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EVALUATION OF ANTIOXIDANT ACTIVITY OF *OCIMUM CANUM* HYDROALCOHOLIC LEAF EXTRACT IN THE PREVENTION OF HEPATIC ISCHAEMIA

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ABSTRACT

The aqueous leaf extracts of *Ocimum canum* were studied for their antioxidant activity. The *in vitro* antioxidant models used were DPPH radical scavenging activity, Hydroxyl peroxide radical scavenging method, reducing power assay 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. Further the antioxidant activity was studied by using an *in vivo* method to prove its potency in preventing ischaemia by incorporating hepatic ischemia in albino rat. The animals were divided into four different groups of six rats in each group. Group-1 was served as Control and received oral saline only once daily for 30 days. Group-2 received (oral saline + RI), Group-3 received *Ocimum canum* hydroalcoholic leaf extract 100mg/kg bwt dose orally for 30 days, Group-4 and Group-5 rats were pretreated with *Ocimum canum* hydroalcoholic leaf extract an oral dose of 200mg/kg bwt and 300 mg/kg bwt for 30 days. The setup for group-3, 4 and 5 was maintained for 30 days and the rats were induced with ischemia on the 29th day. After the experimental period all rats were sacrificed and antioxidant defense system and oxidative stress in hepatic tissue was investigated. The significant results were obtained for all *in vitro* models and *in vivo* models. A significant increase in activity levels of SOD, MDA was found in rats of Group-4 when compared with other group. The results of present study indicate that the hydro-alcoholic leaf extract of *Ocimum canum* has significant antioxidant activity and can prevent ischemia.

INTRODUCTION

Ischemic diseases in the cardiovascular system and CNS account for the majority of morbidity and mortality worldwide, and the incidence is increasing due to an aging population. Cardiovascular diseases represent one of the most common disorders affecting Western societies. There is accumulating evidence to support the notion that oxidative injury plays a critical role in several cardiovascular diseases including myocardial infarction, myocardial I/R (ischemia/reperfusion), atherosclerosis, endothelial dysfunction, restenosis, hypertension as well as cardiomyopathies and heart failure[1, 2]. The 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 plagues 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.

Enhanced oxidant stress during ischemic conditions

The balance of redox is pivotal for normal function and integrity of tissues. 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 [3, 4]. 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 (O_2 −) 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 [5, 6]. 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 [7, 8, 9, 10]. More profound oxidant stress usually occurs when the blood supply is re-established for either ischemic 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

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 [11, 12, 13]. The chemistry of their catalyzing reactions is shown in Fig. 1

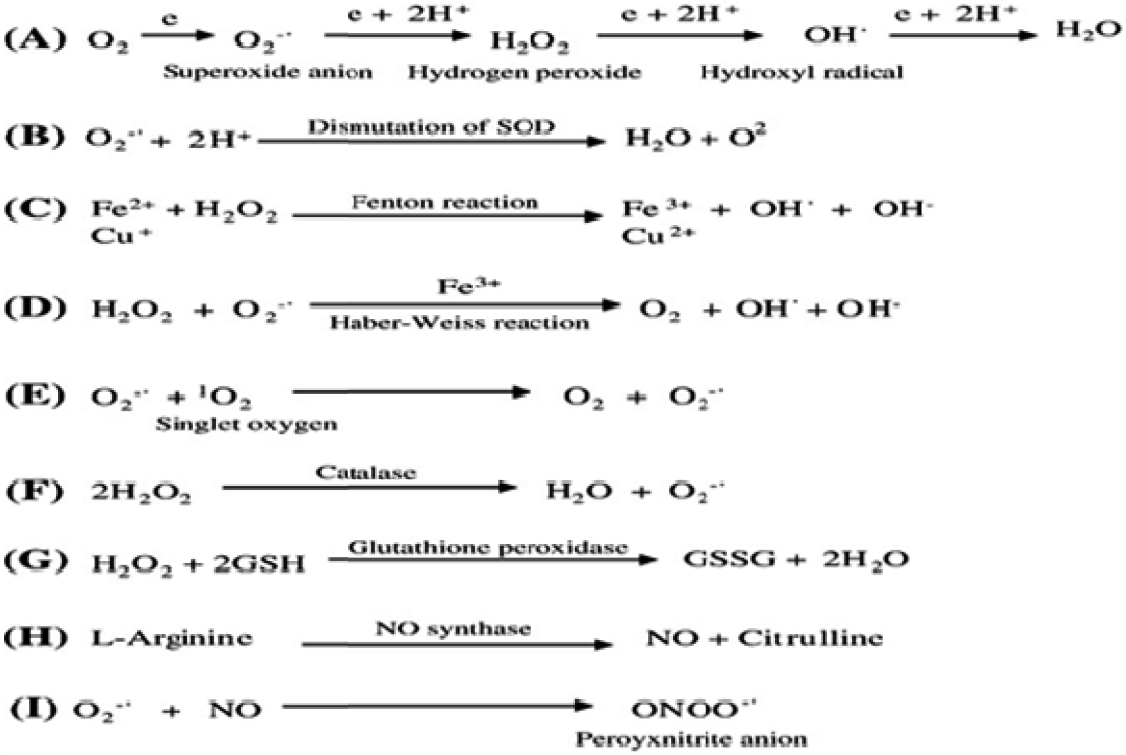

Fig.1. Chemical reactions involved in the formation of reactive oxygen species (ROS) and actions of ROS scavengers. [Common ROS include O_2 −, hydrogen peroxide (H_2O_2), hydroxyl radical (OH) singlet molecular oxygen ($^1O_2$), nitric oxide (NO) and peroxynitrite anion (ONOO−). SOD = superoxide dismutase]

Stroke is the second most common cause of death worldwide and 1/6 of all human beings will suffer at least one stroke in their lives. Furthermore, stroke is the leading causes of adult disability with approximately one third of patients who survive six

months are dependent on others. Because of its huge socioeconomic burden absorbing 6% of all health care budgets and with the fact that life expectancy increases globally one can assume that stroke is already, and will continue to be, the most challenging disease. Ischemic stroke accounts for approximately 80% of all strokes and results from a thrombotic or embolic occlusion of a major cerebral artery (most often middle cerebral artery, MCA) or its branches. Clinical variability of stroke, mainly in terms of causes, duration, localization, and severity of and coexisting systemic diseases, raises the need for very large patient group sizes in clinical research to avoid confounding effects of the diversity[14].

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. Indeed, growing evidence has demonstrated that therapy using antioxidants, free radical scavengers or their mimetics, as well as antioxidant gene transfer can be beneficial by reducing oxidant stress, blocking the activation of signaling mechanisms leading to cellular apoptosis, attenuating tissue damage, and promoting tissue recovery[15]. Thus, antioxidant therapy at an early stage is considered as an adjuvant regimen in a variety of ischemic disorders[16].

Keeping in view the above demographic 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.

MATERIALS AND METHODS

Drugs and Chemicals:-

Hydrogen peroxide, Ascorbic acid, DPPH, Potassium persulphate, H_2SO_4, Potassium Iodide, Mercuric Chloride, BismuthCarbonate, Glacial acetic acid are purchased from Nobel Enterprises, Berhampur, Orissa

Animals:-

Adult rates 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 leaves of *Ocimum canum* were dried for 20 days under the shade to prevent the loss of volatile oils. The powder was extracted with hydro-alcoholic mixture by soxholation. The hydro-alcoholic mixture was prepared by ethanol 47.5% and water in the ratio of 1:1. The filtrate was collected and concentrated on heating mantle to obtain a syrupy mass.

A*) In vitro* Antioxidant Study:-

Ocimum canum aqueous leaf extract was tested for its antioxidant activity using different in vitro models as follows at concentrations of 250, 500, 1000 and 2000µg/ml

a) **DPPH Radical Scavenging Activity (517 nm):**
   500µl of plant extract & 5ml of 0.1mM ethanol solution of DPPH were mixed and vortexed. The mixture was incubated at 27ºc for 20 min in dark room. Thirty minutes later, the absorbance was measured at 517 nm.
b) **Hydroxyl free radical scavenging method (532nm):**
   Mix 0.1 ml EDTA, 0.01ml $FeCl_3$,0.1ml H_2O_2 dissolved in distilled water,0.33 ml of phosphate buffer (50mM,7.4) &0.1 ml of ascorbic acid. Incubate the mixture at 370c for 1 hr. A 1.0 ml of incubated mixture was mixed with 1 ml of 10% TCA & 1ml of 0.5% TBA. Absorbance was measured at 532 nm.

B) *In vivo* Evaluation: - Hepatic Ischemia in the Rat (Yu-Xin Chen*., et al)*

Hepatic Ischemia/Reperfusion Injury in Rats was performed. At the end of the experimental period, the experimental animals were sacrificed; blood was collected retro orbitally or through venous puncture and the serum separated was used for the determination of diagnostic marker enzymes SOD **2.5 Statistical analysis**

The statistical comparison were performed by One way analysis of variance (ANOVA) followed by Student's t-test. Values are expressed in Mean±SD ($P<0.05$). The statistical analysis was done using the latest version of Graph Pad Prism (Version 5.04)

RESULTS:

***In vitro* evaluation:**

Table 1 - DPPH radical scavenging assay method

Concentration	Absorbance
250	0.091
500	0.112
1000	0.131
2000	0.150

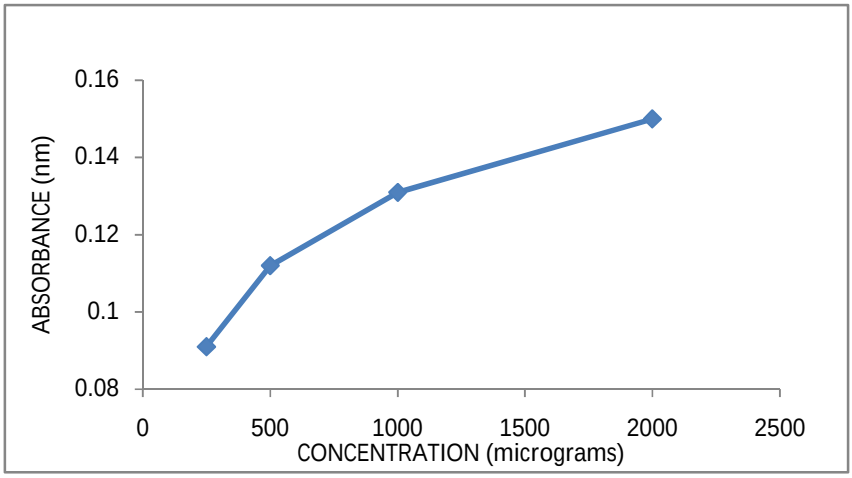

Fig 2 - DPPH radical scavenging activity graph

Table 2 - Hydroxyl peroxide radical scavenging assay method

Concentration	Absorbance
250	0.084
500	0.90
1000	0.999
2000	0.112

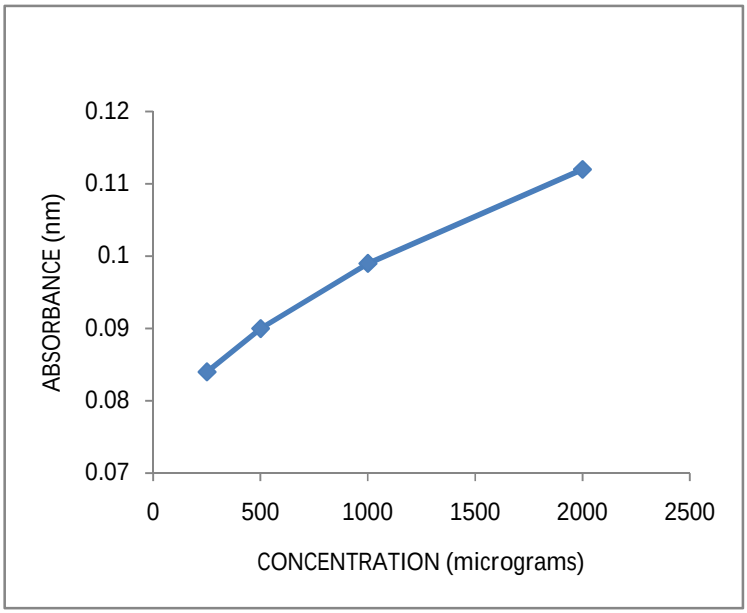

Fig 3 - Hydroxyl peroxide radical scavenging assay graph

Table 3 - *In vivo* evaluation

GROUP	TREATMENT	Hepatic tissue homogenate	
		SOD	MDA
I	CONTROL	91.09+4.2	3.87±0.12
II	IC (Ischemic control)	63.56+2.02	9.56±0.11
III	HAOC + HI	72.22±1.76	6.81±1.17
IV	HAOC + HI	79.06+3.20	5.96±1.93
V	HAOC + HI	82.14+2.90	4.72±0.97

HAOC- Hydroalcoholic leaf extract of *Ocimum canum*

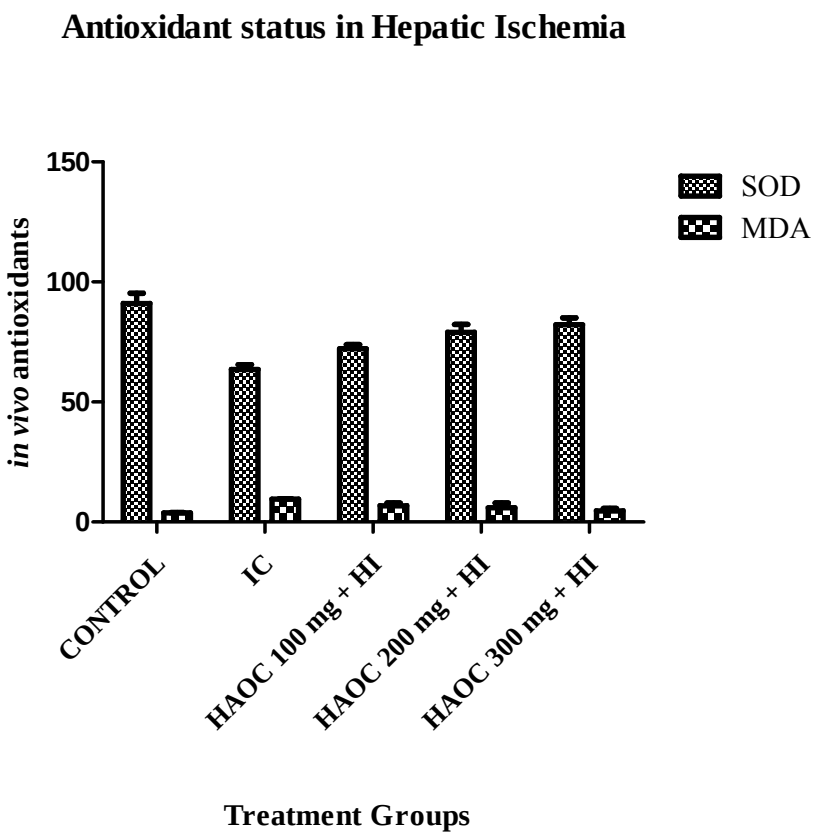

Fig 4 – *In – vivo* evaluation graph

DISCUSSION

Ocimum canum leaf proved to be effective in reducing the extent of hepatic damage given in doses 100 mg/kg, 200 mg/kg and 300 mg/kg body weight by enhancing the endogenous anti-oxidant status in rats. The potential hepato-protective activity of *O.canum* leaf may be due to the presence of therapeutic phytochemicals such as flavones and flavonoids. The hepato-protective effect of *O.canum* leaf is probably

related to strengthening of the hepatic membrane by its membrane stabilizing action, or to a counteraction of free radicals by its antioxidant property.The present study clearly demonstrated the hepatic ischemia induced oxidative stress is evidenced by a significant fall in endogenous anti-oxidant enzyme SOD along with concomitant rise in MDA level *Ocimum canum* leaf extract not only increased the level of SOD but also attuned increase in lipid peroxidation product MDA, in comparison with rats suffering from hepatic ischemia showing a significant rise in SOD level. Ischemia caused by oxidative stress is a major cause of death and disability worldwide. The present study demonstrated that the plant extracts in experimental rats improves endogenous antioxidant defense system. The data of the present study clearly showed plant extract modulated most of the biochemical parameters were maintained to normal status in ischemic reperfusion in rats

CONCLUSION

In conclusion, the results of the present study indicate that the prior administration of *Ocimum canum* hydroalcoholic leaf attenuates hepatic ischemia in experimental rats. Though many antioxidant drugs for the protection against ischemic stroke are in the pipeline yet only few have successfully completed clinical trials. So proper screening of plant source for finding potential antioxidant drugs will definitely fulfill the dearth of suitable drugs for the treatment and protection against ischemia. Also now-a-days many herbal drugs are formulated as pharmaceutical products to impart stability and improve patient acceptability.

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