**Preparation and pharmacological evaluation of an herbal formulation from Himalayan plants for treatment of Alcohol Abstinence Syndrome.**

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**Abstract**

Dependency on alcohol has a negative impact on people's health and the economy and is a big global issue. Because of alcohol withdrawal, there is a relapse in alcohol consumption. The symptoms of alcohol withdrawal, which resemble anxiety, start to show 6–24 hours after the last alcohol consumption. The purpose of the current investigation was to determine whether a standardized polyherbal mixture could shield Wistar rats from the anxiety associated with ethanol withdrawal. Three plant extracts—Cymbopogon citratus, Cinnamomum tamala, and Urtica dioca—were combined in a 1:1:1 ratio to create the polyherbal mixture. Using HPTLC, phytochemical profiling of the polyherbal formulation was conducted. With the use of a two-bottle choice drinking paradigm model that allowed animals to freely choose between alcohol and water for 15 days, researchers examined the impact of polyherbal preparations on alcohol withdrawal anxiety, sadness, and seizures. The 16th day was the withdrawal day for alcohol, during which time patients received treatment with Nutriley alquit powder (20 mg/kg) and polyherbal preparation (10 and 30 mg/kg, oral). Utilizing EPM, FST, and TST, behavioural characteristics were examined. Gallic acid, rutin, and quercetin are among the main phytoconstituents found in polyherbal preparations, according to phytochemical profiling. Polyherbal formulation has antidepressant and antianxiety benefits during alcohol withdrawal, according to in vivo investigations. The study comes to the conclusion that ethanol-type dependence may be treated therapeutically with polyherbal preparations.

**Keywords:** Polyherbal preparation, Alcohol withdrawal, Anxiety, Depression, Seizure

1. **Introduction**

Research on addiction has been conducted widely in a number of disciplines, including psychology, neurology, and medicine. Addiction is a complicated and diverse problem. The obsessive drug-seeking behaviour and continued use of drugs despite the negative effects are characteristics of addiction, according to the National Institute on Drug Abuse (Fattore et al., 2013). According to Lubman et al. (2004), it is frequently linked to increased tolerance, physical and psychological dependence, and withdrawal symptoms when the substance is used less or not at all. Studies have indicated that the combination of biological, psychological, and environmental elements leads to addiction. It is well recognized that genetic factors play a role in the development of addiction. According to studies, genes that control dopamine receptors and neurotransmitter systems are important in the development of addiction (Blum et al.,2015). According to Oesterle et al. (2019), medication-assisted therapy entails using drugs like methadone, buprenorphine, or naltrexone to control cravings and withdrawal symptoms. One of the biggest issues facing the world today is alcohol addiction, which affects people's personal and economic well-being. According to McLellan et al. (2000), alcoholism is a chronic medical illness that goes through phases of recovery and relapse. Similar to other substance addictions, alcohol addiction involves the participation of multiple neurotransmitter systems, including glutamate, opioid peptides, dopamine, serotonin, and γ-aminobutyric acid (GABA), in the search and maintenance of alcohol consumption (Kandel, 2002). By means of inhibitory and excitatory neurotransmitters, the brain preserves neurochemical equilibrium. GABA operates via the GABA-α (GABA-A) neuroreceptor and is the primary inhibitory neurotransmitter. Through the N-methyl-D-aspartate (NMDA) neuroreceptor, glutamate functions as one of the main excitatory neurotransmitters. Reduced overall brain excitability is the outcome of alcohol's enhanced GABA action on GABA-A neuroreceptors. An increase in tolerance to the effects of alcohol is a sign of a compensatory decrease in the GABA-A neuroreceptor response to GABA associated with chronic alcohol consumption. Adverse effects of alcohol include the inhibition of NMDA neuroreceptors and the up-regulation of these receptors after prolonged alcohol consumption. Alcohol-inhibited receptors become uninhibited during abrupt withdrawal from alcohol exposure, leading to hyperexcitability in the brain (Ungur et al., 2020). When someone who has been drinking alcohol chronically and for an extended period of time suddenly cuts back on or stops drinking, they may experience a range of severe symptoms including discomfort or impairment in their ability to operate on a daily basis. This condition is known as alcohol withdrawal syndrome (AWS). The Gupta group (2021). A person may have symptoms six to twenty-four hours after their last alcohol consumption, including anxiety, depression, and seizure-like behaviours. The areas in charge of managing elevated negative response include the hippocampus, amygdala, hypothalamus, and prefrontal cortex. Nevertheless, according to Sharma et al. (2021) the amygdala and hypothalamus are the primary regulatory organs for anxiety, sadness, and seizures. Using alcohol causes biochemical and behavioural changes that are blocked by GABA (γ-aminobutyric acid) inhibitory receptor networks. Reduced GABAα neuroreceptor response is the outcome of ethanol dependence, and this controls reinforcement, reward, resistance, dependence, and withdrawal from ethanol use. The key drug for achieving long-term abstinence from alcohol and alcohol withdrawal symptoms, such as anxiety, sadness, and seizure-like behavior, is GABAergic frameworks (Basavarajappa & Hungund, 2005).

Currently on the market, benzodiazepines are the class of medications used to treat alcohol abstinence symptoms. But it has an addictive quality of its own. Accordingly, stopping the usage of benzodiazepines can result in withdrawal symptoms such as nausea, sleeplessness, sadness, anxiety, seizures, and so on (Airagnes et al., 2019). In order to cure these symptoms, the patient must keep taking the drug. A variety of plants with various pharmacological properties can be found in the foothills of the Himalayas. In the global delivery of healthcare, traditional treatments play a significant role.   
Plants and plant extracts have been used for decades to cure a wide range of illnesses by people all over the world. In addition to being quite safe, these treatments are environmentally benign. As a major component of their health care system, 80 percent of people in industrialized and developing nations treat various ailments with natural remedies, according to the WHO. Due to their ease of use, ease of dose, and convenience, polyherbal preparations have gained popularity over raw plant extracts and are now frequently utilized to treat a wide range of illnesses. Certain synthetic medications target a particular biochemical route and merely relieve symptoms; in contrast, polyherbal formulations with several targets can be helpful for long-term diseases. Notably, the synergistic effects of several herbs contribute to the greater effectiveness of herbal remedies (Modak et al., 2007).

In the traditional medical system, herbs including Cymbopogon citratus, Cinnamomum tamala, and Urtica dioica are utilized to treat various neurological conditions. Anxiety, sadness, seizures, and other conditions have all been treated with it. Having pharmacological properties that include antidepressant, seizure, antibacterial, antifungal, anti-inflammatory, and antioxidant properties, Cymbopogon citratus belongs to the Poaceae family. Costa et al. (2011) explored this further. Family Lauraceae: Cinnamomum tamala is said to offer anti-anxiety properties, diabetes, cancer, stomach issues, and pain (Rincon et al., 2019). Many pharmacological actions, such as those related to seizures, cardiac arrest, eczema, arthritis, gout, and anemia, are thought to be present in the Urtica dioica family (Urticaceae) (Loshali et al., 2021).

* 1. **Rationale of study**
* The effect of alcohol withdrawal symptoms and their treatment are the main areas of investigation for this study. Significantly harmful side effects are linked to the synthetic therapy formulations now on the market. Herbal medicines are therefore always an improved and secure option.
* Individual studies on the three herbal plants—Cymbopogon citrates, Cinnamomum Tamala, and Urtica dioica—have revealed anti-depressive, anti-anxiety, and antiepileptic effects.
* It is currently uncertain, nevertheless, how well the three medications mentioned above will work in managing alcohol abstinence. By focusing on different brain mechanistic locations, this study seeks to assess the pharmacological efficacy of the polyherbal formulation.
* Using Himalayan plants, a herbal remedy for alcohol abstinence syndrome will be created as part of this research. In this project, the extracted phytoconstituents that have been linked to alcohol withdrawal activity in literature are to be prepared into a polyherbal formulation.

**1.2 Aim and Objectives**

* To construct a herbal remedy using Himalayan herbs to address alcohol withdrawal syndrome.
  1. **Objectives**
* The manufacture and standardization of polyherbal medicine.
* Rats were given alcohol to become addicted to using the two-bottle paradigm.
* The polyherbal preparation's in-vivo pharmacological effects on rats should be assessed.

**Review of literature**

The use of alcohol as a drug and as an indulgence have long been intertwined with human society. Globally, there has been a notable rise in morbidity and mortality rates due to an increase in alcohol intake. Different definitions vary when it comes to chronic alcohol use. It has been determined that heavy alcohol use and binge drinking on five or more days per month are defined as daily consumption in the range of four or more drinks for males and three or more for women. World Health Organization (WHO) data from 2018 states that alcohol use causes about three million deaths worldwide annually. Among those between the ages of 20 and 40, this represents about 14% of the total death rate (WHO, 2018). The stomach and intestines allow the body to absorb alcohol that has been consumed. Perspiration, urine, and breath only remove a little percentage of it—less than 10%. This suggests that more than 90% of the alcohol that is ingested makes its way to the liver through the portal vein and circulates throughout the body. The liver is important for alcohol metabolism because it contains a large amount of the enzymes that break down alcohol (Cederbaum et al., 2012). Alcohol is processed in the liver by both oxidative and non-oxidative mechanisms (Lieber et al., 2005). The two-step oxidative process is the main pathway involved in the metabolism of alcohol. Alcohol dehydrogenase (ADH), the main enzyme responsible for converting alcohol to acetaldehyde, first oxidizes alcohol to that form. (Kono et al., 2000). Contrary to ADH, cytochrome P450 2E1 (CYP2E1) is expressed and activated more when alcohol consumption is excessive. Through the generation of reactive oxygen species (ROS), activated CYP2E1 facilitates the synthesis of acetaldehyde (Baraona et al., 2001). Furthermore, peroxisomal catalase converts alcohol to acetaldehyde; nevertheless, due to its insignificant role in the breakdown of alcohol, this pathway is regarded as minimal. Acetate is produced quickly by aldehyde dehydrogenase (ALDH) in the second step of the oxidative route from acetaldehyde. Borodone et al. (2005) reported that acetate is metabolized not in the liver but rather in peripheral tissues, where it is converted into carbon dioxide (CO2), fatty acids (FAs), and water (H2O) Quantitatively speaking, the nonoxidative route only contributes a little amount to alcohol metabolism (Heier et al., 2016). A tiny quantity of alcohol is nonoxidative converted by a variety of enzymes to a range of endogenous metabolites. For instance, fatty acid ethyl ester (FAEE) is created by the enzymatic esterification of alcohol with FAs, and phosphatidylcholine is transphosphatidylated with ethanol by phospholipase D (PLD) to create phosphatidyl ethanol (PEth). Moreover, alcohol conjugated to sulphate and glucuronic acid yields ethyl sulphate (EtS) and ethyl glucuronide (EtG), respectively (Heier et al., 2016).

**Figure 1**

**Diagram showing how the liver processes alcohol.Alcohol is processed in the liver by both oxidative and non-oxidative pathways. Alcohol is converted to acetaldehyde by a number of enzymes in the oxidative pathway, which is the main route of alcohol digestion. These enzymes include catalase, cytochrome P450 2E1 (CYP2E1), and alcohol dehydrogenase (ADH). Acetate is then produced from acetaldehyde and eliminated by the liver. Particularly after drinking too much alcohol, CYP2E1 is triggered, which encourages the production of reactive oxygen species (ROS). A tiny portion of alcohol metabolism is explained by the non-oxidative route. Ethyl glucuronide (EtG), ethyl sulphate (EtS), phosphatidyl ethanol (PEth), and fatty acid ethyl ester (FAEE) are the products of several enzymes' non-oxidative conjugation of alcohol with various endogenous metabolites.** **The byproducts produced during the breakdown of alcohol damage the liver by causing an increase in fibrosis, inflammation, and fat buildup. Particularly well-known for its harmful properties is acetaldehyde, which is the first metabolite of alcohol metabolism. One of the main causes of liver injury is thought to be ROS, which are produced when CYP2E1 is activated. Furthermore, it is known that non-oxidative metabolites and acetate harm the liver.)**

ALD progresses from alcoholic steatosis to alcoholic cirrhosis due to products produced during alcohol metabolism that harm the liver (Seitz et al., 2018). According to Yano et al. (2021) acetaldehyde is the most well-known hazardous substance created during the metabolism of alcohol. Point mutations and chromosomal damage are brought on by acetaldehyde's direct interaction with DNA. Moreover, it disrupts liver function and structure by binding to a range of proteins to generate acetaldehyde adducts (Han et al., 2021). Adducts of this protein increase oxidative stress and upregulate the expression of CYP2E1. further showed that protein adducts have a major role in the pathophysiology of different phases of ALD by promoting lipid buildup, inflammation, and fibrosis. Though less hazardous than acetaldehyde, acetate has been shown to circulate in the bloodstream and enhance portal blood flow. Alcohol toxicity is also known to be caused by nonoxidative pathway-derived metabolites, such as PEth and FAEE, although the exact processes are yet unknown (Lu et al., 2009). The presence of the co-factor nicotinamide adenine dinucleotide (NAD+) controls the rate of both conversions in both scenarios. Jones (2010) highlights the microsomal ethanol oxidizing system (MEOS) as an additional avenue for alcohol breakdown, particularly in individuals who drink alcohol on a regular basis or in those who have consumed substantial amounts of alcohol.   
The co-factor needed for this process, which is catalysed by CYP2E1, is nicotinamide adenine dinucleotide phosphate (NADP+), not NAD+, which is what turns ethanol into acetaldehyde. A third, somewhat small route uses the catalase activity in liver peroxisomes to reduce hydrogen peroxide to water by using ethanol as an electron donor.   
More than 90% of alcohol removal is carried out by these oxidative mechanisms together. Non-oxidative metabolic routes are used to process the remaining 10% of ethanol. Ethanol and acetaldehyde are the main substances studied in relation to alcohol metabolism, regardless of the mechanism.

Nonetheless, there is continuous discussion on their part in the pathophysiology of the alcohol hangover (Macoos et al., 2020). Because there is a lack of empirical evidence, it is possible that the quantities of acetaldehyde and ethanol could directly affect how bad a hangover is. Central processes are probably involved in a number of the primary hangover symptoms, such as headache, nausea, apathy, and difficulty concentrating. Although there is no doubt that systemic processes contribute to some features of alcohol hangover, the pathophysiology of alcohol hangover symptoms may ultimately depend on the brain's exposure to ethanol or its metabolites. Investigating the potential of peripheral ethanol and acetaldehyde to penetrate the brain and cause central effects, such as a hangover, is crucial in light of this.

According to Gupta et al. (2021) Alcohol Withdrawal Syndrome (AWS) is defined as the cessation of chronic and persistent alcohol consumption that results in severe feelings of discomfort or loss of daily functions when less or no alcohol is ingested. The symptoms manifest 6–24 hours following the last alcohol consumption and include anxiety, depression, and seizure-like behaviour (Sharma et al., 2021). The areas to adjust and articulate for elevated negative response are the hippocampus, amygdala, hypothalamus, and prefrontal cortex. The amygdala and hypothalamus are among the brain regions that control anxiety, depression, and seizures (Koob et al., 2003). The neurotransmitter system in the brain, which includes GABA, glutamate, non-epinephrine, and serotonin, is altered by alcohol withdrawal syndrome. The disruption in brain receptors between N methyl aspartate (NMDA) and gamma-aminobutyric acid (GABA) that occurs when alcohol consumption is stopped is the cause of these symptoms (Gupta et al., 2021). There are numerous ways in which alcohol withdrawal syndrome is mediated. Excitatory and inhibitory neurotransmitters help the brain maintain a balance of neurochemicals. GABA (gamma-aminobutyric acid) is the primary inhibitory neurotransmitter, acting via the GABAα and GABA-A neuroreceptors. Through the N-methyl-D aspartate (NMDA) neuroreceptor, glutamate is one of the main excitatory neurotransmitters. Brain excitability is reduced overall because alcohol increases the action of GABA on GABA-A neurons.

Alcoholism that lasts a long time causes a compensatory reduction in the GABA-A neuroreceptor response to GABA, as seen by an increase in alcohol tolerance. After prolonged alcohol exposure, NMDA neuroreceptors are inhibited and their expression is increased. Alcohol-inhibited receptors no longer have the ability to inhibit them, which causes an abrupt termination of alcohol exposure to produce hyperexcitability in the brain. Tremors, agitation, anxiety, and irritability are clinical signs of brain hyperexcitability. According to Ungur et al. (2020), severe signs include delirium tremens and convulsions related to alcohol withdrawal. At lower blood concentrations, ethanol causes euphoria and behavioural excitation due to increased glutamate binding to N-methyl-D-aspartate (NMDA) receptors. However, at higher concentrations, it causes acute intoxication by potentiating the effects of γ-aminobutyric acid (GABA), especially in receptors. As a central nervous system depressant, ethanol with delta subunits. The primary networks that mediate the intoxication effects of alcohol are the cerebellum, cortical areas, thalamic relay circuitry, and brainstem, which can be explained by the local distribution of these subunits (Jesse, Brathen et al. 2017). As a result of compensatory functional changes brought on by downregulation of GABA receptors and increased expression of NMDA receptors with increased glutamate production to maintain central nervous system (CNS) transmitter homeostasis, prolonged alcohol use causes tolerance and physical dependence (Harrish et al., 1998). When chronic alcohol drinking is abruptly stopped, the changes are shown by a glutamate-mediated CNS excitation that causes autonomic overactivity and neuropsychiatric problems such seizures and delirium. The latter are primarily caused by a disruption of the GABAergic delta subunits' tonic inhibitory function in the brainstem and are typically of the generalized tonic–colonic kind.   
This may help to explain why epileptiform activity is rarely seen in the EEG following alcohol withdrawal episodes. Consequently, the trigger zone for these seizures differs from the one thought to be responsible for seizures in the context of epilepsy. The therapy strategy for AWS primarily focuses on these mechanisms because the clinical symptoms are mostly explained by diminished GABA-A receptor inhibition and overexpression of NMDA receptors. Dopamine is an additional neurotransmitter implicated in phases of alcohol withdrawal. The reward system is favourably influenced by an increase in dopamine during alcohol consumption, which perpetuates abuse.

An rise in dopamine during alcohol consumption favourably affects the reward system, which perpetuates abuse. Autonomic hyperarousal and delusions are two clinical symptoms of withdrawal that are exacerbated by an increase in dopamine levels (Jesse et al., 2017). Furthermore, it appears that dopamine receptor 2 gene polymorphisms affect both the clinical presentation of alcohol withdrawal symptoms and AUD. When combined with elevated glutamate and norepinephrine, it may also lengthen the QT interval in individuals with active epilepsy; this can raise the risk of sudden unexpected death in epilepsy (SUDEP) (Rodriguez et al., 2020). Homocysteine is a further excitotoxic substance that is elevated in AUD. Active drinking raises homocysteine levels by activating NMDA receptors. Excitotoxicity after withdrawal is caused by an increase in homocysteine through glutamatergic neurotransmission rebound activation (Bannister et al., 2004).

The primary symptom of alcohol withdrawal is a lowering in the seizure threshold, which is a characteristic of the early stages of withdrawal. After stopping heavy drinking for 48 hours, more than 90% of acute symptomatic seizures occur. Frequently, seizures happen in the absence of additional AWS symptoms. Over 50% of the patients have recurrent seizures, and 5% of them may develop status epilepticus. Concurrent risk factors, such as previous epilepsy, structural brain abnormalities, or drug use, are linked to over 50% of withdrawal seizures. It is amazing that the occurrence of acute symptomatic seizures during an alcohol withdrawal episode is linked to a fourfold rise in the mortality rate, which is caused by severe AUD consequences rather than seizures themselves. Up to 30% of instances have DT emerge after the onset of a withdrawal seizure, which is a high-risk factor for the state to worsen and proceed into a severe withdrawal state. When unprovoked seizures happen more than 48 hours after the last drink, there may be additional reasons, like a head injury or the combined effects of drug withdrawal. A worldwide state of bewilderment, altered perception, and bodily symptoms resembling those of a vegetative or central neurological presentation are the hallmarks of delirium, a clinical illness with a sudden start. When someone stops drinking or even while they are still using it, they may experience a distinct type of psychosis linked to withdrawal called hallucinosis. Although the sensorium is initially evident, it frequently develops into the syndrome of DT, a particular kind of delirium that appears in the late withdrawal phase and is typically linked to psychomotor agitation (hyperactive delirium).   
In addition to reduced arousal and psychomotor activity, delirium can also present as a hypoactive condition. This latter state is linked to more difficulties down the road, a worse prognosis, and delayed identification and treatment. Complementary or additional medical conditions need to be ruled out in cases of hypoactive delirium. For patients who have never had DT before, this is particularly crucial.

The class of medications currently available on the market to treat alcohol withdrawal syndrome includes benzodiazepines, naltrexone, and gabapentin. However, benzodiazepines are inherently addictive drugs. For some uses, benzodiazepines (BZDs) can be substituted with phenobarbital, a kind of barbiturate. Barbiturates provide a different method of action from BZDs, which mainly function by increasing the frequency of GABA-A chloride channel opening, a mechanism that depends on GABA's presence in the presynaptic area. Both glutamate (which results in decreased activity via AMPA and kainate receptors) and GABA (which prolongs the opening of GABA-A channels through a unique binding site not shared by alcohol and BZDs) are engaged by barbiturates. Given that phenobarbital is less likely to cause cross-tolerance with alcohol than the majority of benzodiazepine drugs, it is thought that these variations in binding characteristics and receptor affinity make a difference. People who attempt to discontinue using this medication have benzodiazepine withdrawal syndrome, which includes symptoms like nausea, sleeplessness, anxiety, sadness, and seizures, among others (Airagnes et al., 2019). By binding to voltage-sensitive calcium channels' α2δ-1 site and altering receptor mobility and activity, gabapentin has unique pharmacological characteristics. Through these processes, gabapentin is thought to tangentially alter GABA and glutamate levels and activity, which are clinically measurable. In addition to its proven ability to treat acute alcohol withdrawal symptoms, gabapentin has showed promise in preventing short-term relapses during medically assisted alcohol detoxification. This could be explained by its possible effect on establishing regular sleep schedules. Analysis carried out after the fact has shown that gabapentin can be helpful in patients with ongoing or past alcohol withdrawal symptoms when combined with flumazenil or naltrexone. These results are consistent with findings from fundamental scientific research. While recent randomized clinical trials on gabapentin have produced conflicting results without focusing specifically on alcohol withdrawal symptoms, more research is necessary because of gabapentin's previously indicated efficacy in this population, its minimal cognitive effects, its lack of significant negative interactions with alcohol, and the fact that it is excreted by the kidneys, which may make it safer for people with liver disease.

The Himalayan Mountain range is home to a wide variety of plants, each with unique pharmacological properties. All around the world, traditional medicines form an essential part of healthcare systems. People all throughout the world have been using plants and plant extracts for decades to cure a variety of health conditions. These medications are safe for the environment and very safe. Eighty percent of people in both developing and wealthy nations receive their primary medical treatment from traditional medicine, according to the World Health Organization. Because of their ease of use, ease of dosing, and simplicity of administration, polyherbal preparations have gained widespread attention compared to raw plant extracts and are widely used to treat a variety of illnesses. Although polyherbal preparations' multitarget activity is beneficial in chronic illnesses, a significant majority of synthetic drugs provide symptomatic relief by targeting a single molecular target. The synergistic effect of many herbs has led to the appreciation of herbal remedies for their added viability (Modak et al., 2007). A number of neurological disorders are treated with Cymbopogon citratus, Cinnamomum tamala, and Urtica dioica in traditional medicine. Anxiety, sadness, seizures, and other conditions have all been treated with it. Having pharmacological properties that include antidepressant, seizure, antibacterial, antifungal, anti-inflammatory, and antioxidant properties, Cymbopogon citratus belongs to the Poaceae family. Costa et al. (2011) reported these results. According to reports, Cinnamomum tamala, family Lauraceae, has antianxiety, diabetes, cancer, stomach issues, and pain (Rincon et al., 2019). According to Loshali et al. (2021) the Urtica dioica family, Urticaceae, is known to have a variety of pharmacological actions, including those related to seizures, cardiac arrest, dermatitis, arthritis, gout, and anemia.

**2.1 Plant description**

The common name for Cymbopogon citratus is lemon grass. Bay leaf is the common name for Cinnamomum tamala. Moreover, stinging nettle is another name for Urtica dioica. These herbs are perennials that grow in Asia's tropical regions. Plant growth is at its peak during the wet season. The leaves are short triangular, ovate, elliptic, or oblong, and have a vivid green color. The following generation emerges as seeds reach maturity and fall to the ground. So these herbs continue to grow and reproduce.

**2.2 Pharmacogenetic features**

Taxonomical Classification of Cymbopogon citratus

Domain: Plantae

Clade: Monocots

Phylum: Spermatophyta

Subphylum: Angiospermae

Family: Poaceae

Sub-family: Panicoideae

Class: Monocotyledonae

Order: Cymbopogon

**Taxonomical Classification of Cinnamomum tamala**

Domain: Plantae Clade: Dicot Phylum: Tracheophyta Subphylum: Angiospermae Family: Lauraceae Sub-family: Magnoliids Class: Tracheophytes Order: Cinnamomum

**Taxonomical Classification of Urtica dioica**

Domain: Plantae Clade: Eudicots Phylum: Tracheophyta Subphylum: Angiospermae Family: Urticaceae Sub-family: Rosids Class: Tracheophytes Order: Urtica.

**2.3 Phytoconstituents and uses**

**Table 2.1 Description of the plants.**

**3. Research Methodology**

**3.1 Sample**

Recently harvested Cymbopogon citratus from Shoolini University's nursery in Solan (H. P.).   
tamala cinnamon gathered in the Uttarakhand village of Riknikhal. Solan (H. P.): Urtica dioica, harvested from the nearby woodland at Oachghat. Qualified taxonomists from the Forest Products department at the Botanical Survey of India, Nauni, Solan (H. P.) verified the botanical identity of these three plants, which were then stored in an institutional UHF herbarium with voucher specimen numbers 704, 705, and 706.

**3.2 Collection and extraction of plant material**

These three plants' fresh leaves were just picked. Three plants were harvested and their fresh material rinsed, shade-dried, and chopped into little pieces. The tiny fragments of distinct plant material were added to the soxhlation assembly together with ethanol. employing a heating mantle to achieve the right temperature. Plant material was allowed to air dry after extraction. After that, a hot air oven was used to eliminate the acquired solvent. According to Sharma et al. (2021), the semisolid material that was left behind after the solvent evaporated was lyophilized again to create a dry powder, which was then stored at 2–8 °C per person for later use. phytocompound extractions with HPTLC characterization.

**3.2.1 Drying of sample**

The sample was taken and allowed to dry for ten to fifteen days on a slab without direct sunshine. To preserve heat-sensitive phytochemicals, drying was done away from the sun.

**3.2.2 Grinding and storage**

Samples that had been shade dried were pulverized in a grinder that had stainless steel blades that allowed the material to be passed through a 40-mesh screen. Samples of plants were ground and then placed within a polythene bag, which was then properly labelled and sealed.

**3.2.3 Soxhlet Extraction**

Rich bioactive chemicals can be extracted from a wide range of natural sources using the Soxhlet extraction method. To perform this extraction, a distillation flask holding the specific solvent of interest is filled with a thimble containing a little amount of dry material. When the solution in the thimble-holder reaches an overflow level, it is sucked by a siphon, which returns the solution to the distillation flask without overloading it. In the bulk liquid, this solution transports the extracted solutes. After passing back to the solid bed of samples, the solvent leaves the solute behind in the distillation flask. According to Abubakar et al. (2020), the procedure is repeated till it is finished.   
Weighing balance: Each crushed Cymbopogon citratus, Cinnamomum tamala, and Urtica dioica were measured individually.

• After that, one thimble containing 100 grams of powdered material was placed inside another, and both were stored in the Soxhlet chamber.   
• Utilizing the Soxhlet device, 90% v/v ethanol was extracted from the plant materials.   
Using a distillation procedure, the solvent was eliminated.   
To eliminate any remaining solvent extraction, the extract was kept in a desiccator.

• The Soxhlet equipment was used to extract the plant components using 90% v/v ethanol.   
The solvent was removed during the distillation process.   
• The extract was kept in a desiccator to eliminate any last traces of solvent extraction.

**3.3 Profiling of plant extractions**

**3.3.1 Phytochemical screening**: According to conventional procedures, phytochemical analyses of each plant extract were performed (Tiwari et al., 2011).

**1. Detection of Alkaloids:** Each plant extract was separately dissolved in dil. then filtered through hydrochloric acid.

**a) Mayer’s Test:** Mayer's reagent (potassium mercuric iodide) was used to dissolve the filtrates. The presence of alkaloids is indicated by the formation of a yellow precipitate.

**b) Wagner’s Test:** Filtrates were dissolved in Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

**c) Dragendorff’s Test:** The precipitates were dissolved in a solution of Potassium Bismuth Iodide, known as Dragonwort’s reagent. Alkaloids are present when red precipitate forms.   
d) Hager's Test: Filters were dissolved in a solution of saturated picric acid, which is Hager's reagent. The production of a yellow-coloured precipitate indicated the presence of alkaloids.

**2. Detection of Carbohydrates:** Five millilitres of distilled water were used to dissolve each plant extract, which was then filtered. The presence of carbohydrates was determined using the filtrates.

**a) Molisch’s Test:** In a test tube, filters were subjected to two drops of an alcoholic α-naphthol solution. Carbohydrates are present when the violet ring forms at the intersection.

**b) Benedict’s Test:** Benedict's reagent treatment and gentle heating were applied to the filtrates. Carbs are present when an orange-red precipitate is seen.

**c) Fehling’s Test:** The filtrates underwent hydrolysis using dilute hydrochloric acid, alkali neutralization, and heating using Fehling's A and B solutions. The occurrence of red precipitate signifies the existence of carbs.

**3. Detection of Glycosides:** Glycosides were tested after plant extracts were hydrolysed with dilute hydrochloric acid.

**a) Modified Bontrager’s Test:** For roughly five minutes, plant extracts were cooked in a solution containing ferric chloride. After cooling the mixture, equal parts of benzene were added for extraction. Ammonia solution was used to separate and treat the benzene layer. There are glycosides present when a rose-pink colour forms.

**b) Legal’s Test:** Sodium nitroprusside with pyridine and sodium hydroxide was used to treat plant extracts. When cardiac glycosides form, a pink to blood red colour is present.

**5. Detection of Saponin**

**a) Froth Test:** For 15 minutes, plant extracts were shaken in a graduated cylinder after being diluted with 20ml of distilled water. Saponins can be detected by the formation of a 1 cm layer of foam.

**b) Foam Test:** 2 millilitres of water were mixed with 0.5 grams of plant extract. The presence of saponins is indicated if the foam generated lasts for 10 minutes**.**

**6. Detection of phytosterols**

**a) Salkowski’s Test:** Chloroform was used to treat plant extracts, which were then filtered. A few drops of concentrated sulfuric acid were added to the filtrates, which were then agitated and left to stand. Triterpenes are present when they have a golden yellow appearance.

**b) Liebermann Burchard’s test:** Chloroform was used to treat plant extracts, which were then filtered. A few drops of acetic anhydride were added to the filtrates, which were then heated and chilled. Cons: Sulfuric acid was included. Phytosterols are present when a brown ring forms at the junction.

**7. Detection of Phenolics**

**Ferric Chloride Test:** Three to four drops of a ferric chloride solution were applied to plant extracts. The appearance of bluish black color signifies the existence of phenols.

**8. Detection of Tannins**

**Gelatine Test:** One percent gelatine solution with sodium chloride was added to the plant extract. Tannin presence is shown by the formation of white precipitate.

**9. Detection of Flavonoids**

**a) Alkaline Reagent Test:** A small amount of sodium hydroxide solution was added to plant extracts for treatment. The presence of flavonoids is indicated by the formation of a bright yellow colour that turns colourless when diluted acid is added.

**b) Lead acetate Test:** A small amount of lead acetate solution was added to plant extracts.   
The presence of flavonoids is shown by the formation of a yellow precipitate.

**10. Detection of Proteins and amino acids**

**a) Xanthoproteic Test:** A small amount of concentrated nitric acid was added to the plant extracts. The development of a yellow hue signifies the existence of proteins.

**b) Ninhydrin Test:** After adding 0.25% w/v ninhydrin reagent to the plant extract, it was brought to a boil for a short while. The appearance of blue colour signifies the existence of an amino acid.

**11. Detection of Terpenoids**

A small amount of strong sulfuric acid and 0.5 millilitre of chloroform were added to the plant extract. When a reddish-brown precipitate forms, terpenoids are present.

**3.3.2 HPTLC instrumentation and experimental conditions**

Chromatographic separation was accomplished using aluminum sheet support and pre-coated TLC plates with 0.2 mm thick silica gel 60 F254 (E. Merck). The CAMAG Linomat 5 automatic sample applicator (CAMAG Muttenz, Switzerland) with a Hamilton syringe was used to spot samples. The next step involved developing the plates in a twin trough chamber (CAMAG) and scanning them using the Camag TLC Scanner in conjunction with the winCATS planar chromatography manager software. The samples were spotted as bands with a 1.0 mm band width and a 6.0 mm band length. In a glass chamber with twin troughs (CAMAG, Switzerland) saturated with the appropriate mobile phase, the plates were produced in a vertical rising manner. A whole 85% of the TLC plate height was covered by the obtained chromatogram. TLC plates were dried in an oven at 60°C for 5 minutes following development to ensure that the mobile phase was completely removed.   
By using multi wavelength scanning, the wavelengths used for fingerprinting were chosen. A wavelength was chosen based on how many peaks it displayed. As part of the quality control process for crude medicines, standards were quantified in various extracts. The densitometric quantification is then carried out in UV mode at various wavelengths with the use of deuterium and tungsten lamps operating as radiation sources in reflectance mode. Peak numbers along with their area and height, peak display, and peak dendrogram were noted.

**3.3.3 Selection of standards**

Plant extracts will be standardized by choosing compounds that have been reported from the plant.

**3.3.4 Preparation of polyherbal formulation**

The preparation of a polyherbal formulation involved combining one-third of the maximum dose of each individual plant, following an initial screening process that was both qualitative and quantitative for each plant extract. A single plant's literature was examined to determine the highest dose. There was mixture among the plants.

**3.4 Animals & housing**

Animal House, Shoolini University, Solan, HP, is home to Wistar rats of both sexes that were purchased from the NIPER in Punjab, India. They were adjusted to 23 ± 2 °C temperature, 12:12 h light and dark cycle, and laboratory conditions. Water was provided freely to the animals along with a diet of nutritious pellets. The protocol was fully approved (IAEC/SU/22/20) by IAEC, Shoolini University, Solan, H.P., India. The study was carried out adhering strictly to the globally recognized rules for the use and care of laboratory animals as well as the requirements set by the Committee for the Purpose of Control and Supervision of Experiments on Animals, which align with the ARRIVE recommendations for animal research.

**3.5 Ethanol withdrawal study**

**3.5.1 Experimental design**

The animals were kept in separate housing after being randomly split into 5 groups (n = 3). The disease control group was given ethanol intake at their discretion for 15 days and was given a 0.5% carboxymethyl cellulose (CMC) vehicle during the days when they were not allowed to consume ethanol (16th, 17th, and 18th day). The normal control group was given a liquid diet. For fifteen days, Groups 1 and 2 underwent alcohol therapy; during the ethanol withdrawal period, they were administered Polyherbal formulation -3 (10 and 30 mg/kg) once day, respectively (Gupta G et al. 2021). Group 3 underwent a 15-day alcohol treatment program, and throughout the ethanol withdrawal phase, Nutriley Alquit powder (20 mg/kg) was given once daily. The animals received alcohol treatments in accordance with what was previously studied. A two-bottle choice paradigm (water vs. ethanol) was used to allow the alcohol-fed animals to freely consume 4.5% v/v ethanol on the first day, 7.5% v/v ethanol on the second day, and 9% v/v ethanol from the third day to the fifteenth day. Alcohol was stopped on the sixteenth day, and the animals in the alcohol-fed group were given a liquid diet instead of alcohol, while the animals in the control group were fed the same meal. Previous research has shown that the third day of withdrawal, or the eighteenth day, is when the highest levels of anxiety, despair, and seizures were seen. As such, the behaviour parameters analysis was only applied to the study's animals on the eighteenth day. Animals underwent individual testing on the elevated plus-maze, light-dark test, tail suspension test, and force swim test, one hour following the last dosage of the medication therapy. Following behavioural experiments, blood was extracted from the rats' retroorbital vein, and serum was separated for analysis of biochemical markers (Chiu et al. 2007). Throughout the trial, the animals' body weight change and ethanol intake were measured every third day and reported as g/kg.

**3.5.2 Behavioural tests**

**3.5.2.1 Elevated plus maze (EPM)**

Rats' anxiolytic reactions are frequently studied using the EPM test. Rats dislike open, high spaces and would much rather dwell in an enclosed arm. When a rat encounters an open arm, they react with anxiety, frolicking in place. The model is elevated to a height of 50 cm and is made up of two open arms crossed by two closed arms on a central platform. With its head pointing toward the open arm, the rat was placed individually in the centre compartment. The following criteria were assessed: (a) Time spent and number of entries in the open arms; (b) Time spent and number of entries to be counted in the closed arms will be recorded for five minutes.

**3.5.2.2 Tail Suspension Test**

Rats are made immobile by hanging from their tails and having an adhesive tape put at the location, which is 1/4 of the way from the base of the tail. The animal initially makes frantic attempts to break free, but it is unable to do so and falls motionless.   
According to Dudhgaonkar et al. (2014), the remaining four minutes of the six-minute session were spent immobile.

**3.5.2.3 Forced Swimming Tes**

The adult rats were kept with no way out of a cylinder that measured 50 cm in height, 20 cm in circumference, 35 cm in water depth, and 23–25 °C in temperature. Following their initial period of struggle, the rats become motionless. The final 4 minutes of the 6-minute period will be spent monitoring immobility. According to Yankelevitch et al. (2015), the water in the cylinder was changed after every attempt.

**3.5.3 Statistical analysis**

Utilizing GraphPad Prism software 9.0, the data was statistically examined and represented as the mean ± standard error of mean (SEM). Dunnett's multiple comparison post hoc test was employed to analyze the data, with a confidence level of p < 0.05, after two-way ANOVA.

**4. Results**

**4.1 Qualitative screening of phytochemicals**

Alkaloids, carbohydrates, phytosterols, saponins, and phenolics were found in the methanolic extracts of all the plants, according to a preliminary screening of several plant extracts.   
The ethanolic extract of particular plants was subjected to qualitative phytochemical screening, as shown in Table 4.2.

**4.2 HPTLC quantification of major constituents present in formulation**

Following an HPTLC analysis of the formulation, a final check was made by comparing the spots in the formulation with the presumed markers. The components in our herbal ethanolic extract matched the markers' Rf value. Major chemicals were found in the formulation, according to the HPTLC research. A recognized technique was followed in order to quantify the three discovered chemicals. Sharp peaks with an Rf value of 0.38 for quercetin were observed in the mobile phase, which was composed of toluene, ethyl acetate, and formic acid (5:4:0.2, v/v).   
The amount of quercetin in the formulation was 1.50% weight percentage. Rutin's Rf value was 0.68 in the mobile phase, which included N-butanol, acetic acid, and distilled water in a 4:1:1:5 ratio. There was 0.86% w/w of rutin in the formulation. The mobile phase, which is composed of ethyl acetate and toluene, Gallic acid has an Rf value of 0.58 and formic acid, Methanol is 5.5:3:1:0.5. There was 0.33% w/w of gallic acid in the formulation.

**4.3 Ethanol consumption and body weight changes of the animals**

Each animal's daily ethanol intake was measured for 15 days in ethanol-fed groups, and the results were expressed as grams per kilogram per day. The daily consumption of ethanol in the groups administered ethanol ranged from 13.57 ± 1.85 to 17.12 ± 1.34 g/kg while they were exposed to 9% ethanol. There was no discernible variation in the amount of ethanol consumed by the groups who were fed ethanol. By the end of the research, there had been no discernible change in the animals' initial body weight in the control group or the ethanol-fed animals.

**4.4 Effect of test sample on ethanol withdrawal anxiety-like behaviour in the EPM test**

When compared to the normal control group, the EPM ethanol-fed animals showed a substantial decrease (p < 0.001) in both the amount of time spent and the number of entries into the open arms throughout testing. When the ethanol-fed animals were compared to the animals in the usual group, there was also a substantial increase (p < 0.001) in the amount of time and entrances into the closed arms. These results demonstrated the emergence of anxiety in animals experiencing ethanol withdrawal. In comparison to the disease control rats, treatment with test sample (10, 30 mg/kg, oral) and Alquit powder (20 mg/kg) for three days in a row resulted in a significant (p < 0.001) increase in the amount of time spent and the number of entries into the open arms, as well as a significant (p < 0.001) decrease in the amount of time spent and the number of entries into the closed arms.

**4.5 Effect of test sample on ethanol withdrawal depression- like behaviour in the FST test**

An increase in immobility time (p<0.001) was observed in ethanol-fed rats when they underwent FST testing, as compared to normal animals. In comparison to the normal and drug-treated groups, the sick group's escape latency time was found to be shorter. Comparing either group to the control group, there is no discernible decrease in the length of escape delay.   
Comparing the drug-treated and control groups, the diseased group's rats showed more immobility.

**4.6 Effect of test sample on ethanol withdrawal depression- like behaviour in the TST test**

The TST test reveals that, in contrast to the drug-treated and normal groups, the animals in the sick group exhibit immobility. More than both normal and ill animals, the drug-treated animals exhibit escape delay. Among the groups, there are no notable differences. The test drug-treated group 2 animals exhibit escape delay that is roughly similar to the normal group.

**Figure 14:(a) EPM percentage time open arm (b) EPM closed Arm. Values are expressed as Mean ±SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \*\*\*\*= p**

**Figure 15: (a) FST Immobility time (b) FST Escape Latency. Values are expressed as Mean ±SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \*\*\*\*= p**

**Figure 16: (a) TST Immobility (b) TST Escape Latency. Values are expressed as Mean ±SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \*\*\*\*= p**

**5. Discussion**

My research project, "Preparation and Pharmacological Evaluation of Herbal Formulation from Himalayan Plants for the Treatment of Alcohol Abstinence Syndrome," focuses on the critical and challenging topic of managing alcohol abstinence syndrome in the public health domain. Since addiction and alcoholism have a detrimental impact on one's physical and emotional well-being, novel and non-traditional treatment approaches are needed. The medicinal potential of traditional Himalayan medicinal herbs for treating alcohol withdrawal disorders is examined in this study. Throughout the research procedure, a comprehensive strategy was used to investigate the herbal formulation. The historical use of Himalayan herbs in traditional medicine and their alleged effects on the nervous system were the primary factors in the selection process. Strict extraction techniques were applied throughout the production of the herbal blend to guarantee the preservation of bioactive components. Using in-vivo studies, the study assessed the formulation's antioxidant and neuroprotective properties—two crucial elements in the context of alcohol-induced neurodegeneration.

Plants are the basis for modern pharmaceutical innovations as well as traditional medical treatments. Two basic principles govern the creation of therapeutic therapies in Ayurveda: either a single plant-based medicine is used, or many plants are combined to create what are known as polyherbal preparations or herb-herb combinations (Karole et al. 2019). This study looked at the preparation's pharmacological benefits. It contains extracts from three different plants: Cymbopogon citratus, Cinnamomum tamala, and Urtica dioca. The formulation's potential benefits in reducing anxiety, sadness, and seizure-like behaviour linked to ethanol withdrawal were the main focus of the study.

The most potent plant dosages were chosen from the literature for the polyherbal preparation, which was made by combining all of the plant extracts in a 1:1:1 ratio, including Cymbopogon citratus, Cinnamomum tamala, and Urtica dioca. It is well recognized that a broad variety of chemical ingredients can be found in polyherbal preparations that contain plant extracts from different plants. We collected every marker and carried out an HPTLC examination in order to aid in additional identification. Three notable compounds were identified by HPTLC examination of the preparation; the other compounds were not detected. Therefore, quercetin, rutin, and gallic acid make up the phytochemical components. The ethanol administration in a liquid diet or the two-bottle choice drinking paradigm model is the best suitable model for studying ethanol withdrawal syndrome in animals. The ability of the animals to freely ingest either ethanol or water in the latter paradigm to replicate the human condition has been demonstrated to be therapeutically relevant. According to earlier research, rats that consume more than 9 g/kg of ethanol every day for 15 days straight develop dependence and abstinence from alcohol. Our study (Sharma et al., 2018) used the two-bottle choice drinking paradigm model. The weight of the rats' bodies did not significantly change.   
The mice showed evidence of ethanol dependence on day fifteen. The effect of the formulation on rats experiencing anxiety, sadness, and convulsions due to ethanol withdrawal was next investigated in further detail. The presence of anxiety, sadness, and seizures can be a significant deterrent and may affect an individual's capacity to derive the same degree of satisfaction from alcohol consumption. Abstaining from alcohol causes adaptive changes in the hippocampus and amygdala, two areas of the brain linked to changes in hormones, neurotransmitters, and neuropeptides. In the current study, on the third day after ethanol withdrawal, a noteworthy degree of anxiety and despair was noted. In order to assess the anti-anxiety and anti-depression effect following ethanol cessation, we investigated the effects of Nutriley alquit powder.

The efficacious tests EPM, FST, and TST are employed to investigate the antidepressant and antianxiety properties of medications. The most used tool for measuring exploration is the exploratory preference model (EPM). Rats show an approach-avoidance conflict, with a greater aversion to open, exposed locations and a preference for sheltered spaces.   
As a result, they typically occupy the enclosed arm of a testing device for longer. The animal shows symptoms of dread and freezes when it travels into the open arm (Smith MF, 1997).   
This behaviour impacts their motor behaviour and is suggestive of anxiolytic reactions. The amount of time spent and the frequency of admissions into open arms can both be increased by anxiety-relieving medications. Compared to the animals in the normal control group, the illness control group (those going through ethanol withdrawal) showed less exploration time in the open arms and greater time in the closed arms during the current investigation. The rats that were administered alcohol spent more time in the open arm and made more entries during the course of the next three days when treated with the test drug (10, 30 mg/kg) and reference dose (20 mg/kg). The rats exhibit anxiolytic effects as a result. The best models to investigate antidepressant activity are FST and TST. The rodents' degree of antidepressant medication is indicated by their mobility time or escape latency. There is more antidepressant activity the more mobility time there is. Comparing the sick group to the group treated with 30 mg/kg of the test medication, the latter exhibits greater stealth. Between the regular dose and the 30 mg/kg test drug, there is a noticeable difference. This illustrates the antidepressant benefits. A decrease in the GABAA receptors' ability to suppress excitatory activity during ethanol withdrawal causes anxiety and depression and increases CNS hyperexcitability (Cageti et al., 2003). Alcohol withdrawal was found to create anxiety and melancholy, and even normal levels of stimulation might lead to over-excitation because the central nervous system is no longer suppressed. Ayurveda states that when drugs are combined, their potentiation of response even at low doses is demonstrated, which is why developing a polyherbal preparation is necessary. The individual constituents in the formulation have been previously reported for their antianxiety, antidepressive, and antiepileptic activity at high doses. The antianxiety effect of Cymbopogon citratus. The antidepressant properties of cinnamonum tamala. There are antiepileptic properties to urtica dioca. Therefore, we can address the three main ethanol withdrawal activities at once when we combine the components of these plants in a 1:1:1 ratio. Furthermore, promising outcomes from in-vivo studies on animal models of alcohol abstinence syndrome were obtained. Among the withdrawal symptoms that the herbal medicine effectively reduced were anxiety, melancholy, tremors, and convulsions. These results suggest that alcohol dependency and withdrawal are connected brain circuits and neurotransmitter systems that the herbal formulation may change. The results of the study have several implications for the field of addiction medicine. In line with the growing acceptance of complementary and alternative medicine, a herbal medication made from Himalayan plants has been developed as a possible treatment for alcohol abstinence syndrome. If further clinical trials demonstrate this herbal composition's efficacy, it could offer a comprehensive and natural way to manage alcohol withdrawal, reducing the need for synthetic medications with uncertain negative effects. However, it is imperative to acknowledge the limitations of the study. The majority of the research was conducted using animal models, and although these models provide useful information, the findings might not instantly translate to human responses. Clinical trials involving human participants are therefore necessary to verify the safety and efficacy of the herbal mixture in real-world conditions.

**6. Conclusion**

According to the current study's findings, a polyherbal formulation protects rats from alcohol abstinence syndrome. We therefore came to the conclusion that, because the polyherbal formulation lowers ethanol withdrawal anxiety and depression-like behaviour, it may have therapeutic promise for treating ethanol-type dependence. To sum up, this investigation into the pharmacological assessment and formulation of a herbal remedy derived from Himalayan plants for the management of alcohol abstinence syndrome has yielded significant findings regarding the possible medical advantages of conventional herbal remedies. The investigation of alternative and complementary therapies is required because alcohol abstinence syndrome, which is typified by a variety of upsetting symptoms upon alcohol discontinuation, continues to pose a serious threat to public health. The formulation created for this study has shown encouraging results in reducing the withdrawal symptoms related to quitting alcohol. It is made up of carefully chosen Himalayan plants with well-known therapeutic qualities. After a stringent set of preclinical assessments, encompassing both in vitro and in vivo investigations, the herbal mixture demonstrated exceptional effectiveness in mitigating withdrawal symptoms, easing anxiety and depression, and bolstering the return of regular physiological processes throughout the crucial withdrawal stage. The comprehension of herbal medicine and its possible use in the treatment of alcohol abstinence syndrome has been enhanced by the integration of scientific approaches and traditional knowledge in this study. To learn more about the formulation's mechanism of action, possible drug interactions, long-term safety, and ideal dosing schedules, additional research is necessary. Furthermore, in order to verify the herbal formulation's safety and effectiveness in an actual environment, human subjects’ clinical trials are required. In summary, the results of this study make a substantial contribution to the expanding body of research that suggests herbal formulations made from Himalayan plants may be a successful supplemental treatment for alcohol abstinence syndrome. This research opens the door for the creation of innovative treatment approaches that can lessen the burden of alcohol-related disorders and enhance the general wellbeing of those who are affected by them by embracing the synergy between conventional wisdom and contemporary science. Such developments in natural medicine have the potential to promote integrative healthcare methods and create a more salubrious society in the long run.

**7. Recommendations and Future Direction:**

The research study named "Preparation and Pharmacological Evaluation of Herbal Formulation from Himalayan Plants for the Treatment of Alcohol Abstinence Syndrome" yielded the following conclusions, which can be put into practice:

* Verifying the safety and effectiveness of the herbal formulation in human subjects requires conducting carefully planned and supervised clinical trials. To find the best dosage for the formulation, as well as any potential negative effects, these trials ought to include a wide and representative sample of people suffering from alcohol abstinence syndrome.
* In-depth research on the herbal formulation's underlying mechanisms of action will improve our comprehension of how it delivers its medicinal benefits. In-depth research on biochemistry and pharmacology that examine receptor interactions, signaling cascades, and neurotransmitter modulation can help achieve this.
* An analysis of the herbal formulation in comparison to current standard treatments for alcohol withdrawal syndrome can yield important information about its potential as a supplemental or alternative therapy. Comparative research like this one will make it easier to determine the special advantages and restrictions of the herbal formulation over prescription drugs.
* A thorough evaluation of safety is essential, encompassing an analysis of possible medication interactions, long-term effects of use, and the influence of the formulation on different organs and systems. The safety profile of the formulation will be determined in part by safety information gathered from clinical trials and preclinical models.
* Both the herbal formulation's therapeutic efficacy and repeatability depend on maintaining a consistent level of quality and composition. Throughout the preparation and manufacturing process, standardization techniques and quality control measures are to be executed.

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