EVALUATION OF METHANOLIC LEAF EXTRACT OF *Cymbopogon citratus* ON ALCOHOL WITHDRAWAL SYNDROME IN WISTAR RATS

A Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI – 600 032.

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

G.S.SRI BHARATHI

261925403

Under the guidance of

Dr. V.LALITHA, M. Pharm., Ph. D.,

Associate Professor,

Department of Pharmacology

OCTOBER- 2021

NANDHA COLLEGE OF PHARMACY

ERODE – 638 052. TAMIL NADU.

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GUIDE CERTIFICATE

This is to certify that the work entitled **"EVALUATION OF METHANOLIC LEAF EXTRACT OF *Cymbopogon citratus* ON ALCOHOL WITHDRAWAL SYNDROME IN WISTAR RATS"** submitted to The Tamil Nadu Dr. M.G.R Medical University Chennai, in partial fulfillment of the Degree of Master of Pharmacy programme in Pharmacology, was carried out by **G.S.SRI BHARATHI**, in the Department of Pharmacology, Nandha college of Pharmacy, Erode, under my direct supervision and guidance in our laboratory.

Place : Erode

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EVALUATION CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"EVALUATION OF METHANOLIC LEAF EXTRACT OF *Cymbopogon citratus* ON ALCOHOL WITHDRAWAL SYNDROME IN WISTAR RATS"** submitted to The Tamilnadu Dr.M.G.R. Medical university, Chennai was carried out by **Ms. G.S. Sri Bharathi (261925403),** in Nandha college of pharmacy, Erode for the fulfillment of the degree of **Master of Pharmacy** in **Pharmacology** under direct supervision of **Dr.V.Lalitha, M.Pharm., PhD.,** Associate Professor, Department of Pharmacology, Nandha college of pharmacy, Erode**.**

This work is original and has not been submitted in part or full for the award of any other degree or diploma of any university .

INTERNAL EXAMINER **EXTERNAL EXAMINER**

DECLARATION CERTIFICATE

The work presented in this thesis entitled **"EVALUATION OF METHANOLIC LEAF EXTRACT OF *Cymbopogon citratus* ON ALCOHOL WITHDRAWAL SYNDROME IN WISTAR RATS"** was carried out by me in the Department of Pharmacology, Nandha College of Pharmacy, Erode, under the direct supervision and guidance of **Dr.V.Lalitha, M.Pharm., PhD**., Associate Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode -52.

This work is original and has not been submitted in part or full for any other degree or diploma of any university.

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Place: Erode

Date:

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ABBREVIATIONS

ABBREVIATION	EXPANSION
AWS	Alcohol Withdrawal Syndrome
ICMR	Indian Council of Medical Research
CNS	Central Nervous System
GABA	Gamma-Aminobutyric acid
NMDA	N-methyl-d-aspartate
ROS	Reactive Oxygen Species
MDA	Malondialdehyde
SOD	Superoxide Dismutase
CAT	Catalase
GPx	Glutathione Peroxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
LPO	Lipid Peroxidation
PUFA	Polyunsaturated Fatty acids
GSH	Glutathione
AUD	Alcohol use disorder
ICU	Intensive care unit
DT	Delirium tremens
BZD	Benzodiazepines
CIWA	Clinical Institute Withdrawal Assessment
MECC	Methanolic extract of *Cymbopogon citratus*
IAEC	Institutional Animal Ethics Committee
TP	Total Protein
TBARS	Thiobarbituric acid reactive substances
TBA	Thiobarbituric acid

TCA	Trichloroacetic acid
DTNB	Dithiobisnitrobenzoic acid
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
ANOVA	Analysis of Variance

INTRODUCTION

ALCOHOL WITHDRAWAL SYNDROME

Alcohol use is a pervasive problem that is taking an increasing toll on the world's population. The World Development Report found that the alcohol related disorders affects 5-10% of the world's population each year and accounted for 2% of the global burden of disease. Globally alcohol consumption has increased in recent decades, with most of the increase in developing countries. Increase is more in countries where use of alcohol is traditionally less on population level and methods of prevention, control or treatment are not easily available. ICMR bulletin estimated 62 million alcoholics in India which is as big as that of the population of France [1].

The alcohol withdrawal syndrome (AWS) is a set of signs and symptoms that typically develops in alcohol dependent people within few hours of their last drink. It may occur unintentionally if abstinence is enforced by illness or injury, or deliberately if the person voluntarily stops drinking because of an alcohol-related illness [2].

SYMPTOMS OF AWS

The syndrome's signs and symptoms are similar to, but not identical to, those of autonomic hyperactivity, which is the opposite of the effects of alcohol intoxication. They indicate a central nervous system (CNS) homoeostatic readjustment to the neuroadaptation that occurs with prolonged alcohol intoxication. They range from mild to severe in severity.

For diverse purposes, the symptoms of AWS have been categorized in various ways. Autonomic hyperactivity symptoms are included in all classifications; however, classifications differ on whether seizures, hallucinations, and delirium are late and important symptoms of AWS, consequences of poorly controlled AWS, or different clinical disorders.

A popular classification of the symptoms of AWS is listed in table 1. The first consists of those of autonomic hyperactivity which appear within hours of the last drink and usually peak

within 24–48 h. The most common features are tremulousness, anxiety, sweating, nausea, vomiting, and agitation.

The second sets of symptoms are those of neuronal excitation, which include epileptiform seizures (usually grand mal) that usually occur within 12–48 h of abstinence. The third set of symptoms comprises delirium tremens (or alcohol withdrawal delirium), which develops in a very few cases; it is characterized by auditory and visual hallucinations, confusion and disorientation, impaired attention, clouding of consciousness, and pronounced autonomic hyperactivity. If untreated, death may occur from respiratory and cardiovascular collapse [1].

Table 1: Classification of Symptoms of AWS [3]

Autonomic symptoms	Motor symptoms	Awareness symptoms	Psychiatric symptoms
Tachycardia	Hand tremor	Insomnia	Illusion
Tachypnea	Tremulousness of body	Irritability	Delusion
Dilated pupils	Seizures	Disorientation	Hallucination
Elevated blood pressure	Ataxia	Delirium	Paranoid ideas
Elevated body temperature	Gait disturbances	Agitation	Anxiety
Diaphoresis	Hyper-reflexia		Instability
Nausea and vomiting	Dysarthria		Combativeness
Diarrhea			

Based on the time of onset of the symptoms, it can be classified chronologically as mentioned in figure 1:

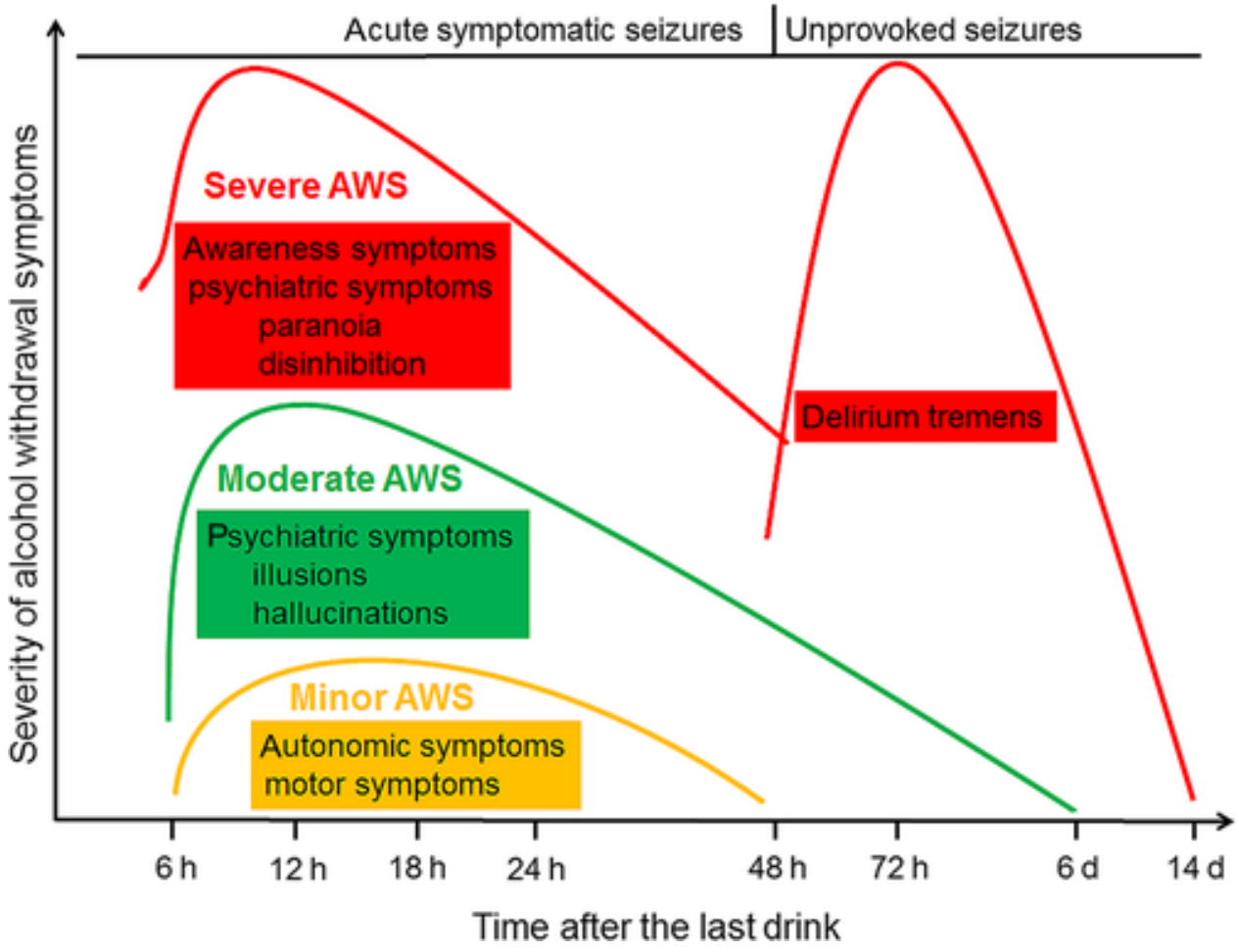

Figure 1: Chronological development of various symptoms of AWS.

The symptoms of AWS can also be classified based on their severity as mild, moderate and severe symptoms. The list of that classification in mentioned in table 2.

Table 2: Classification of symptoms of AWS based on severity [4]

Stage	Time of onset after last drink	Signs and symptoms
I – Minor Withdrawal Symptoms	6–12 h	Tremors, diaphoresis, nausea/vomiting, hypertension, tachycardia, hyperthermia, tachypnea
II – Alcoholic Hallucinosis	12–24 h	Dysperceptions: Visual (zoopsy), auditory (voices) and tactile (paresthesia)
III – Alcohol Withdrawal seizures	24–48 h	Generalized tonic-clonic seizures (with short or no postictal period)
IV – Delirium Tremens	48–72 h	Delirium, psychosis, hallucinations, hyperthermia, malignant hypertension, seizures and coma

According to Clinical institute withdrawal assessment of alcohol scale, the symptoms after alcohol withdrawal may be scored as follows [5]:

a) Nausea and vomiting

0- No nausea no vomiting

1-4- Mild nausea with no vomiting to intermittent nausea with dry heaves

5-7- Constant nausea, frequent dry heaves and vomiting

b) Tremor

0- No tremor

1-4- Not visible but can be felt to moderate tremor

5-7- Moderate to severe tremor

c) Paroxysmal sweats

0- No sweat visible

1-4- Barely perceptible sweating, palms moist to beads of sweat obvious on forehead

5-7- Drenching sweats

d) Anxiety

0- No anxiety

1-4- Mild to moderate anxious

5-7- Equivalent to acute panic states as seen in severe delirium or acute schizophrenic reactions

e) Tactile disturbances

0- None

1- Very mild itching, pins and needles, burning or numbness

2- Mild itching, pins and needles, burning or numbness

3- Moderate itching, pins and needles, burning or numbness

4- Moderately severe hallucinations

5- Severe hallucinations

6- Extremely severe hallucinations

7- Continuous hallucinations

f) Agitation

0- Normal activity

1-4- Greater than normal to moderately fidgety and restless

5-7- Paces back and forth during most of the interview, or constantly thrashes about

g) Auditory disturbances

0- Not present

1- Very mild harshness or ability to frighten

2- Mild harshness or ability to frighten

3- Moderate harshness or ability to frighten

4- Moderately severe hallucinations

5- Severe hallucinations

6- Extremely severe hallucinations

7- Continuous hallucinations

h) Visual disturbances

0- Not present

1- Very mild sensitivity

2- Mild sensitivity

3- Moderate sensitivity

4- Moderately severe hallucinations

5- Severe hallucinations

6- Extremely severe hallucinations

7- Continuous hallucinations

i) Headache, fullness in head

0- Not present

1- Very mild

2- Mild

3- Moderate

4- Moderately severe

5- Severe

6- Very severe

7- Extremely severe

j) Orientation and clouding of sensorium

0- Oriented and can do serial additions

1- Cannot do serial additions or is uncertain about date

2- Disoriented for date by no more than 2 calendar days

3- Disoriented for date by more than two calendar days

4- Disoriented for place/person

PATHOPHYSIOLOGY OF AWS [6]

Each neuron releases one or a few unique type of neurotransmitters. Inhibitory neurotransmitters transiently decrease the response of other neurons to further stimuli, whereas excitatory neurotransmitters produce the opposite effect. The main neurotransmitters involved in the progression of AWS are GABA and Glutamate.

The effects of alcohol consumption on these neurotransmitters vary depending on the duration of alcohol administration:

1. Short term alcohol consumption
2. Long term alcohol consumption.

Short term alcohol consumption

Short-term alcohol consumption depresses brain function by altering the balance between inhibitory neurotransmission and excitatory neurotransmission. Especially, alcohol can act as a depressant by increasing inhibitory neurotransmission, by decreasing excitatory neurotransmission, or through a combination of both.

The amino acid GABA is the main inhibitory neurotransmitter in the brain. Acting through a receptor subtype called $GABA_A$, GABA leads to a state of sedation and reduced anxiety. Short-term alcohol consumption increases the inhibitory effect of $GABA_A$ receptors. Alcohol might produce sedative effects also by reducing excitatory neurotransmission.

The major excitatory neurotransmitters in the brain are the amino acids aspartate and glutamate, which act through both NMDA receptors—so named because they respond to the synthetic chemical N-methyl-d-aspartate—and non-NMDA receptors. Short-term exposure to intoxicating concentrations of alcohol appears to inhibit both NMDA and non-NMDA receptor activity, resulting in sedation.

Long term alcohol consumption

Although short-term alcohol exposure may increase $GABA_A$ receptor function, prolonged drinking has the opposite effect. This decrease in $GABA_A$ function may result from a reduction in receptor levels or a change in the protein composition of the receptor, leading to reduced sensitivity to neurotransmission. Similarly, glutamate receptors appear to adapt to the inhibitory effects of alcohol by increasing their excitatory activity.

Alcohol withdrawal effects

The $GABA_A$ and NMDA receptor systems together could be responsible for a main portion of the alcohol withdrawal syndrome.

GABA's role in withdrawal is related to reduce inhibitory function. As previously described, long-term alcohol use may lead to a decrease in $GABA_A$ receptor function. In the absence of alcohol, the decreased activity of inhibitory GABA neurotransmission might contribute to the anxiety and seizures of withdrawal. These symptoms are treated, at least in part, using medications that increase $GABA_A$ receptor function, such as diazepam (Valium) and other sedatives.

Increased NMDA receptor activity significantly increases the amount of calcium that enters nerve cells. Although calcium is essential for nerve cell function, an excess of this substance within neurons has been reported to produce cell toxicity or death. In fact, repeated cycles of alcohol consumption and abstinence may cause calcium-related brain damage.

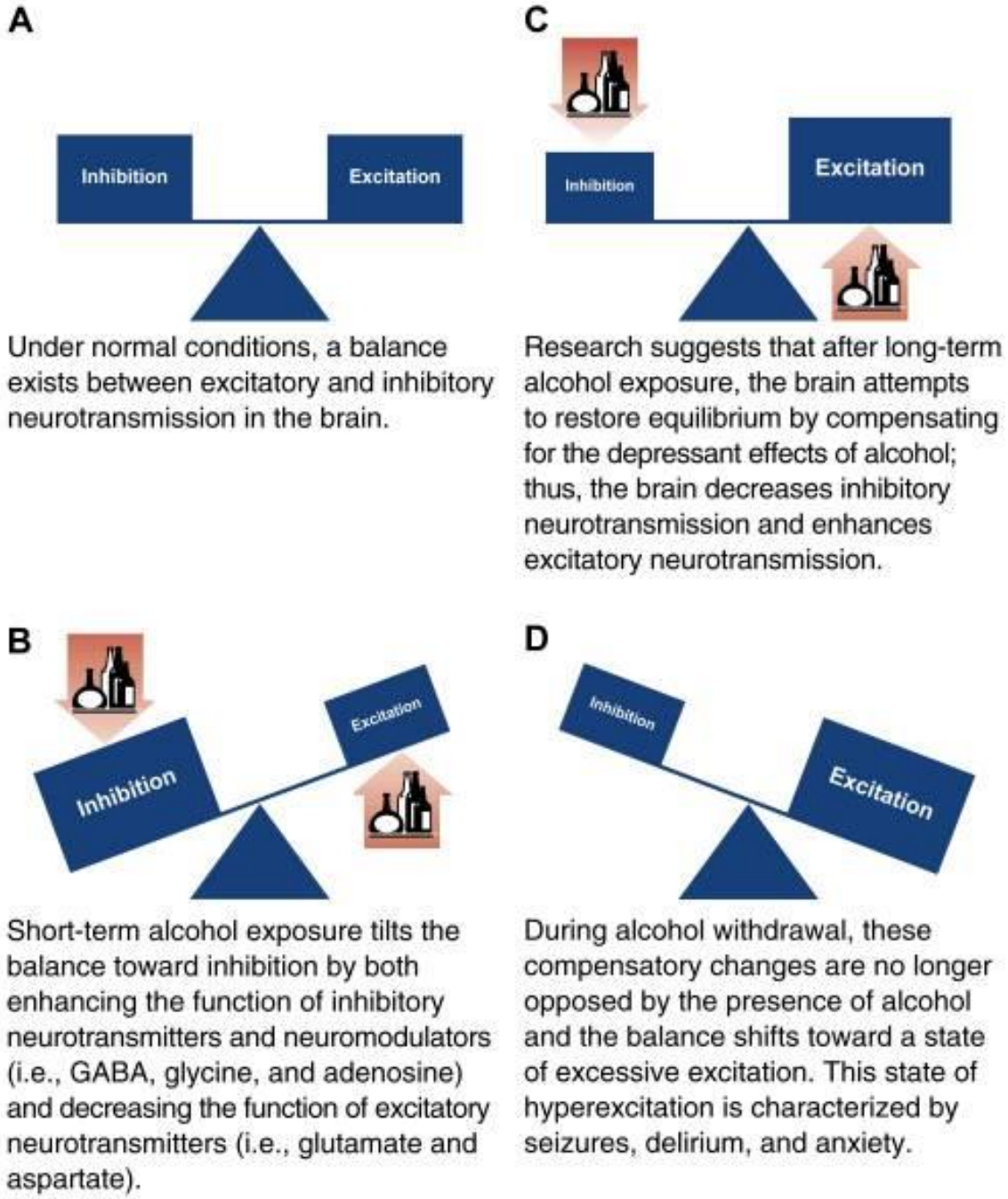

Figure 2: Schematic representation of alcohol's effects on the balance of inhibitory and excitatory neurotransmission in the brain.

OXIDATIVE STRESS IN ALCOHOLICS

Many routes of alcohol-induced organ damage have been discovered to involve oxidative stress. It's a condition marked by high levels of reactive oxygen species (ROS) generation and ineffective scavenging due to inadequate antioxidant defences. The liver is the primary organ impacted by alcohol, and several studies have shown that alcoholics experience increased oxidative stress [7]. Alcohol-induced oxidative stress is the result of the combined production of reactive oxygen species [ROS; e.g. malondialdehyde (MDA), an index of lipid peroxidation] and impairment of antioxidant defenses [e.g. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), which are involved in the elimination of ROS] [8].

Generation of free radicals

Biological free radicals include reactive oxygen species, reactive nitrogen species, reactive sulfur species, free radicals obtained from xenobiotics.

1) **Superoxide anion radical (O_2-)**

It is generated from NADPH oxidase and from mitochondria.

i) NADPH oxidase is present in the lysosomal cell membrane. It steals electron from O_2 resulting in the formation of superoxide anion radical (O_2). It is converted to hydrogen peroxide and is a spontaneous reaction which is known as respiratory burst. This hydrogen peroxide may react with the chlorine in the presence of myeloperoxidase to form hypochlorous acid or it may produce hydroxyl radicals, by the Fenton reaction which uses the metal ion Fe^{3+}. They are capable of killing bacteria.

ii) From Mitochondria: Ubiquinone, which is a terminal acceptor of electron, is converted to semiquinone (free radical). By reacting with O_2, it forms (O_2-)superoxide radical and with H_2O_2, it forms hydroxyl radical.

2) Hydrogen peroxide

It is formed by the dismutation of superoxide by the enzyme superoxide dismutase.

$$O_2^- + O_2^- \xrightarrow{SOD} H_2O_2$$

Hydrogen peroxide is generated from:

i) Aminoacid oxidases: Flavin is a co- enzyme required for the oxidative deamination of aminoacid. The reduced flavin attacks molecular oxygen to form hydrogen peroxide.

ii) Xanthine oxidase: Xanthine oxidase catalyses the convertion of hypoxanthine to xanthine in the presence of xanthine oxidase and hydrogen peroxide which comes from molecular oxygen.

iii) Peroxisomes: Peroxisomes is the site of β-oxidation of fatty acids. β- oxidation of fatty acids is catalysed by acetyl co-enxyme-A dehydrogenase. During this process, a co-enzyme called FAD which donates two electrons gets reduced to $FADH_2$. Again it is converted to FAD. During that process it gives out O_2 and H_2O[9].

3) Hydroperoxy radical

They are highly lipophillic and capable of initiating lipid peroxidation.

Lipid peroxidation

Lipid peroxidation is a self-perpetuating widespread process through which lipid components of the membranes from cell organelles are converted into lipid peroxides an event which strikingly contribute towards the formation of lipofusion pigment[10]. The membrane debris derived from cell membrane system including organelles, while lysosomic ROS are the end-result of a complex oxidative chain of molecular event mainly initiated in mitochondria during energy production. Fenton- type reaction between H_2O_2 and Fe(II) take place leading to the production of the harmful hydroxyl radicals, which are capable of initiating processes of lipid peroxidation[11]. Malondialdehyde is the major reactive aldehyde resulting from the peroxidation

of biological membrane polyunsaturated fatty acids (PUFA). MDA, a secondary product of LPO, is used as an indicator of tissue damage by a series of chain reactions.

MDA is also a by-product of prostaglandin biosynthesis. It reacts with thiobarbituric acid and produces a red- coloured product. MDA is a mutagenic and genotoxic agent that may contribute to the development of human cancer.

ANTIOXIDANT ENZYMES

1) Superoxide dismutase (SOD)

SODs are a family of metalloenzymes that converts superoxide to hydrogen peroxide (H_2O_2) and represents the first line of defense against oxygen toxicity.

$$2O_2^- + 2H \xrightarrow{SOD} H_2O_2 + O_2$$

Three forms of SOD have been described. The first isoform, containing copper and zinc at its acive site (Cu/Zn SOD-1), is found in the cytoplasm of cells. Another isoform, containing manganese at its active site is located in mitochondria (Mn SOD-2). The third isoform is present in extracellular fluids such as plasma (Cu/Zn SOD-3). SOD is a stress protein, which is synthesized in response to oxidative stress. It was found that the traces of copper, zinc and manganese metals are essential for maintaining the antioxidant activity of SOD [11].

2) Glutathione peroxidase(GPx)

GPx is one of the major enzymes responsible for the degradation of hydrogen peroxide and organic peroxides in the brain. GPx catalyses the oxidation of GSH to GSSG at the expense of H_2O_2. There are two isoforms been identified, selenium-dependent which are highly active towards H_2O_2 and organic hydroperoxides and selenium independemt GPx. GPx activity has been reduced in selenium deficiency [12].

3) Catalase (CAT)

It is a heme-containing protein present in most cells.

$$2\ H_2O_2 + 2\ H_2O \xrightarrow{\quad CAT \quad} O_2$$

Catalase is 104 times faster than GPx. It consists of four protein subunits, each containing a heme Fe (III)- protoporphyrin group bound to its active site. GPx and CAT were found to be important in the inactivation of many environmental mutagens [13].

4) Glutathione (GSH)

GSH is a ubiquitous tri-peptide formed from three aminoacids glutamate, glycine and cysteine and synthesized by two ATP- dependent enzymatic reactions. GSH has major intracellular antioxidant molecule. It plays a critical role in detoxification of peroxides and electrophilic toxins as a substrate for GSH peroxidase and GSH transferase. It was shown that depletion of GSH enhances cerebral ischemic injury in rats [14].

DIAGNOSIS [15]

AWS is diagnosed by physical examination and evaluation of blood alcohol level. The blood alcohol level varies according to the 37 frequency and quantity of alcohol intake, gender, and body weight. Commonly, females have shown high blood alcohol level compared to males, both of them consuming the same quantity of alcohol. The physiological effects produced by different blood alcohol levels are shown in table 3.

Table No. 3: Blood alcohol levels and their effects

Blood alcohol level	Effect
10-40 mg/dl	Relaxation, mild euphoria, increased social interaction
50-70 mg/dl	Euphoria, motor impairment
80 mg/dl	Impairment in driving
80-120 mg/dl	Emotional swings and depression
120-150 mg/dl	Motor function and speech are all severely affected
150-200 mg/dl	Appear drunk. Visual impairment
200-300 mg/dl	Vomiting, symptoms of alcohol intoxication
300-400 mg/dl	Severe alcohol intoxication, total loss of consciousness
400-500 mg/dl	Fatal and may be comatose
>500 mg/dl	Highly dangerous. Fatal blood alcohol level

PREVALENCE [3]

An estimated 76.3 million people worldwide have alcohol use disorders (AUDs), and these account for 1.8 million deaths each year. It is estimated that up to 42% of patients admitted to general hospitals, and one-third of patients admitted to hospital intensive care units (ICU) have Alcohol use disorders. Alcohol withdrawal syndrome occurs in about 8% of hospitalized AUD inpatients. A complicated AWS includes epileptic seizures or/and delirium tremens (DT), the occurrence of which may be as high as 15% in AUD patients. Patients with delirium have a high rate of comorbidities, and their mortality rate is comparable to that of patients with severe malignancies. However, with early detection and appropriate treatment, the expected mortality is in the range of 1% or less.

MANAGEMENT OF AWS [16]

An ideal drug for AWS should not interact with alcohol, should suppress the 'drinking behavior' without producing cognitive or/and motor impairment and it should not have a potential for abuse.

Pharmacological strategies for treatment of AWS

The treatment for AWS depends on the symptoms developed. There are several classes of drugs recommended for the treatment of alcohol withdrawal syndrome:

- Benzodiazepines
- Antipsychotic agents
- Antiepileptic agents
- α2- agonistic agents
- Anesthetic agents

Benzodiazepines

At present BZDs represent the 'gold-standard' in the treatment of AWS. Furthermore, BZDs are the only drugs that have been shown to be effective in reducing the development of complicated forms of AWS, with an 84 % reduction in the incidence of seizures, DT, and the accompanying risk of mortality. The efficacy of BZDs in the treatment of AWS seems to be mediated by their stimulation of $GABA_A$ receptors with alcohol mimicking effects. Mostly diazepam(5 - 20 mg) and chlordiazepoxide (50 – 100 mg) are being used.

Barbiturates

Barbiturates have a narrow therapeutic window, a risk of severe sedation, and they interact with the clearance of many medications, hence their use in the treatment of AWS has been limited. Barbiturates, on the other hand, have a specific application in the ICU setting, for patients who require high doses of BZDs to manage AWS symptoms or who are developing DT. When phenobarbital is used with BZDs, BZD binding to the $GABA_A$ receptor is increased, perhaps increasing BZD efficacy.

Propofol

In the ICU setting, propofol is a promising drug. The antagonist impact on the NMDA receptor, GABAA stimulation, and brief duration of effect are the key benefits, allowing for a quick assessment of a patient's mental health following withdrawal. Propofol is an effective treatment choice for patients with severe DT who are unable to manage their symptoms with high dosages of BZDs.

α_2-Agonists, β -blockers and neuroleptics

α_2-Agonists (Eg: clonidine and dexmedetomidine), β-blockers(Eg: atenolol) and neuroleptics(Eg: Haloperidol) have been tested and are presently used as adjunctive treatment for AWS. These medicines are not indicated as monotherapy due to their lack of efficacy in avoiding severe AWS and the potential of masking AWS symptoms. They should only be used as a supplement to BZD therapy in patients with co-morbidities and to manage neuro-autonomic aspects of AWS that are not sufficiently controlled by BZD administration.

Carbamazepine

Carbamazepine is a tricyclic anticonvulsant able to produce a GABAergic effect and to block NMDA receptors. It has been proven to be an effective drug in the treatment of AWS, at least in mild to moderate forms, producing an effect superior to placebo and non-inferior to BZDs.

OBJECTIVES OF CLINICAL MANAGEMENT OF ALCOHOL WITHDRAWAL SYNDROME(MAW) [17]

Detoxification or MAW is a part of an overall treatment pathway, and failure to consider ongoing treatment for the newly abstinent drinker often leads to relapse. The goals of the MAW episode are to safely achieve physical withdrawal from alcohol, prevent (or treat) severe withdrawal phenomena such as seizures or delirium tremens, and optimize physical and mental health.

Knowledge of the usual level of drinking and the time of the last alcoholic drink helps to measure the extent and severity of the AWS. Patients may under-report their consumption (quantity and/or frequency), and so validation by other reliable sources is helpful. It is also important to enquire about the outcome of past attempts to stop and the use of other psychoactive substances.

CURRENT MANAGEMENT

Alcohol withdrawal seizures (<50%) occurrence rate increased with concurrent risk factors such as prior epilepsy, brain lesions, and use of drugs. Benzodiazepines (intravenous diazepam and lorazepam) are, effectively treated against alcohol withdrawal seizures. The benzodiazepine dose is calculated based on average alcohol intake per day.

Alcohol (in g) = Volume of liquor (ml) × 0.008 × (%) ethanol content in the liquor (w/v).

In the case of mild to moderate, AWS are treated with supportive therapy including intravenous rehydration, multivitamin, electrolyte supplements, and thiamine especially for Wernicke-Korsakoff syndrome (thiamine deficiency in the brain due to Alcohol Withdrawal). A banana bag is an IV fluid containing vitamins and minerals that are preferably used to treat alcoholics. The bags contain vitamins (thiamine, folic acid), and magnesium sulfate used to correct the chemical alterations in the human body induced by alcohol.

Some of the treatment regimens used in alcohol withdrawal states are fixed-dose regimen (Diazepam 60 mg/ Chlordiazepoxide 125 mg), loading dose regimen (Diazepam 20 mg), symptom triggered treatment (CIWA-Ar score 8 or more Chlordiazepoxide), symptom monitored loading dose (Diazepam 20 mg/2 h till CIWA-Ar score becomes 10) and rapid loading with close monitoring (Diazepam).

Benzodiazepine withdrawal after prolonged usage may also lead to protracted withdrawal syndromes. However, for severe symptoms scored 5-7 in CIWA, allopathic medicines are the first line choice of drugs. Whereas, herbal medicines can be used to treat mild to moderate symptoms scored 1-4 in CIWA categories [15].

ANIMALS USED [15]

Mostly male Wistar albino rats are preferred over the female (some studies are carried out in females and both sexes). A female alcohol-preferring rat transgenic animal model and C57BL/6 transgenic mouse that is specific to alcohol withdrawal syndrome are also used.

Cymbopogon citratus

Lemongrass (Poaceae) is a perennial grass which is evenly dispersed and found in the tropic regions, South and Central America widely used for their pleasant taste and therapeutic properties. It is commonly known as lemongrass or citronella but due to its distribution, it has several other names. Cymbopogon originated from the Greek word "kymbe - pogon" meaning boat-beard (due to its flower spike configuration) and citratus (Latin) means lemon-scented leaves. It is part of Poaceae family. Findings have reported more than 55 species. About three species *Cymbopogon citratus* (West Indian grass), *Cymbopogon flexuosus* (East Indian or Malabar grass), and *Cymbopogon pendulus* (Jammu grass) are widely distributed [18].

The consumption of infusions and decoctions made from *C. citratus* has been a common practice in various countries since the discovery of the medicinal value of the plant throughout recorded history. It is most frequently consumed for recreational and medical/therapeutic purposes, much like green, black, and red teas, herbal tea blends, and coffee. Many consumers prefer *C. citratus* tea to other beverages, because of its physicochemical characteristics, including taste, distinctive lemony smell, color, strength, and intensity, while many others consumes *C. citratus* tea or decoction for physiological reasons. The pharmacokinetics of its constituents and the mechanism of action of its isolated compounds may substantiate the rationale behind its use in traditional and Ayurvedic medicine in many part of the world [19].

PLANT PROFILE

Cymbopogon citratus

Family name : Poaceae(Gramineae)

Synonyms : *Andropogon citriodorum, Acroceras citratus*

Common name : West Indian Lemongrass, Serai, Fever grass, Oil grass

Plant division : Angiosperm

Maximum height : 0.6- 1.2m

Native habitat : Terrestrial

Plant Features:

Foliage

Leaf blades are light green and strap-shaped (up to 0.9 m long, 2.5 cm wide). Crushed leaves exude a lemony scent.

Stems

A pseudostem formed from tightly-overlapping leaf sheaths on non-flowering shoots is 12-25 cm long and 1-2 cm across, bulbous and thickening towards base of plant. It is fragrant when crushed and yellowish-brown or reddish.

Flowers

Numerous brownish florets held on compound drooping panicles up to 0.5 m across, rarely produced in cultivation.

Figure 3: *Cymbopogon citratus* plant

Figure 4 : *Cymbopogon citratus* leaf

Fruits

Simple fruit, Spikelets with hairy awns, rarely observed [20].

Parts used

Leaf and whole plant.

Phytochemicals present

Flavonoids, Tannins, Saponins, Steroids, Terpenoids, Coumarins [21]

Part used in this study

Leaf.

ESTABLISHED PHARMACOLOGICAL ACTIVITIES

Anti-microbial activity: The ethanolic extracts of the leaves of Lemon grass showed potential antibacterial property against *Staphylococcus aureus*. Flavonoids and Tannins found in the extract are reported to be responsible for its activity.

Anti-fungal activity: *Candida albicans* is an important pathogen of human infections; moreover, other species can be associated with some infections. The anti-fungal activity of lemongrass and citral against *Candida species* was studied and the study reported that lemongrass oil and citral have a potent in vitro activity against Candida spp.

Anti-protozoan activity: The family Trypanosomatidae harbours protozoans that are agents of important illnesses in humans, animals and in plants. This family also includes some lower trypanosomatids such as Blastocrithidia, Crithidia, and Herpetomonas, monoxenous protozoans usually found in insect hosts. The essential oil extracted from *Cymbopogon citratus* showed anti-protozoan activity against *Crithidia deanei*.

Anti-oxidant activity: The role of phenolic acid and flavonoids as natural anti-oxidants and free radical scavenger has been of interest due to their pharmacological behavior. Phenolic acids present in the plant showed the anti-oxidant profile.

Anti-diarrheal activity: In practice, the whole stalk and the leaf of lemongrass are boiled and the decoction is drunk to relieve the diarrhea. In view of its popular use in traditional medicine system, the anti-diarrheal efficacy of *C. citratus* stalk decoction and its main chemical constituent citral, was studied.

Anti-mutagenic activity: The ethanolic extract of lemongrass was found to possess anti-mutagenic properties towards chemical induced mutation in Salmonella typhimurium strains TA98 and TA100.

Anti-Inflammatory activity: Anti-Inflammatory Activity of *Cymbopogon citratus* leaf infusion in lip polysaccharide stimulated dendritic cells was studied and used for the treatment of inflammatory diseases, in particular of the gastrointestinal tract.

Anti-malarial activity: In vivo antimalarial activity of essential oil obtained from *Cymbopogon citratus* on mice infected with *Plasmodium berghei* was studied.

Anti-nociceptive activity: Essential oil of *C. citratus* possesses a significant anti-nociceptive activity. Comparing the results Obtained with three different experimental models of nociception viz., hot-plate, acetic acid-induced writhing in mice, and formalin test, essential oil acts both at the peripheral and central levels.

Anti-hepatotoxic activity: The aqueous leaf extracts of *Cymbopogon citratus* showed anti-hepatotoxic action against cisplatin induced hepatic toxicity in rats. Hence the extracts have the potential to be used for the management of hepatopathies and as a therapeutic adjuvant in cisplatin toxicity [22].

Anti-obesity and antihypertensive activity: Lemon grass has been incorporated in hypolipidemic and hypoglycemic drugs. In folk and Ayurvedic medicine, it has been used to regulate glucose, lipid and fat level in the blood serum which could prevent obesity and hypertension, usually taken as tea. The plant has been used to maintain blood glucose through secretion of insulin (hyperinsulinemia). It reduces blood pressure which could lead to hypertension. Citral isolated from *C. citratus* has function as endotheliumin dependent vaso-relaxation through the blockage of Ca2+ influx and prostacyclins (PGI2) channel.

Anti-HIV activity: Citronella oil isolated from *C. citratus* leaf was reported to effectively cure mouth thrush caused by *Candida albicans* in HIV/AIDS patients within 1–5 days.

Antidiabetic activity: Diabetes is one of the lethal diseases of the twentieth century. It inhibits the pancreas from production of adequate insulin and could prevent the regulation of blood sugar. The in-vivo antidiabetic potency of *C. citratus* was investigated via molecular docking at dosage rate of 400 and 800 mg. The extracts show pronounced reduction in the level of insulin , glucose and triglycerides. The in-vitro antidiabetic potential of *C. citratus* was investigated against Type II diabetes via α-amylase and α-glucosidase inhibitory assays. The inhibition of 99.9% (1 mg/mL) and EC50 (0.31 mg/mL) was identified for α-glucosidase and α-amylase, respectively[18].

LITERATURE REVIEW

Fabio Attilia *et al.*, (2018) mentioned Alcohol withdrawal syndrome (AWS) as a medical emergency, rare in the general population, but very common among alcoholic individuals, which could lead to severe complications when unrecognized or late treated. It represents a clinical condition which can evolve in few hours or days following an abrupt cessation or reduction of alcohol intake and is characterized by hyperactivity of the autonomic nervous system resulting in the development of typical symptoms. The Clinical Institute Withdrawal Assessment of Alcohol Scale, revised version (CIWA-Ar), is the tool for assessing the severity of AWS. The support to patient with AWS includes pharmacological intervention as well as general support, restoration of biochemical imbalances and specific therapy. Regarding the pharmacological treatment, benzodiazepines represent the gold standard, in particular long-acting benzodiazepines, administered with a gradual reduction up to cessation [23].

Ekkasit Kumarnsit *et al.*, (2007) reported that the administration of the aqueous extract of *Mitragyna speciosa* at a dose of 300 mg/kg significantly inhibited ethanol withdrawal induced behaviors that included rearing, displacement and head weaving. The results also showed that at doses of 100, 300 and 500 mg/kg *M. speciosa* showed antidepressant activity without effect on the spontaneous motor activity [24].

Ilke Coskun *et al.*, (2006) investigated the effects of *Hypericum perforatum* on ethanol withdrawal syndrome in ethanol-dependent rats. Adult male Wistar rats were subjects. Ethanol (7.2% v/v) was given to rats by a liquid diet for 15 days. *Hypericum perforatum* extract (HPE) (25–200 mg/kg) and saline were injected to rats intraperitoneally just before ethanol withdrawal. HPE (25–200 mg/kg) produced some dose dependent and significant inhibitory effects on locomotor hyperactivity at second and sixth hour of ethanol withdrawal. In addition, it significantly reduced the number of stereotyped behaviors at the same dose range. HPE (50 and 100 mg/kg) produced some significant inhibitory effects on tremor and audiogenic seizures during withdrawal period [25].

Meilan Xue *et al.*, (2021) investigated the neuroprotective activity of fucoidan in alcohol exposure and withdrawal mice and the underlying mechanisms. C57BL/6J mice were

used to establish the murine model of chronic alcoholism and withdrawal over 10 weeks of alcohol exposure. After fucoidan treatment, the depression-like behaviors in mice with alcoholism were improved, and 5-hydroxytryptamine and brain derived neurotrophic factor levels were increased both in serum and brain tissues. Fucoidan treatment attenuated the increase of lipopolysaccharide induced by alcohol, and decreased tumor necrosis factor-α and interleukin-1β levels. In hippocampus fucoidan inhibited microglia cell activation, and down-regulated the levels of Toll-like receptor 4 and its downstream protein factors. In addition, fucoidan regulated the structure of gut flora and made the composition of microbiota more similar to that of the normal control mice with increased abundances of *Prevotella* and *Alloprevotella*. Oral administration of fucoidan could alleviate the depression-like behaviors of mice with alcoholism through the gut-microbiota-brain axis [26].

Lalit Sharma *et al.*, (2018) evaluated the effects of *Oscimum sanctum* L (O. *sanctum),* on alcohol withdrawal syndrome in Wistar rats. Liquid diet with 7.2%, v/v ethanol was administered to the rats for 21 days. After alcohol withdrawal, rats were examined at 6th and 24th hour for major withdrawal signs that included anxiety and hyper locomotor activity. *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 24th hour. On the last day of the protocol, liver, kidney and brain were isolated and preserved in formalin for further histopathological examination. Findings from the study revealed that *O. Sanctum* leaf extract treatment at doses 100, 200 and 300 mg/kg, oral had a significant protective effect on signs and symptoms of ethanol withdrawal in alcohol-dependent rats. However, no remarkable pathological and microscopic alterations were observed in histopathological examination. *O. sanctum* seems to be an active drug for the treatment of alcohol abstinence syndrome [27].

Girdhari Lal Gupta *et al.*, 2019 evaluated the beneficial effects of *Bacopa monnieri* extracts (BME) in alcohol abstinence-induced anxiety-like behavior and the underlying mechanism of action subsequent to long-term voluntary drinking of alcohol. For the assessment of the effects of BME, Wistar rats were exposed to voluntary ingestion of 4.5%, 7.5% and 9% v/v alcohol for 15 days. The doses (100, 200, and 500 mg/kg) of BME and diazepam (2 mg/kg)

were administered via gavage for three consecutive days in the alcohol abstinence period on the days 16, 17, and 18. The HPLC analysis demonstrated that BME contained 9.9% bacoside-A as a major component. The results revealed that BME at the doses of 200 mg/kg and 500 mg/kg alleviated anxiety-like behavior which was escalated during alcohol abstinence. However, BME (100 mg/kg) exhibited insignificant protection against alcohol abstinence-induced syndrome. The escalated levels of alcohol-intake biomarkers were also reversed by BME at the dose of 200 mg/kg and 500 mg/kg. The down-regulation of Gabra1, Gabra4, and Gabra5 gene expression following alcohol abstinence were also reversed with a higher dose of BME (200 and 500 mg/kg) treatment. These results show that BME abrogates anxiety-like behavior by modulating alcohol markers and Gabra1, Gabra4, Gabra5 gene expression of GABAA receptor signaling pathway in rats [28].

Girdhari Gupta *et al.*, (2008) investigated the role of *Withania somnifera* in acute ethanol and withdrawal from chronic ethanol consumption using elevated plus maze paradigm in rats. Acute administration of ethanol (1.5-2 g/kg, ip) triggered anxiolytic effect and withdrawal from prolonged ethanol (9% v/v ethanol, 15 days) consumption elicited enhanced behavioral despair (anxiety). Acute administration of WS (50 mg/kg, oral) potentiated the anxiolytic action of subeffective dose of ethanol (0.5 or 1 g/kg, ip). Moreover, the ethanol withdrawal anxiety was markedly antagonized in dose dependent manner by WS at 200 and 500 mg/kg or higher dose of ethanol (2.5 g/kg). However, co-administration of subeffective doses of WS (50 mg/kg, oral) and ethanol also attenuated withdrawal-induced anxiety due to chronic ethanol (9% v/v ethanol, 15 days) consumption. The results suggest the protective effect of WS in the management of ethanol withdrawal reactions [29].

Rebeca Vargas Antunes Schunck *et al.*, (2017) investigated the effects of a commercial extract of *Passiflora incarnata* in the analgesia induced by alcohol withdrawal syndrome in rats. Male adult rats received by oral gavage: (1: water group) water for 19 days, 1 day interval and water (8 days); (2: *P. incarnata* group) water for 19 days, 1 day interval and *P. incarnata* 200 mg/kg (8 days); (3: alcohol withdrawal group) alcohol for 19 days, 1 day interval and water (8 days); and (4: *P. incarnata* in alcohol withdrawal) alcohol for 19 days, 1 day interval and *P. incarnata* 200 mg/kg (8 days). The tail-flick and hot plate tests were used as nociceptive

response measures. Confirming previous study of our group, it was showed that alcohol-treated groups presented an increase in the nociceptive thresholds after alcohol withdrawal, which was reverted by *P. incarnata*, measured by the hot plate test. Besides, alcohol treatment increased brain-derived neurotrophic factor and interleukin-10 levels in prefrontal cortex, which was not reverted by *P. incarnata*. Considering these results, the *P. incarnata* treatment might be a potential therapy in the alcohol withdrawal syndrome [30].

Muthuswamy Umamaheswari *et al.*, (2012) investigated the effect of the various fractions of hydromethanolic extract of the leaves of *Vitex negundo*(Verbenaceae) against ethanol-induced cerebral oxidative stress in rats. Cerebral oxidative stress was induced by the administration of 20% ethanol (5 ml/100gbw) for a period of 28 days. The petroleum ether (PEF), chloroform (CF), ethyl acetate (EAF) and residual (RF)fractions at a dose of 200 mg/kg bw orally were simultaneously administered with ethanol for 28 days. α-tocopherol at a dose of 100 mg/kg orally was used as the standard. Histopathological examination of the brain tissue of the ethanol treated animals showed marked gliosis. Simultaneous administration of the fractions prevented the enzymatic leakage and elevation of serum uric acid, triglycerides and lipoprotein levels. All the fractions (except the residual fraction) prevented the rise in lipid peroxidation and enhanced the antioxidant enzymes. Further, histopathological examination revealed that the fractions of *V. Negundo* offered a significant protection against ethanol toxicity in rat brain. The activity exhibited by the chloroform fraction is comparable to that of the standard. The study revealed that the leaf of *V.negundo* has protective action on the brain, which could be attributed to its antioxidant potential [31].

Selevich *et al.*, (1999) confirmed that Saltwort plants (salsocollin) ameliorated plasma contents of total lipids, triacylglycerols, and phosphatidylcholine in rats with alcohol intoxication, but had no effect on cholesterol and total phospholipid levels. Salsocollin did not prevent the increase in the levels of total lipids and triacylglycerols 3 days after ethanol withdrawal. During abstinence, salsocollin potentiated symptoms of ethanol withdrawal (7 days later) in relation to the content of total phospholipids, but normalized the levels of phosphatidylcholine, phosphatidylethanolamine, and total lipids [32].

Ruby B *et al.*, (2012) evaluated the effect of Ashwagandha (ASW) in attenuation of alcohol withdrawal in ethanol withdrawal mice model. Alcohol dependence was induced in mice by the oral, once-daily administration of 10% v/v ethanol (2 g/kg) for one week. Once the animals were withdrawn from alcohol, the efficacy of ASW (200mg/kg and 500mg/kg) in comparison with diazepam (1 mg/kg) in the attenuation of withdrawal was studied. 6 hours after the last ethanol administration, seizure threshold was measured in all the groups by administering the convulsant drug, PTZ with a subconvulsive dose of 30 mg/kg i.p. Compared to ethanol group, ASW (500 mg/Kg) has suppressed the PTZ kindling seizures in ethanol withdrawal animals [0% convulsion], FST has shown decreased immobility time and OFT has exhibited increase in the number of line crossing activity by mice which may be the consequence of anxiolytic activity of ASW similar to that of diazepam [33].

Nandkishor Ramdas Kotagale *et al.*, (2018) reported that *Withania somnifera* (WS) inhibited acquisition and expression conditioned place preference, self-administration and withdrawal anxiety of psychostimulants. Animals had given free access to ethanol uninterrupted for 21 days through liquid diet. Withaferin A (5, 10 and 20 mg/kg) was injected (ip) either during the development of ethanol dependence phase (days 15 – 21 or 30 min before ethanol withdrawal assessment. Withaferin A treatment 30 min before 24 h post- ethanol withdrawal assessment did not alter the scores of somatic behavioral signs in ethanol abstinence animals. However, withaferin A (10 and 20 mg/kg, ip) from day 15-21 prevented the ethanol withdrawal-induced elevated scores of somatic behaviors, hyperlocomotion, depressive behavior, and anxiety. Withaferin A treatment did not influence the blood ethanol levels in dependent and withdrawn animals. However, withaferin A administration attenuated the elevated plasma corticosterone and ACTH levels in ethanol-withdrawn rats, suggesting withaferin A induced anti-stress effect and stabilization of HPA axis activity could have facilitated the inhibitory effect of withaferin A on ethanol withdrawal syndrome [34].

Joshi *et al.*, (2005) evaluated the protective effects of Quercetin alcohol abstinence induced anxiety and convulsions. Chronic administration of ethanol (2 g/kg, p.o.) on days 1–6 and its withdrawal produced an anxiogenic reaction in mice as assessed in the mirrored-chamber test. Daily administration of quercetin (25 or 50 mg/kg, p.o.) prior to ethanol for 6 days

prevented withdrawal-induced anxiety in mice. However, acute administration of a single dose of quercetin (50 mg/kg) to animals withdrawn from ethanol, i.e., on day 7, did not prevent withdrawal-induced anxiety. Ethanol withdrawal also induced a significant increase in the loco motor activity of mice indicating an anxiogenic response. Daily administration of quercetin (25 or 50 mg/kg, p.o.) prior to ethanol for 6 days prevented withdrawal-induced increased locomotor activity. Ethanol withdrawal also sensitized the convulsogenic reaction to pentylenetetrazole (PTZ). A non-convulsive dose (40–60 mg/kg) of PTZ produced full-blown convulsions and increased mortality in ethanol-withdrawn mice. Both acute and chronic administration of quercetin (25 or 50 mg/kg, p.o.) produced a significant protection against ethanol withdrawal-induced reduction in PTZ threshold in mice. The result suggests the protective effect of this safe drug, quercetin, in the management of ethanol withdrawal reactions [35].

Hicham El Mostafi *et al.*,(2020) investigated the potential neuroprotective effect of argan oil (AO) on adolescent intermittent ethanol intoxication (IEI), induced voluntary ethanol consumption, and withdrawal syndrome in rats. Animals were treated with ethanol (i.p., 3 g/kg body weight) in intermittent doses (2 days on; 2 days off, from postnatal day 30-43), with/without oral AO pre-treatment (10 mL/kg/day bw, from postnatal day 21-121). A 2-bottle free access test was performed over 10 weeks to assess 10% ethanol consumption. Behavioral signs of withdrawal were observed after 2, 6, 24, 48, and 72 h after ethanol removal. Anxiety like behaviors in the elevated plus maze and the light/dark box tests were also evaluated at 72 h of withdrawal. It was found that AO pre-treatment significantly decreased the voluntary ethanol consumption induced by adolescent IEI. In addition, by establishing low ethanol consumption, AO pre-treatment counteracts negative effects of ethanol withdrawal and anxiety-like behaviors in ethanol-treated rats after 72 h of abstinence. Low ethanol drinking in the AO-supplemented rats was associated with inhibition of oxidative stress and neurodegeneration in the rats brains [36].

Bo Jiang *et al.*,(2021) reported the protective effects of *Puerariae flos* extract on EtOH withdrawal models. Sixty male Kunming mice were involved which were randomly divided into five groups (intact control, EtOH group (35-day EtOH exposure), EtOH withdrawal group (28-day exposure + 7-day withdrawal), EtOH withdrawal group + positive control (Deanxit) group,

and EtOH withdrawal group + PFE group). Deanxit and PEF ameliorated depressive and anxiety behaviors (vs. withdrawal group). Hippocampal BDNF expression was significantly downregulated by EtOH exposure and upregulated by withdrawal. Deanxit and PEF significantly upregulated the BDNF expression. The hippocampal CA1 neuronal density significantly decreased by EtOH exposure but unchanged by withdrawal and treatments. The plasma CRH, ACTH, and CORT levels show a significant enhancement by EtOH exposure and reduced by withdrawal. They were further reduced by Deanxit and PEF. The protective effects of PEF on EtOH chronic withdrawal mouse models were verified. The results of the study also indicated a complicated scenario of neuropsychological behaviors, hippocampal BDNF expression, and hypothalamic–pituitary–adrenal axis which are affected by the timing of EtOH exposure and withdrawal [37].

Wu Yiyan *et al.*, (2014) investigated the effect of aqueous extract of the *Schizandra chinensis* fruit (AESC) on anxiety-like behavior and the levels of norepinephrine and 3-methoxy-4-hydroxyphenylglycol (a metabolite of norepinephrine) in different brain regions during ethanol withdrawal in rats. Male Sprague-Dawley rats were treated with 3 g/kg of ethanol (20%, w/v) or saline by daily intraperitoneal injection for 28 days followed by three days of withdrawal. During withdrawal, rats were given AESC (100 mg/kg or 300 mg/kg P.O.) once a day for three days. Rats undergoing ethanol withdrawal exhibited substantial anxiety-like behavior, which was characterized by both the decrease in time spent in the open arms of the elevated plus maze and the increased level of corticosterone secretion, which were greatly attenuated by doses of AESC in a dose-dependent manner. The HPLC analysis revealed that ethanol withdrawal significantly increased norepinephrine and 3-methoxy-4- hydroxy-phenylglycol levels in the hypothalamic paraventricular nucleus, while not significantly altering them in the hippocampus. Similar to the results from the elevated plus maze test, the AESC significantly inhibited the elevation of norepinephrine and its metabolite in the hypothalamic paraventricular nucleus in a dose-dependent manner [38].

Li Bo Li et al., (2019) evaluated the effects of Semen *Ziziphi Spinosae* (SZS) on ethanol withdrawal anxiety and the involvement of amygdaloid CRF/ CRFR1 and N/OFQ/NOP pathways. Male Sprague Dawley rats received intraperitoneal injections of 2 g/kg EtOH (20%

v/v) once daily for 28 d followed by a 3-d withdrawal. During EtOHW, the rats were given once-daily intragastric treatments of a methanol extract of SZS (MESZS, 60 or 180 mg/kg/d). MESZS increased the distance traveled in the center zone of the OF and dose-dependently elongated the duration of staying in the center zone in EtOHW rats. MESZS increased both the number of entries into and the time spent in the open arms of the EPM by EtOHW rats. And, MESZS inhibited the over secretion of plasma CORT during EtOHW. EtOHW enhanced CRF and CRFR1 gene and protein expression in the central nucleus of the amygdala (CeA), which were inhibited by 180 mg/kg/d MESZS. EtOHW increased amygdaloid NOP mRNA and protein expression but spared N/OFQ mRNA expression, and 180 mg/kg/d MESZS further promoted these increases. Additionally, a post-MESZS intra-CeA infusion of either CRF or the selective NOP antagonist UFP-101 abolished the expected anxiolytic effect of 180 mg/kg/d MESZS [39].

Pravinkumar Bhutada *et al.*, (2010) investigated the influence of berberine treatment on the development and expression of ethanol dependence was tested by using the ethanol withdrawal-induced hyper excitability paradigm. Mice were provided with a nutritionally balanced control liquid diet as the sole nutrient source on day 0; from day 1–4 (ethanol, 3% v/v), from day 5–7 (ethanol, 6% v/v) and from day 8–10 (ethanol, 10% v/v) was incorporated into the liquid diet. On day 11, the ethanol liquid diet was replaced with nutritionally balanced control liquid diet, and ethanol withdrawal-induced hyper excitability signs were recorded. The results revealed that acute administration of berberine (10 and 20 mg/kg, i.p.) dose-dependently attenuated ethanol withdrawal-induced hyper excitability signs, and these results were comparable to diazepam (1.25 and 2.5 mg/kg, i.p.). Further, chronic administration of berberine (10 and 20 mg/kg, i.p.) to the ethanol diet fed mice markedly attenuated the ethanol withdrawal-induced hyper excitability signs. In conclusion, the results and evidence suggest that berberine exhibited an inhibitory influence against ethanol withdrawal-induced hyper excitability signs, which could be mediated through its neuromodulatory action [40].

Hoseyn Fatolahi *et al.*, (2019) evaluated the effect of training and curcumin on the PON-1 activity and lipid profile after alcohol withdrawal in male Wistar rats. For this study, 32 male Wistar rats were divided into four groups based on alcohol consumption. After four days of alcohol consumption program and six days of alcohol withdrawal, curcumin

and training intervention (swimming) was performed for fourteen days. Blood samples were collected for laboratory analysis. In order to investigate inter-group differences, a two-way analysis of variance and the LSD post hoc test was used. Independently, the training increased the activity of the PON-1 (p=0.001). Further, the interaction of exercise and curcumin had a significant increase in the activity of PON-1 (p=0.02). Moreover, the training independently increased the concentration of HDL (p=0.01). Additionally, the interaction of exercise and curcumin showed a significant effect on the increasing of HDL (p=0.01).
The findings of this study confirm that combining short-term training of swimming and curcumin increases the activity of PON-1 and the concentration of HDL in the male Wistar rats. Therefore, in order for reducing the complications of alcohol withdrawal period, we can use training + curcumin in improving lipid profile and increasing PON-1 activity [41].

Swaroopa Maralla *et al.*,(2013) investigated the effects of daily oral administration of ginger extract for 6 weeks on kidney functions in withdrawal rats to evaluate the ameliorating effects in alcohol induced-withdrawal rats. Rats (130-150gm) were divided into 4 groups; normal control rats, alcoholic control rats, ethanol withdrawal rats and ethanol withdrawal rats pretreated with ginger. Ginger extract was administered orally for 6 weeks to pre-treated rats, and they were compared with the normal and alcoholic groups, respectively. The treatment with ginger extract had significant effect on Plasma Electrolyte Profiles. Low plasma sodium level and increased plasma potassium levels were observed in ginger pretreated ethanol withdrawal group. The plasma creatinine, urea and uric acid levels were significantly reduced in this group compared to alcoholic control rats and ethanol withdrawal rats. It is concluded that the consumption of ginger produced a significant anti- nephrotoxic effect in ethanol withdrawal rats. In addition, ginger is showing properties of anti-hypertensive drugs and is capable of improving impaired kidney function in ethanol withdrawal rats [42].

Dania Cheaha *et al.*, (2014) aimed to identify surrogate biomarkers that represent intact biological or ethanol withdrawal processes and response to pretreatment with fluoxetine, a selective serotonin reuptake inhibitor, with quantitative methods. Adult male Wistar rats implanted with electrodes over the frontal and parietal cortices were rendered dependent on

ethanol via modified liquid diet (MLD) containing ethanol. Then, ethanol-containing MLD was replaced with isocaloric ethanol-free MLD to induce ethanol withdrawal symptoms. One-way ANOVA confirmed significant increases in locomotor activity and time spent in awake state and decreases time spent in non-rapid eye movement (NREM) sleep and REM-sleep during ethanol withdrawal period. Fast Fourier Transformation also revealed predominant increases in gamma spectral powers within both the frontal and parietal cortices during ethanol withdrawal. However, these changes, except sleep-wake disturbances, were significantly attenuated by fluoxetine pretreatment (10 mg/kg). The present study supports the hypothesis that serotonergic hypo function may underlie most of ethanol withdrawal symptoms and proposes that electroencephalographic patterns are valid biomarkers for ethanol withdrawal evaluation and treatment [43].

Sundarrajan T *et al.*, (2016) evaluated alcohol withdrawal symptoms with leaf extract of *Sesbania grandiflora* Linn. The alcohol withdrawal syndrome is a group of symptoms observed in persons who stop consumption of alcohol. Milder forms of the syndrome include tremulousness, seizures, and mental confusion. In chronic alcoholism associated with delirium tremens, involves, hallucinations, and affect the autonomic nervous system over activity. The induced alcohol dependence in mice by the administration of oral dose of ethanol (2g/Kg) 10% v/v once a daily for seven days alcohol withdrawal syndrome are evaluated by of both loco motor activity using actophotometer and anti-depression activity with forced swim test. Evaluation of loco motor activity of *Sesbania grandiflora* Linn extract (100 and 200 mg/kg) and imipramine (15mg/kg) were studied by observing its effect on actophotometer. This exhibited slight increase in loco motor activity when compared with the positive control animals that received ethanol. For antidepressant activity the extract (200 mg/kg) was found to be effective and it exhibited activity similar to that of the standard drug of imipramine [44].

Ehsan Mohebbi *et al.*, (2020) evaluated the effect of clavulanic acid on the symptoms of ethanol withdrawal in rats. Alcohol dependence was induced by the gavage of ethanol (10% v/v, 2 g/kg), twice daily for 10 days. Clavulanic acid (10, 20, 40, and 80 mg/kg) was administered concurrently with ethanol (sub-acute study), or a single dose after ethanol withdrawal (acute study). Six hours after the last dose of ethanol were assessed. The number of

entries and time spent on the open arms of EPM decreased during the withdrawal state. Motor coordination and loco motor activity were significantly decreased. In the sub-acute study, clavulanic acid 80 mg/kg increased time spent and the number of entries to the open arms of EPM, in withdrawn animals. Both motor incoordination and loco motor activity reduction were normalized by clavulanic acid (10, 20, 40 and 80 mg/kg). Withdrawal-induced PTZ kindling seizure was also suppressed by all of the doses. MDA increased, while GSH decreased after withdrawal. Clavulanic acid attenuated such changes [45].

Gagan Shah *et al.*, (2010) reported the anti-anxiety effect of *Cymbopogon citratus.* The Methanolic extract of *Cymbopogon citratus* leaves at the dose of 200 mg/kg increased the percentage of time-spent and the percentage of arm entries in the open arms of the elevated plus-maze (EPM) and decreased the percentage of time-spent in the closed arms of EPM. Moreover, it prolonged the ketamine-induced latency to sleep but had no sufficient effects on total sleeping time induced by ketamine. Also the loco motor activity was affected but not to the same extent as observed for diazepam. The anxiolytic effects of methanolic extract of *Cymbopogon citratus* leaves may be related to their content of flavonoids [46].

Ditte Dencker *et al.*,(2017) investigated the potential therapeutic benefit of a ketogenic diet in managing alcohol withdrawal symptoms during detoxification. Male Sprague Dawley rats fed either ketogenic or regular diet were administered ethanol or water orally, twice daily for 6 days while the diet conditions were maintained. Abstinence symptoms were rated 6, 24, 48, and 72 hours after the last alcohol administration. Maintenance on a ketogenic diet caused a significant decrease in the alcohol withdrawal symptoms' "rigidity" and "irritability" [47].

AIM AND OBJECTIVE

AIM

The aim of the present study is to evaluate methanolic leaf extract of *Cymbopogon citratus* on Alcohol withdrawal syndrome in Wistar rats.

OBJECTIVE

Alcohol withdrawal syndrome is a group of symptoms that occurs following the cessation of alcohol after a period of excessive alcohol use. These symptoms occur due to alcohol-induced chemical imbalances in the brain which result in excessive neuronal activity if the alcohol is withheld.

Benzodiazepines are the most commonly used medications to treat alcohol withdrawal syndrome. But, benzodiazepine withdrawal after prolonged usage may also lead to protracted withdrawal syndromes. It may also cause increased risk of excessive sedation, memory and motor deficits and respiratory depression. Barbiturates are also preferred in the treatment of AWS either alone or along with BZDs. But due to its long half-life, they increase the chance of respiratory insufficiency and coma so that intubation and mechanical ventilation is often required. Neuroleptics are usually used in the treatment of hallucinations and delirium. Their risk of development of seizure and prolongation of QT interval limit its use.

However, for severe and serious symptoms scored 5-7 in CIWA, allopathic medicines are the first line choice of drugs. Whereas, Ayurvedic medicines can be used to treat mild to moderate symptoms scored 1-4 in CIWA categories. The ayurvedic medicines has several advantages such as minimum or no side effects, low of cost, can be continued for a long period of time and it has no withdrawal effects.

Lemon grass (*Cymbopogon citratus*) family Poaceae is a widely used herb in tropical countries, especially in Southeast Asia. The essential oil of the plant is used in flavor, fragrancing and aromatherapy, medicinal tea, culinary herb, and treatment for skin diseases. It is known as a source of ethno medicines.

C. citratus is used in different parts of the world in the treatment of digestive disorders, fevers, menstrual disorder, rheumatism and other joint pains.

Based on its reported anti-anxiety activity, *Cymbopogon citratus* plant is selected in this study and evaluated for its efficacy in treating alcohol withdrawal syndrome in wistar albino rats [46].

PLAN OF WORK

The present study involved the following steps:

- Collection and authentication of the leaves of *Cymbopogon citratus* (Family: Poaceae)
- IAEC approval for use of animals in the study
- Preparation of Methanolic leaf Extract of *Cymbopogon citratus* (MECC)
- Phytochemical screening
- Induction of alcohol withdrawal syndrome
- Administration of MECC (200 and 400 mg/kg) and Diazepam to groups respectively
- Observation of behavior parameters using Open field test, Elevated plus maze, Rota rod, Actophotometer and Forced swim test.
- Blood collection for estimation of AST, ALT and ALP.
- Animal Sacrifice and isolation of brain and liver
- Estimation of tissue protein, malondialdehyde, Antioxidant enzymes (SOD, CAT and GPx), non-enzymatic antioxidants (GSH) in brain and liver homogenates.
- Statistical analysis.

MATERIALS AND METHODS

COLLECTION, IDENTIFICATION AND EXTRACTION

COLLECTION AND IDENTIFICATION OF PLANT

The leaves of *Cymbopogon citratus* was collected from the outskirts of Erode in the month of October (Figure 5). The plant was identified and authenticated as *Cymbopogon citratus* by V. Sampath Kumar, Scientist D in Tamilnadu Agricultural university, Coimbatore [BSI/SRC/5/23/2020/Tech].

EXTRACTION

The collected leaves were shade dried for 4 days. The dried leaves were minced and 100g of the minced leaves were macerated in 600ml of methanol for 3 days with occasional shaking. The mixture was then filtered through muslin cloth and the filtrate was collected (Figure 6). It was then concentrated using rotary evaporator at 40^{o}C. The final extract obtained was used for phytochemical analysis and the extract was dissolved in normal saline to suitable concentration for evaluation of its effect against alcohol withdrawal syndrome.

Figure 5: Leaf collection

Figure 6: Extract preparation

ANIMALS

Male Wistar albino rats (180-220g) were used for the study. All experimental procedures and animals were approved by IAEC proposal No: NCP/IAEC/2021-22/01. The animals were obtained from animal house of Kerala veterinary and animal science university, Mannuthy. On arrival, the animals were placed at animal house in Nandha college of pharmacy, Erode. The animals were randomly allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70%. A 12:12 light : day cycle was followed. All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines.

DRUGS AND CHEMICALS

Ethanol, methanol, concentrated sulphuric acid, Bovine serum albumin, Thiobarbituric acid, Trichloroacetic acid, sodium chloride, phenol reagent were obtained commercially and are of analytical grade. The equipments used were manufactured by Merck specialties, Private limited, Ambernath.

PHYTOCHEMICAL SCREENING[48],[49],[50],[51]

Chemical tests were carried out for the fruit and peel extract of *C.citratus*, for the presence of phytochemical constituents (Figure 7).

1) **Test for Flavonoids**

Alkaline reagent test:

To 1 ml of the prepared extract, 2ml of 2% NaOH solution was added and few drops of dilute Hydrochloric acid was added. Appearance of an intense yellow color, which becomes colorless on addition of diluted acid indicates the presence of flavonoids.

Lead acetate test:

To 1ml of the prepared extract, few drops of 10% lead acetate solution was added. Appearance of yellow precipitate indicates the presence of Flavonoids.

2) Test for aldehydes

Fehling Test :

To 2 ml of the extract solution few drops of Fehling's solution A and Fehling's solution B was added and boiled for 5 min. on boiling water bath. Yellow or red color develops.

3) Test for Terpenoids:

To 2 ml of the extract, 2 ml of chloroform was added and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish color indicates the presence of terpenoids.

4) Test for Tannin:

About 2 ml of the ethanolic extract was stirred with 2 ml of distilled water and few drops of FeCl3 Solution were added. Formation of green precipitate was indication of presence of tannins.

Figure 7: Phytochemical screening of the prepared extract

5) Test for Phenolic compounds:

Ferric chloride test:

To the extract, few drops of 5% ferric chloride solution was added. Appearance of dark green or bluish black color indicates the presence of phenolic compounds.

Iodine test:

To 1ml of the extract, few drops of dilute iodine solution was added. Appearance of transient red color indicates the presence of phenolic compounds.

Lead acetate test:

The plant extract was dissolved in 5ml of distilled water and 3ml of 10% lead acetate solution was added to it. Appearance of white precipitate indicates the presence of phenolic compounds.

Potassium dichromate test:

To few ml of plant extract, few drops of potassium dichromate solution was added. Appearance of a dark color indicates the presence of phenolic compounds.

6) Test for Saponins:

5 ml of ethanolic extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

7) Test for alkaloids:

Dragendorff Test:

To 2 ml of the extract solution, Dragendorff reagent (potassium bismuth iodide solution) was added. Orange brown precipitate is formed.

8) Unsaturated steroids:

Development of a greenish color when 2 ml of the extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid, indicates the presence of unsaturated steroids.

9) Steroid derivatives:

Production of red color in the lower chloroform layer when 2 ml of the extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid which indicate the presence of steroid derivatives.

EXPERIENTAL PROCEDURE

I. ALCOHOL WITHDRAWAL SYNDROME EXPERIMENTAL DESIGN[38,45]

Alcohol dependence was induced by administration of 10 % alcohol for 28 days. Rats were randomly divided into five groups (n = 6). The alcohol dependence was induced for all groups except group I. The control group (group I) was fed with saline for 31 days. (2) The withdrawal group (group II) was fed 10 % ethanol for 28 days. (3) The rats treated with alcohol for 28 days and Diazepam (2mg/kg for 22-28 days, group III). Diazepam was used in this group as a positive control to treat the neuropsychological symptoms. The rats treated with alcohol for 28 days and MECC at a dose of 200 mg/kg and 400 mg/kg for 22-28 days (group IV and V) respectively. After withdrawal of ethanol on the 29^{th} day, behavioral assessments were carried out on the 6^{th} , 24^{th} hour and 72^{nd} hour after withdrawal of alcohol on the 29^{th} day. Diazepam, MECC 200 mg/kg and MECC 400 mg/kg were administered to groups III, IV and V respectively for 3 days (Day 29-31). The blood samples of the animals were collected on the 32^{nd} day for biochemical assessments. Finally, the animals were sacrificed with pentobarbital 40 mg/kg and then the brain and liver tissue samples were collected for estimation of antioxidants level and histopathological studies.

Table 4: Grouping of animals for the experimental study

GROUPS	DRUG TREATMENT	DOSE	NUMBER OF ANIMALS
I	Normal control (Vehicle)	-	6
II	Ethanol induced (Negative control)	3ml (10% v/v) (p.o)	6
III	Standard control (Ethanol+ Diazepam)	2mg/kg (i.p)	6
IV	Test I (Ethanol + MECC)	200 mg/kg (p.o)	6
V	Test II (Ethanol + MECC)	400 mg/kg (p.o)	6

BEHAVIOURAL ASSESSMENT

1) OPEN FIELD TEST

The open-field box was a cubic chamber (60×60×40 cm), with a white floor divided into 16 equal squares. It was placed in a quiet room with dim light. Rats were delivered to the testing room, 30 min prior to the experiment. Each animal was gently placed in the center of the box and allowed to move freely over a 5-min period. The number of lines crossed by four paws in the open field apparatus was recorded. The apparatus was cleaned before each trial.

2) ELEVATED PLUS MAZE

The EPM apparatus was constructed of two open (10 cm wide, 50 cm long with 5 cm wall sides) and two closed (10 cm wide, 50 cm long with 40 cm wall sides) arms, made of wood, arranged at 90 ° angles (plus-shape) and intersected by a center platform (10×10 cm). The apparatus was raised 40 cm above the floor. Each animal was observed individually, for a 5-min test period.

The total time spent on the open and closed arms was recorded. The maze was cleaned between tests.

3) ROTAROD TEST

The rotarod test was performed to evaluate the motor coordination of animals. Briefly, one day before the test, rats were pre-trained on the rotarod apparatus twice at 5 rpm speed for at least 5 min, until they reached a stable baseline performance. On the day of the experiment, rats were evaluated on the rod rotating at an accelerating speed (from 4 to 40 rpm), over five min. The time taken for the falling of the rat (latency time) from the rotating rod, was recorded and the mean value of time (seconds on the rod) from the two or three testing trials was then calculated [45].

4) LOCOMOTOR ACTIVITY

The spontaneous loco motor behavior of the animals was recorded individually for 5 min using a digital actophotometer. The count of the movement of the animal cutting a beam of light which falls on the photocell was recorded digitally [27].

5) FORCED SWIM TEST

In forced swim test rats were individually forced to swim in a transparent vertical glass cylinder (45 cm high, 20 cm in diameter) filled with 27°C water to a depth of 20 cm. The duration of immobility of the animal (in sec) was measured for 6 min. 'Immobility' was defined as floating and treading water just enough to keep the nose above water. The water was changed for each animal [52][53].

BIOCHEMICAL ASSESSMENT

Preparation of Brain homogenate:

Reagents used: 0.9% NaCl, Phosphate buffer pH 7.4 (Dissolve 6.8g of potassium dihydrogen orthophosphate + 1.56g of sodium hydroxide in 930ml water. Adjust pH to 7.4 with sodium hydroxide and dilute to produce 1000ml).

Instruments used: Homogenizer, Centrifuge.

Procedure: After isolation, homogenate of brain (10% w/v) was prepared in phosphate buffer (pH 7.4). The whole brain samples were rinsed with ice cold saline (0.9% sodium chloride) and homogenized in phosphate buffer (pH 7.4). The homogenates were centrifuged at 800 g for 5 min. The supernatant thus obtained was recentrifuged at 10,500 g for 20 min to get the supernatant (Figure 8), which was used for the estimation of total protein (TP), determination of end product of lipid peroxidation(MDA), enzymatic antioxidants like superoxide dismutase(SOD), catalase(CAT), glutathione peroxidase (GPx) and the non-enzymatic antioxidant reduced glutathione(GSH) [54][55][56] .

Preparation of liver homogenate:

Reagents used: 0.9% NaCl, 0.25M sucrose solution.

Instruments used: Homogenizer, Centrifuge.

Procedure: After isolation, homogenate of liver (10% w/v) was prepared in 0.25M sucrose solution. The whole liver samples were rinsed with ice cold saline (0.9% sodium chloride) and homogenized in 0.25M sucrose solution. The homogenates were centrifuged at 7000g for 10 mins at 4^0C. The supernatant thus obtained was used for the estimation of total protein (TP), determination of end product of lipid peroxidation(MDA), enzymatic antioxidants like superoxide dismutase(SOD), catalase(CAT), glutathione peroxidase(GPx) and the non-enzymatic antioxidant reduced glutathione(GSH)[57][58].

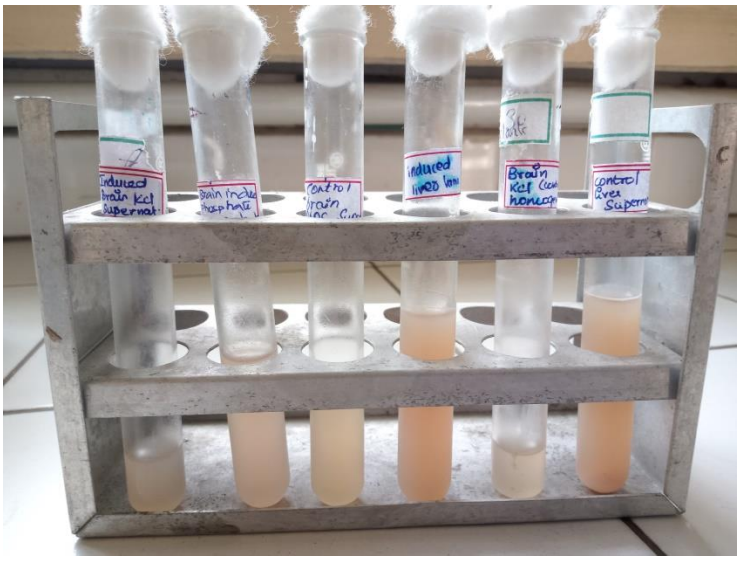

Figure 8: Brain and liver homogenate

Estimation of total protein(TP)

Reagents used: Prepared homogenate, Alkaline copper solution, Phenol reagent, Distilled water.

Instruments used: UV-Visible spectrophotometer.

Procedure: The amount of total protein present in the brain/liver homogenate was estimated by the Lowry's method. To 0.1 ml of the brain/liver homogenate, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 mins. Then, 0.4ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for color development. Reading was taken against blank prepared with distilled water at 610 nm in UV-Visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed as µg/mg brain/liver tissue [59][60][61].

Measurement of MDA levels:

Reagents used: Prepared homogenate, Thiobarbituric acid, Trichloroacetic acid, Hydrochloric acid.

Instruments used: Centrifuge, UV-Visible spectrophotometer.

Procedure: Lipid peroxidation is evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and hydroperoxides(HP). About 0.1 ml of the brain/liver homogenate was treated with 2ml (1:1:1 ratio) of TBA- TCA- HCL reagent(Thiobarbituric acid 0.37%, 0.25N HCl and 15% TCA) and placed in a water bath for 15 mins, cooled and centrifuged at 1000g at room temperature for 10 mins. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The values are expressed as nmoles of MDA /mg protein [62][63][64].

Determination of enzymatic antioxidants:

Estimation of Superoxide dismutase:

Reagents used: Prepared homogenate, 30mM Carbonate buffer pH 10.2, distilled water, 45mM epinephrine.

Instruments used: UV-Visible spectrophotometer.

Procedure: SOD activity was determined by the inhibition of auto catalyzed adrenochrome formation in the presence of the homogenate at 480 nm. The reaction mixture contained 150µl of brain/liver homogenate, 1.8ml of the carbonate buffer(30mM, pH 10.2) and 0.7 ml of distilled water and 400µl of epinephrine (45mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate [65][66][67].

Estimation of Catalase:

Reagents used: 25mM potassium phosphate buffer, prepared homogenate, H_2O_2

Instruments used: UV-Spectrophotometer

Procedure: The catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25mM potassium phosphate buffer pH 7.0 and 0.1 ml of brain/liver homogenate and was incubated at 37^0C for 15 mins and the reaction was started with the addition of 0.1 ml of 10 mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of the enzyme activity. Activity was expressed as µmoles/mg tissue protein.

Measurement of Glutathione Peroxidase(GPx)

Reagents used: 0.4M Tris buffer, Sodium azide, H_2O_2, Glutathione, Prepared homogenate, Trichloro acetic acid.

Instruments used: UV-Spectrophotometer

Procedure: The reaction mixture consists of 0.2 ml of 0.4 M Tris buffer, 0.1ml of sodium azide, 0.1ml of hydrogen peroxide, 0.2ml of glutathione and 0.2ml of supernatant incubated at 37^0C for 10 mins. The reaction was arrested by the addition of 10% TCA and the absorbance was measured at 340nm. Activity was expressed as nmoles /mg brain/liver protein [68][69][70].

Determination of non enzymatic antioxidant

Measurement of Reduced Glutathione levels(GSH)

Reagents used: Prepared homogenate, TCA, Ellman's reagent, 0.2M Phosphate buffer pH8.0.

Instruments used: UV-Spectrophotometer

Procedure: The method was based on the reaction of reduced glutathione with dithiobisnitrobenzoic acid(DTNB) to give a compound that absorbs at 412nm. To the homogenate, 0.1ml of 10% TCA was added and centrifuged. About 0.1 ml of supernatant was treated with 0.5 ml of Ellman's reagent and 3.0 ml of phosphate buffer (0.2 M, pH 8.0) and the absorbance was read at 412 nm. Activity was expressed as nmoles /mg brain/liver protein [71][72].

Determination of ALT, ALP and AST

The blood collected was centrifuged at 3000g for 10 mins and the serum was separated. Serum biochemical analysis was performed using a biochemical auto-analyzer device the assessment of the biochemical parameters (alanine aminotransferase ALT, aspartate aminotransferase AST, Alkaline phosphatase ALP)[73][74][75].

Statistical Analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results are expressed as mean ± SEM of six animals in each group. P values < 0.05 were considered significant.

RESULTS

I. Phytochemical screening of methanolic leaf extract of *Cymbopogon citratus.*

The phytochemical contituents present in the MECC extract are denoted in table 5. Phytochemical screening of the methanolic leaf extract of *Cymbopogon citratus* confirmed the presence of flavonoid, aldehyde, terpenoid, tannin, phenolic compounds, saponin, alkaloid, unsaturated steroids and steroid derivatives.

Table 5: Phytochemical constituents in methanolic leaf extract of *Cymbopogon citratus*

PHYTOCHEMICALS	MECC
Flavonoid	+
Aldehyde	+
Terpenoid	+
Tannin	+
Phenolic compound	+
Saponin	+
Alkaloid	+
Unsaturated steroid	+
Steroid derivatives	+

+ - Present

II. Effect of methanolic leaf extract of *Cymbopogon citratus* on open field test in animals induced with alcohol withdrawal syndrome

Effect of methanolic leaf extract of *Cymbopogon citratus* on open field test in animals induced with alcohol withdrawal syndrome are denoted in table 6 and figure 9. The alcohol withdrawal animals showed a significant ($P < 0.01$) increase in the number of lines crossed by animals when compared to normal control. Treatment with Diazepam produced a significant ($P < 0.01$) decrease in the number of lines crossed when compared to the alcohol withdrawal animals and which was even less when compared to the normal control. Animals treated with MECC (200 mg/kg) produced significant ($P< 0.01$) decrease in the number of lines crossed in the 6^{th} and 24^{th} hour when compared to the alcohol withdrawal animals and produced also significant ($P < 0.05$) decrease in the 72^{nd} hour. Animals treated with MECC (400 mg/kg) produced a significant ($P < 0.01$) decrease in the number of lines crossed when compared to the alcohol withdrawal animals.

Table 6: Effect of methanolic leaf extract of *Cymbopogon citratus* on open field test in animals induced with alcohol withdrawal syndrome

GROUP	NO. OF LINES CROSSED		
	6^{th} Hour	**24^{th} Hour**	**72^{nd} Hour**
Normal control	44.6 ± 4.94	43.8 ± 2.30	46.2 ± 3.36
Withdrawal group	79.4 ± 2.86[a]	70.6 ± 4.92[a]	56.7 ± 2.78[a]
Diazepam	33.8 ± 3.62[c]	30.5 ± 2.76[c]	27.2 ± 4.92[c]
MECC (200 mg/kg)	60.6 ± 4.08[c]	54.9 ± 4.38[c]	49.6 ± 2.64[d]
MECC (400 mg/kg)	49.7 ± 3.34[c]	45.7 ± 3.62[c]	48.2 ± 3.58[c]

Values are mean ± SEM; n=6. [a]$P < 0.01$, when compared to control animals. [c]$P < 0.01$, [d]$P < 0.05$ compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

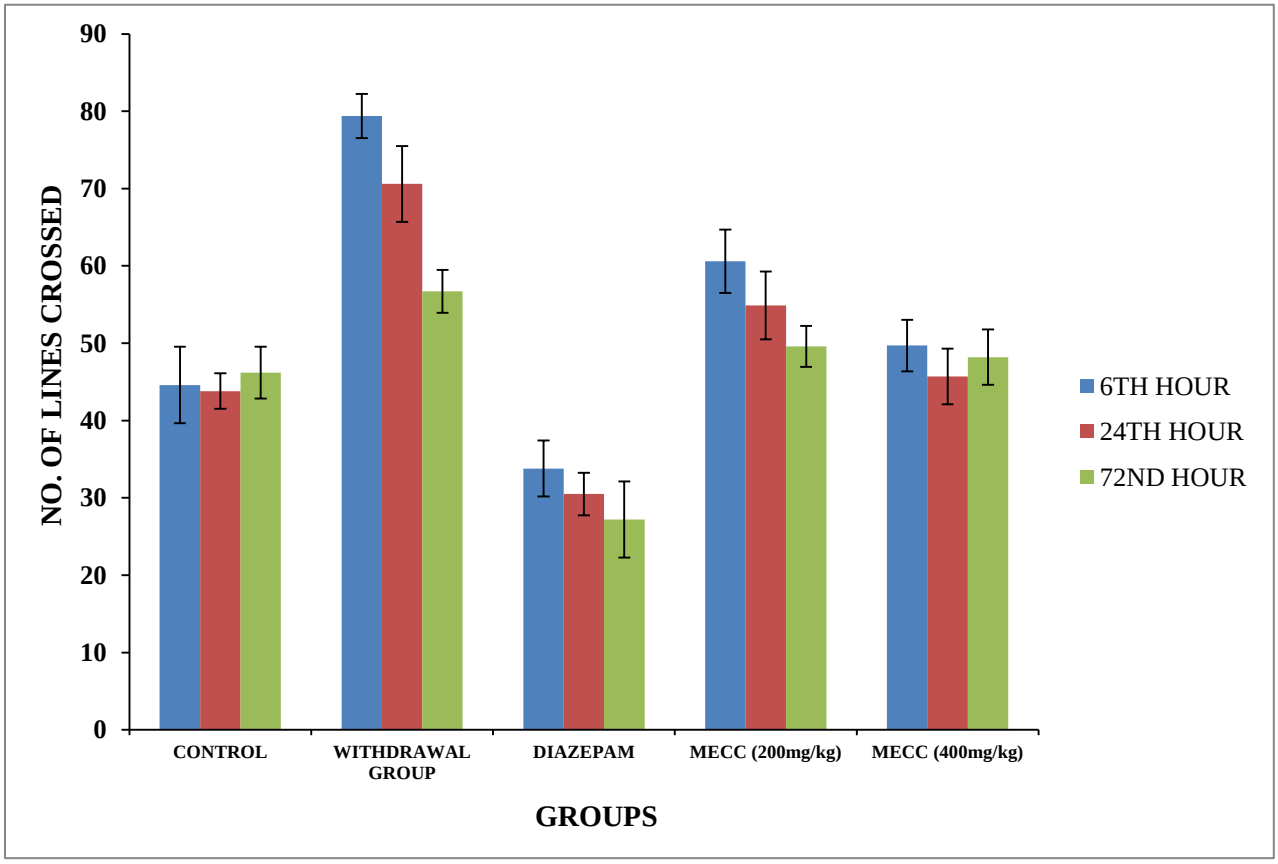

Figure 9: Number of lines crossed in open field test

III. Effect of methanolic leaf extract of *Cymbopogon citratus* on Rota Rod test in animals induced with alcohol withdrawal syndrome

The animals of the alcohol withdrawal group showed a significant ($P < 0.01$) decrease in the latency time to fall from rota rod apparatus when compared to normal control. Treatment with Diazepam did not produce a significant increase in the latency time. It further decreased the latency time ($P < 0.05$) to fall when compared to the alcohol withdrawal animals. Animals treated with MECC (200 and 400 mg/kg) produced significant ($P < 0.01$) increase in latency time to fall from rota rod apparatus when compared to the alcohol withdrawal animals in dose dependant manner (Table 7 and Figure 10).

Table 7: Effect of methanolic leaf extract of *Cymbopogon citratus* on Rota Rod test in animals induced with alcohol withdrawal syndrome

GROUP	LATENCY TIME TO FALL (SEC)		
	6th Hour	**24th Hour**	**72nd Hour**
Normal control	40.6 ± 1.8	42.8 ± 1.2	48.2 ± 1.8
Withdrawal group	18.2 ± 1.4[a]	26.7 ± 1.9[a]	32.9 ± 1.6[a]
Diazepam	15.4 ± 1.2[d]	22.9 ± 1.6[d]	30.1 ± 1.5[ns]
MECC (200mg/kg)	22.7 ± 1.6[c]	30.6 ± 1.8[c]	39.2 ± 1.9[c]
MECC (400mg/kg)	29.9 ± 1.8[c]	36.5 ± 1.4[c]	46.5 ± 1.4[c]

Values are mean ± SEM; n=6. [a]$P < 0.01$, when compared to control animals. [ns]$P > 0.05$, [c]$P < 0.01$, [d]$P < 0.05$ compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

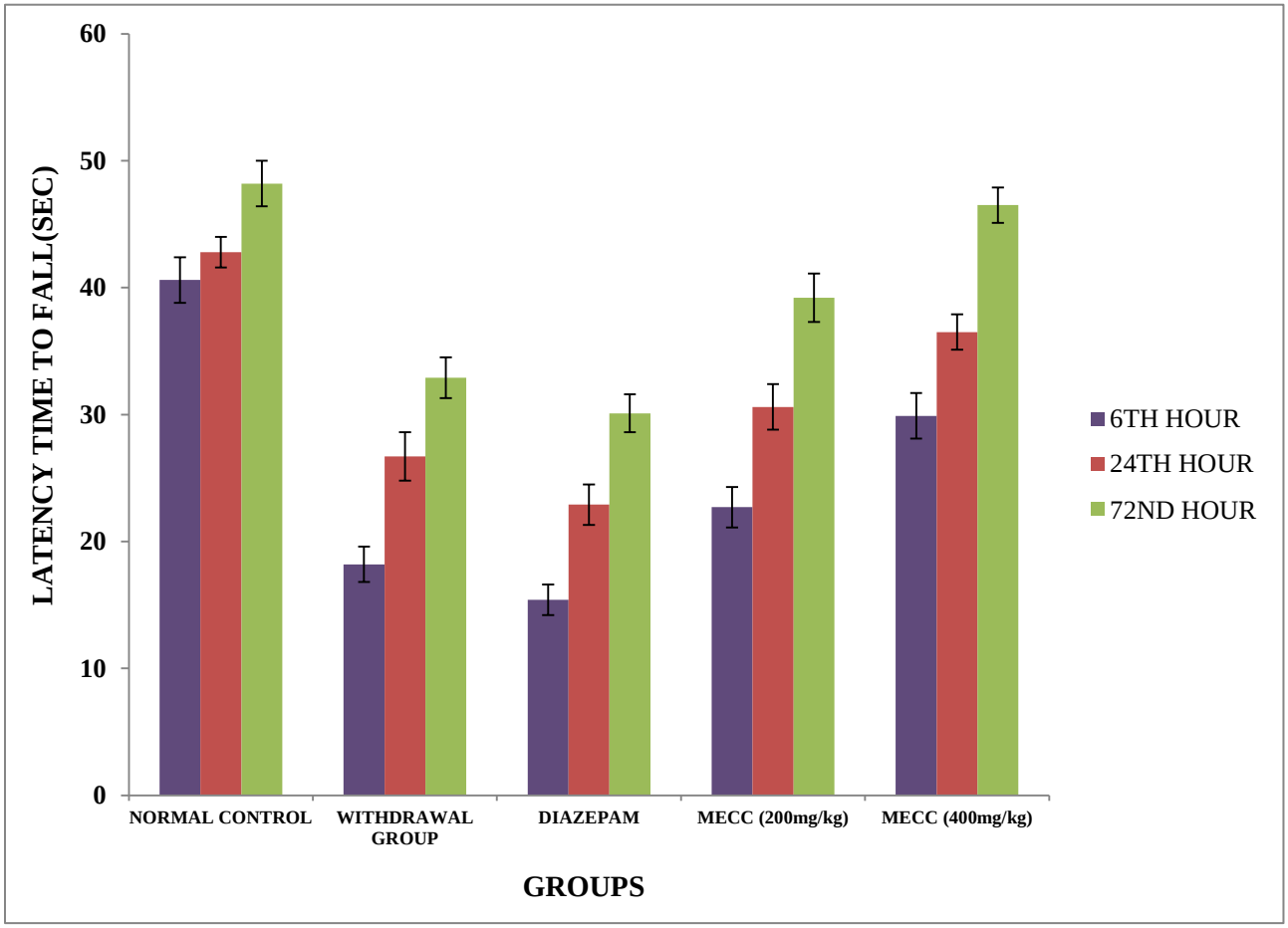

Figure No.10: Latency time to fall in Rota Rod apparatus

IV. Effect of methanolic leaf extract of *Cymbopogon citratus* on Elevated Plus Maze test in animals induced with alcohol withdrawal syndrome

The effect of MECC on elevated plus maze test in animals induced with alcohol withdrawal syndrome is shown in table 8 and figure 11. There was a significant ($P < 0.01$) decrease in the number of open arm entries in elevated plus maze by animals of withdrawal group when compared to normal control. Treatment with diazepam produced a significant ($P < 0.01$) increase in the number of open arm entries when compared to the ethanol withdrawal group. Animals treated with MECC (200 mg/kg) produced significant ($P < 0.01$) increase in the number of open arm entries when compared to the alcohol withdrawal animals in the 6^{th} hour. It produced significant ($P < 0.05$) increase in the number of open arm entries in the 24^{th} and 72^{nd} hour. Animals treated with MECC (400 mg/kg) also produced a significant ($P < 0.01$) increase in number of open arm entries when compared to the alcohol withdrawal animals.

Table 8: Effect of methanolic leaf extract of *Cymbopogon citratus* on Elevated Plus Maze test in animals induced with alcohol withdrawal syndrome

GROUPS	NO. OF OPEN ARM ENTRIES		
	6^{th} Hour	24^{th} Hour	72^{nd} Hour
Normal control	3.8 ± 0.3	4.1 ± 0.2	4.4± 0.2
Withdrawal group	2.4 ± 0.2[a]	2.8 ± 0.4[a]	3.2 ± 0.1[a]
Diazepam	4.6 ± 0.4[c]	4.4 ± 0.3[c]	4.6 ± 0.2[c]
MECC (200mg/kg)	3.2 ± 0.3[c]	3.4 ± 0.4[d]	3.8 ± 0.3[d]
MECC (400mg/kg)	3.4 ± 0.5[c]	3.8 ± 0.4[c]	4.0 ± 0.2[c]

Values are mean ± SEM; n=6. [a]$P < 0.01$, when compared to control animals. [c]$P < 0.01$, [d]$P < 0.05$ compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

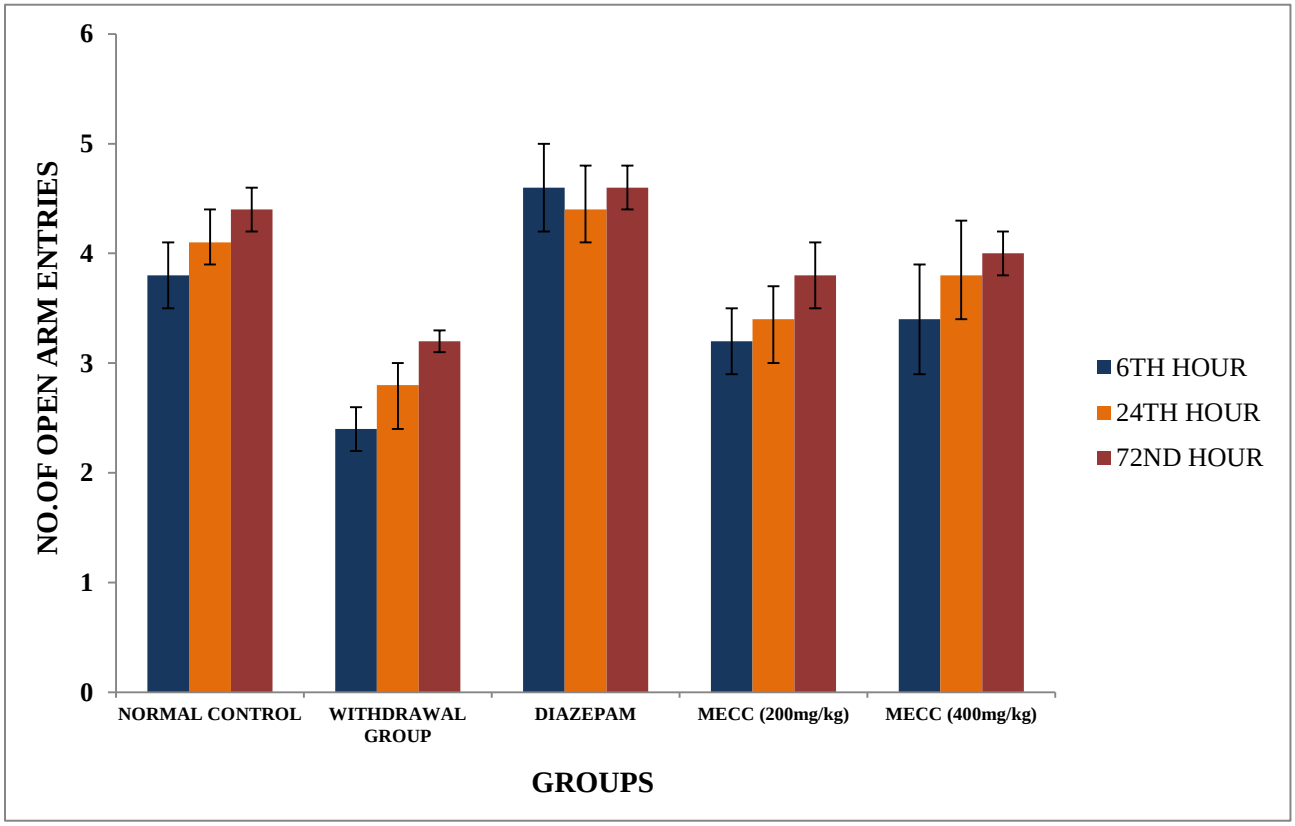

Figure 11: Number of open arm entries in Elevated Plus Maze apparatus

Time spent in open arm in Elevated Plus Maze apparatus.

The time spent in open arm in EPM is listed in table 9 and figure 12). The alcohol withdrawal animals produced a significant ($P < 0.01$) increase in the time spent in open arm in elevated plus maze when compared to normal control. Treatment with Diazepam produced a significant ($P < 0.01$) decrease in the time spent in open arm when compared to the ethanol withdrawal group. Animals treated with MECC(200 mg/kg and 400 mg/kg) produced significant ($P < 0.01$) decrease in the time spent in open arm when compared to the alcohol withdrawal animals in a dose dependant manner.

Table 9: Time spent in open arm in Elevated Plus Maze apparatus.

GROUPS	TIME SPENT IN OPEN ARM (SEC)		
	6th Hour	24th Hour	72nd Hour
Normal control	31.8 ± 1.2	32.9 ± 1.2	30.7 ± 1.3
Withdrawal group	94.2 ± 1.4[a]	89.3 ± 1.4[a]	69.3 ± 1.2[a]
Diazepam	58.9± 1.8[c]	52.8 ± 1.6[c]	45.1 ± 1.4[c]
MECC (200 mg/kg)	62.7 ± 1.4[c]	59.6 ± 1.2[c]	50.8 ± 1.6[c]
MECC (400 mg/kg)	59.2 ± 1.2[c]	58.8 ± 1.8[c]	47.4 ± 1.6[c]

Values are mean ± SEM; n=6. [a]P<0.01, when compared to control animals. [c]P<0.01 compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

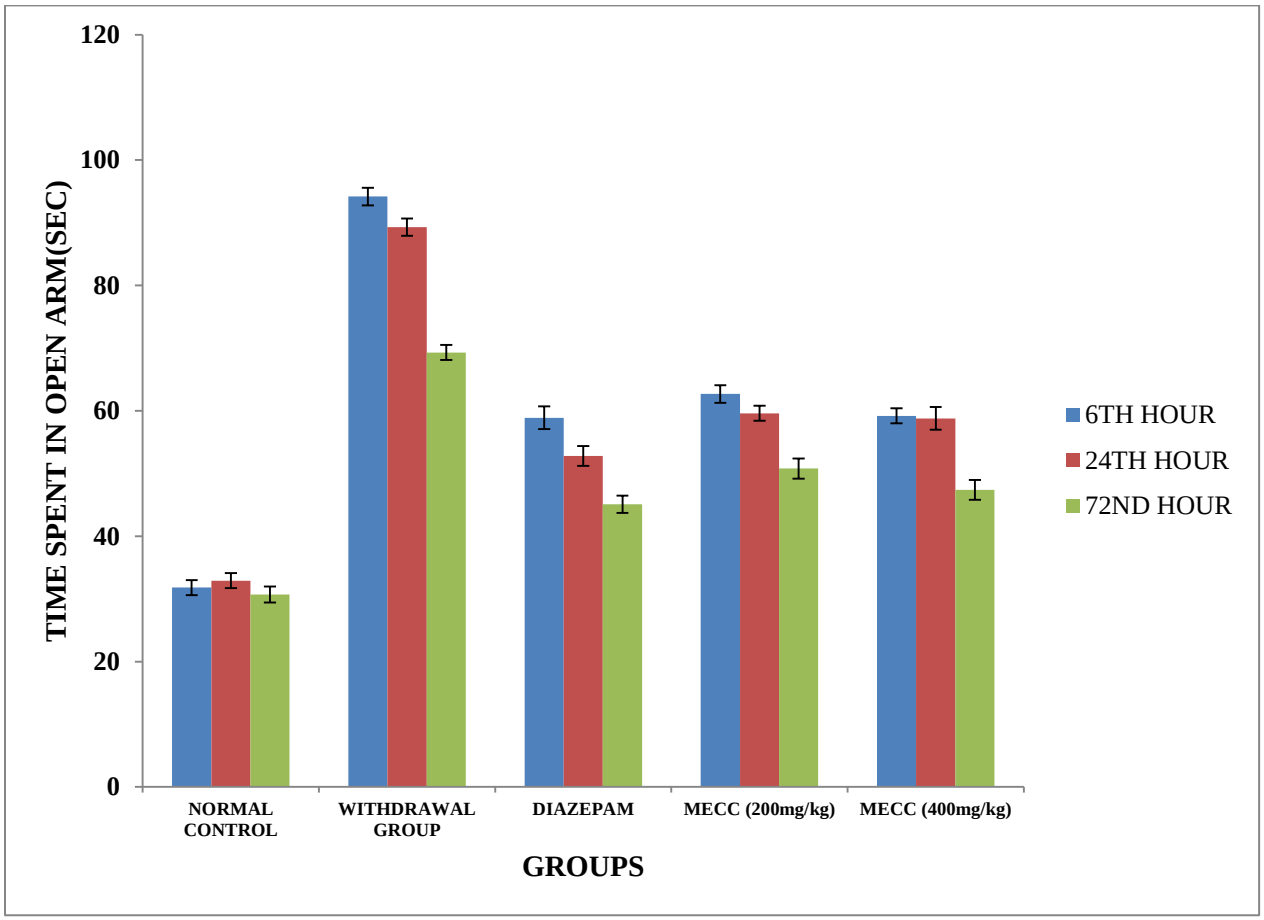

Figure 12: Time spent in open arm in Elevated Plus Maze apparatus.

V. Effect of methanolic leaf extract of *Cymbopogon citratus* on Forced Swim test in animals induced with alcohol withdrawal syndrome

There was a significant ($P < 0.01$) increase in the immobility time in forced swim test by animals of withdrawal group when compared to normal control in the 6^{th} and 24^{th} hour. Whereas, It also produced significant ($P < 0.05$) increase in immobility time in the 72^{nd} hour. Treatment with Diazepam produced a significant ($P < 0.01$) decrease in the immobility time when compared to the ethanol withdrawal group. Animals treated with MECC(200 mg/kg) produced significant ($P < 0.01$) decrease in the immobility time when compared to the alcohol withdrawal. Animals treated with MECC (400 mg/kg) also produced a significant ($P < 0.01$) decrease in the immobility time when compared to the alcohol withdrawal animals. Whereas, it produced less significant ($P < 0.05$) decrease in the immobility time in the 72^{nd} hour. The results are listed in table 10 and figure 13.

Table 10: Effect of methanolic leaf extract of *Cymbopogon citratus* on Forced Swim test in animals induced with alcohol withdrawal syndrome

GROUPS	IMMOBILITY TIME (SEC)		
	6^{th} Hour	**24^{th} Hour**	**72^{nd} Hour**
Normal control	106.4 ± 3.2	108.5 ± 2.0	112.6 ± 3.2
Withdrawal group	143.8 ± 2.4[a]	130.1 ± 3.2[a]	122.9 ± 2.8[b]
Diazepam	81.2 ± 3.6[c]	89.8 ± 2.2[c]	92.7 ± 3.6[c]
MECC (200mg/kg)	94.6 ± 2.8[c]	99.4 ± 1.8[c]	102.8 ± 2.4[c]
MECC (400mg/kg)	100.4 ± 2.4[c]	106.1 ± 3.6[c]	108.3 ± 3.8[d]

Values are mean ± SEM; n=6. [a]P<0.01, [b]P<0.05, when compared to control animals. [c]P<0.01, [d]P<0.05 compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

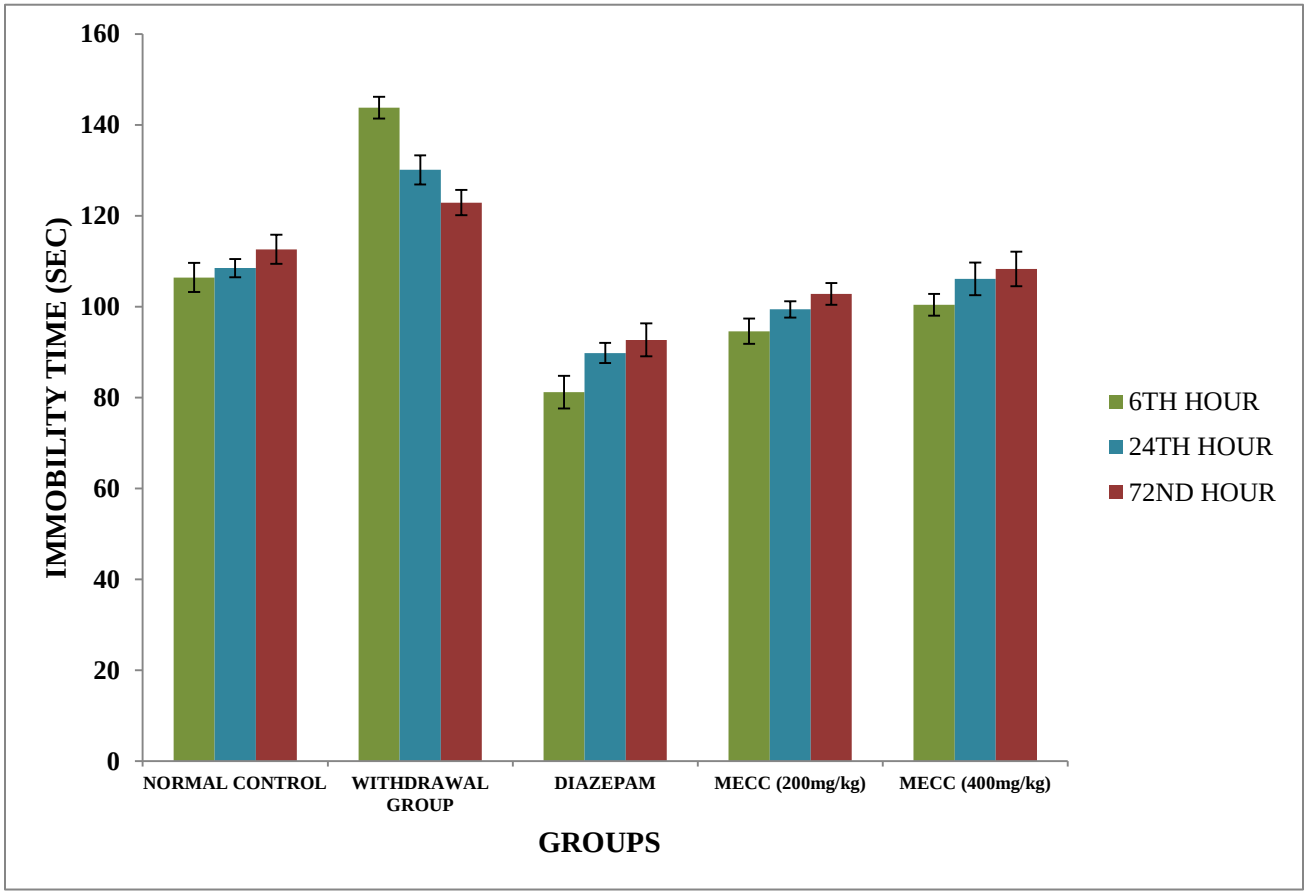

Figure 13: Immobility time in Forced swim Test

VI. Effect of methanolic leaf extract of *Cymbopogon citratus* on MDA level in rat brain and liver induced with alcohol withdrawal syndrome.

The malondialdehyde level was significantly increased ($P < 0.05$) in the withdrawal group animals when compared to the normal control. Treatment with Diazepam did not produce any significant ($P > 0.05$) decrease in MDA when compared to the ethanol withdrawal group. Animals treated with MECC (200 mg/kg) produced significant ($P < 0.05$) decrease in MDA in both brain and liver when compared to the alcohol withdrawal. Animals treated with MECC (400 mg/kg) also produced a significant ($P < 0.05$) decrease in MDA in both brain and liver when compared to the alcohol withdrawal animals(Table 11 and Figure 14).

Table No.11: Effect of methanolic leaf extract of *Cymbopogon citratus* on MDA level in rat brain and liver induced with alcohol withdrawal syndrome

GROUP	MDA (nmoles MDA/mg protein)	
	BRAIN	LIVER
Normal control	0.76 ± 0.06	4.12 ± 0.18
Withdrawal group	1.08 ± 0.10^{b}	6.38 ± 0.24^{b}
Diazepam	0.93 ± 0.08^{ns}	6.94 ± 0.12^{ns}
MECC (200mg/kg)	0.81 ± 0.06^{d}	4.85 ± 0.20^{d}
MECC (400mg/kg)	0.78 ± 0.04^{d}	4.57 ± 0.16^{d}

Values are mean ± SEM; n=6. $^{b}P < 0.05$, when compared to control animals. $^{ns}P > 0.05$, $^{d}P < 0.05$ compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

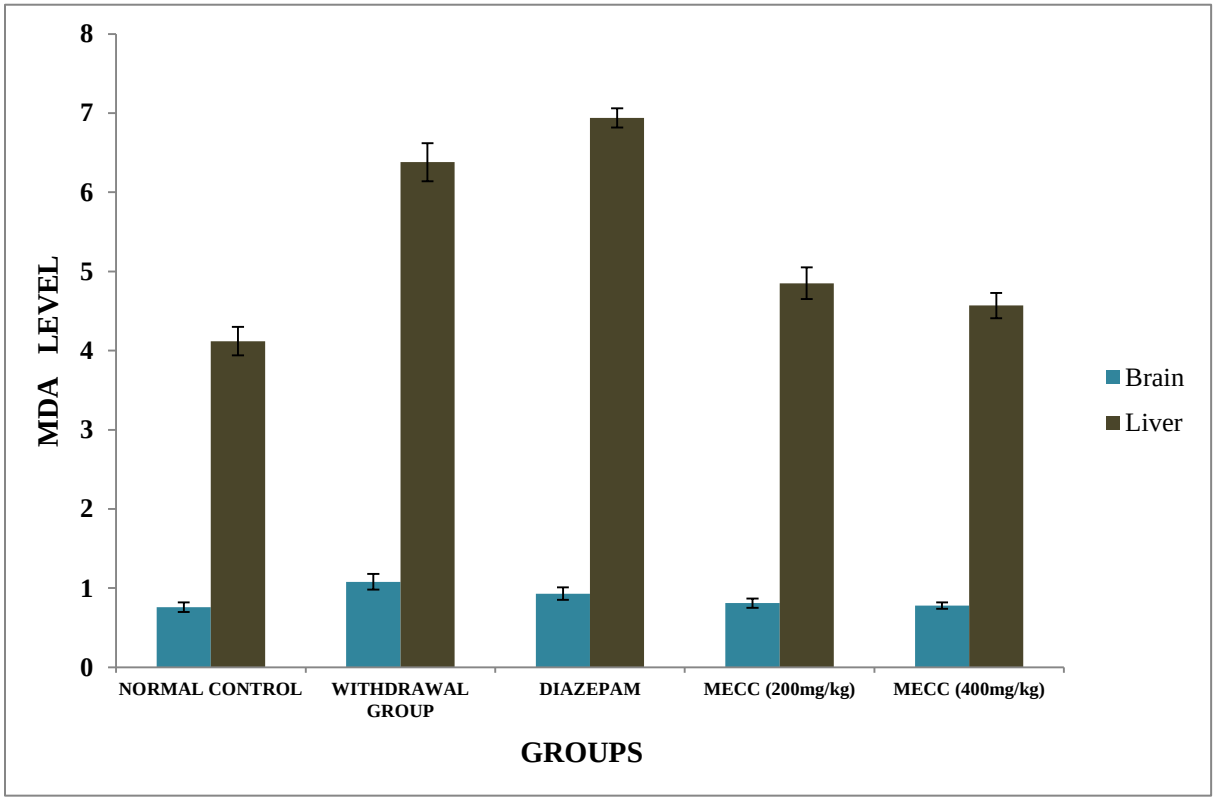

Figure No. 14: MDA level in brain and liver homogenate

VII. Effect of methanolic leaf extract of *Cymbopogon citratus* on SOD level in rat brain and liver induced with alcohol withdrawal syndrome

Effect of MECC on SOD level in rat brain and liver induced with alcohol withdrawal syndrome is shown in table 12 and figure 15. There was a significant ($P < 0.01$) decrease in SOD level in the brain and liver of animals of withdrawal group when compared to normal control. Treatment with Diazepam showed a significant ($P < 0.05$) elevation in brain SOD level (2.58 nmoles/mg protein), a significant ($P < 0.05$) reduction in liver SOD level (4.16 nmoles/mg protein) when compared to the ethanol withdrawal group. Animals treated with MECC (200 mg/kg) produced significant($P < 0.01$) increase in SOD in both brain and liver when compared to the alcohol withdrawal. Animals treated with MECC(400 mg/kg) also produced a significant($P < 0.01$) increase in SOD in both brain and liver when compared to the alcohol withdrawal animals.

Table 12: Effect of methanolic leaf extract of *Cymbopogon citratus* on SOD level in rat brain and liver induced with alcohol withdrawal syndrome

GROUP	SOD (nmoles/ mg protein)	
	BRAIN	LIVER
Normal control	3.82 ± 0.14	5.58 ± 0.26
Withdrawal group	2.29 ± 0.20[a]	4.49 ± 0.22[a]
Diazepam	2.58 ± 0.28[d]	4.16 ± 0.28[d]
MECC (200mg/kg)	2.97 ± 0.36[c]	4.89 ± 0.14[c]
MECC (400mg/kg)	3.26 ± 0.18[c]	5.25 ± 0.28[c]

Values are mean ± SEM; n=6. [a]$P < 0.01$, when compared to control animals. [c]$P < 0.01$, [d]$P < 0.05$ compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

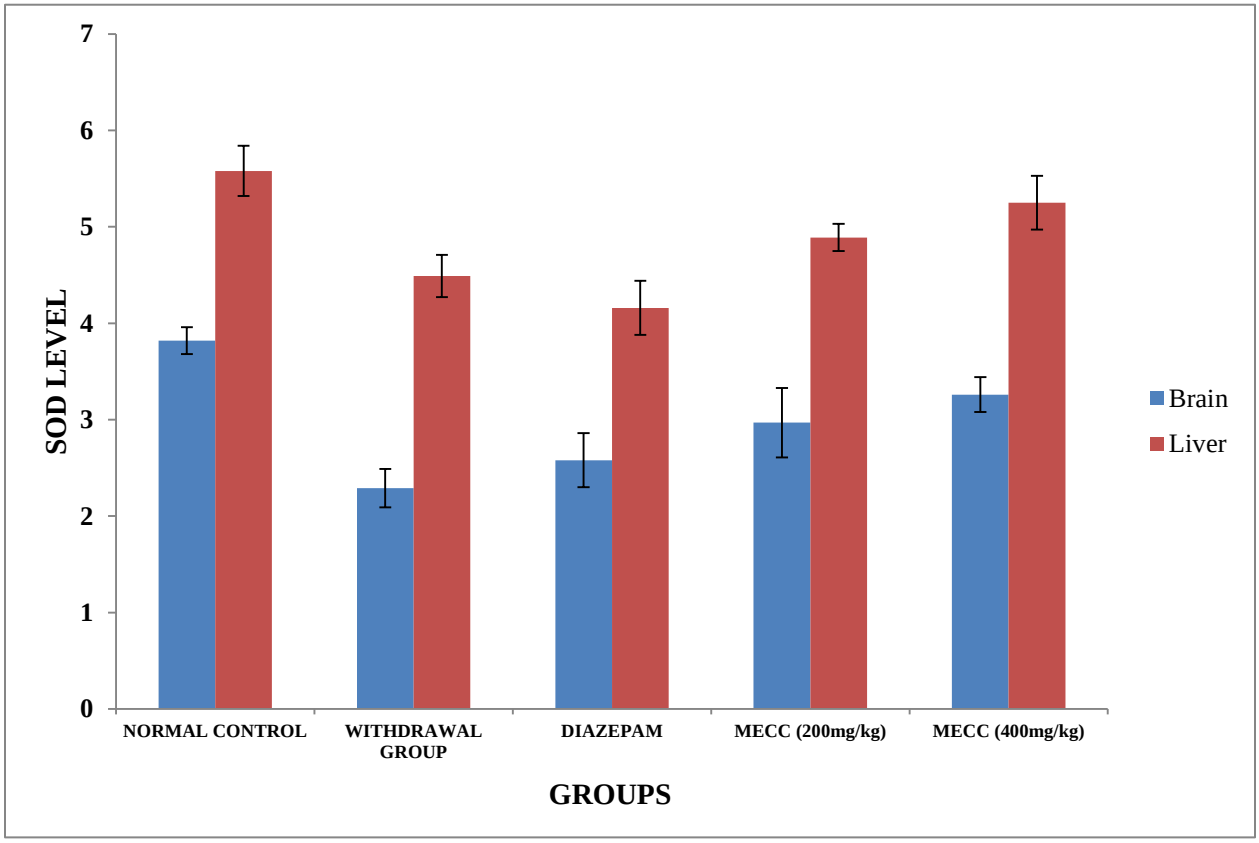

Figure 15: SOD level in brain and liver homogenate

VIII. Effect of methanolic leaf extract of *Cymbopogon citratus* on CAT level in rat brain and liver induced with alcohol withdrawal syndrome.

The animals of the withdrawal group produced a significant ($P < 0.01$) decrease in CAT level in the brain and liver of animals of withdrawal group when compared to normal control. Treatment with Diazepam produced significant ($P < 0.05$) increase in CAT level in liver(36.12 µmoles / mg protein) when compared to the ethanol withdrawal group. Whereas, in brain it produced significant ($P < 0.01$) increase CAT levels(27.12 µmoles / mg protein). Animals treated with MECC(200 mg/kg) produced significant ($P < 0.01$) increase in CAT in both brain and liver when compared to the alcohol withdrawal. Animals treated with MECC(400 mg/kg) also produced a significant ($P < 0.01$) increase in CAT in both brain and liver when compared to the alcohol withdrawal animals(Table 13 and Figure 16).

Table 13: Effect of methanolic leaf extract of *Cymbopogon citratus* on CAT level in rat brain and liver induced with alcohol withdrawal syndrome.

GROUP	CAT (µmoles / mg protein)	
	BRAIN	LIVER
Normal control	32.17 ±1.8	53.14 ± 2.6
Withdrawal group	25.15 ± 2.4[a]	40.17 ± 2.2[a]
Diazepam	27.12 ± 2.6[c]	36.12 ± 1.8[d]
MECC (200mg/kg)	29.30 ± 1.2[c]	45.16 ± 2.4[c]
MECC (400mg/kg)	30.86 ± 2.6[c]	49.35 ± 2.6[c]

Values are mean ± SEM; n=6. [a]$P < 0.01$, when compared to control animals. [c]$P < 0.01$, [d]$P < 0.05$ compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

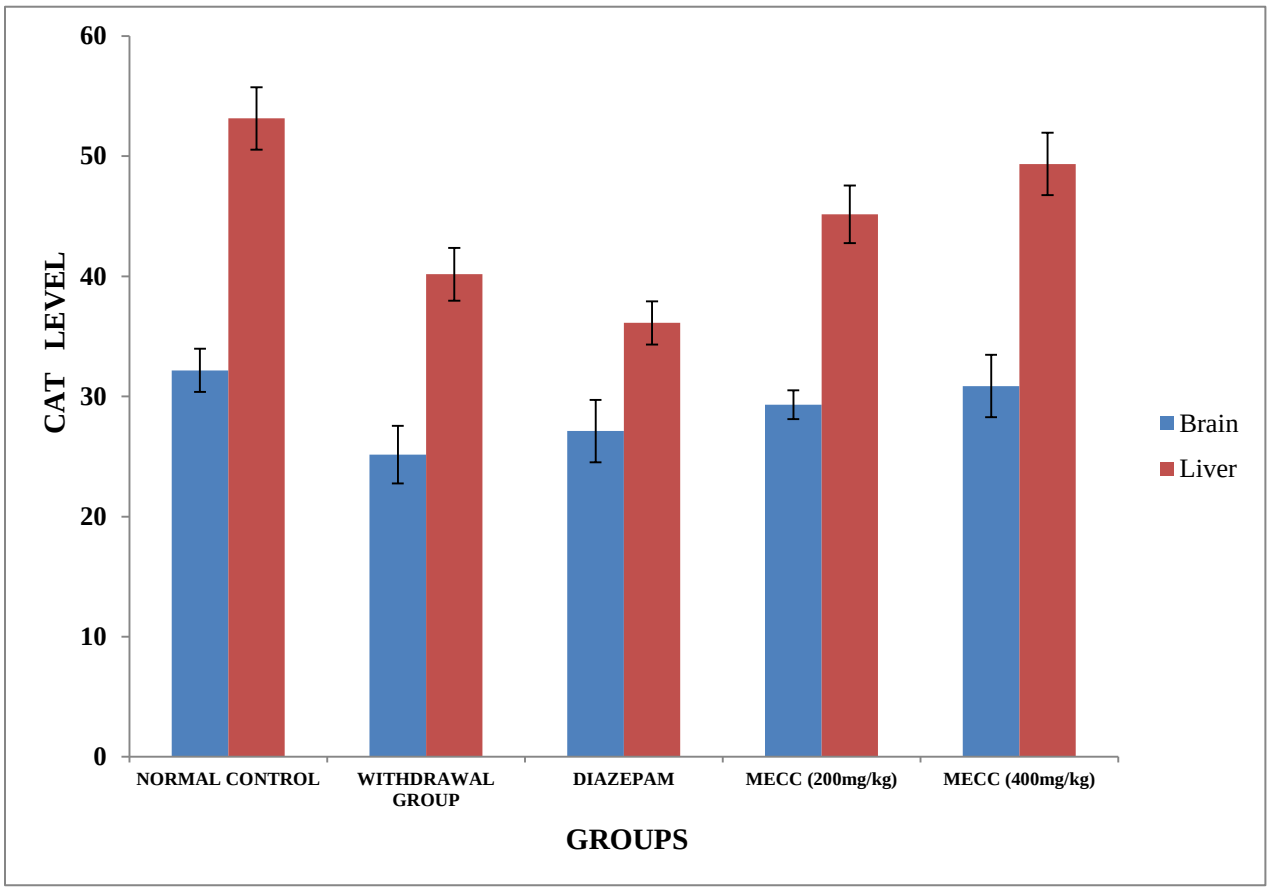

Figure 16: CAT level in brain and liver homogenate.

IX. Effect of methanolic leaf extract of *Cymbopogon citratus* on GPx level in rat brain and liver induced with alcohol withdrawal syndrome.

The results of the effect of MECC on GPx level in rat brain and liver induced with alcohol withdrawal syndrome is listed in table 14 and figure 17. There was a significant ($P < 0.01$) decrease in GPx level in the brain and liver of animals of withdrawal group when compared to normal control. Treatment with Diazepam produced significant ($P < 0.05$) increase GPx level in liver when compared to the ethanol withdrawal group. In brain it produced significant ($P < 0.01$) increase in GPx levels. Animals treated with MECC (200 mg/kg and 400 mg/kg) produced significant ($P < 0.01$) increase in GPx in both brain and liver when compared to the alcohol withdrawal in a dose dependant manner.

Table 14: Effect of methanolic leaf extract of *Cymbopogon citratus* on GPx level in rat brain and liver induced with alcohol withdrawal syndrome

GROUP	GPx (nmoles/ mg protein)	
	BRAIN	LIVER
Normal control	6.24 ± 0.42	11.02 ± 0.38
Withdrawal group	3.12 ± 0.24[a]	6.24 ± 0.42[a]
Diazepam	4.29 ± 0.48[c]	5.91 ± 0.36[d]
MECC (200mg/kg)	5.06 ± 0.24[c]	7.64 ± 0.48[c]
MECC (400mg/kg)	5.82 ± 0.46[c]	9.28 ± 0.26[c]

Values are mean ± SEM; n=6. [a]P<0.01, when compared to control animals. [c]P<0.01, [d]P<0.05 compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test

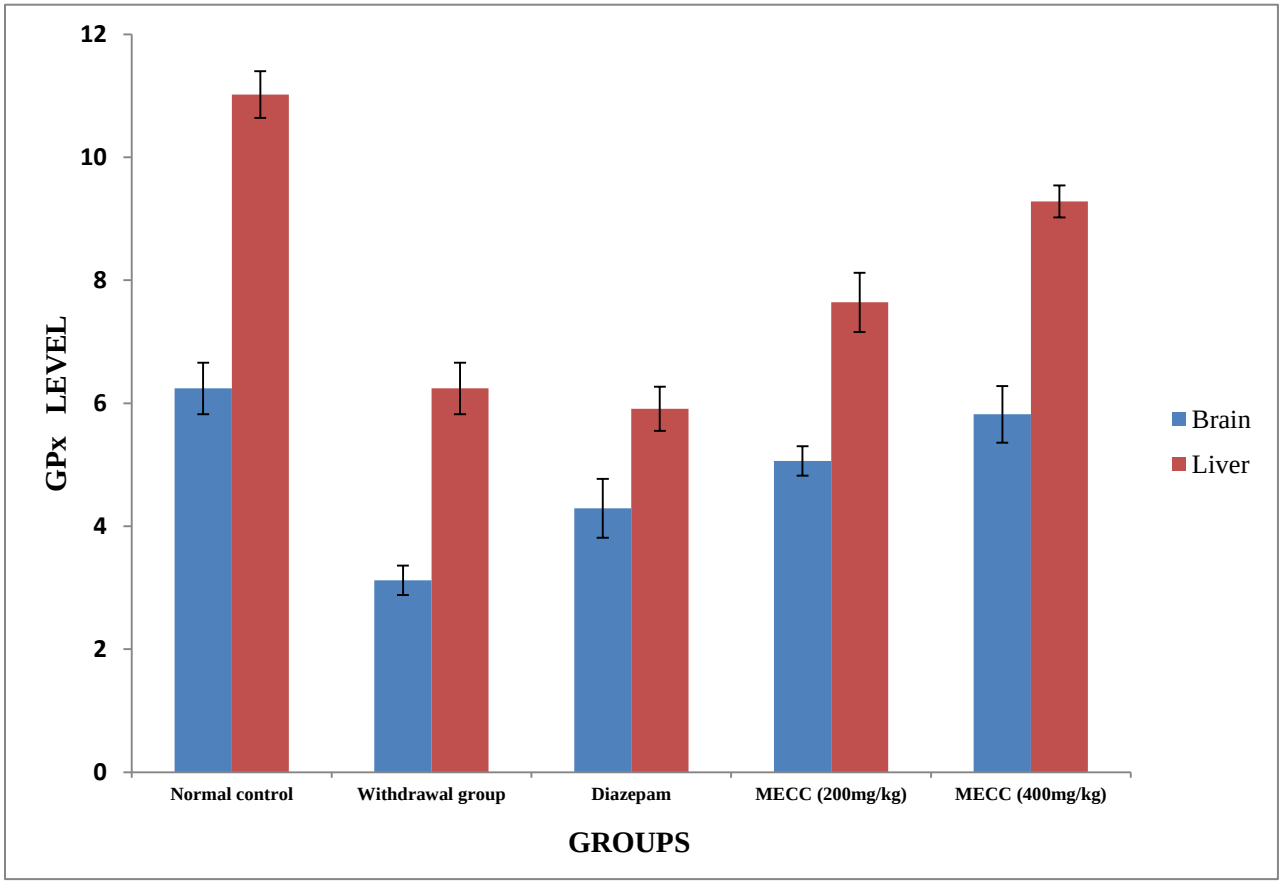

Figure 17: GPx level in brain and liver homogenate.

X. Effect of methanolic leaf extract of *Cymbopogon citratus* on GSH level in rat brain and liver induced with alcohol withdrawal syndrome.

There was a significant ($P < 0.01$) decrease in GSH level in the brain and liver of animals of withdrawal group when compared to normal control. Treatment with Diazepam produced significant ($P < 0.01$) increase in GSH level when compared to the ethanol withdrawal group in liver and brain. Animals treated with MECC(200 mg/kg) produced significant ($P < 0.01$) increase in GSH in both brain and liver when compared to the alcohol withdrawal. Animals treated with MECC(400 mg/kg) also produced a significant ($P < 0.01$) increase in GSH in both brain and liver when compared to the alcohol withdrawal animals (Table 15 and Figure 18).

Table 15: Effect of methanolic leaf extract of *Cymbopogon citratus* on GSH level in rat brain and liver induced with alcohol withdrawal syndrome.

GROUP	GSH nmoles/ mg protein)	
	BRAIN	LIVER
Normal control	5.22 ± 0.28	7.38 ± 0.14
Withdrawal group	3.04 ± 0.14[a]	5.56 ± 0.16[a]
Diazepam	3.96 ± 0.32[c]	4.82 ± 0.14[c]
MECC (200mg/kg)	4.28 ± 0.28[c]	6.24 ± 0.26[c]
MECC (400mg/kg)	4.66 ± 0.36[c]	6.86 ± 0.18[c]

Values are mean ± SEM; n=6. [a]P<0.01, when compared to control animals. [c]P<0.01, compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test

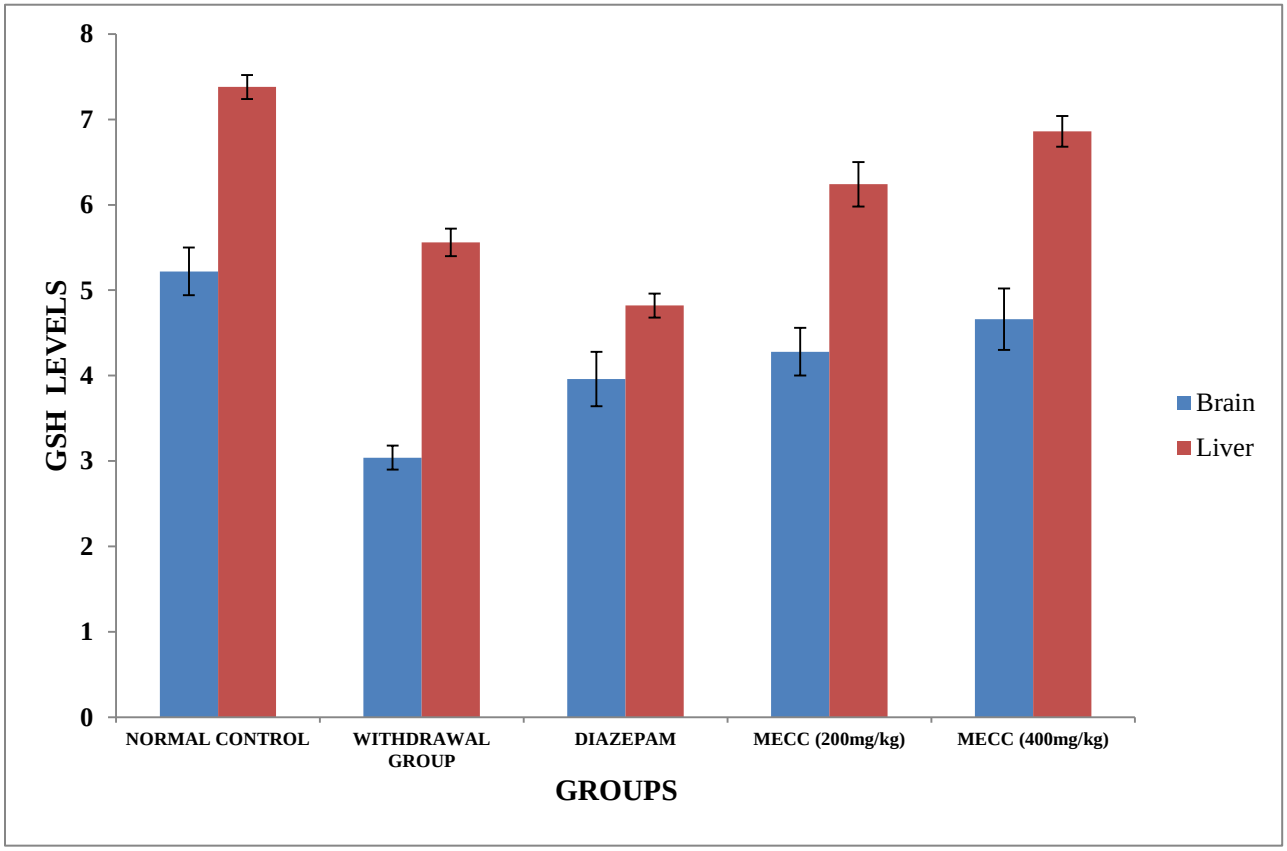

Figure 18: GSH level in brain and liver homogenate.

XI. Effect of methanolic leaf extract of *Cymbopogon citratus* on hepatic serum biomarker enzymes in rat induced with alcohol withdrawal syndrome.

The animals of withdrawal group shown a significant ($P < 0.01$) increase in hepatic biomarker level in the serum when compared to normal control. Treatment with Diazepam produced significant ($P < 0.01$) increase in hepatic biomarker level when compared to the ethanol withdrawal group in liver and brain. Animals treated with MECC(200 mg/kg) produced significant ($P < 0.01$) increase in GSH in both brain and liver when compared to the alcohol withdrawal. Animals treated with MECC(400 mg/kg) also produced a significant ($P < 0.01$) increase in GSH in both brain and liver when compared to the alcohol withdrawal animals. The results of the effect of MECC on hepatic serum biomarker enzymes in rat induced with alcohol withdrawal syndrome is shown in table 16 and figure 19.

Table 16: Effect of methanolic leaf extract of *Cymbopogon citratus* on hepatic serum biomarker enzymes in rat induced with alcohol withdrawal syndrome.

GROUP	AST (U/L)	ALT(U/L)	ALP(U/L)
Normal control	62.5 ± 2.2	48.24 ± 2.6	128.24 ± 3.4
Withdrawal group	102.2 ± 1.4[a]	92.98 ± 3.2[a]	181.76 ± 5.6[a]
Diazepam	148.5 ± 2.6[c]	97.72 ± 1.8[c]	195.28 ± 4.2[c]
MECC (200mg/kg)	91.8 ± 1.8[c]	62.16 ± 1.2[c]	159.24 ± 2.6[c]
MECC (400mg/kg)	81.5 ± 2.4[c]	55.92 ± 2.8[c]	146.18 ± 4.4[c]

Values are mean ± SEM; n=6. [a]P<0.01, when compared to control animals. [c]P<0.01, compared to alcohol withdrawal animals. Data were analyzed by one way ANOVA followed by Dunnett's test.

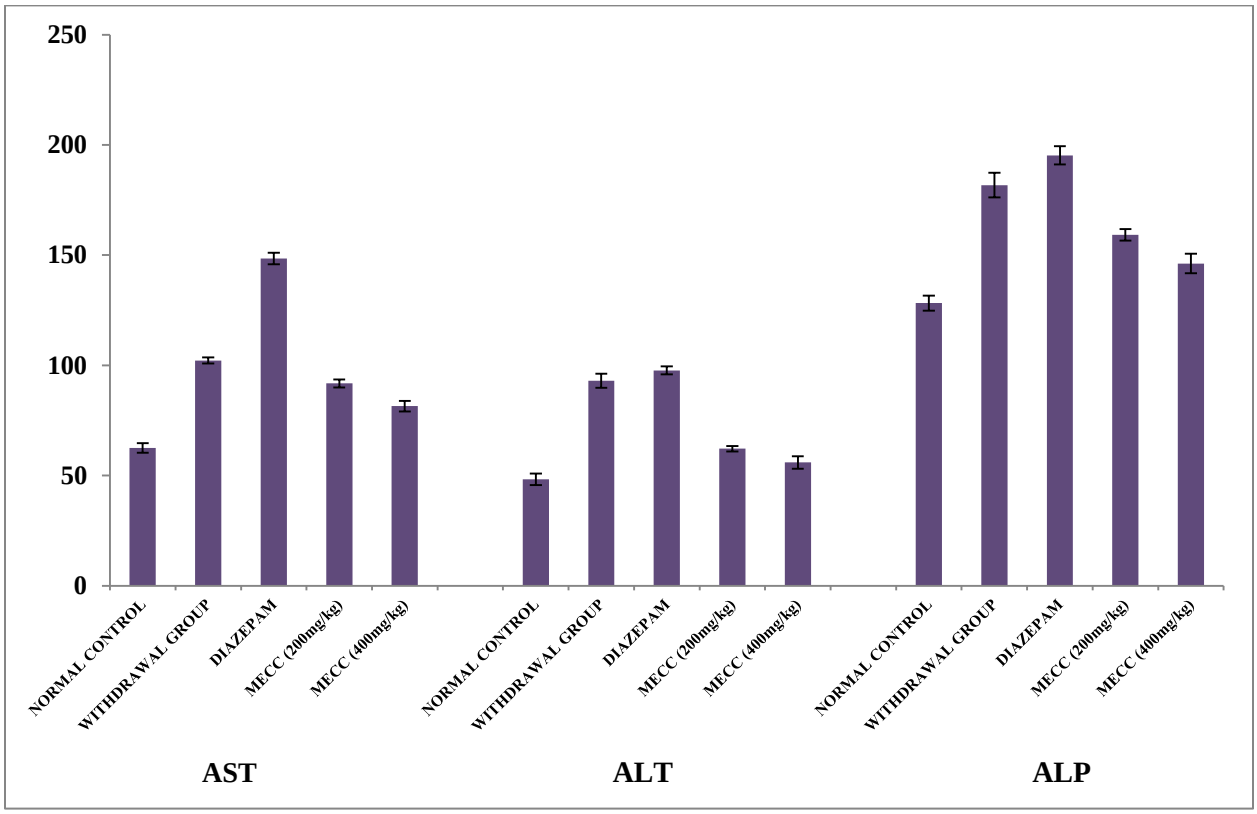

Figure No. 19: AST, ALT and ALP level in serum

XII. Effect of methanolic leaf extract of *Cymbopogon citratus* on liver of alcohol treated rats.

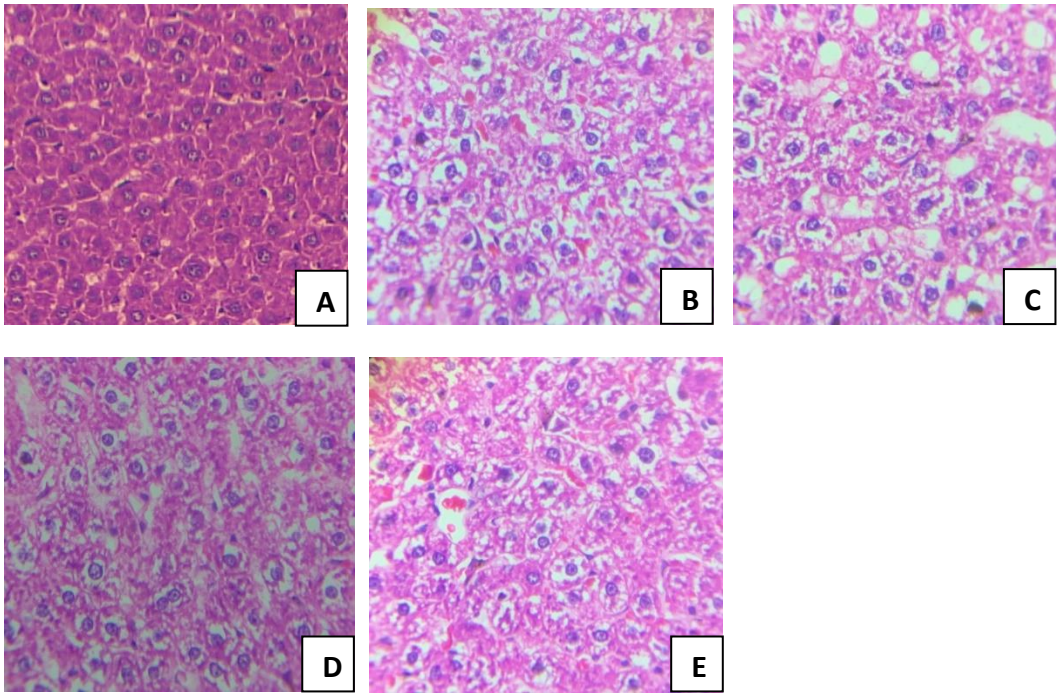

Figure 20: Histopathology of rat liver

(A) Normal control-Normal Hepatocytes, sinusoids, hexagonal or pentagonal hepatic lobules. (B)Withdrawal group- Distorted hepatic lobules, dissociation of hepatic cords, sinusoidal dilatation, pyknotic nuclei. (C)Diazepam treated- Distorted hepatic lobules, dissociation of hepatic cords, more sinusoidal dilatation, pyknotic nuclei. (D)MECC(200mg/kg)- Less distortion of hepatic lobules, less sinusoidal dilatation. (E) MECC(400mg/kg)- Retain hepatic lobules, less sinusoidal dilatation, retain hepatic cords when compared to withdrawal group.

XIII. Effect of methanolic leaf extract of *Cymbopogon citratus* on brain of alcohol treated rats.

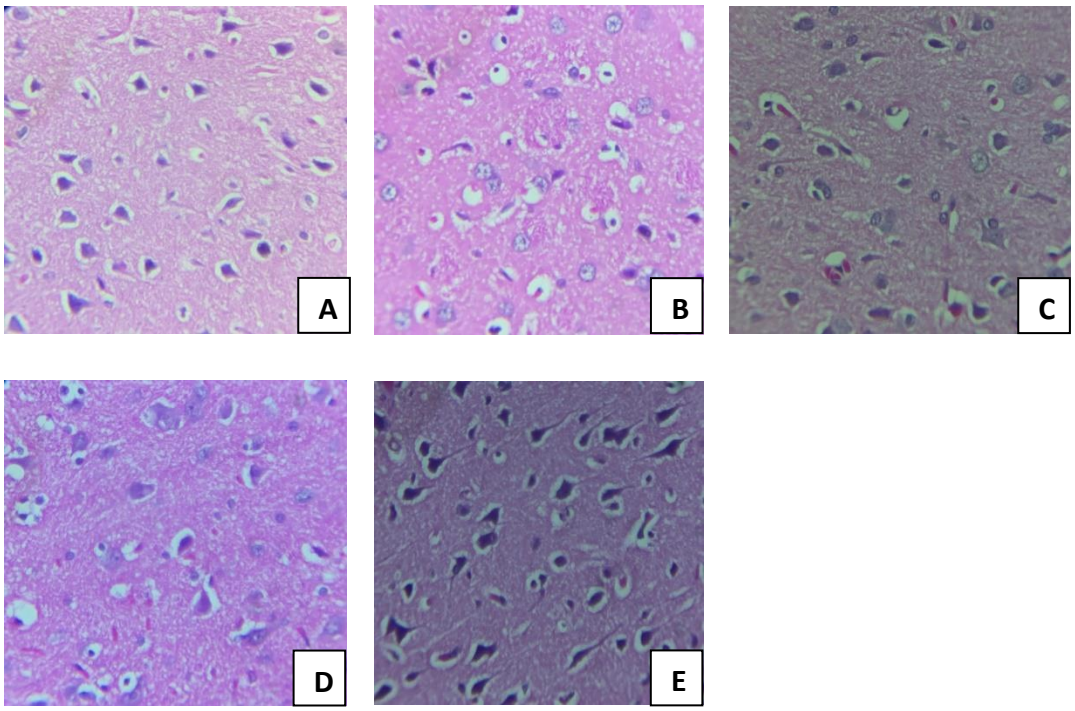

Figure 21: Histopathology of rat brain

(A)-Normal control-Normal and organized neuronal cells. (B)-Withdrawal group- Appearance of Microglia, Alzheimer's type II astrocytes, neuronal degradation. (C) Diazepam treated- Significantly less neurodegradation, apprearence of Alzheimer's type II astrocytes. (D)-MECC 200mg/kg- Less neurodegradation. (E)-MECC 400mg/kg- Significantly less neurodegradation.

DISCUSSION

A huge population worldwide have alcohol use disorder. Alcohol withdrawal syndrome is a set of symptoms that occurred by an individual cessation of alcohol after a prolonged period of excessive use. Generally, alcohol consumption produces sedative effect. But, on withdrawal it produces excitatory effects.

Acute alcohol consumption produces sedative effects by inhibiting the excitatory neurotransmission and increasing the inhibitory neurotransmission. The main neurotransmitters involved in this pathway are GABA and Glutamate. GABA is a major inhibitory neurotransmitter and glutamate is a major excitatory neurotransmitter.

On chronic alcohol consumption, the effect reverses as the body tries to balance the neurotransmitters. As a result, the excitatory neurotransmission increases and the inhibitory neurotransmission decreases.

On alcohol withdrawal, this effect continues leading to the symptoms of AWS such as tremulousness, anxiety, sweating, nausea, vomiting, and agitation. Benzodiazepines are the widely used class of drugs for the treatment of AWS. But, benzodiazepine withdrawal after prolonged usage may also lead to protracted withdrawal syndrome. However, for severe symptoms scored 5-7 in CIWA, allopathic medicines are only the first line choice of drugs. Whereas, herbal medicines can be used to treat mild to moderate symptoms scored 1-4 in CIWA categories. Herbal medicines have many advantages such as minimum or no side effects, low cost can be continued for a long period.

Cymbopogon citratus (Poaceae) is a perennial grass which is evenly dispersed and found in the tropic regions, South and Central America widely used for their pleasant taste and therapeutic properties. It has various proven pharmacological actions such as anti-microbial, anti-fungal, anti-anxiety, anti-diarrhoeal, anti-obeity, anti-hepatotoxic and anti-malarial activity.

The methanolic leaf extract of *Cymbopogon citratus* was prepared by maceration process. The phytochemical screening of the prepared extract confirmed the presence of flavonoid,

aldehyde, terpenoid, tannin, phenolic compounds, saponin, alkaloid, unsaturated steroids and steroid derivatives.

Alcohol withdrawal syndrome was induced in the rats by administering ethanol for 29 days following by withdrawal. Diazepam, MECC 200 mg/kg and MECC 400 mg/kg were administered to their respective groups. The behavioral parameters were assessed on the 6^{th}, 24^{th} and 72^{nd} hour after withdrawal.

In open field test, the withdrawal group animals shown increase in the number of lines crossed in the apparatus when compared to the normal control, due to hyper locomotion parameter caused by alcohol withdrawal. On administration of diazepam, it significantly reduced the number of lines crossed when compared to the withdrawal group. But, it was even less when compared to the normal control as diazepam produces depressive effect. MECC 200 mg/kg and 400 mg/kg also reduced the number of lines crossed when compared to the control and it was nearly similar to the normal control.

In rotarod test, the latency time to fall from the apparatus is decreased in the withdrawal group when compared to the normal control due to impaired muscle co-ordination. Administration of diazepam, even more reduced the latency to fall as it produces sedative and depressive effect. Administration of MECC 200 mg/kg and 400 mg/kg increased the latency time to fall, which is similar to that of the normal control. Thus, counteracting the effect produced by ethanol withdrawal.

In elevated plus maze test, ethanol withdrawal decreases the number of open arm entries when compared to that of the normal control. The administration of diazepam increased the number of open arm entries by reducing the anxiety. MECC 200 mg/kg and 400 mg/kg also increased the number of open arm entries.

In elevated plus maze test, the time spent in open arm was found to be increased in the ethanol withdrawal group when compared to the normal control. Administration of diazepam decreased the time spent in open arm when compared to the withdrawal group. MECC 200 mg/kg and 400 mg/kg also decreased the time spent in of open arm.

In forced swim test, the immobility time spent by the animal was increased in the withdrawal group when compared to the normal control as ethanol withdrawal produces depressive and muscle incoordination. Administration of diazepam decreased the immobility time when compared to that of the withdrawal group. Administration of MECC 200 mg/kg and 400 mg/kg also decreased the immobility time when compared to that of the withdrawal group.

Malondialdehyde (MDA), final product of polyunsaturated fatty acids peroxidation in the cells was measured. Ethanol withdrawal group possess increase in MDA level in both brain and liver when compared with that of the normal control. Diazepam did not produce and significant decrease in the MDA level in both brain and liver.

The antioxidants, such as SOD, CAT and GPx were measured both in brain and liver homogenates of the animals. The ethanol withdrawal group possess significant decrease in the levels of these antioxidants in both brain and liver when compared to that of the normal control. Administration of diazepam produced slight increase in these anti-oxidants in brain as it possess sedative effects. But, in liver it further decreased the level of antioxidants when compared to that of the withdrawal group as it possesses liver toxicity. MECC 200 mg/kg and 400 mg/kg increased the level of these antioxidants both in brain and liver.

Reduced glutathione (GSH), as an antioxidant modulator was also measured. The ethanol withdrawal group possesses significant decrease in the level of GSH in both brain and liver when compared to that of the normal control. Administration of diazepam, produced slight increase in GSH in brain as it possess sedative effects. But, in liver it further decreased the level of GSH when compared to that of the withdrawal group as it possess liver toxicity. MECC 200 mg/kg and 400 mg/kg increased the level of GSH both in brain and liver.

The hepatic biomarkers such as ALT, AST and ALP were monitored. The ethanol withdrawal group shown significant increase in these biomarkers level due to liver toxicity. Administration of diazepam further increased the hepatic biomarker levels as it also possess hepatotoxicity. Administration of MECC 200 mg/kg and 400 mg/kg reduced the hepatic biomarker levels showing hepatoprotective effects.

The brain and liver samples were subjected to histopathological studies. From histopathology of liver it can be concluded that, withdrawal group shown distorted hepatic lobules, dissociation of hepatic cords, sinusoidal dilatation, pyknotic nuclei. Administration of diazepam further elevated the hepatotoxicity. MECC 200 mg/kg and 400 mg/kg shown protective and therapeutic effects on the liver histology. From histopathology of brain it can be concluded that the withdrawal group shown appearance of microglia, Alzheimer's type II astrocytes, neuronal degradation. Diazepam administration minimized these effects. Administration of MECC 200 mg/kg and 400 mg/kg also reduced these effects and shown therapeutic efficacy.

These behavioral assessment and biochemical assessment shows that *Cymbopogon citratus* has potential therapeutic effects against alcohol withdrawal syndrome. It can be used to treat mild to moderate level of AWS symptoms.

SUMMARY

Alcohol withdrawal syndrome refers to a set of symptoms that occurs within few hours following the cessation of alcohol. Some of the symptoms are anxiety, tremor, hyper locomotion, sweating, nausea, vomiting, and agitation. On severe cases, it may lead to Delirium tremens and Seizures. Benzodiazepines are the popular class of drugs used in the treatment of AWS. But on prolonged usage it may also cause addiction. So, in mild to moderate symptoms of AWS herbal medicines can be used to suppress those symptoms which is of less or no side effect.

Wistar albino rats were used in this study. For which permission was granted from the Institutional Animal Ethics Committee[NCP/IAEC/2021-22/01].

Cymbopogon citratus, commonly known as lemongrass is a perennial herb with therapeutic activities such as anti-obesity, anti-diabetic, anti-hepatotoxic, anti-anxiety and anti-microbial activity. It is selected to evaluate its activity against AWS. The plant was identified and authenticated by the Tamilnadu Agricultural University, Coimbatore[BSI/SRC/5/23/2020/Tech].

The leaves of the plant were collected and the methanolic extract was prepared by maceration process. Phytochemical screening confirmed the presence of flavonoid, aldehyde, terpenoid, tannin, phenolic compounds, saponin, alkaloid, unsaturated steroids and steroid derivatives.

Alcohol withdrawal syndrome was induced in the wistar rats by administering 10% ethanol for 28 days followed by withdrawal. Diazepam(2mg/kg ip.,) was used as a standard in this study. MECC 200mg/kg and MECC 400 mg/kg were administered to group IV and group V respectively from day 22 to 28.

The behavioral parameters were assessed using apparatus such as open field, rota rod, elevated plus maze, forced swim test on the 29th day. The ethanol withdrawal groups shown anxiety, muscle incoordination, depression and hyper locomotion. Which was attenuated by diazepam. But diazepam administration produced sedative effects. Administration of the MECC produced results similar to that of normal control.

Then blood was collected on the 31st day and the biochemical parameters such as AST, ALT and ALP were assessed in the serum. The withdrawal group shown increased level of these biomarkers due to hepatotoxicity. Diazepam further increased the hepatic toxicity thus showing increased level of these biomarkers. MECC administration decreased the level of these biomarkers.

Animals were sacrificed on the 31st day and the brain and liver were isolated. The brain and liver homogenates were prepared and MDA, SOD, CAT, GSH, GPx were measured. The withdrawal group shown decrease in the level of these antioxidants. Diazepam administration increased the level of these antioxidants in brain. But, in liver it further decreased the level of antioxidants when compared to that of the withdrawal group. MECC (200mg/kg and 400mg/kg) produced significant increase in the level of these antioxidants.

The brain and liver samples were histopathologically analysed. The histopathology reports also supports the therapeutic and protective effects of *Cymbopogon citratus*.

CONCLUSION

Alcohol withdrawal syndrome is a group of symptoms that occurs following the cessation of alcohol after a period of excessive alcohol use. These symptoms occur due to alcohol-induced chemical imbalances in the brain which result in excessive neuronal activity if the alcohol is withheld. Herbal medicines can be used for a very long period of time to treat them without possessing any side effects. Based on evidence from previous studies, *Cymbopogon citratus* was chosen and evaluated for its efficacy against Alcohol withdrawal syndrome in Wistar rats.

From the biochemical and behavioral results it can be concluded that the methanolic leaf extract of *Cymbopogon citratus* possess anti-oxidant activity and it also attenuates the symptoms of Alcohol Withdrawal Syndrome in Wistar rats.

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CERTIFICATE

This is to certify that the work embodied in this thesis entitled **"EVALUATION OF METHANOLIC LEAF EXTRACT OF *Cymbopogon citratus* ON ALCOHOL WITHDRAWAL SYNDROME IN WISTAR RATS"** submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, was carried out by **Ms. G.S.SRI BHARATHI,** Department of Pharmacology, Nandha College of Pharmacy, Erode for the partial fulfillment for the degree of **MASTER OF PHARMACY** in Pharmacology under the supervision of **Dr. V.LALITHA, M.Pharm., Ph.D.,** Associate Professor, Department of Pharmacology, Nandha College of Pharmacy, Erode.

The work is original and has not been previously formed the basis for the award of any other Degree, Diploma, Associateship, Fellowship or any other similar title and the dissertation represent entirely an independent work on the part of the candidate.

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CERTIFICATE

This is to certify that the project **Proposal No: NCP/IAEC/2021-22/01** entitled **"Evaluation of role of methanolic extract of *Cymbopogon citratus* on Alcohol Withdrawal Syndrome in wistar rats"** submitted by Dr./Mr./Ms **G.S. Sri Bharathi** has been approved/recommended by the IAEC of Nandha College of Pharmacy in its meeting held on 12/08/2021 and **Wistar Albino Rat : 30** (Number and Species of animals) have been sanctioned under this.

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पौधे प्रमाणीकरण प्रमाणपत्र /PLANT AUTHENTICATION CERTIFICATE

The plant specimen brought by you for authentication is identified as *Cymbopogon citratus* - POACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

03/2/2020

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