JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

EVALUATION OF ALCOHOL WITHDRAWAL ACTIVITY OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM* LINN

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Abstract
The ethanol extract of *Ocimum sanctum* was investigated for in vivo alcohol withdrawal effects. *Ocimum sanctum* L, a significant medicinal plant, affects Wistar rats experiencing alcohol withdrawal techniques. The rats were fed a liquid diet containing 7.2% ethanol by volume for a period of 21 days. Animals in the control group were given sucrose as a liquid diet that is isocaloric. Rats were observed at 6th and 24th hours post-alcohol withdrawal for indicators of severe withdrawal, such as anxiety and hyperlocomotor activity. Ethanol withdrawal anxiety was tested using an elevated plus maze, a light and dark model, and the hyperlocomotor activity was measured using an actophotometer. *O. sanctum leaf* extract (100, 200, and 300 mg/kg, oral) and diazepam (2 mg/kg, i.p.) were administered to the treatment group animals 30 min before alcohol withdrawal estimation. Drug treatment was also given 30 min before the second observation at the 24th hour. On the last day of the protocol, rats were sacrificed by cervical dislocation. liver, kidney, and brain were isolated and preserved in formalin for further histopathological examination.

Keywords: Elevated plus maze, light and dark model, Actophotometer

Introduction

Over 1.5 million people receive treatment for alcoholism or are admitted to a general hospital each year as a result of the health problems brought on by alcohol dependence. These individuals experience alcohol withdrawal (AW), as does a significant portion of the general population who quit drinking without medical assistance. AW is a clinical syndrome that affects individuals who are used to drinking alcohol regularly and who either cut back on their intake or give it up entirely. These individuals have a central nervous system (CNS) that has adapted to the ongoing presence of alcohol in the body and makes up for the depressing effects of alcohol on brain function and neuronal communication. As a result, withdrawal syndrome results from abruptly lowering the alcohol intake because the brain stays hyperactive, or overexcited.[1]

In 5% to 10% of individuals, the symptoms progress to severe forms characterized by seizures, cardiac arrest, and death. The symptoms of withdrawal usually start 6–24 hours after the most recent ethanol intake [2, 3].Epileptic seizures and/or delirium tremens (DT), which can occur in as many as 15% of AUD patients, are examples of a complex AWS. [4, 5] Patients with dementia exhibit elevated levels of comorbidities, and their death rate is similar to that of individuals with extremely serious cancerous illnesses.

However, the anticipated mortality is 1% or less with early discovery and appropriate treatment. Patients who are referred to neurological departments or are hospitalized due to epileptic seizures, dementia,

polyneuropathy, gait abnormalities, or coma frequently experience AUDs [6]. However, diagnosis and therapy are frequently postponed until severe symptoms manifest.The main aim is to raise awareness of AWS's early clinical symptoms as well as the proper diagnosis and treatment of this significant disorder in a neurological context.

The activation of the hypothalamus and amygdala in different limbic areas is thought to have a significant role in regulating anxiety-like responses associated with ethanol withdrawal [7].Consuming ethanol causes changes in the nervous system and behavior that are mediated via the excitatory neurotransmitter N-methyl-D-aspartic acid (NMDA) and the GABAA receptor. Systems of inhibitory receptors. GABA levels decrease with ethanol dependency. neuroreceptor response and excitatory NMDA receptor up-regulation, which influence drug discrimination, tolerance, dependence, withdrawal, and reinforcing reward associated with ethanol consumption. The NMDA/glutamatergic and GABAergic systems may be key pharmacological targets for attaining sustained alcohol abstinence [8, 9]. In present treatments for the ethanol withdrawal syndrome, there are very few, such as benzodiazepines, acamprosate, naltrexone, and disulfiram, but these medications can only debilitate ethanol craving and have no effect on withdrawal symptoms.

Plants are a major source of medicine, and between 25 and 30 percent of all medications in use have their origins in plants [10]. The plants in the genus are among the very therapeutic plants. The Ocimum family Because Lamiaceae have so many different medical characteristics, they are exceedingly important. Ocimum sanctum, often known as *O. sanctum* or *tulsi*, has been utilized for thousands of years due to its various healing qualities and has a long history of medical use [11, 12]. Eugenol, cubenol, rosmarinic acid, sitosterol, luteolin, borneol, cardinene, linolenic acid, gallic acid, palmitic acid, oleic acid, stearic acid, carnosic acid, vallinin, vitexin, rientin, vallinin acid, circineol, vitamin C, vitamin A, iron, and phosphorus are among the chemical compounds that have been isolated from different plant parts.

Ursolic acid is the main ingredient that has been linked to anti-fertility effects [13, 14]. The *O. sanctum* leaf extract contains another ingredient called estragol, which may induce uterine contractions [15]. *O. sanctum* leaves may reduce blood sugar levels, according to a study [16]. In addition to its minimal negative effects, *O. sanctum* has been shown to have immunomodulatory, analgesic, anti-inflammatory, antidiabetic, antistress, anticancer, hepatoprotective, anticonvulsant, antihyperlipidemic, neuroprotective, and memory-enhancing properties.

Materials and Method:

Chemicals used:

The chemicals used for this experimental study are ethanol, petroleum ether, sucrose, vitamin A, diazepam, ascorbic acid, phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, sodium dihydrogen phosphate, DPPH, methanol, and distilled water.

Collection of Plant material:

The leaves of the Ocimum sanctum plant were shade dried. We ground the entire plant into a coarse powder using a mechanical grinder, followed by sieving. numbered 60 and kept in a sealed container. Petroleum ether (30°–40°C) was used to defatten the coarsely ground plant material. The Soxhlet apparatus used 70% v/v ethanol (hydroalcoholic) at 50°C for 48 hours to extract the defatted, air-dried plant powder. A rotary evaporator (Heidolph, Germany) is used to recover the solvent through evaporation at lower pressure. The semisolid bulk has undergone additional lyophilization (New Brunswick) for 24 hours at -40°C. The plant's dry powder is kept at -20°C until it is needed again. [17]

''Figure 1'' 1 Leaves of *Ocimum sanctum*

Animals:

Wistar rats (220-250 g) were purchased from Mahaveera Enterprises, Hyderabad [Reg. no.: 146/1999/CPCSEA], India, and housed (two animals per cage) at the Animal House. They were acclimatized to laboratory conditions maintained at a temperature of 23±2, controlled humidity conditions, and light and dark cycles (12:12 h). Animals were fed with a food pellet diet and water ad libitum. Experimental procedures were carried out after the approval from the Institutional Animal Ethics Committee (IAEC-2024/SVIPS/MPC/007) and as per CPCSEA (Committee for the Purpose of Control And Supervision of Experiments on Animals) norms (Regd.No:1362/PO/Re/S/10/CPCSEA) at Sri Vasavi Institute of Pharmaceutical Sciences, Pedatadepalli, Andhra Pradesh, India.

Study Design:

The rats were housed separately in five groups, each consisting of four animals (n=4): disease, three treatment, and standard control. Treatment for alcohol was administered in accordance with previous research's recommendations for a liquid diet [19]. The rats were fed a liquid meal at will, with or without alcohol; no other water or chow was provided. According to earlier research, a liquid meal including 925 mL of cow milk, 25–75 mL of alcohol (ethyl alcohol 96.5%), 17 g of sugar, and 5,000 IU of vitamin A was made. The animals were given a liquid food devoid of alcohol for 7 days at the start of the study, and then 2.4% ethanol was added to the liquid diet for 3 days. After that, the ethanol content was raised to 4.8% for the following 4 days and 7.2% for the following 21 days. After 21 days, alcohol was removed from the liquid diet. The rats received saline, diazepam (2 mg/kg, i.p.), and O. sanctum leaf extract (100, 200, and 300 mg/kg, oral) 30 minutes before the alcohol withdrawal test. At the 6^{th} and 24th hours of the withdrawal phase, tests were conducted to assess alcohol withdrawal symptoms such as anxiety and hyperlocomotor activity employing an actophotometer, a light and dark model, and an elevated plus maze. The animals were put back in their original cages in between evaluation periods. A second dose of the test medication was given to each group 24 hours after the first. Rats in the control group were fed a liquid diet containing sucrose, which is a calorie-dense alternative to alcohol, and were assessed in tandem with the disease control groups [20]. Every observation was made during the light phase. A fresh liquid diet was offered every day at 9:00 a.m. Rats' body weight and ethanol consumption were measured every day and reported as g/kg/day. Animals were sacrificed by liver and cervical dislocation after the treatment was finished. For additional histological research, the kidney and brain were separated and kept in formalin.

In vivo Alcohol withdrawal effect:
Elevated Plus maze:

The apparatus is made up of a central platform that is raised by 50 cm and two open and two closed arms positioned opposite each other. Each animal was positioned separately on the central platform, facing in the direction of the open arm. For five minutes, the observer recorded the number of entries in the open

arms, the total time spent, and the number of entries in the closed arms, as well as the total time spent. The observations were recorded. [23, 24]

Light and dark model:

A rectangular box with two compartments, one dark and one light, connected by a 7.5 cm x 7.5 cm gap between the walls, makes up the model. Each animal was positioned separately in the middle of the light chamber, facing the dark chamber's entrance. The observer recorded how many people entered the light and dark chamber and how long they stayed there for five minutes. [25]

Actophotometer:

A digital actophotometer was used to capture each animal's spontaneous locomotor movement for five minutes. A digital record was made of the number of times the animal moved, cutting a beam of light that fell on the photocell. [25]

Histopathology:

The brain, liver, and kidney of rats were isolated after they were sacrificed via cervical dislocation. Organs that had been isolated were preserved in 10% neutral buffered formalin. The tissues' 5 µm histological sections were Hematoxylin and eosin staining was used, and the samples were further inspected under a microscope to assess for cellular damage or alteration. [27]

Statistical analysis

The Student's t-test was used to examine the differences in body weight between the alcohol-control rats and the animals in the normal group. The *O. sanctum* effects in the elevated plus maze, light and dark model, and locomotor activities were determined using a two-way ANOVA (analysis of variance) and Bonferroni's test. A significance threshold of $P < 0.001$ was established.

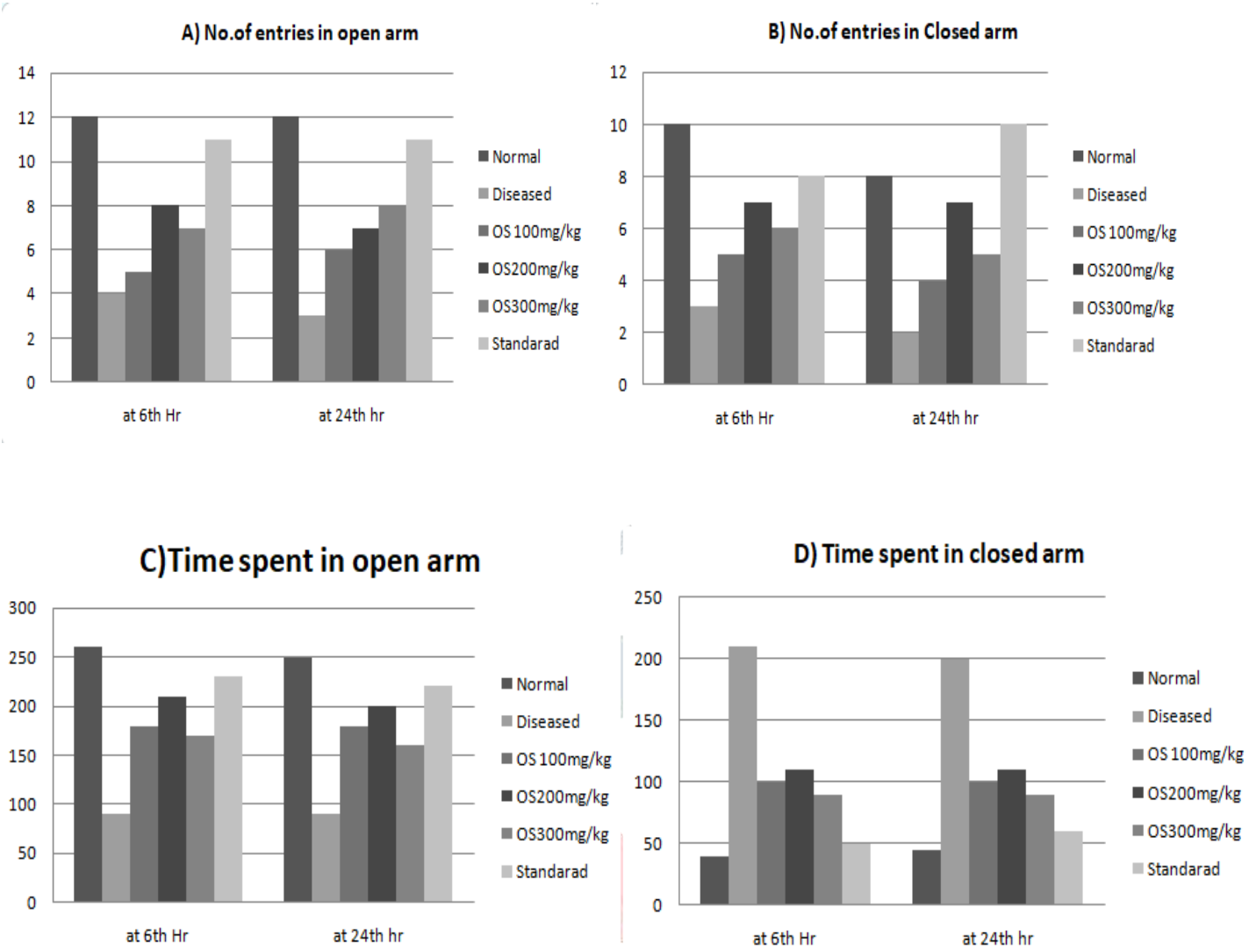
Result and Discussion

Ethanol consumption and body weight changes of the animals:

The daily alcohol intake of rats fed ethanol ranges from 13.28±0.89 to 16.41±0.64 g/kg, with no discernible variation between the groups. A body weight of 225.71±4.56 was recorded.
At the start of the study, the control group weighed 231.30±5.49 g, while the ethanol-fed group weighed 262.34±5.06 g and 242.14±6.01 g at the conclusion of the study.
By the end of the trial, the rats in the ethanol control group had gained 4.6% of their body weight, while the rats in the control group had gained 16.2%.

Elevated Plus Maze Test:

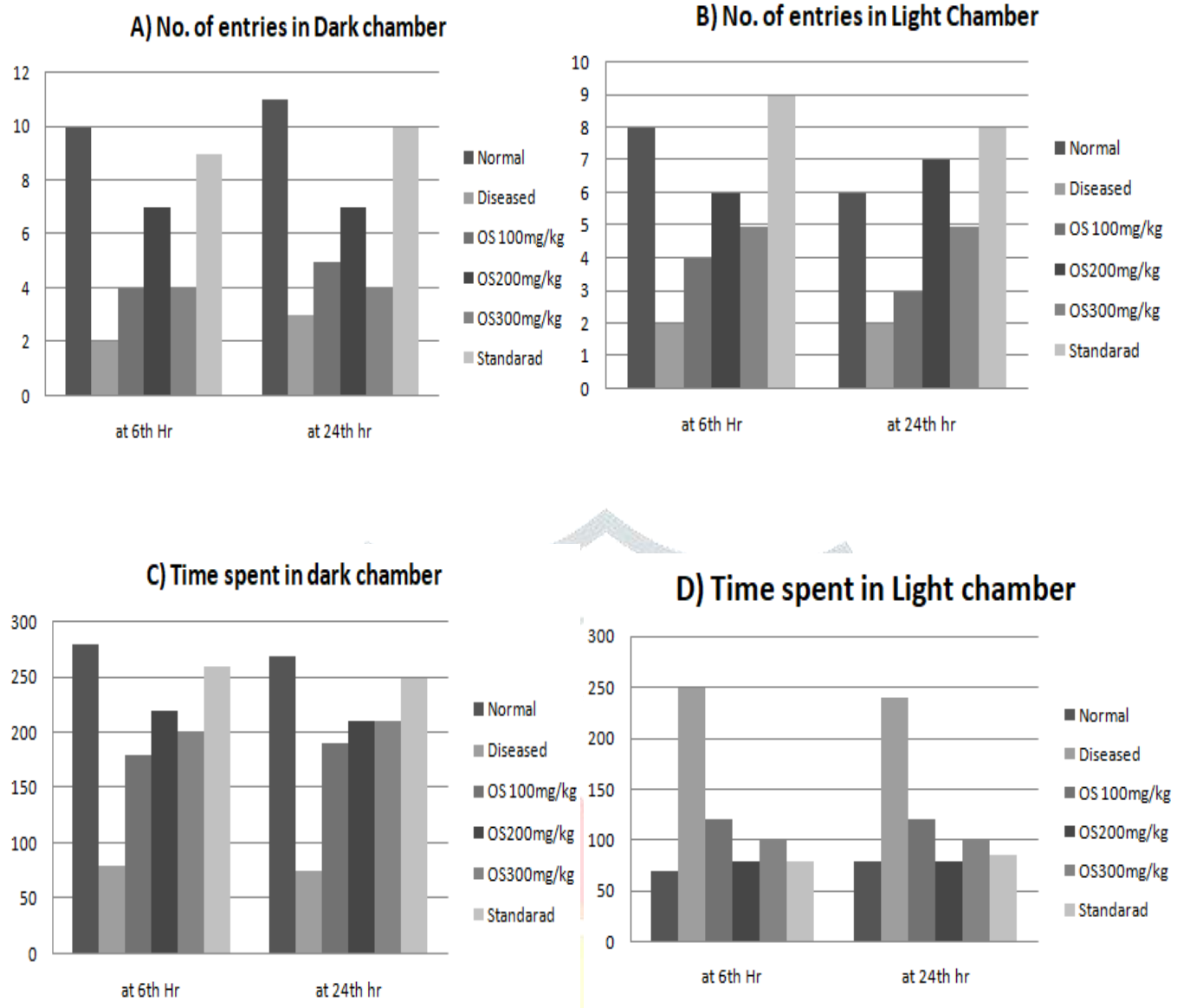
Rats administered ethanol showed a marked reduction in the amount of time spent in open arms as well as the frequency of entrances into the open arms and closed arms when compared to the normal control group; nevertheless, it also demonstrated that Figure 2a-d shows a notable increase in the amount of time spent in the closed arms on the sixth and twenty-fourth hours of the withdrawal period. At the sixth and twenty-fourth hours of alcohol withdrawal, treatment with O. sanctum leaf extract (100, 200, and 300 mg/kg, oral) resulted in a notable increase in the amount of time spent in open arms, as well as the number of entrances into both closed and open arms; nevertheless, it demonstrated a notable reduction in the amount of time spent in closed arms in comparison to the diseased rats under control (Figure 2).



''Figure 2'' Effect of drug treatment on alcohol withdrawal anxiety when tested on the elevated plus maze model. A: No. of entries in open arm; B: No. of entries in closed arm; C: Time spent in the open arm; D: Time spent in the closed arm. Results were presented as mean ± SD analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test. a: P < 0.001 vs. NC, b: P < 0.001 vs. DC, c: P < 0.001 vs. diazepam after the 6th hour. a': P < 0.001 vs. NC, b: P < 0.001 vs. DC, c': P < 0.001 vs. diazepam after the 24th hour.

Light and dark model:

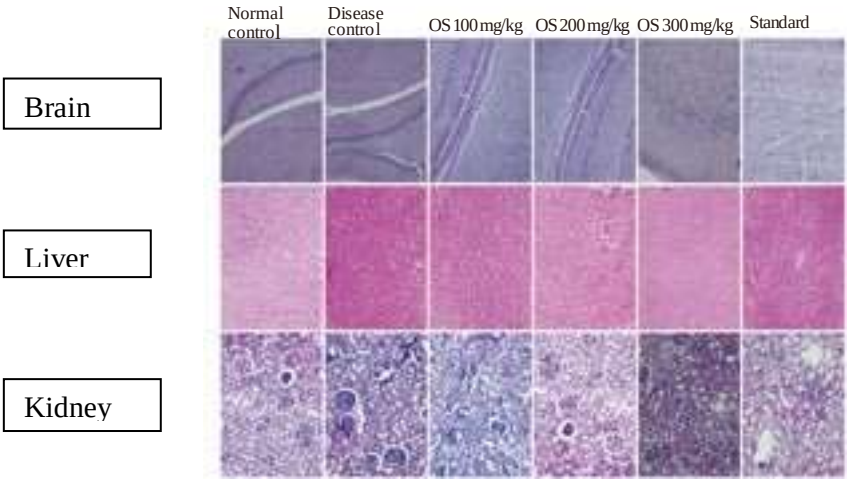
Rats fed ethanol demonstrated a significant decrease in the amount of time spent in open arms and the number of entries into both closed and open arms when compared to the normal control group. However, at the 6th and 24th hours of the alcohol withdrawal, they demonstrated a significant increase in the amount of time spent in the dark chamber (Figure 3a-d). Treatment with O. sanctum leaf extract (100, 200, and 300 mg/kg, orally) at the 6th and 24th hours of alcohol withdrawal produced a significant increase in the time spent in the open arms and in the number of entries into the open arms and closed arm while it showed a significant decrease in the time spent in the dark chamber when compared to the disease control rats (Figure 3).



''Figure 3'' Effect of drug treatment on alcohol withdrawal anxiety when tested on a light and dark model. A: No. of entries in open arm; B: No. of entries in closed arm; C: Time spent in the open arm; D: Time spent in the closed arm. Results were presented as mean ± SD analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test. a: P < 0.001 vs. NC, b: P < 0.001 vs. DC, c: P < 0.001 vs. diazepam after the 6th hour. a': P < 0.001 vs. NC, b': P < 0.001 vs. DC, c': P < 0.001 vs. diazepam after the 24th hour.

Histopathology:

Alcohol-treated animals did not exhibit any pathological changes associated with alcohol consumption or withdrawal. Normal glial cells were found in the brain (Figure 4), the liver had normal hepatocytes with central and portal veins , and the kidney had normal nephrons free of glomerular congestion, interstitial bleeding, and vacuolar degeneration of tubular cells. The alcohol-treated rats' liver, kidney, and brain showed no histopathological alterations



''Figure 4'' Histology of brain, liver, and kidney (H&E, x100).

Discussion

The findings from our study revealed that the ethanolic leaf extracts of *Ocimum sanctum* have been extensively studied for their inhibitory effects of the *O. sanctum* leaf extract on ethanol withdrawal symptoms in alcohol-dependent rats. The ethanolic extraction process helps in obtaining a wide range of bioactive compounds such as flavonoids, phenolic acids, and tannins, which are known for their potent antioxidant effects. A pertinent and reliable paradigm for ethanol intake in rats is the administration of ethanol together with a liquid diet. According to earlier research, rats that consume more than 9 g/kg of ethanol per day for 15 straight days develop dependence, and those that abstain have a variety of symptoms. Major indicators of alcohol withdrawal, including hyperlocomotor activity and withdrawal anxiety, were noted in this study. At the sixth and twenty-fourth hours of abstinence, the incidence of alcohol withdrawal anxiety in the rats administered alcohol was very similar to that seen in earlier research. Similar to several earlier findings, the current investigation shows that O. sanctum significantly reduces anxiety at doses of 100, 200, and 300 mg/kg. Prior research documented the anti-anxiety properties of O. sanctum in normal rats, but our work was the first to show that it also had anti-anxiety effects in animals that abstained from alcohol. In rats given ethanol withdrawal, O. sanctum at doses of 100, 200, and 300 mg/kg had a noteworthy anti-anxiety effect. As a typical medication, diazepam (2 mg/kg, i.p.) was administered. When compared to the rats in the normal group, the alcohol-dependent rats exhibited a marked increase in hyperlocomotor activity on the sixth and twenty-fourth hours of alcohol abstinence. This shift in the animals' body weight is consistent with past research because alcohol alters the absorption, metabolism, and excretion of vital nutrients and reduces the release of digestive enzymes.

The primary organs that are impacted by alcohol are the liver, kidney, and brain; however, histological analysis revealed that the treated rat did not exhibit any pathological alterations associated with alcohol dependence or withdrawal. The progression of the symptoms of alcohol withdrawal is thought to be significantly influenced by the glutamatergic system and NMDA receptors. The hydroalcoholic leaf extract of O. sanctum includes components such as flavonoids, tannins, steroids, terpenoids, and glycosides, according to the phytochemical analysis. According to the literature, luteolin, gallic acid, valine, and β-sitosterol all have well-established anti-anxiety qualities. Because O. sanctum has been shown to have inhibitory effects on the reuptake of neurotransmitters like noradrenaline, serotonin, and dopamine and to modify neuronal excitability via GABAergic and glutamatergic mechanisms, these phytoconstituents may therefore play a protective role in the ethanol abstinence syndrome and may lessen the effects of ethanol deprivation.

Conclusion

In conclusion, *O. sanctum* leaf extract seems to be pharmacologically active by suppressing ethanol withdrawal signs and symptoms and may have therapeutic potential in treating ethanol dependence.

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