPPH test provides information on the activity of the test compounds with a stable free radical. This assay determines the scavenging of stable radical species of DPPH by antioxidants. The degree of reduction in absorbance measurement by Ocimum canum is indicative of the radical scavenging (antioxidant) power of the plant. The hydroxyl radical scavenging activity is measured as the percentage inhibition of hydroxyl radicals generated in the Fenton’s reaction mixture by studying the competition between deoxyribose and the extract for hydrogen radicals generated from Fe²⁺-ascorbate/EDTA/H₂O₂ systems. The hydroxyl radicals attack deoxyribose which eventually results in TBARS formation. From the present results, it is observed that the leaf extract of O. canum have good hydroxyl radical scavenging activity as reflected in terms of percentage inhibition.

This study showed no significant difference between the biochemical measurements of group 1 and group 2. Hepatic I/R rats (group 3) showed a significant elevation of serum index of hepatotoxicity (ALT). ALT, AST and ALP levels were significantly higher in the I/R group when compared with those of the control group (p < 0.001). OC treatment reversed these values significantly.

Similarly, in the I/R group, increased lactate dehydrogenase activity, as an index of generalized tissue damage, was reversed significantly by OC treatment (p < 0.01) (Fig.4).

The liver MDA, which is an index of tissue lipid peroxidation, was found to be significantly higher in the I/R group (27.70±2.51 nmol/g), however treatment with OC decreased the elevated MDA level significantly back to the control level (17.28±2.87 nmol/g protein) (Fig. 5A). The levels of liver SOD lowered significantly after hepatic I/R compared with the sham group (SOD: 30.45±3.19 U/g protein vs 20.43±2.44 U/g protein, P = 0.000), after administration of Ocimum canum hydro-alcoholic leaf extract 100, 200 and 400mg/kg, SOD activity in liver was elevated (20.43±2.44 U/g protein vs 25.37±2.48 U/g protein, 27.68±2.86 U/g protein, 31.75±2.37 U/g protein, P = 0.706, P = 0.014, P = 0.014) (Figure 5B).

After 60 minutes of continuous ischemia, large confluent areas of tissue lysis with blood congestion in the sinusoids and leukocyte infiltrates were observed (Fig 6C). In the liver treated with OC 100, limited and focal areas of hepatocyte necrosis were also observed (Fig. 6D), whereas the parenchyma was almost normal after OC 200 and OC 300 treatment respectively (Fig 6E and 6F).

**Discussion**

The antioxidant activity of Ocimum canum is due to the presence of phenolics. The main phenolics reported in basil are phenolic acids and flavonol-glycosides [30, 31]. Nevadensin and salvigenin are the two flavonol-glycosides found in O. canum Sims.

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. The present study showed that the hydro-alcoholic extract have the proton-donating ability and can serve as free radical inhibitors or scavenger of DPPH, acting possibly as primary antioxidant. O. canum also have better hydroxyl radical scavenging activity than deoxyribose as reflected in terms of percentage inhibition.

The current data demonstrate that temporary blockade of hepatic blood supply yielded structural and functional alterations in the liver. The antioxidant, OC, on the other hand, reduced the severity of injury. Oxygen radical-initiated lipid peroxidation may contribute to the impaired cellular function and necrosis associated with reperfusion of ischemic tissues [32]. In the present study, I/R caused significant increases in the hepatic malondialdehyde levels, end products of lipid peroxidation. Our results demonstrated that OC treatment abolishes the increase in malondialdehyde, probably in part by
scavenging the very reactive hydroxyl and peroxy radicals. Previous studies have justified that flavonoid glycosides of Ocimum basilicum (Labiatae) decreased ulcer index, and inhibited gastric acid and pepsin secretions in aspirin-induced ulcers in rats [33]. P. Caturvedi et al in 2008 [34] demonstrated the antioxidant properties O. canum exhibiting its hepatoprotective abilities against alcohol-induced oxidative stress. Superoxide dismutase scavenges the superoxide ions produced as cellular by-products of ethanol metabolism. The reduced SOD activity in the I/R group resulted in the accumulation of superoxide radicals and the production of oxidative stress. The Ocimum canum hydro-alcoholic leaf extracts (i.e. OC 100, OC 200 and OC 300) were found to have good scavenging activity, mainly via superoxide anions, at all concentrations tested. The results show significant differences between the normal control and I/R groups (P>0.001), but no significant difference from the OC -treated groups. The results of this study clearly demonstrated that temporary blockade of hepatic blood supply yielded structural and functional alterations in the liver. On the other hand OC treatment improved I/R-induced impairment in the liver functions.

Conclusion

In conclusion, it was found that the plant based antioxidant Ocimum canum hydro-alcoholic leaf extract decreased oxygen free radicals during hepatic IR injury in rats. The present study clearly indicates that Ocimum canum is a rich source of phyto-constituents having immense antioxidant potential. Ocimum canum is rich in flavonoid glycoside, thus the results indicate that hydro-alcoholic leaf extract of Ocimum canum have potent antioxidant activity, achieved by scavenging abilities observed against DPPH and Hydroxyl radical assay. Also, evaluation of liver enzymes and histopathological findings of liver tissue indicated that Ocimum canum had beneficial effects on the liver, so Ocimum canum can be considered a preventive treatment agent in hepatic IR injury.

References

Illustrations

Illustration 1

Fig.1 Ocimum canum - Wild basil, from Yamasaki Lab. Plant Photo Gallery, by kind permission from Kazuo Yamasaki
Illustration 2

Table 1:- Study on DPPH scavenging activity in Ocimum canum leaves

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Ascorbic acid (% scavenging activity)</th>
<th>O. canum (% scavenging activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>90.2±0.004</td>
<td>46.8 ± 0.005</td>
</tr>
<tr>
<td>500</td>
<td>91 ±0.009</td>
<td>56.7 ± 0.003</td>
</tr>
<tr>
<td>1000</td>
<td>92.4 ±0.005</td>
<td>72 ± 0.005</td>
</tr>
<tr>
<td>2000</td>
<td>93±0.007</td>
<td>79.2 ± 0.004</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three separate experiments; Statistical comparison has been done by student’s t-test.
Illustration 3

Fig 2:- Study on DPPH scavenging activity in Ocimum canum leaves at 517 nm
Illustration 4

Table 2:-Study on Hydroxyl Radical Scavenging Activity in Ocimum canum leaves

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Ascorbic acid (% scavenging activity)</th>
<th>O. canum (% scavenging activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>250</td>
<td>85.1±0.012</td>
<td>49.52 ± 0.023</td>
</tr>
<tr>
<td>500</td>
<td>87.4±0.019</td>
<td>57.22 ± 0.021</td>
</tr>
<tr>
<td>1000</td>
<td>88.1±0.018</td>
<td>66.45 ± 0.027</td>
</tr>
<tr>
<td>2000</td>
<td>89.7±0.021</td>
<td>74.64 ± 0.024</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of three separate experiments; Statistical comparison has been done by student’s t-test
Illustration 5

Fig 3: Study on Hydroxyl Radical Scavenging Activity in Ocimum canum leaves

[Graph showing hydroxyl radical scavenging activity with concentration on the x-axis and % inhibition on the y-axis, comparing ascorbic acid and Ocimum canum leaves.]
Illustration 6

Table 3:-Level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in Serum &nbsp;

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>ALT (IU/L) (Mean ± SEM)</th>
<th>AST (IU/L) (Mean ± SEM)</th>
<th>LDH ((U/L) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>Naive</td>
<td>90.40±11.45</td>
<td>196.23±13.32</td>
<td>1197.74±134.58</td>
</tr>
<tr>
<td>GROUP II</td>
<td>Sham-operated control</td>
<td>97.52±10.79</td>
<td>198.71±14.95</td>
<td>1395.47±125.60</td>
</tr>
<tr>
<td>GROUP III</td>
<td>Ischemia/reperfusion (I/R)</td>
<td>212.12±26.06a</td>
<td>324.21±23.32a</td>
<td>2010.96±140.19a</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>OC control</td>
<td>87.53±16.62</td>
<td>195.38±10.53</td>
<td>1145.53±113.53</td>
</tr>
<tr>
<td>GROUP V</td>
<td>OC 100mg/kg + I/R</td>
<td>186.31±17.45b</td>
<td>287.64±17.73b</td>
<td>1740.31±131.76b</td>
</tr>
<tr>
<td>GROUP VI</td>
<td>OC 200mg/kg + I/R</td>
<td>152.42±18.19</td>
<td>224.35±13.04</td>
<td>1478.47±125.60</td>
</tr>
<tr>
<td>GROUP VII</td>
<td>OC 300mg/kg + I/R</td>
<td>94.62±12.15</td>
<td>197.38±11.02</td>
<td>1206.56±126.78</td>
</tr>
</tbody>
</table>

a P < 0.01 vs. naive, sham and OC groups.

b P < 0.05 vs. I/R + OC.
Illustration 7

Figure 4: Level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in Serum 4 (A)

Illustration 8

4 (B)
Illustration 9

4 (C)

![Graph showing Serum LDH levels for different treatment groups](image-url)
Illustration 10

Table 4:- The renal tissue oxidant and antioxidant enzyme levels of the groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>MDA (nmol/g protein) (Mean ± SEM)</th>
<th>SOD (U/g protein) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I</td>
<td>Naive</td>
<td>17.20±3.93</td>
<td>33.65±3.76</td>
</tr>
<tr>
<td>GROUP II</td>
<td>Sham-operated control</td>
<td>18.69±2.08</td>
<td>30.45±3.19</td>
</tr>
<tr>
<td>GROUP III</td>
<td>Ischemia/reperfusion (I/R)</td>
<td>27.70±2.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.43±2.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP IV</td>
<td>OC control</td>
<td>15.60±1.44</td>
<td>35.70±2.56</td>
</tr>
<tr>
<td>GROUP V</td>
<td>OC 100mg/kg + I/R</td>
<td>24.87±4.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.37±2.48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GROUP VI</td>
<td>OC 200mg/kg + I/R</td>
<td>19.45±1.83</td>
<td>27.68±2.86</td>
</tr>
<tr>
<td>GROUP VII</td>
<td>OC 300mg/kg + I/R</td>
<td>17.28±2.87</td>
<td>31.75±2.37</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.01 vs. naive, sham and OC groups.

<sup>b</sup> P < 0.05 vs. I/R + OC.
Illustration 11

Figure 5: The renal tissue oxidant and antioxidant enzyme levels of the groups. 

- **A**: MDA
- **B**: SOD

Illustration 12

5 (B): SOD
Illustration 13

Liver histological examination
Table 5:- Histological injury scores* of liver tissue

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Portal inflammation</th>
<th>Necrosis</th>
<th>Vacuolar degeneration</th>
<th>Sinusoidal dilatation</th>
<th>Vascular congestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IR</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
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<tr>
<td>IR + OC 100mg/kg</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IR + OC 200mg/kg</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IR + OC 300mg/kg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Injury Scores: None [0], Mild [1], Moderate [2] and Severe [3]
Illustration 14

Figure 6: OC pre-treatment prevent histological lesions of the liver following reperfusion injury
A-NORMAL; B-SHAM; C-I/R; D-OC 100; E-OC-200; F-OC 300
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