

# Evaluation of Anxiolytic Activity of Ethanolic Leaf Extract of *Gallium Odoratum* Using Mice

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**Abstract:** The anti anxiety activity of ethanolic leaves extract of *Gallium odoratum* was determined by experimental animal models. The anxiolytic activity of the plant was studied using diazepam(3 mg/kg, orally) as standard and two different doses of extract (250 mg/kg, 500 mg/kg, oral) by using elevated plus maze, open field model, light-dark model, rota rod model. Phytochemical screening indicates the presence of alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, steroids, and glycosides. From acute oral toxicity studies, reported that ethanolic extract of *Gallium odoratum* did not produce any toxicity or behavioral changes or mortality upto an oral dose of 2000 mg/kg in animals. The plant extract showed anxiolytic activity confirmed by number of entries and time spend in open arm increased in elevated plus maze, number of squares crossed and number of rearing was increased in open field model, time spent in light chamber and number of transition between two chambers was increased in light- dark model, time spent on rota rod and locomotor activity was increased in rota rod model. The above studies have been concluded that the extract exhibit anxiolytic effect in experimental mice. So it supports the use of *Gallium odoratum* leaves extract as anxiolytic agents. Future studies will be focused on the neurobiological mechanism of action and possible interaction on *Gallium odoratum* with classical neurotransmitters and phytoconstituent responsible for the observed central effects has to be isolated and identified.

## INTRODUCTION

### ANXIETY

Anxiety is a psychological and physiological state which is characterized by somatic, emotional, cognitive, and behavioural components. The root meaning of the word anxiety is to vex or trouble in either presence or absence of psychological stress, anxiety can create feelings of fear, worry, nervousness, and dread. Anxiety is considered to be a normal reaction to a stressor. It may help an entity to deal with a demanding situation by prompting them to cope with it. When anxiety becomes excessive, it may fall under the category of an anxiety disorder [1].

Anxiety is a generalized mood condition that can occur without an identifiable triggering stimulus. As such it is renowned from fear, which is an appropriate emotional response to a perceived threat. Additionally, fear is related to the unambiguous behaviours of escape and avoidance, whereas anxiety is related to situations perceived as uncontrollable or unavoidable. Another view defines anxiety as a future-oriented mood state in which one is ready or prepared to attempt to cope with upcoming negative events, fear and anxiety were said to be differentiated into four domains: (1) duration of emotional experience, (2) temporal focus, (3) specificity of the

threat, and (4) motivated direction.[1]

Fear was defined as short lived, present focused, geared towards a specific threat, and facilitating escape from threat; while anxiety was defined as long acting, future focused, broadly focused towards a diffuse threat, and promoting caution while resembling a potential threat[2].

The physical effects of anxiety may comprise heart palpitations, tachycardia, muscle weakness and tension, fatigue, nausea, chest pain, shortness of breath, stomach aches, or headaches. As the body prepares to deal with a threat, blood pressure, heart rate, perspiration, blood flow to the major muscle groups are increased, while immune and digestive functions are subdued (The fight or flight response)[2].

Anxiety is a common sentiment in humans and can be uttered with words. We do not know animals words but can understand that they have a variety of feelings, including anxiety, by observing their behaviour. Attempts have been made to analyse anxiety by observing animals

behaviour in an artificial environment, which were classified into two different types: with the use or without the use of punishment. Geller and Seifter experimentally created a conflict state in rats by using food pellets (positive reinforcer) and electrical foot shock (punishment). Through lever pressing hungry rats were trained to take food pellets with and without an electric foot shock[3].

#### VARIOUS EFFECTS OF ANXIETY

The emotional effects of anxiety may include "feelings of apprehension or dread, trouble concentrating, feeling tense or jumpy, anticipating the worst, irritability, restlessness, watching (and waiting) for signs (and occurrences) of danger, and, feeling like your mind's gone blank as well as nightmares/bad dreams, obsessions about sensations, trapped in your mind feeling, and emotion like everything is scary[3].

The cognitive effects of anxiety may include thoughts about suspected dangers, such as fear of dying. "You may fear that the chest pains are a lethal heart attack or that the shooting pains in your head are the consequences of a tumour or aneurysm. You feel an intense fear when you think of dying, or you may imagine of it more often than normal, or can't get it out of your mind[3].

The behavioural effects of anxiety may include pulling out from situations which have provoked

anxiety in the past. Anxiety can also be experienced in ways which include changes in sleeping patterns, nervous habits, and increased motor tension like foot tapping[3].

An evolutionary psychology explanation is that augmented anxiety serves the purpose of increased vigilance regarding potential threats in the environment as well as increased tendency to take proactive actions concerning such possible threats. This may cause false positive reactions but also avoid real threats. This may clarify why anxious people are less likely to die due to accidents[4].

Research upon adolescents who has infants had been highly apprehensive, vigilant, and fearful finds that their nucleus accumbens is more sensitive than that in other people when selecting to make an action that resolute whether they received a reward. This suggests a link between circuits liable for fear and also reward in anxious people. As researchers note "a sense of 'responsibility, or self agency, in a context of uncertainty (probabilistic outcomes) drives the neural system underlying appetitive motivation (i.e; nucleus accumbens) more strongly in temperamentally inhibited than no inhibited adolescents[4].

Neural circuitry connecting the amygdala and hippocampus is thought to underlie anxiety. When people are confronted with unpleasant and potentially harmful stimuli such as foul odours or tastes, PET-scans show increased blood flow in the amygdala. In these studies, the participants also reported moderate anxiety. This might indicate that anxiety is a protective mechanism intended to avert the organism from engaging in potentially harmful behaviours[5].

Anxiety may be induced by some drugs especially cocaine. The underlying mechanism is unclear but studies disclose the importance of sympathetic system in the initiation of anxiety. Nor epinephrine is synthesised from dopamine in the presence of Dopamine-B-hydroxylase (DBH), Dbh knock out gene mice studies were done to study the neither importance of nor epinephrine in cocaine induced anxiety[5].

Although single genes have little effect on complex traits and interact heavily both between themselves and with the peripheral factors, research is underway to unravel possible molecular mechanisms underlying anxiety and co-morbid conditions. One candidate gene with polymorphisms that influence anxiety is PLXNA2[6].

Neuroanatomical, physiological and behavioural data suggest a possible role of the neuropeptide tuberoinfundibular-peptide-of- 39-residues (TIP39)

in fear memory. At the outset, TIP39 is synthesized by neurons projecting to brain areas enriched in its receptor, the parathyroid-hormone 2 receptor (PTH2-R). These areas comprise prefrontal cortex and amygdala. To explore this possibility, long-term fear memory is assessed in TIP39 knockout (TIP39-KO), PTH2-R knockout (PTH2-R-KO) and wild-type (WT) mice after exposure to an aversive event. For the aversive event, they used a foot shock. In many rodent studies, foot shock has been regarded as equivalent to the traumatic event that leads to fear-related psychopathologies in liable people[6].

#### MAJOR TYPES OF ANXIETY

Generalised Anxiety Disorder  
Panic Disorder  
Obsessive-compulsive disorder  
Post-Traumatic Stress Disorder(PTSD)  
Social Phobia or Social Anxiety disorder[7].

#### GENERALIZED ANXIETY

Overwhelming anxiety, if not treated early, can subsequently become a generalized anxiety disorder (GAD), which can be identified by symptoms of overstated and excessive worry, chronic anxiety, and constant, irrational thoughts. The anxious thoughts and feelings felt while suffering from GAD are difficult to control and can cause serious mental anguish that interferes with normal, daily functioning[8].

The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) includes specific criteria for diagnosing generalized anxiety disorder. The DSM-IV states that a patient must experience chronic anxiety and excessive worry, almost daily, for at least 6 months due to a number of stressors (such as work or school) and experience three or more defined symptoms including, restlessness or feeling keyed up or on edge, being easily fatigued, difficulty concentrating or mind going blank, irritability, muscle tension, sleep disturbance (difficulty falling or staying asleep, or restless unsatisfying sleep)[8]. If symptoms of chronic anxiety are not addressed and treated in adolescence then the risk of developing an anxiety disorder in adulthood increases significantly. "Clinical worry is also associated with risk of comorbidity with other anxiety disorders and depression" which is why immediate treatment is so important[8].

#### OBSESSIVE-COMPULSIVE DISORDER

Obsessive-compulsive disorder (OCD) is a type of anxiety disorder primarily characterized by repetitive obsessions (distressing, persistent, and intrusive thoughts or images) and compulsions (urges to perform specific acts or rituals). It affects roughly around 3% of the population worldwide. The OCD thought pattern may be likened to superstitions insofar as it involves a belief in a causative relationship where, in reality, one does not exist. Often the process is entirely illogical; for example, the compulsion of walking in a certain pattern may be employed to alleviate the obsession of impending harm. And in many cases, the compulsion is entirely inexplicable, simply an urge to complete a ritual triggered by nervousness. In a slight minority of cases, sufferers of OCD may only experience obsessions, with no overt compulsions. A much smaller number of sufferers experience Only compulsions[9].

#### PANIC DISORDER

In panic disorder, a person suffers from brief attacks of intense terror and apprehension, often marked by trembling, shaking, confusion, dizziness, nausea, difficulty breathing. These panic attacks, defined by the APA as fear or discomfort that abruptly arises and peaks in less than ten minutes, can last for several hours and can be triggered by stress, fear, or even exercise; the specific cause is not always apparent. In addition to recurrent unexpected panic attacks, a diagnosis of panic disorder requires that said attacks have chronic consequences: either worry over the attacks' potential implications, persistent fear of future attacks, or significant changes in behaviour related to the attacks. Accordingly, those suffering from panic disorder experience symptoms even outside specific panic episodes. Often, normal changes in heartbeat are noticed by a panic sufferer, leading them to think something is wrong with their heart or they are about to have another panic attack. In some cases, a heightened awareness (hypervigilance) of body functioning occurs during panic attacks, wherein any perceived physiological change is interpreted as a possible life-threatening illness (i.e: extreme hypochondriasis)[9].

#### PANIC DISORDER WITH AGORAPHOBIA

A person experiences an unexpected panic attack, then has substantial anxiety over the possibility of having another attack. The person fears and avoids whatever situation might induce a panic attack. The

person may never or rarely leave their home to prevent a panic attack they believe to be inescapable, extreme terror[9].

### PHOBIAS

The single largest category of anxiety disorders is that of phobic disorders, which includes all cases in which fear and anxiety is triggered by a specific stimulus or situation. Between 5% and 12% of the population worldwide suffer from phobic disorders. Sufferers typically anticipate terrifying consequences from encountering the object of their fear, which can be anything from an animal to a location to a bodily fluid to a particular situation. Sufferers understand that their fear is not proportional to the actual potential danger but still are overwhelmed by the fear.[9]

### AGORAPHOBIA

Agoraphobia is the specific anxiety about being in a place or situation where escape is difficult or embarrassing or where help may be unavailable. Agoraphobia is strongly linked with panic disorder and is often precipitated by the fear of having a panic attack. A common manifestation involves needing to be in constant view of a door or other escape route. In addition to the fears themselves, the term agoraphobia is often used to refer to avoidance behaviours that sufferers often develop. For example, following a panic attack while driving, someone suffering from agoraphobia may develop anxiety over driving and will therefore avoid driving. These avoidance behaviours can often have serious consequences; in severe cases, one can be confined to one's home[10].

### AIM

The aim is to evaluate the anxiolytic activity of ethanolic leaves extract of *Galium odoratum* in mice by using elevated plus maze, open field model, light-dark model and rota rod test. Medicinal plants have become the focus of intense study recently in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore.

Extensive research has been carried out for an alternative, more specific, cost free and safe therapy for natural anxiolytic drugs as well as new antidepressant principles from Mother Nature and hence identification of such medicinal plant and provision of scientific validation is the need of the hour. Therefore, based on the exhaustive literature

survey, the objective of the present work focused on the search of herb, which will found more suitable among the reported herbs/ herbal preparations, towards the control of anxiety condition based on their traditional claim of the selected herbs.

### OBJECTIVES

- To find out toxicity of *Galium odoratum* by acute oral toxicity study.
- To study the anxiolytic activity of *Galium odoratum* plant extract in experimental animals.
- To estimate the various phytochemical parameters.
- To compare the anti-anxiety activity of *Galium odoratum* leaves extract against standard drug diazepam(3 mg/kg) .

### PLAN OF WORK

The present study involved the following steps:

- Ethanolic leave extract of *Galium odoratum* purchased from kshipra biotech private limited from Madhya Pradesh.
- Authentication of plant by kshipra biotech private limited.
- Phyto pharmaceutical analysis of the leaf extracts of *Galium odoratum*.
- IAEC & CPCSEA approval for use of animal in the experimental study.
- Acute toxicity study.
- Anxiolytic screening methods (exploratory behaviour)
  1. Elevated plus maze model
  2. Open field test
  3. Light-dark exploration
  4. Rota rod test

### PLANT PROFILE



Figure 1 : Leaves and flowers of *Galium odoratum*  
Botanical name: *Galium odoratum*.

Synonyms : Sweet woodruff, bedstraw  
Family : Rubiaceae  
Kingdom : Plantae  
Native : Northern , central Europe

Parts used in this study: leaves

Herbs : flowers, leaves, whole plant

## MATERIALS AND METHODS

Plant extract purchase:

The leaves extract of *Gallium odoratum* (powder form) were purchased from kshipra biotech private limited organic certified company from indore (Madhya Pradesh).(ISO 9001:2015-WHO-GMP).

The plant herb(leave) was authenticated and confirmed and certified by kshipra biotech private limited organic certified company from indore (Madhya Pradesh - ISO 9001: 2015-WHO-GMP).

### PRELIMINARY PHYTOCHEMICAL TESTS

Phytochemicals are natural compounds found in plants that contribute to their medicinal and therapeutic properties.*Gallium odoratum* commonly known as sweet woodruff, is a plant known for its traditional use in folk medicine. The leaf of gallium odoratum believed to contain a diverse array of phytochemicals, which may contribute to its histological activities. The following preliminary phytochemical screening were performed [47,49].

Alkaloids Mayer's test

Add a few drops of Mayer's reagent or Wagner's reagent to a portion of the extract. The formation of a cream-colored or yellow precipitate indicates the presence of alkaloids.[48]

Flavonoids

Ferric chloride test

Add a few drops of concentrated hydrochloric acid to the extract, followed by the addition of magnesium turnings. A red, pink, or purple coloration indicates the presence of flavonoids.[48]

Tannins

Ferric chloride test

Add a few drops of ferric chloride solution to the extract. A blue-black or greenish-black coloration indicates the presence of tannins.[48]

Phenols

Ferric chloride test

Add a few drops of ferric chloride solution to the extract. The development of a bluish-green coloration suggests the presence of phenols.[48]

Saponins

Foam test

Shake a portion of the extract vigorously with water and observe the formation of stable froth. Persistent frothing indicates the presence of saponins.[48]

Terpenoids

Salkowski test

Mix the extract with chloroform and add concentrated sulfuric acid carefully along the sides of the test tube. The development of a reddish-brown coloration in the interface indicates the presence of terpenoids[48]

Steroids

Liebermann-Burchard test

A small amount of the sample is dissolved in a suitable solvent. Acetic anhydride is added to the solution. Sulfuric acid is then added dropwise. The color changes observed in the reaction can help identify the type of steroid present.[48]

Glycosides

Keller-Killiani test

A small amount of the sample is mixed with concentrated sulfuric acid. The appearance of a violet or purple color indicates the presence of glycoside. The intensity of the color may be compared with a standard color chart to estimate the concentration. [48]

Experimental animals

The mice weighing between (25-30g) were housed in clean polypropylene cages and maintained under standard conditions of light (12 hours with alternative day/night cycles), relative humidity (60-70%) and temperature ( $26 \pm 1^{\circ} \text{C}$ ). The animals were fed daily with rodent pellet diet and tap water ad-libitum under strict hygienic conditions[3].

Animal approval

The study was conducted after obtaining the approval from institutional animal ethical committee(IAEC), and the experimental procedures were in accordance to the guidelines of IAEC (688/PO/Re/S/02/CPCSEA), proposal No : NCP /IAEC / 2024-25/03.

Acute toxicity study

The study was conducted to indicate if ethanolic extract of *Gallium odoratum* induced any toxicity in experimental animals by increasing dose concentration upto 2000mg/kg.

Experimental groups of rat was treated orally with

ethanolic extract of *Gallium odoratum* leaves at doses of (2000~mg/kg). The procedure was followed as per OECD 423(acute oral toxicity) guidelines (OECD/OCDE. 2002). The extract was administered Orally by oral canula at a dose of 2000 mg/kg body weight. Rat were kept under observed for 14 days to register possible mortality[3].

Table 1: Animal distribution in acute toxicity study

GROUPS	DOSE(mg/kg)/ORA L	ANIMAL REQUIRED
I	5	3
II	50	3
III	300	3
IV	2000	3

pharmacological screening methods [1,2,3]

#### EXPLORATORY BEHAVIOUR TESTS

1. Elevated plus maze model
2. Open field test
3. Light-dark exploration
4. Rota rod test

#### METHODOLOGY[1,2,3]

##### Procedure:

Animal were weighed, numbered and divided into four groups, each consisting six mice. One group were used as control (saline), second for standard drug (diazepam)treatment. third and fourth group for *Gallium odoratam* extract treatment. (250,500mg/kg) as per acute toxicity test result ) administered through orally by oral canula.test drug administered 30min prior to experiment by orally.[1]

#### ELEVATED PLUS MAZE

The elevated plus maze was first proposed as an animal model of anxiety by handley and mithani constructed of black-colored wooden planks consisting of two close arms.covered with a removable lid or closed arms opposite to each other. During the experiment each mouse were placed in the central compartment facing one of the open arms.The number of entries and the time spent in open arms were recorded for 5 min.an entry was counted when all paws of the mouse entered an open or closed arm. An increase in open arms entries and increase in time spent in open arms were interpreted as an index of potential anxiolytic activity[1,2,45].

If increase open arm exploration time after plant extract(test) administered indicates - decrease anxiety – Anxiolytic activity .

#### Open field test

A dark colored wooden box(60×60×30cms) with its floor carved into 16 equal sized squares(15×15cms) and 40w lamp appended 100cms above for illumination used. Before dropping the individuals mice in one of the corner of the box(i.e 45min prior) respective groups were administered with respective treatment(normal saline, diazepam 3mg/kg(oral), plant extract(two doses as per acute toxicity study) and then recorded number of central squares crossed peripheral squares cross end and number of rearing for 5 min[2].

##### Parameters/observation:

- No of square line crossings
- No of rearing
- No of grooming

#### Light-dark exploration

The apparatus (45×21×21) consist of two plastic boxes . One light compartment painted white and brightly illuminated and the other were dark painted black and dimly illuminated .The mice was allowed to move from one box to the other through an open door between the two boxes.The mice were put into the light box then transition between the light and the dark box and time spent in the light box and dark box were recorded for 5 min.(before and after drug administration. [1,3]

Anxiolytic activity - More crossings between two chambers - more locomotor activity after treatment with test substance - locomotor activity

Anxiolytic – if increase in time spent in the light chamber than black chamber - anxiolytic activity produced by test compounds(plant extract).

#### Rotarod test(motor coordination)

Rota rod apparatus consisted of a base platform and an iron rod of 3 cm diameter and 30 cm length,with a non-slippery surface. This rod were divided into two equal sections by two disks, this enabling two mice to walk on the rod at the same time at the speed of 25 rpm. Animals of either sex were taken. They were fasted for 12 hr before starting the experiment.animal was divided into four groups, each group consist of six animals. group 1 received saline (control), group 2 received standard (diazepam), group 3 and 4 received test (sample extract) . Then the animals were trained to remain for 5min on the rod rotating at a speed of 25rpm on the previous day of experiment.on the next day either vehicle and extract were administered orally and their ability to remain on the rotating rod were assessed before and after 30mins of

the oral administration. The fall of time from the rotating rod was noted for each animal.[3]

If Increase time spent on rota rod without falling from rod - anxiolytic activity.

GROUPS	DRUG TREATMENT	DOSE/ROUTE	NUMBER OF ANIMALS
I	Control (saline)	1ml/kg/oral	6
II	Standard (diazepam)	3mg/kg/oral	6
III	Test-1 (Leaves extract)	250mg/kg/oral	6
IV	Test-2 (Leaves extract)	500mg/kg/oral	6

Table no: 2 –animal distribution in pharmacological screening methods

### RESULTS AND DISCUSSION

#### phytochemical screening

Preliminary phytochemical screening of every plant is essential to know the presence of the active biocomponent that would be responsible for the biological activity. On screening the phytoconstituents it was found that, the presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, saponins, glycosides, terpenoids, and steroids as shown in Figure 2 and Table 3

Phytochemicals like flavonoids, tannins, and saponin, anthraquinone may be responsible for the treatment of anxiety which are reported in some traditional uses of medicine.



Figure no 2: preliminary phytochemical screening

S.NO	Phytoconstituents	Present /Absent
1	Alkaloids	Present
2	Flavonoids	Present
3	Carbohydrate	Present
4	Tannins	Present
5	Phenols	Present
6	Saponins	Present
7	Glycosides	Present
8	Terpenoids	Present
9	Steroids	Present

Table no: 3 - Preliminary phytochemical screening

#### Acute toxicity

The dose of 2000mg/kg of *Gallium odoratum* plant extract had no adverse effect on the behavioural response of the tested rat up to 14 days of observation. Physical observations indicated no signs of changes in the skin, eyes mucous membrane, behavioural patterns, salivation, and diarrhea of the rat. There was no mortality observed and recorded weight loss in normal. Based on the above observation fix the doses 250 and 500 mg/kg for anxiolytic activity. The effect of ethanolic leaf extract of gallium odoratum on general behavioural observation in control and treated rat of acute toxicity studies.

#### Elevated plus maze

Diazepam(3-mg/kg)significantly augmented number of open arm entries. Time spent in open arms and the number of rearing in open arm. They showed a reduction in the time spent in closed arm. Plant extracts treated mice exhibit significant increase in the time spent on open arm and number of open arm entries was increased as shown in the table no:4

S.NO	Treatment	Dose(mg/kg)/oral	Time spent in open arm (seconds/5min)	Entries in open INTRarm Counts/5min)
1	Control(saline)	1ml/kg	28.86±4.40	3.97±0.40
2	Diazepam(positive control)	3mg/kg	87.6±4.98**	8.60±0.9**
3	Plant extract	250mg/kg	46.7±3.20**	4.30±0.44*
4	Plant extract	500 mg/kg	80.6±4.16**	8.21±0.51**

Table no: 4 - Effect of plant extract on animals behaviours by elevated plus maze test

\*p<0.05, \*\* p<0.05 as compared to control. Data where analyzed by one way ANOVA followed by dunnetts test.

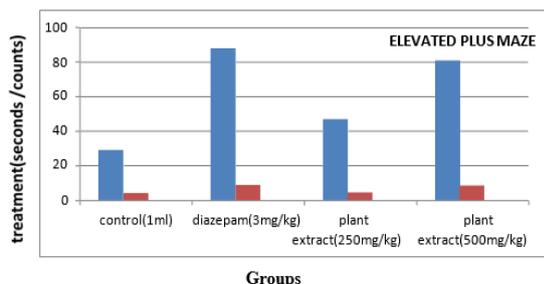


Figure no: 3 – Time spent in open arm / number of open arm entries

➤ number of open arm entries(counts)/5min  
 ➤ time spent in open arm(seconds)/5min

Open field model

In the open field test, plant extract showed significant increase in number of rearings, number of squares crossed and number of assisted rearing during 5 min intervals of test as compared with control as show in table no:5

S.NO	Treatment	Dose(mg/kg)/oral	No of squares crossed (Counts/5min)	No of rearing (counts/5min)
1	Control(saline)	1ml/kg	15.2±3.63	6.5±0.40
2	Diazepam(positive control)	3mg/kg	50.6±7.90**	15.5±0.3**
3	Plant extract	250mg/kg	33.8±3.25*	7.22±0.3
4	Plant extract	500 mg/kg	45.3±4.14**	10.30±0.4**

Table no: 5 - Effect of plant extract on animals behaviours by open field test

\*p<0.05, \*\* p<0.05 as compared to control. Data where analyzed by one way ANOVA followed by dunnett's test.

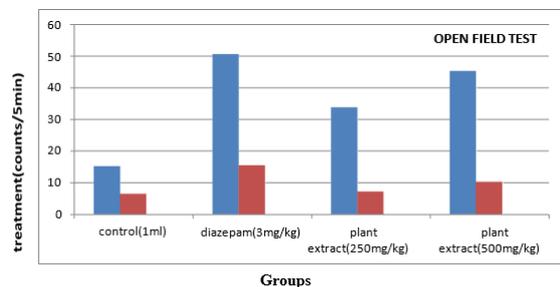


Figure no 4 – Number of squares crossed / number of rearing

➤ number of squares crossed(counts/5min)  
 ➤ Number of rearing (counts/5min)

Light and dark test

*Gallium odoratum* at the dose of 250 mg/kg and 500mg/kg, and diazepam (3-mg/kg) induced a significant increment of the time spent by mice on the illuminated side of the apparatus compared to the respective control group (P<0.05 P <0.01), without

significantly affecting other parameters .

S.NO	Treatment	Dose(mg/kg)/oral	Time spent in the light box (Seconds/5min)	No of transition (Counts/5min)
1	Control(saline)	1ml/kg	40.66±10.05	10.5±3.5
2	Diazepam(positive control)	3mg/kg	121.30±15.01**	24 ±10.5**
3	Plant extract	250mg/kg	70±5.05**	13.5±4.7*
4	Plant extract	500 mg/kg	110±30.05**	20.2±5.1**

Table no : 6 - Effect of plant extract on animals behaviours by light-dark test

\*p<0.05, \*\* p<0.05, p<0.01 as compared to control. Data where analyzed by one way ANOVA followed by dunnett's test.

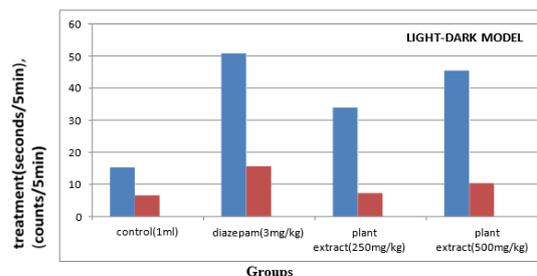


Figure no :5 – Time spent in light box / number of transition

➤ time spent in light box(seconds/5min)  
 ➤ Number of transition (counts/5min)

Rota rod test

The data shows that on average the mice treated with 250 and 500mg/kg of the ethanolic extract of *Gallium odoratum* were able to maintain equilibrium on the rotating rod and stayed shorter than diazepam without falling in 5 min of duration (Table 8). Whereas diazepam (at 3 mg/kg only) showed a significant increase in the locomotor score when compared to other groups.

S.NO	Treatment	Dose(mg/kg)/oral	Time spent in rod without falling from rod (seconds/5min)	Locomotor activity (time of running (seconds/5min))
1	Control(saline)	1ml/kg	15.2±5.05	13.02±1.01
2	Diazepam(positive control)	3mg/kg	60.02±4.5**	56.8±2.00**
3	Plant extract	250mg/kg	33.08±3.03**	30.06±3.05**
4	Plant extract	500 mg/kg	38.03±4.5**	40.04±2.09**

Table no: 7 - Effect of plant extract on animals behaviours by rotarod test

\*\* p<0.01 as compared to control. Data where analyzed by one way ANOVA followed by dunnett's test .

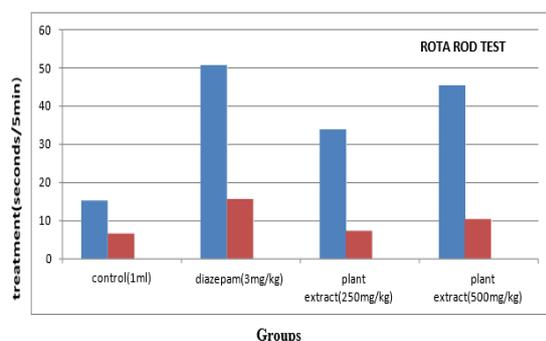


Figure no : 6 – Time spent in rod without falling from rod / locomotor activity

➤ locomotor activity = time of running(seconds/5min)

➤ time spent in rod without falling from rod(seconds/5min)

## SUMMARY

Anxiety disorder are among the most common mental, emotional, panic, restlessness, tension, fear, behavioral problems affecting one eighth of the total population in worldwide. Anxiety represents a heterogenous group of disorder, probably with no single unifying etiology for anxiety. Various psychodynamic, psychoanalytic, behavioral, cognitive and may be due to some drug use including caffeine and alcohol, genetic and biological theories have been proposed to explain the etiology of anxiety disorders. One of the reason for anxiety is GABA and serotonin (5HT), corticotropin releasing factor (neurotransmitters) abnormalities in our central nervous system.

Pharmacologic treatment of anxiety through the ages has included different drugs. The first class of drugs developed (barbiturates) was highly effective, unfortunately the barbiturates can cause respiratory arrest and have a narrow therapeutic index. So the benzodiazepines were developed as a safer alternative to barbiturates.

Due to adverse effects associated with the currently available drugs, patients on anxiolytic drugs usually terminate the treatment before full recovery. In addition, one third of patients in controlled studies are unresponsive to any one of the medication. There is a critical need for development of newer anxiolytic agents with less side effects than available drugs.

We need highly effective treatment for anxiety when compare with already available treatments. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research has progressed constantly around the world wide and demonstrating the pharmacological effectiveness of

different plant species in a variety of animal models. Several plant extracts obtained from the plants are employed in order to balance emotions, improve physical and mental well being.

*Gallium odoratum* plant is a spreading perennial with charming early spring blooms and delicate fragrance, is known by several common names including sweet woodruff, bedstraw. It was formerly placed in the genus *Asperula* in the family *Rubiaceae*. Native is northern and central Europe and north Africa, it was traditionally used as a herbal remedy during the middle ages it was used externally on wounds and cuts and also taken internally to treat digestive and liver problems.

I selected ethanolic leaves (herb) extract of *Gallium odoratum* (sweet woodruff) plant herb for the evaluation of anxiolytic activity. Because no one conduct anxiolytic activity experiment on this *Gallium odoratum* plant so I selected this plant herb for my experiment.

This plant not available in our state so I purchased ethanolic leaves extract of *Gallium odoratum* from Indore Madhya Pradesh (Kshipra Biotech Private Limited Company) in powder form.

The plant herb was authenticated and analysed by Kshipra Biotech Private Limited Company and they provide certificate for identification. The ethanolic plant extract (powder form) was easily solubilized in water. Phytoconstituents of plant responsible for therapeutic activity we want to know about what are the phytoconstituents present in our plant extract so we want to conduct phytochemical screening tests.

Phytochemical screening tests of plant extract was conducted. The chemical constituents of *Gallium odoratum* include alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, steroids, glycosides was identified by phytochemical screening tests.

If we start to the experiment on animals we should get approval from CCSEA and IAEC. The study was conducted after obtaining the approval from institutional animal ethical committee (IAEC). The experimental procedures were in accordance to the guidelines of IAEC (688/PO/Re/S/02/CCSEA), proposal No : NCP/IAEC/2024-25/03.

Acute oral toxicity study was followed as per OECD guidelines – 423. The extract of plant was administered orally upto a dose of 2000mg/kg body weight. Animal were kept under observed for 14 days to register possible mortality. It was not produce any mortality during oral acute toxicity studies.

Acute oral toxicity study was determined by using four group of animals (rat (180 to 200 g weight),

select four group of animals for the acute oral toxicity studies, each group contain three animals. the rat were fasted for 3hr prior to the experiment. The plant extract was administered using oral canula. first dose is 5 mg/kg(1mg /200g), second dose is 50mg/kg (10mg/200g), third dose is 300mg/kg(60mg/200g), fourth dose is 2000mg/kg(400 mg/200 g) as per OECD guidelines 423.

From this studies, reported that ethanolic extract of *Gallium odoratum* did not produce any toxicity or behavioral changes or mortality upto an oral dose of 2000mg/kg in rat.

Four group of animal was selected for each method of anxiolytic activity screening tests, each group contain six animals (mice 25 g ). Control (1ml/kg), diazepam 3mg/kg, plant extract 250 mg kg, plant extract 500mg/kg . First group of animasl receive 1ml of water, second group of animals receive 3mg/kg (0.075 mg /25 g) of diazepam (standard drug), third group of animals receive 250 mg/kg (6.25mg/25g) of plant extract, fourth group of animal receive 500mg/kg (12.5mg/25g) of plant extract. The plant extract and diazepam was diluated with water depends on dose then administered to the animals by the oral route.

3mg of diazepam was dissolved in 40 ml of water (each ml contain 0.075 mg of diazepam/25g of mice) 250 mg of plant extract was dissolved in 40 ml of water (each ml contain 6.25mg of plant extract /25 g of mice

500mg of plant extract was dissolved in 40 ml of water (each ml contain 12.5 mg of plant extract /25 g of mice.

Four anxiolytic screening methods were used for this experiment

1. elevated plus maze
2. open field model
3. light and dark model
4. rota rod test

In elevated plus maze, before and after drug /plant extract administered, observed time spent in open arm and number of open arm entries in 5min of intervals. Time spent in open arm and number of entries were increased. Anxiolytic activity of the compound *Gallium odoratum* extract (250mg/kg, 500mg/kg) were similar to the those of diazepam(3mg/kg). The plant extract induce specific anxiolytic activity.

In open field model, before and after drug/plant extract administered, observed number of squares

crossed and number of rearing in 5 min of intervals. Number of squares crossed and number of rearing was increased .

In light and dark method , before and after drug administered, observed time spent in light box and black box in 5min of intervals. Time spent in light chamber and number of transition between two chambers were increased.

In rota rod model, time spent on rota rod and locomotor acivity were increased after plant extract administration. The plant extract induced specific anxiolytic activity.

Acute oral toxicity and Anxiolytic activity of *Gallium odoratum* plant extract did not proved in previous studies. I compared anxiolytic activity of plant extract with benzodiazepen derivatives (diazepam 3mg/kg). I tested the acute oral toxicity of the *Gallium odoratum* plant extract (ethanolic extract) in rat and anxiolytic effects of this plant extract in mice using elevated pluz maze, open field test, light and dark box test,rota rod test(anxiolytic screening methods).

## CONCLUSION

The present work exhibits that the *Gallium odoratum* extract had anxiolytic activity in mice. There is no reports of scientific evaluation of its anxiolytic activity. Our study shows that the *Gallium odoratum* extract had marked effect on the anxiety related behavioural parameters on exposure to the elevated plus maze test, open field test, light-dark model test, rota rod test in mice. *Gallium odoratum* extract causes an anxiolytic behavior comparable with the effect of diazepam. Future studies will be focused on the neurobiological mechanisam of action and possible interaction on *Gallium odoratum* with classical neurotransmitters and phytoconstituent responsible for the observed central effects has to be isolated and identified.

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