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Pharmacological Screening For Neuroprotective Effect Of Origanum Sipyleum Plant Leaves Extract In Rat Model Of Ischemic Stroke

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ABSTRACT
The present study was designed to evaluate the beneficial effects of Extract of Origanum sipyleum (EOS) pre-supplementation on the middle cerebral artery occlusion (MCAO) model of ischemic stroke. Ischemic stroke was induced in the rats by inserting an intraluminal suture for 90 min, followed by reperfusion injury for 24 hours. The animals were assessed for Neurobehavioural changes which were accompanied by decreased acetylcholinesterase activity, increased oxidative stress in terms of enhanced lipid peroxidation, and lowered thiol levels in the MCAO animals. On the other hand, pre-supplementation with WS (200 and 400 mg/kg body weight) for 21 days to MCAO animals was effective in restoring the acetylcholinesterase activity, lipid peroxidation, thiols, and attenuated MCAO-induced behavioral deficits. EOS significantly reduced the cerebral infarct volume and ameliorated cerebral ischemia. The results of the study showed a protective effect of EOS supplementation in ischemic stroke and are suggestive of its potential application in stroke management.
Keywords; Cerebral ischemia Origanum sipyleum Antioxidant Behaviour, Oxidative stress

I. INTRODUCTION

Stroke is the third leading cause of death in developed countries. So yet, there is no neuroprotective treatment that is effective in treating stroke. It has long been known that oxidative stress contributes to neuronal death after a stroke. Superoxide, hydroxyl, hydrogen peroxide, and peroxynitrite radicals are reactive oxygen species (ROS) that are known to be produced by ischemic reperfusion (IR) injury. Neuronal death after cerebral ischemia is caused in large part by these radicals. Superoxide and hydroxyl radicals can damage cell membranes by initiating lipid peroxidation. Inducible nitric oxide synthase (iNOS) expression is upregulated following IR injury. Overproduction of nitric oxide (NO) is the result. When an excess of NO reacts with superoxide, a strong radical called peroxynitrite is generated. This radical is responsible for the death of neurons during cerebral ischemia. A fast hydroxylation and nitration of aromatic residues of nucleotides and amino acids in the cytosol and nucleus is possible with peroxynitrite. These reactions cause the cellular machinery to break down, which in turn causes neurons to die. Several antioxidants have been discovered to reduce reactive oxygen species (ROS)-mediated reactions and safeguard neurons against reperfusion-induced neuronal death in animal models of cerebral ischemia. The active components, which include curcumin and quercetin among others, have antioxidant action, according to multiple in vitro and in vivo tests. The cytoprotective activity was demonstrated in various experimental situations, including those involving cardiac and renal ischemia[1].

Stroke continues to rank high among global health hazards due to the high rates of morbidity, death, and disability it causes (World Health Organization, 2010). Stroke is projected to overtake all other causes of death and disability in adults in developing countries as the global population ages. It is already becoming more common, and it will only become worse from here on out. Younger adults also have a higher stroke mortality rate, which may be attributable to the increased stress and strains experienced by modern people. The burden of a person's incapacity to work after a stroke falls on their families and society as a whole. Scientists in this area have persisted in their pursuit of better medications for the treatment and prevention of stroke. Clinical trials have not been successful for a few of these medications. Tissue plasminogen activator (t-PA) is an exception. Although t-PA has received FDA approval as a thrombolytic medication for acute ischemic stroke, its effectiveness is short-lived and it carries the risk of bleeding, which limits its use[2].

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Various degrees of circulatory malfunction are linked to distinct types of brain ischemia. Countless experimental models have been devised to explain this difference, including transient or permanent interruptions of blood flow, either totally or partially, on a local or global scale. The potential use of these models in determining the origins of injuries and developing treatment strategies for people with cerebrovascular disease is the main area of focus when thinking about their clinical significance. The pathophysiology of the model needs to represent the essential features of the real-world situation for the experimental results to be effectively transferred to clinical practice. Due to damage and therapeutic ideas that were suitable for the model rather than the disease, numerous prior clinical trials were unsuccessful. The reliability of experimental results is at risk at three stages:(1) selecting an appropriate animal model to generate ischemia;(2) determining an appropriate method to quantify ischemic injury; and (3) appropriately evaluating outcome data. This evaluation outlines the most prominent strategies in these specific areas[3].

The use of animal models is crucial in the study of human diseases. For the last several decades, researchers have relied heavily on animal models of stroke to learn about ischemia and create new medications. Over 40% of neuroprotection research has used the MCAO model because it is considered to be the most accurate representation of human ischemic stroke. Furthermore, an increase in brain infection rates is likely to occur in the craniotomy-prepared cerebral ischemia model. As a result, MCAO in rats utilizing the intraluminal suture approach is a widely acknowledged and standardized animal model because of its easier procedure and consistent infarct volume. In addition, the link between brain trauma and cerebral ischemia is not well-established[4].

Reperfusion begins by reducing blood flow and then moves on to reopening blood vessels and reoxygenating tissues all at once. Tissue damage could get worse even if the initial hypoxia is alleviated. Similar to global hypoxic injury, this occurs in stroke-related focal ischemia injury. In this review, we compile the extensive basic science literature on ischemia-reperfusion injury, including its causes and therapies. We also consider how multimodal imaging has recently allowed us to study the timing of serial perfusion, allowing the precise measurement of changes in tissues, and how this relates to acute ischemic stroke since no comprehensive study has yet examined the dynamic nature of reperfusion injury in humans. Possible protections against ischemia-reperfusion damage in humans may be possible as a result of this[5].

Plant Profile
Botanical Name: Origanum sipyleum L.
Synonyms: O. sipyleum
Common name Bayirqayi
Plant Family: Lamiaceae
Plant Form: Shrub[6]

Fig: Leaves of Origanum sipyleum

Figure 1.4 leaves and Plant of Origanum sipyleum

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Botanical Description

Habit- A perennial semi-shrub that stands at a height of 80 centimeters and features pink blooms and more than one stem that is rigid. Stem- bark whitish, stems and branches terete or obsoletely 4 gonous, glabrous. **Leaves-** 3.5-7 by 1-2.5 inches, elliptic, acuminate, opposite, entire, bristle- tipped, entire, lineolate, glabrous or more or less pubescent beneath, base tapering into the petiole, main nerves about 5 pair, petioles 0-0.75 inch long, becoming shorter upwards, usually with 3 (sometimes 2 or 4) divaricate acicular spines in the axils[7]. **Inflorescence-** Axillary or terminal spikes. **Flowers**- Sessile, often solitary in the lower axils, becoming spicate above bracts foliaceous, oblong or oblong lanceolate, acute, bristle tipped nearly glabrous, bracteoles 0.5 inch long. **Seeds-** 2 orbicular, compressed, hairy. (Sattya Narayan Talukadr, Md. Bokhtiar Rahman and Sudip Paul, 2015)[8].

Chemical Constituents:

From the aerial parts Origanum sipyleum contains the Flavonoids, tannis, and anthracenes[9].

Uses

Used as folk tea and medicine. Recently, the use of spices and herbs as antioxidants and antimicrobial agents in foods has gained an increasing importance. Antioxidants have been widely used as food additives to protect against oxidative deterioration of food. Spices have been traditionally used in different types of food to improve flavor.[10] The genus Origanum,which is classified under the Lamiaceae family, this genus is comprised of a collection of herbaceous plants that are distinguished by its culinary and medicinal use[12].

This particular species is only found in Western Anatolia, grows flowers from April to October, and has a lifespan of approximately three to four years when it is cultivated in conditions that are appropriate to its growth. This species has been utilized for the manufacture of essential oil, as well as for the creation of medicinal tea and food additives[13]. The O. sipyleum plant is widely used as a spice in the central region of Anatolia, whereas in the western region of Anatolia, it is employed for the treatment of coughs and gastrointestinal diseases. A tea made from the infusion of the leaf and flower is also utilized for the treatment of common colds in the region of inner-western Anatolia[14].

Experiment Work: -

To Study the effect of Origanum sipyleum leaves extract on the ischemic animal model. The experimental work was planned as follows: -

PART I

IA. Collection of plant material of Origanum sipyleum leaves.
IB. Processing of sample
IC. Extraction of dried leaves of Origanum sipyleum with ethanol by Soxhlet apparatus.
ID. Phytochemical Screening.
IE. Experimental design.

PART II

IIA. Acute oral toxicity study.
IIB. Induction of cerebral ischemia by middle cerebral artery occlusion.
IIC. Collection of blood.
IID. Protein estimation.
IIE. Estimation of antioxidant activity.
IIF. Collection of brain sampling
IIG. Statistical analysis

PART I

IA. Collection of plant material of Origanum sipyleum leaves: -

The leaves of Origanum sipyleum belonging to family Lamiaceae were collected in the month of September 2023 from Garwhwal, Uttrakhand, India.

IB. Processing of sample

Origanum sipyleum plant leaves were dried for 2 days at room temperature in the shade, then dried at 40–50 °C for 3–4 hours, yielding a coarse powder that was then ground. This powder was stored in an airtight container and used for extraction.

IC. Extraction of dried leaves of Origanum sipyleum with ethanol by Soxhlet apparatus: -

For extraction of Origanum sipyleum warm the extraction solvent in a flask with a round bottom using a heating source like a mantle. The temperature at which extraction occurs is dependent on the solvent employed. Due to the increase in temperature, the solvent in the bottom flask evaporates into the Soxhlet apparatus's thimble chamber uses a porous "thimble" made of strong filter paper or cellulose condenser, and eventually drips back into the sample thimble. When the liquid reaches the syphon arm, it is drained back into the bottom flask, and the clear

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solution in the syphon tube shows that the process is finished. After the extraction procedure was finished, a semisolid mass was obtained by evaporating the solvent at low pressure in a Rota evaporator.

Fig: Soxhlet apparatus for plant extraction

ID. Phytochemical Screening: -

Testing for phenol: -

To determine the presence of phenols by observing the formation of a colorful complex when the 2ml of dissolved extract is coupled with a 10% of ferric chloride solution that has been recently produced. Almost all phenols result in a solution that is bluish or even black.

The detection of alkaloids: -

Dragendroff's test: - Which is performed by adding 2ml of the reagent to 1ml of extract. A precipitate was produced, and the presence of alkaloids was revealed by the orange-brown coloring of the precipitation.

Mayer's test: - 3-4 drops of Mayer's reagent are added to the 1ml of filtrate. It was discovered that the extract included alkaloids due to the presence of a white precipitate in the sample.

Wagner's test: - 3-4 drops of Wagner's reagent are mixed with the 1ml of extract. precipitate that is reddish-brown in color will be generated

Tannin tests include:

a) Test with 5% FeCl3: The chemical was applied to a test solution comprised of two to three milliliters, and the solution did not take on a dark blue-black hue.

b) The lead acetate test: After treating 1 ml of the test solution with 2 ml lead acetate solution, a white precipitate was generated, which confirmed the presence of tannins in the solution.

Test for lipids

Fatty triglycerides and sodium hydroxide are the two components that are utilized in the saponification process, which results in the production of glycerol and soaps when mixed with ethanol.

The acrolein test is designed to determine whether or not acrylic aldehyde, a molecule that has a pungent odor, is produced. Acrolein is frequently produced when lipids are heated with potassium bisulphate. This is a common occurrence.

Assessment of iodine by Hubel

Di-halo adducts are produced as a result of the reaction that takes place between unsaturated fatty acids and bromine or iodine. There is a direct correlation between the amount of bromine that is consumed and the increase in unsaturation. An indirect method for determining whether or not unsaturated fatty acids are present is to add 2ml of alkaline potassium permanganate to 1ml of extract and observe whether or not the fatty acids are able to undergo partial oxidation.

Test for amino acid

a) Ninhydrin Test: - 3-4 drops of Ninhydrin fluid were added to the extract; the characteristic purple color of this fluid indicates the presence of amino acids.

b) xanthoproteic test: - fill a test tube that is boiling with 2 ml of extract and 1 ml of concentrated hydrogen nitrate. bring out the color by heating it for two minutes over a flame. It is a Now, while the solution is being submerged in cold water, gradually add enough NaOH at a concentration of forty percent to make it extremely alkaline. the distinct change in color indicate the presence of amino acid.

Test for saponin

a) 1 ml of extract was mixed with 20 ml milliliters of distilled water in a graduated cylinder, and the mixture was swirled for fifteen minutes under the microscope. The presence of saponin can be determined by the establishment of a consistent foam.

b) In step one milliliter of the extract was combined with one milliliter of the lead acetate solution, which had a concentration of one percent. It is possible to determine the presence of saponin by observing the formation of white precipitates.

Test for flavonoids

a) 2 ml of ferric chloride with 1 ml of extract, a brilliant green color shows the presence of flavonoids.

b) 1 ml of filtrate with 2 ml 10% lead acetate test, gives brown precipitation.

c) 1 ml of filtrate with 2 ml of dilute NaOH shows the development of golden yellow precipitation.

Test for resins

a) The existence of resins is verified by the HCl test, and the medication is then subjected to treatment with hydrochloric acid, which causes it to take on a pink pigmentation.

b) Extract mixed with a ferric chloride solution; the ferric chloride test reveals a color change that is described as being greenish-blue in appearance. The presence of resins is therefore certainly indicated by this.

Test for steroids

a) Salkowaski Assessment

1 ml of filterate in 2 ml of chloroform, adding 2 m of strong sulphuric acid through the side of the test tube was also performed. The test tube was shaken for a few minutes before examination. During the process, the chloroform layer developed a reddish hue, which served as evidence that steroids were present.

b) Liebermann-Burchard test

A reddish ring can be created at the junction, as indicated by this test, by combining a chloroform solution of steroids with acetic anhydride and strong sulfuric acid, and then allowing the combination to stand for a period of time.

Experimental Design

Animal Grouping is as follows:

Group 1: Normal Group (Carboxy Methyl Cellulose)

Group 2: Negative Control Group (MCAO)

Group 3: MCAO+ Extract low dose 200mg/kg

Group 4: MCAO+ Extract high dose 400 mg/kg

Group 5: MCAO+ Vit-E 10mg/kg

The animals were categorized into five groups, each consisting of six rats. Six rats from each group underwent to localized brain ischemia, followed by the assessment of biochemical parameters after 24 hours of reperfusion. The initial group served as the control group and received a regular treatment (vehicle treated). In other words, the rats were given the vehicle (carboxy methyl cellulose) orally 60 minutes before being subjected to 90 minutes of MCAO (middle cerebral artery occlusion) followed by 24 hours of reperfusion. The second group was subjected to MCAO. The third and fourth groups of rats received oral doses of 200 mg/kg and 400 mg/kg of EOS, respectively. The fifth group was used as a control group and received standard treatment. In this group, rats were given Vitamin E (10 mg/kg, oral) 60 minutes before being subjected to MCAO. The subjects were administered either a vehicle or medicines once daily for 15 consecutive days before the commencement of the experiment

PART II

Acute oral toxicity study (OECD423)

The procedures for the oral toxicity test were carried out by the guidelines that were established by OECD Law 423. In each advancement, there is a methodical utilization of three animals of the same gender throughout the board. You may readily estimate the toxicity of the test chemical by looking at the animal mortality rate or the dosage required to cause injury (50, 300, or 2000 mg/kg body weight). Both of these methods are straightforward alternatives. In comparison to other methods, this one is superior because it only requires a limited number of animals to provide meaningful results.

The utilization of data that has been cleaned up and structured through the application of a universal categorization that is segmented is what this method makes use of. In this study, ethanol-based extracts of EOS were given orally at a dosage of 2,000 milligrams per kilogram of body weight. The inclusion of this component was initially voluntary, but it was subsequently made obligatory in order to identify any adverse effects that may have been affecting the rats that were being observed for their water consumption. A second experiment that was quite identical to the first one was conducted for a period of 72 hours and involved three additional rats. This experiment monitored the skin tone, salivation, bowel movement, sleep patterns, tremors, spasms, and effects on the cardiovascular, autonomic, and central neurological systems.

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It was the responsibility of three different creatures to make each movement possible. From the beginning, you have the option of beginning with any one of the following five doses that have already been determined based on your weight: 5, 50, 300, or 2000 mg/kg.

Middle cerebral artery occlusion induced focal cerebral ischemia

This is an example of localized brain damage due to reduced blood flow generated by a slightly altered version of the middle cerebral artery occlusion (MCAO) technique, as previously explained by Longa et al. To summarize, the rats are given atropine sulfate at a dose of 0.5 mg/kg through injection into the abdominal cavity before being anesthetized with 350 mg/kg through injection into the abdominal cavity. The midline incision was made on the nape of the neck. The trachea became visible after the left common carotid artery. After identifying the confluence of the internal and external carotid arteries, all ligaments and muscles surrounding the left external carotid artery were detached. The external carotid artery underwent surgical intervention and subsequently was incised. Following sandpaper smoothing, the end of a 3-0 nylon monofilament was coated with a 0.01% solution of poly-L-Lysine. Subsequently, it was introduced into the external carotid artery and progressed up to a distance of 21 mm or until encountering resistance. Once occlusion was achieved, ligature was employed to securely fasten the monofilament. The incision was sealed with sutures. Occlusion was administered for a period of 2 hours. Once the rats were given anesthesia, the stitched wounds were opened and the entire filament was carefully removed in order to prepare for reperfusion. Each rat received a 1 milliliter intraperitoneal injection of a sterile 5% dextrose solution at 4 and 12 hours after MCAO. In order to remove rodents from the study, a neurological score below 3 indicates that the rats did not show any immediate neurological problems after reperfusion. If the brain is isolated after sacrifice, the detection of a blood clot in the circle of willis served as an indication of subarachnoid hemorrhage. Unless stated otherwise, all measures necessitated the euthanization of rats and subsequent extraction of their brains after 22 hours of reperfusion[89].

Collection of blood

In the final day of the procedure, a retro orbital sinus puncture was performed on the rat with the use of a small amount of ether anesthesia. It was inevitable that blood would be drawn. This process of centrifuging the samples took a total of ten minutes to complete. Following that, samples of serum were taken and utilized for a variety of biochemical examinations respectively. These included determining LDL, HDL, and VLDL levels, as well as lipid profiles (triglyceride and total cholesterol) by utilizing Erba kits in accordance with the directions provided in their leaflets respectively. Additionally, tests for antioxidant enzymes including glutathione and superoxide dismutase were carried out using the aforementioned methods.

Protein estimation

Quantification of the protein content in the brain homogenate was accomplished using the Biuret method. In 100 mL, then mixed 4.5 g of sodium potassium tartrate with 40 mL of a sodium hydroxide solution with a concentration of 0.2 N. next added half a gram each of potassium iodide and cupric sulphate pentahydrate. After mixing 50 µL of supernatant with 2.9 mL of saline and 3 mL of Biuret reagent, the mixture is incubated at room temperature for 10 minutes to prepare the test solution. A PERKIN ELMER UV/VIS spectrophotometer was used to measure the optical density at 540 nm. The protein content (mg/mL) in the supernatant was determined by using bovine serum albumin (BSA) to create a standard graph that correlates protein concentration with optical density.

Calculating decreased glutathione levels (GSH)

The Ellman (1959) method was used to measure GSH. To separate the proteins, an equal amount of serum was combined with 10% TCA and centrifuged. 0.4 ml of double-distilled water, 0.5 ml of DTNB, and 2 ml of phosphate buffer (pH 8.4) were added to 0.01 ml of this supernatant. After vortexing the mixture, the absorbance at 412 nm was measured. The value of GSH concentration was given in nmol/mg protein.

Estimation of superoxide dismutase (SOD)

SOD activity serum of all the rats were evaluated by utilizing the technique of Kakkar et al. (1984), based on inhibition of the synthesis of nicotinamide adenine dinucleotide, PMS and NBT formazan. In summary, 40 ml of double-distilled water, 30 l of 0.3 mM NBT, 10 l of 0.96 mM PMS, and 90 ml of 30 mM sodium tetra pyrophosphate buffer (pH 8.3) were added to 10 ml of

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homogenate. The addition of 20 l of 0.72 mM NADH started the reaction. At 560 nm, absorbance was measured. The expression for a single enzyme unit was 50% inhibition of NBT reduction/min/mg protein. The units used to express the results were SOD/min/mg protein.

Calculating the catalase activity

Serum catalase activity was measured in a UV recording spectrophotometer by detecting the decrease in absorbance at 240 nm caused by the breakdown of H202, in accordance with the Beers and Sizer method as stated by 18. 0.1 ml of serum in phosphate buffer (50 mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer pH 7.0 made up the reaction mixture (3 ml). For H_2O_2 at 240 nm, an extinction coefficient of 40.0M^{-1} cm^{-1} was computed. The catalase specific activity was reported in terms of moles of H_2O_2 decreased per minute per milligram of protein.

Methodology for Brain Sampling

After the brains were taken 24 hours after the reperfusion, they were cleaned in ice-cold 0.90% saline, blotted on filter paper, weighed, and homogenized in cold phosphate buffer (0.1 M, pH 7.4). After centrifuging the homogenates for 20 minutes at 4 ◦C at 8500 × g, the supernatant was stored on ice until it was analyzed. The AChE biochemical assay was carried out in compliance with the reference.

The procedure for measuring the amount of **acetylcholinesterase in brain** tissue was somewhat modified from that outlined by Ellman et al. The assay combination included 0.05 ml of acetylthiocholine iodide (AChI), 0.1 ml of dithio-bis-nitrobenzoic acid (DTNB), 2 ml of sodium phosphate buffer (0.1M, pH 8.0) containing 0.1% BSA, and 0.1 ml of supernatant. The absorbance was measured using a UV-VIS Spectrophotometer (Perkin Elmer Lambda 20, USA) for two minutes at one-minute intervals at 412 nm. The amount of acetylthiocholine iodide hydrolyzed every minute per milligram of protein was what was used to express the acetylcholinesterase activity.

Statistical Analysis

All data were expressed as the mean ± SEM. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by tukeys test, $P<0.05$ was considered significant.

II. RESULTS

Phytochemical Screening

Sr. No.	Metabolite	Tests	Ethanolic Extract
1	Phenols	i) Ferric Chloride Test	+
2	Alkaloids	Dragendroff's Test Mayer's Test Wagner's Test	+ + +
3	Tannins	Ferric Chloride Test Lead Acetate Test	+ +
4	Lipids	i) Acrolein Test	-
5	Amino acids	i) Ninhydrin Test	-
6	Saponins	i) Foam Test	-
7	Flavonoids	Ferric Chloride Test Lead Acetate Test	+ +
8	Resins	HCL Test Ferric Chloride Test	+ +
9	Steroids/Terpenes	Salkowaski Test Liebermann-Burchard Test	- -

Table4.1 : Phytochemical screening of Origanum sipyleum

Although the phytochemical composition of ethanolic extracts of **Origanum sipyleum** is identical

Acute Toxicity Study

EOS was shown to be non-toxic in an acute oral toxicity study following OECD guidelines 423. Animals given 2000 mg/kg EOS showed no signs of lethargy, toxicity, or aberrant

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behavior. Per the GHS categorization system, the LD50 for EOS is more than 2,000 mg/kg (OECD, 2001). Since the ED50 is often defined as being 10% of the LD50, the ED50 of EOS was determined to be 200 mg/kg, and 400 mg/kg was also chosen for the investigation. The results of the studies conducted on rats are included in the following table.

Table4.2 : Acute toxicity study of EOS at a dose of 2000 mg/kg

Parameters	1st hr	2nd hr	3rd hr	4th hr
Piloerection	-	-	-	-
Edema	-	-	-	-
Urine stains	-	-	-	-
Alopecia	-	-	-	-
Loss of writhing reflex	-	-	-	-
Circling	-	-	-	-
Nasal sniffing	+	+	+	+
Lacrimation	-	-	-	-
Seizures	-	-	-	-
Righting reflex	+	+	+	+
Grip strength	+	+	+	+
Eye closure at touch	+	+	+	+
Rearing	+	+	+	+
Straub tail	-	-	-	-

+=Present/affected, -=Absent/not affected

Effect of Extract of Origanum sipyleum Leaves on Body Weight

This study observed a significant increase in body weight of MCAO-treated groups compared to the normal control group. The significant decrease in body weight of EOS treated groups compared to the MCAO group was noticed in 200 and 400 mg/kg. Also, vitamin E significantly lowered the body weight compared to the MCAO group and restored it when compared to the normal control group.

Table4.3 : Effect of Extract of Origanum sipyleum leaves on Body Weight

Group	Body Weight (grams)	
	Before treatment	After Treatment
Normal Control (NC)	201±5	213±3
Middle carotid artery occlusion (MCAO)	203±9^{ns}	315±10$^{@}$
Extract of Origanum sipyleum leaves (EOS Low)	202±4^{ns}	213±6**
Extract of Origanum sipyleum leaves-high (EOS High)	199±5^{ns}	216±5**
Vitamin E (Vit E)	198±6^{ns}	217±4**

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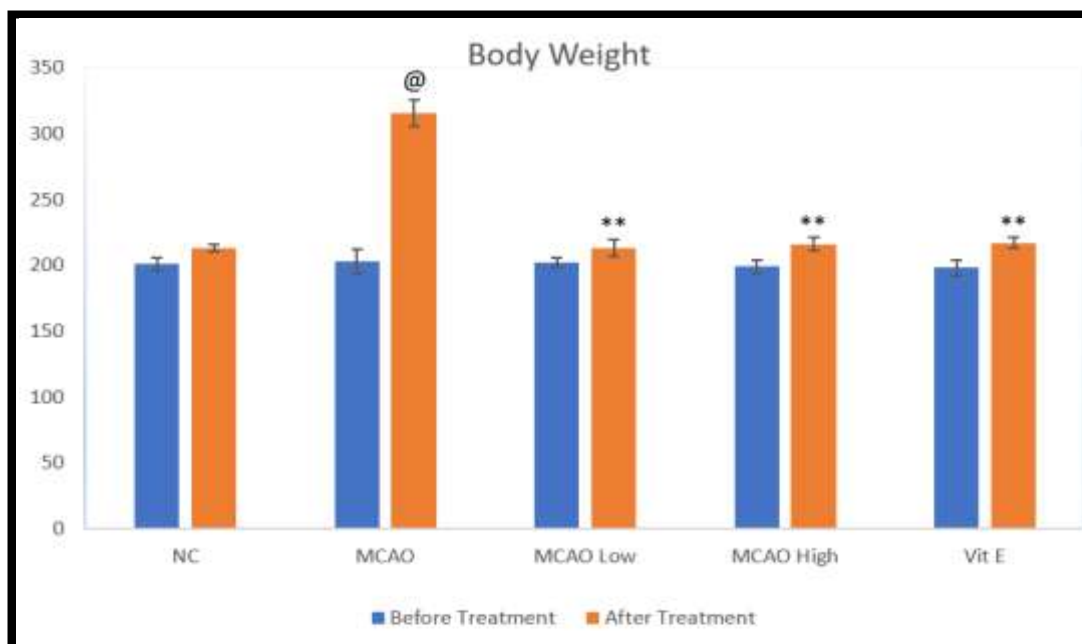

Figure 4.1: Effect of Extract of Origanum sipyleum leaves on Body Weight

Values are expressed in Mean ±SEM (n=6)
P<0.001@ Significant increase in body weight was observed compared to normal control group.
P<0.001** Significant decrease in body weight was observed compared to negative control group.
P>0.05ns Compared with Diseased control group.
Table No. 4.3 & Figure No. 4.1 shows. There was significant (p<0.01) increase in body weight of diseased control group as compared to normal control group. There was significant (p<0.01) decrease body weight of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

Effect of Extract of Origanum sipyleum leaves on AChE level

A significant increase in AChE activity in the brains of MCAO-treated groups compared to the normal control group. The significant decrease in AChE activity in the brain of EOS-treated groups compared to the MCAO group was ameliorated in 200 and 400 mg/kg. Also, vitamin E significantly lowered the AChE activity compared to MCAO and restored when compared to the normal control group.

Table 4.4: Effect of Extract of Origanum sipyleum leaves on AChE level

Group	AChE level (mmol/min/mg of protein)
Normal Control (NC)	0.168±0.019
Middle carotid artery occlusion (MCAO)	0.345±0.022 @
Extract of Origanum sipyleum leaves-low (EOS Low)	0.21±0.015 **
Extract of Origanum sipyleum leaves-high (EOS High)	0.181±0.012 **
Vitamin E (Vit E)	0.171±0.018 **

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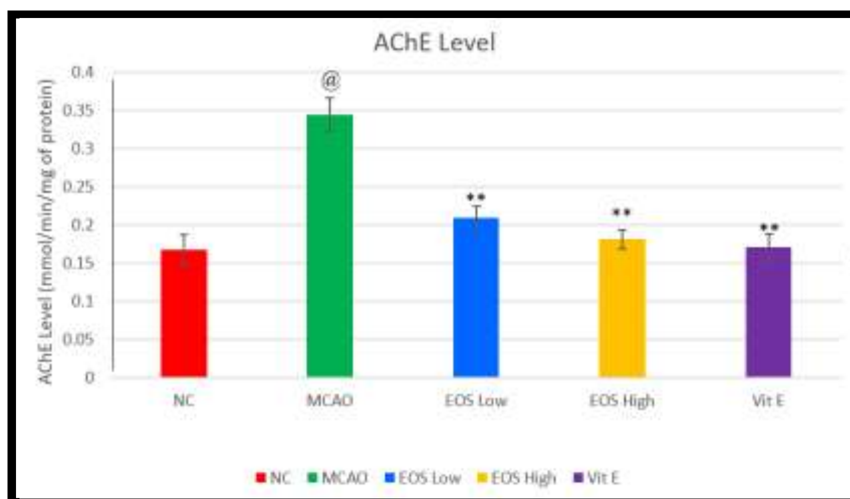

Table 4.4: Effect of Extract of Origanum sipyleum leaves on AChE level

All values are expressed as Mean ± SEM@ (n=6) p<0.01 Significant increase in AChE Level was observed compared to normal control group. **p< 0.01 significant decrease in AChE level was observed compared diseased control group.

Table No. 4.4 & Figure No. 4.2 shows the effect of Origanum sipyleum on AChE activity in cerebral ischemic stroke rats. There was significant (p<0.01) increase in brain AChE activity of diseased control group as compared to normal control group. There was significant (p<0.01) decrease in brain AChE activity of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

Effect of Extract of Origanum sipyleum leaves on Lipid Profile

A significant decrease in HDL in the serum of MCAO-treated groups compared to the normal control group is observed. A significant increase in HDL levels in the serum of EOS 200 and 400 mg/kg treated groups compared to the MCAO group was noticed. Also, vitamin E significantly raised the HDL level compared to MCAO and restored when compared to the normal control group. A significant increase in LDL, VLDL, TG, and TC levels in the serum of MCAO-treated groups compared to the normal control group is observed. A significant decrease in LDL, VLDL, TG and TC levels in the serum of EOS 200 and 400 mg/kg treated groups compared to the MCAO group was noticed. Also, Vit E significantly lowered the LDL, VLDL, TG and TC level compared to MCAO and restored when compared to the normal control group.

Table 4.5: Effect of Extract of Origanum sipyleum leaves on HDL, LDL and VLDL level

Group	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal Control (NC)	44.2±5	22.4±2	14.2±1
Middle carotid artery occlusion (MCAO)	14.25±6@	59.3±4@	22.35±2@
Extract of Origanum sipyleum leaves- low (EOS Low)	31.4±2**	37.4±3**	16.4±2**
Extract of Origanum sipyleum leaves- high (EOS High)	39.78±3**	32.4±5**	15.5±1**
Vitamin E (Vit E)	40.17±5**	29.1±4**	14.8±2**

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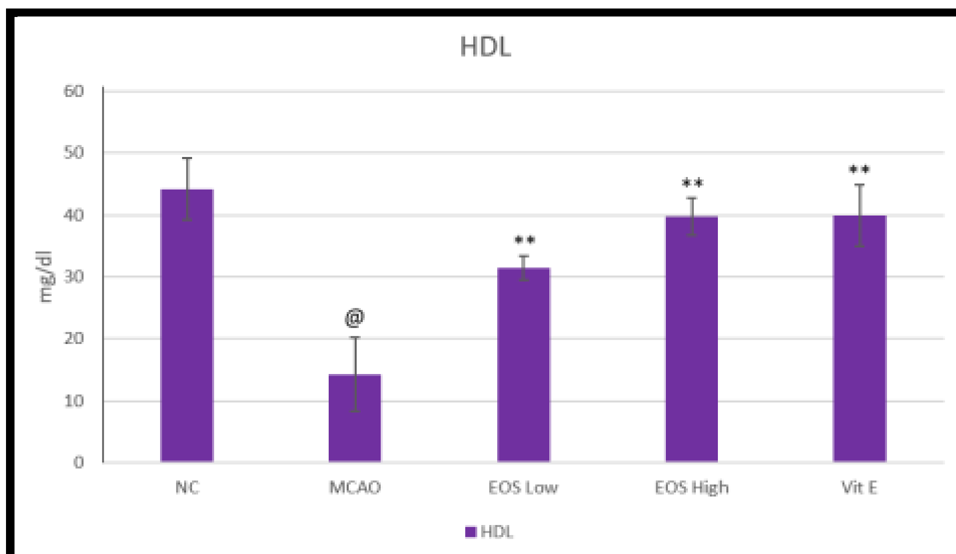

Figure 4.3: Effect of Extract of Origanum sipyleum leaves on HDL

All values are expressed as Mean ± SEM@ (n=6) p<0.01 Significant decrease in HDL Level was observed compared to normal control group. **p< 0.01 significant increase in HDL level was observed compared diseased control group.

Table No. 4.5 & Figure No. 4.3 shows. There was significant (p<0.01) decrease in HDL level of diseased control group as compared to normal control group. There was significant (p<0.01) increase HDL level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

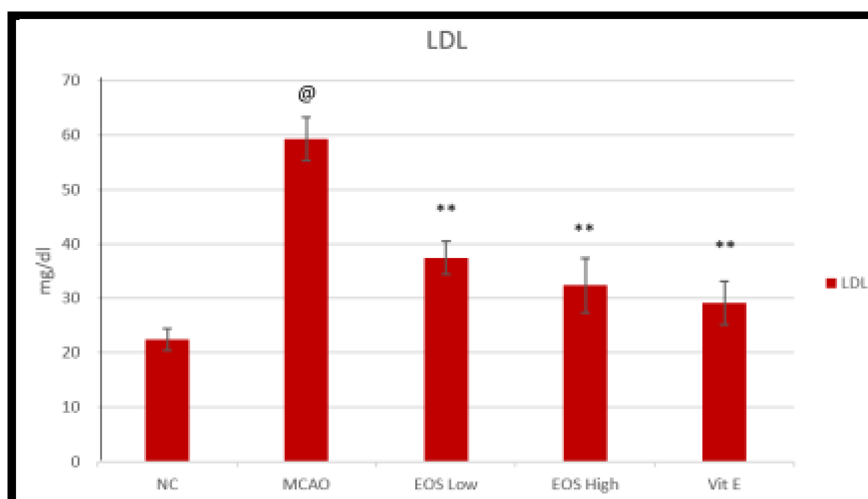

Figure 4.4: Effect of Extract of Origanum sipyleum leaves on LDL level

All values are expressed as Mean ± SEM@ (n=6) p<0.01 Significant increase in LDL level was observed compared to normal control group. **p< 0.01 significant decrease in LDL level was observed compared diseased control group.

Table No. 4.5 & Figure No. 4.4 shows. There was significant (p<0.01) increase in LDL level of diseased control group as compared to normal control group. There was significant (p<0.01) decrease lDL level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

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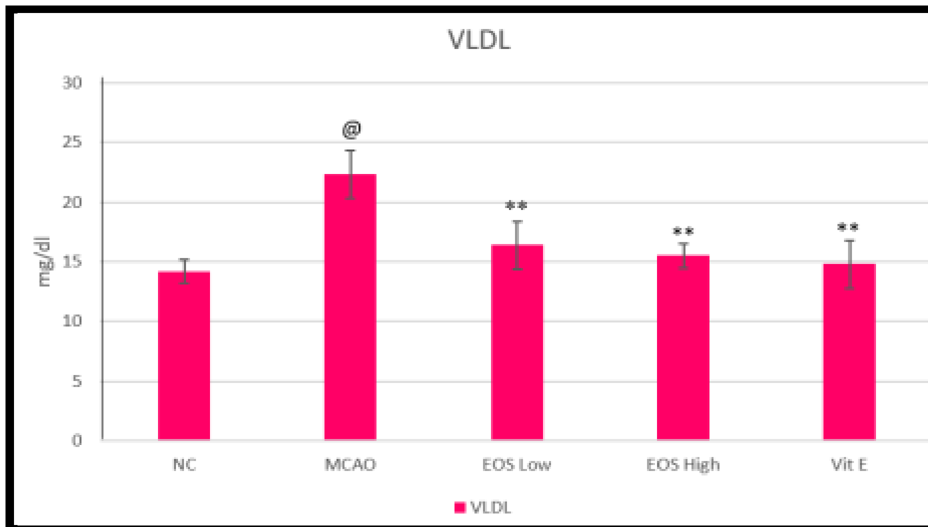

Figure 4.5: Effect of Extract of Origanum sipyleum leaves on VLDL level

All values are expressed as Mean ± SEM[@] (n=6) $p<0.01$ Significant increase in VLDL level was observed compared to normal control group. **p< 0.01 significant decrease in VLDL level was observed compared diseased control group.

Table No. 7.5 & Figure No. 7.5 shows. There was significant ($p<0.01$) increase in VLDL level of diseased control group as compared to normal control group. There was significant ($p<0.01$) decrease lDL level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

Table 4.6: Effect of Extract of Origanum sipyleum leaves on TG and TC levels

Group	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)
Normal Control (NC)	59.4±4	54.2±3
Middle carotid artery occlution (MCAO)	102.5±5[@]	94.6±7[@]
Extract of Origanum sipyleum leaves-low (EOS Low)	71.2±4**	74.2±5 **
Extract of Origanum sipyleum leaves-high (EOS High)	62.5±2**	61.5±6 **
Vitamin E (Vit E)	53.4±4**	52.4±4 **

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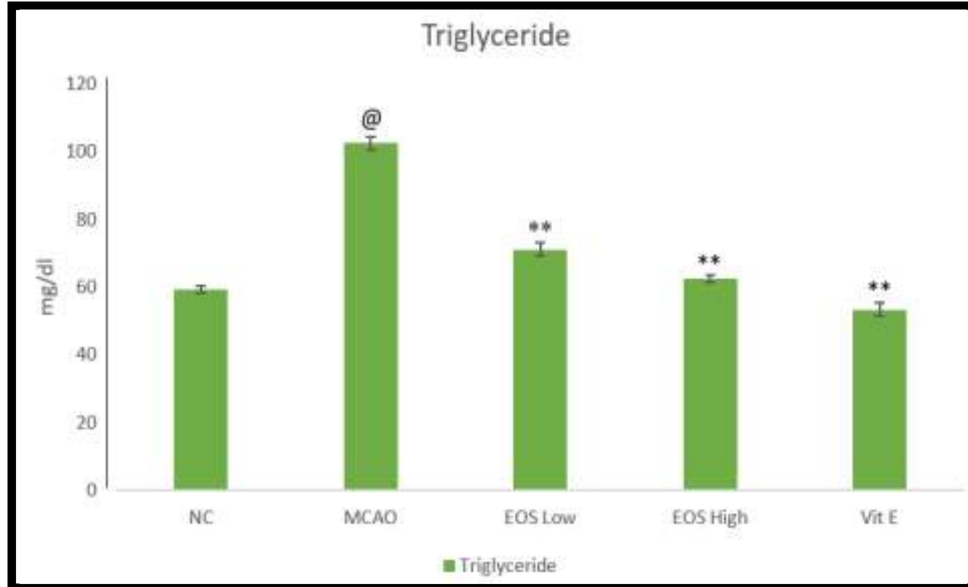

Figure 4.6: Effect of Extract of Origanum sipyleum leaves on TG level

All values are expressed as Mean ± SEM[@] (n=6) p<0.01 Significant increase in Triglyceride level was observed compared to normal control group. **p< 0.01 significant decrease in Triglyceride level was observed compared diseased control group.

Table No. 4.6 & Figure No. 4.6 shows. There was significant (p<0.01) increase in Triglyceride level of diseased control group as compared to normal control group. There was significant (p<0.01) decrease Triglyceride level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

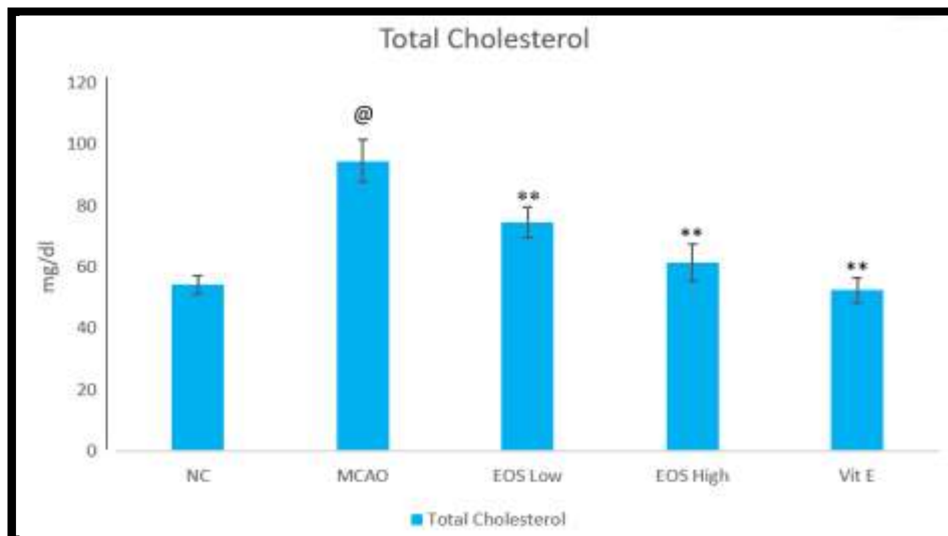

Figure 4.7: Effect of Extract of Origanum sipyleum leaves on TG, TC levels

All values are expressed as Mean ± SEM[@] (n=6) p<0.01 Significant increase in Cholesterol level was observed compared to normal control group. **p< 0.01 significant decrease in Cholestero level was observed compared diseased control group.

Table No. 4.6 & Figure No. 4.7 shows. There was significant (p<0.01) increase in Cholesterol level of diseased control group as

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compared to normal control group. There was significant (p<0.01) decrease Cholesterol level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

Effect of Extract of Origanum sipyleum leaves on superoxide dismutase activity

There was 50% decrease in the SOD activity in the serum of rats. Ischemia induced decrease in the SOD activity was reverted by EOS (200 and 400 mg/kg) treatment.

Effect of Extract of Origanum sipyleum leaves on catalase and GSH activity

Catalase and GSH were estimated in the serum of rats, there is significant increase in the catalase and GSH level in MCAO group compared to normal control group. EOS treatment (200 and 400 mg/kg) inhibited the IR induced decrease in catalase and GSH in a statistically significant manner (p < 0.01).

Table 4.7: Effect of Extract of Origanum sipyleum leaves on SOD, Catalase and GSH level

Group	SOD	Catalase	GSH
Normal Control (NC)	15.2±0.24	3.7±0.35	2.7±0.35
Middle carotid artery occlusion (MCAO)	4.5±0.5 @	0.89±0.14 @	0.79±0.14 @
Extract of Origanum sipyleum leaves-low (EOS Low)	7.2±0.54 **	1.8±0.21 **	1.6±0.21 **
Extract of Origanum sipyleum leaves-high (EOS High)	11±0.89 **	2.54±0.23 **	2.2±0.23 **
Vitamin E (Vit E)	13.4±0.56 **	3.1±0.34 **	2.5±0.34 **

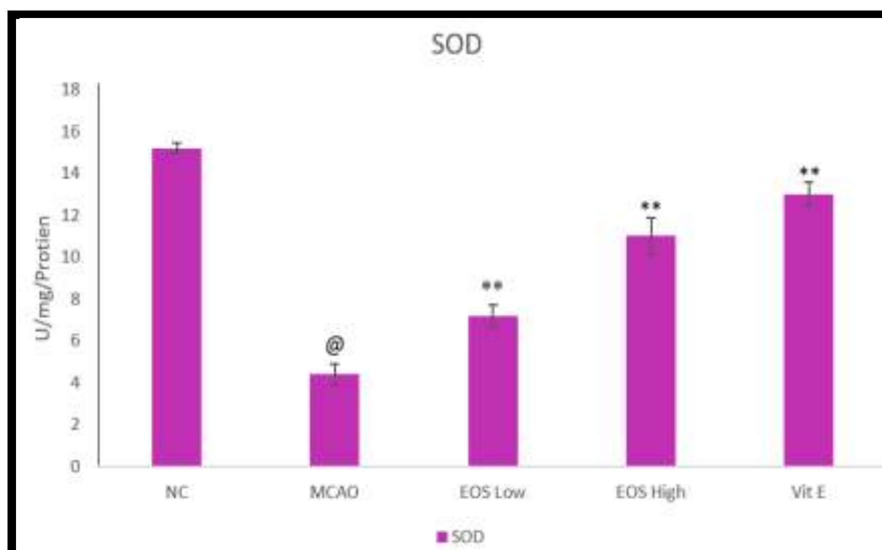

Figure 4.7: Effect of Extract of Origanum sipyleum on SOD

All values are expressed as Mean ± SEM @ p<0.01 Significant decrease in SOD was observed compared to normal control group. **p< 0.01 significant increase in SOD was observed compared diseased control group.

Table No. 4.7 & Figure No. 4.7 shows. There was significant (p<0.01) decrease in SOD of diseased control group as compared to normal control group. There was significant (p<0.01) increase SOD level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

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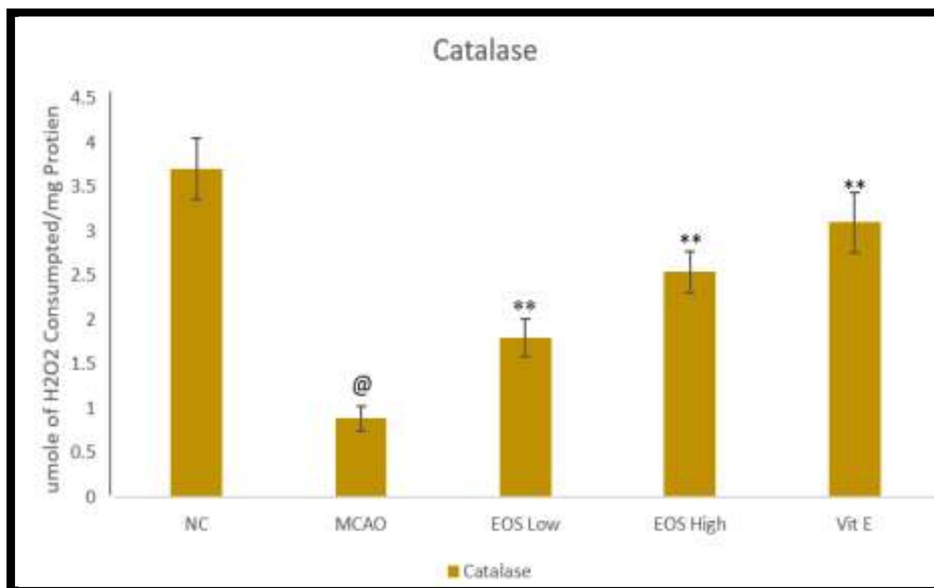

Figure 4.8: Effect of Extract of Origanum sipyleum on Catalase

All values are expressed as Mean ± SEM @ p<0.01 Significant decrease in Catalase was observed compared to normal control group. **p< 0.01 significant increase in Catalase was observed compared diseased control group.

Table No. 7.7 & Figure No. 7.8 shows. There was significant (p<0.01) decrease in Catalase of diseased control group as compared to normal control group. There was significant (p<0.01) increase Catalase level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

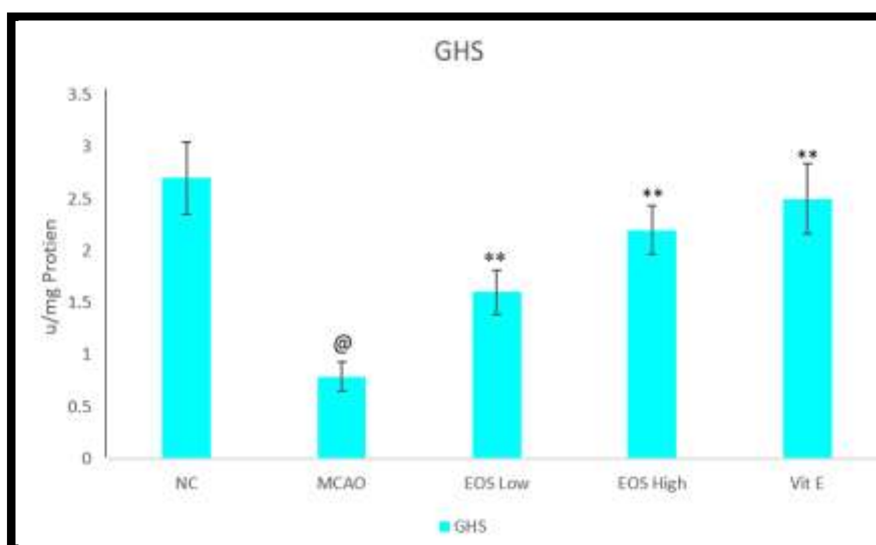

Figure 4.9: Effect of Extract of Origanum sipyleum leaves on GSH level

All values are expressed as Mean ± SEM @ p<0.01 Significant decrease in Catalase was observed compared to normal control group. **p< 0.01 significant increase in Catalase was observed compared diseased control group.

Table No. 4.7 & Figure No. 4.9 shows. There was significant (p<0.01) decrease in GSH of diseased control group as compared to normal control group. There was significant (p<0.01)

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increase GSH level of EOS 200 mg/kg, EOS 400 mg/kg and VitE 10 mg/kg.

III. DISCUSSION

In an experimental stroke paradigm, the objective of this study was to investigate the effects of EOS pre-supplementation on behavioral abnormalities and biochemical changes that are associated with neuronal injury. The results of several behavioral tests indicated that the damage caused by MCAO-induced ischemia reperfusion substantially impacted both the motor and cognitive functions of the subjects[15].

Based on the findings of the phytochemical analysis (Tables 1), the EOS plants were discovered to contain a wide range of compounds that were favorable to the organism. On the basis of the findings of the acute toxicity test, we came to the conclusion that the plant extract did not pose any health risks. A dose of the extract of up to 2,000 mg/kg did not result in any adverse effects. With the use of this statistics, the dosage can be determined more accurately[16].

As a result of its beneficial effects in treating a wide variety of disorders without causing any adverse effects, herbal medicines are gaining popularity in today's society. With the help of an animal model of focal cerebral ischemia, the purpose of this study was to determine whether or not ethanol extracts of Origanum sipyleum are effective in preventing and treating stroke episodes[17].

Acetylcholinesterase, also known as AChE, is an enzyme that must be present in both the neuromuscular junction and the synapse in order to prevent the transmission of nerve impulses. The anomalies in cholinergic transmission are negatively associated with the degree of ischemia that is present. Individuals who suffer from ischemia have a high concentration of AChE in their brains. MCAO has a neurotoxic effect that is characterized by an increase in the activity of acetylcholinesterase (AChE), which makes the hydrolysis of acetylcholine easier to accomplish[18]. Inhibiting acetyl-cholinesterase (AChE) is an essential stage in the process of developing and manufacturing anti-ischemic drugs. Natural chemicals, such as essential oils, have been shown to have a significant inhibitory effect on acetylcholinesterase (AChE), according to a number of reports. According to the findings of our experiment, the group of rats that were given MCAO had a higher level of AChE activity than the rats that were used as the control group. As a result of the antioxidant and neuroprotective qualities that EOS possesses, the activity of AChE was significantly reduced as a result of the treatment[19].

As a potential therapeutic agent and a method for comprehending ischemia responses, free radicals have garnered a great deal of attention in recent years. Reactive oxygen species (ROS) pose a threat to the life of neurons because they initiate an attack on the lipid-rich membranes of the brain and then propagate that attack to lipid peroxidation. This, in turn, encourages the glycation of proteins, the deactivation of enzymes, and alterations in the structure and function of DNA damage. Both ischemic and hemorrhagic strokes are associated with an increase in the generation of free radicals as well as a breakdown in a physiological system that is normally responsible for eliminating excess free radicals after being exposed to brain damage. The formation of free radicals in a brain that has been ischemic is contingent on the intensity, duration, location, and timing of the ischemia, as well as the presence or absence of reperfusion. An excessive amount of free radicals, which can cause damage to DNA, lipids, and proteins, can cause damage to neurons, glial cells, blood vessels, and nerve fibers. This damage can also occur in neurological tissue. In order to alleviate the damage that was already there and to prevent ischemia-reperfusion damage, a number of different approaches, including anti-inflammatory medicines, free radical scavengers, and anti-apoptotic medications, were tested[20].

According to the findings of the current study, the potent antioxidant EOS significantly reduced the neurobehavioral impairments that were present in mice just before they were treated with EOS. Specifically, this is due to the fact that EOS is capable of scavenging free radicals, which are thought to be the origin of the behavioral abnormalities that were seen in the experimental animals used in the study. Antioxidant treatment has been shown to improve a range of behavioral outcomes, including motor coordination competence, according to research that was conducted in the past. There were substantial alterations observed in the flexion tests as well as spontaneous motor activity in the group that was subjected to ischemia. This could be due to the fact that regions of the brain that are essential for motor control, such as the hippocampus and the cortex, are especially susceptible to oxidant stress as a result of increased free radical production brought on by cerebral ischemia[21].

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As a result of the brain's relatively low levels of antioxidative enzymes such as SOD, GSH-Px, and CAT, as well as its high concentration of oxidizable unsaturated fatty acids, which are highly susceptible to lipid peroxidation caused by free radicals, the brain is extremely vulnerable to reactive oxygen species (ROS) that are produced by ischemia-reperfusion. In the brain, these reactive oxygen species (ROS) are responsible for oxidative damage to DNA, lipids, and proteins, which eventually results in malfunction and cell death. After reperfusion, there is a sudden availability of molecular oxygen, which is utilized as a substrate for xanthine oxidase in the process of nucleotide metabolism[22]. This results in an increase in the creation of hydrogen peroxide and superoxide than there would have been otherwise.In the process of cerebral ischemia-reperfusion, endoplasmic reticulum (EOS) has the potential to inhibit the action of xanthine oxidase, which would suggest that it might prevent the production of superoxide and other reactive oxygen species (ROS). The ability of EOS to serve as a free radical scavenger has been demonstrated in a number of studies to be effective in preventing LDL oxidation and lipid peroxidation[23].

We found that EOS was able to enhance the LDL/HDL ratio, which is consistent with the findings of other researchers. According to the available evidence, the topical use of EOS has the potential to slow the course of atherosclerosis and reduce serum cholesterol levels. In addition, an EOS reduces the levels of cholesterol in rats by preventing the oxidation of lipids and by boosting the activity of antioxidant enzymes[24].

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