Assessment of the anxiolytic and CNS depressant effects of eichhornia crassipes

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Abstract:

Anxiety is a significant medical issue that has been extensively researched in recent years, but there are still many unanswered questions about its causes and the mechanisms behind pain relief, especially for long-lasting anxiety. The absence of a comprehensive understanding is a significant obstacle in the advancement of more effective anti-anxiety medications. While there are numerous synthetic drugs available in the market, they often come with various side effects and unwanted consequences. That's why there is an increasing amount of research being conducted for the development of safer drugs to treat anxiety. Since herbal drugs are free from side effects and considered safer than synthetic drugs, researchers prioritize the development of drugs from natural sources.

Based on the literature review, it is evident that eichhorni crassipes has a broad range of therapeutic effects and is cultivated in various regions of India. In this context, the purpose of this study is to assess the anxiolytic and CNS depressant effects of the whole plant extract of Eichhorni crassipes on various animal models.

Key words: eichhorni crassipes, anxiety, elevated plus maze method.

Introduction:

Stress is a common occurrence that everyone experiences. When stress becomes extreme, it is harmful for the body and, hence need to be treated. Stress is involved in the pathogenesis of a variety of diseases that include psychiatric disorders including diabetes mellitus, male impotence, congestive dysfunction, peptic ulcer, hypertension and ulcerative colitis [1,2]. Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal treat [3]. It is an emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined future threat. Anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioural components [3]. These components combine to create an unpleasant feeling that is typically associated with uneasiness, apprehension, fear, or worry [4]. Anxiety is a broad emotional state that frequently arises without a specific cause or trigger. As such, it is distinguished from fear, which occurs in the presence of an observed threat. Additionally, fear is related to the specific behaviours of escape and avoidance, whereas anxiety is the result of threats that are perceived to be uncontrollable or unavoidable [5,6]. The normal fear response to threatening stimuli comprises several components, including defensive behaviours, autonomic reflexes, arousal and alertness, corticosteroid secretion and negative emotions [7]. In anxiety states, these reactions occur in an anticipatory manner, independently of external events [8]. The distinction between a 'pathological' and a 'normal' state of anxiety is not clear-cut but represents the point at

which the symptoms interfere with normal productive activities [9,10]. Despite (or perhaps because of) this loose distinction, anxiolytic drugs were until recently among the most widely used drugs in general practice. They have lost popularity due to their uncertain advantages and clear risks [11].

Anxiety disorders are clinically recognized as follows:

1: Generalised anxiety disorder (a continuous state of excessive anxiety without a specific cause or focus) [12].

2: Panic disorder is characterized by sudden episodes of intense fear accompanied by physical symptoms like sweating, rapid heartbeat, chest pain, trembling, and difficulty breathing. Even individuals without any pre-existing conditions can experience such attacks when exposed to sodium lactate infusion, and there seems to be a genetic predisposition to this phenomenon [13].

3: Phobias (intense fears of particular objects or situations, such as snakes, open spaces, flying, social interactions) [14].

4: Post-traumatic stress disorder (anxiety caused by remembering past stressful events) [15].

5: Obsessive compulsive disorder (compulsive behaviour driven by irrational anxiety, e.g. Fear of contamination) [16].

The categorization of drugs that alleviate anxiety and induce sleep [17].

• Benzodiazepines

• Buspirone.

• B-adrenoceptor antagonists

• Zolpidem

• Barbiturates

• Miscellaneous other drugs

Anxiety affects one-eighth of the total population worldwide and has become an important area of research in psychopharmacology during this decade [18]. Benzodiazepines (bzds) are the major class of compounds used in anxiety and they remain the most commonly prescribed treatment for anxiety. However, the realization that bzds have a narrow safety margin has prompted many researchers to evaluate new compounds in the hope of identifying other anxiolytic drugs with fewer unwanted side effects [19, 20].

Central nervous system depression or CNS depression refers to physiological depression of the central nervous system that can result in decreased rate of breathing, decreased heart rate, and loss of consciousness possibly leading to coma or death [21]. CNS depression most often results from the use of depressant drugs such as alcohol, opioids, barbiturate, benzodiazepines, general anaesthetics, and anticonvulsants such as valproate used to treat epilepsy [22].

A central nervous system activity refers to physiological depression of the central nervous system, general or local anaesthesia, relaxation of skeletal muscles, or anticonvulsant activities [23,24]. Many depressants and anaesthetics acting on the central nervous system do so by increasing the activity of a particular neurotransmitter known as gamma-amino butyric acid (GABA), although other targets such

as the n-methyl d-aspartate (NMDA) receptor, μ opioid receptor and cb1 cannabinoid receptor can also be important, depending on which drug is involved [25].

Skeletal muscle relaxants act peripherally at neuromuscular junction or in the cerebrospinal axis to reduce muscle tone [26]. CNS depressants and the CNS depression produced by these agents could be classified pharmacologically as major tranquillizers like chlorpromazine [27,28].

MATERIALS

In December 2024, the whole plants of Eichhorni crassipes were collected from its natural habitat in Jagdalpur, Bastar district of Chhattisgarh. The entire plant was thoroughly cleaned and dried. To prevent the degradation of volatile oil, it was shade dried. Approximately 100 grams of powder was mixed with ethanol and water and left to macerate. The extracts were dehydrated and turned into a fine powder. The extracts were analysed for their phytochemical properties and tested for their potential medicinal effects.

Animals:

Twelve healthy albino rats, weighing between 150-200 grams, were chosen for the study and obtained from the animal house of the royal college of pharmacy and health sciences (RCPHS), Berhampur. These animals were housed in the pharmacology laboratory of the Royal College of Pharmacy and Health Sciences in Berhampur. All the animals were kept in polypropylene cages with husk bedding and maintained at a room temperature and relative humidity with a 12:12 light: dark cycle. The animals were provided with a standard diet suggested by ICMR and had access to pure drinking water whenever they desired. The work was conducted following the necessary approval from the institutional animal ethics committee (IAEC) of the Royal College of Pharmacy and Health Sciences in Berhampur.

Chemicals:

For chemical analysis of plant.

Wagner's reagent, Mayer’s reagent, Dragendroff's reagent, and Benedict’s reagent are all names for the same chemical compound.

For pharmaceutical research:

Diazepam (campose) is a medication produced by Ranbaxy ltd, derived from the whole plant extracts of eichhornia crassipes.

Instruments:

The elevated plus maze, light and dark model, and actophotometer are all different models of the Rolex watch.

Methods:

1: **Phytochemical investigation**:

Various extracts obtained from the extraction process were examined for the presence of phytochemical constituents, using the qualitative phytochemical analysis method. The subsequent chemical tests were conducted.

Experiments for alkaloids.

• Wagner’s reagent test:

When exposed to alkaloid, the substance forms a reddish brown precipitate. It was prepared by dissolving 1.27 grams of the substance. of iodine and 2 grams. In a 5ml solution of potassium iodide, the total volume was increased to 200 ml.

• Mayer’s reagent test:

Another method of detecting alkaloids is through a different approach. To create the reagent, measure out 1.36 grams of the substance. The solution of mercuric chloride was prepared by dissolving it in distilled water. In a separate container, dissolve 5 grams of potassium iodide in 60 millilitres of distilled water. After combining the two parts, the volume was adjusted to 200 ml. When alkaloids are present, a white to buff precipitate is observed.

• **Dragendroff’s Reagent Test:**

When using alkaloids, this reagent produces a reddish-brown colored solid. To create this reagent, 14 grams of sodium iodide was heated with 5.2 grams of the substance. Add bismuth carbonate to 50 ml of glacial acetic acid and let it sit for a few minutes. After being permitted to remain standing overnight, the precipitate of sodium acetate was removed through filtration. To a volume of 40 ml of filtrate, 160 ml of acetate and 1 ml of water were combined. The stock solution was kept in a bottle with a golden hue. During the experiment, 20 ml of acetic acid was added to 10 ml of stock solution, and the final volume was adjusted to 100 ml by adding water.

• Tests for carbohydrates

• Benedict’s test:

In this method of test for monosaccharide, 5 ml of benedict's reagent and 3 ml of test solution when boiled on a water bath and brick red precipitate appears at the bottom of the test tube confirms the presence of the compounds.

Tests for sugar alcohols.

• Keller-killiani test:

A small amount of the drug was extracted from glacial acetic acid, and then a few drops of ferric chloride and concentrated sulphuric acid were added. When the two layers of the skin come into contact, a reddish brown colour is created at the point of intersection, and the upper layer turns bluish green.

Tests for flavonoids.

• Test with NaOH:

To detect flavonoids, the extract was initially dissolved in water. The water was purified and the resulting solution was treated with sodium hydroxide. The colour yellow indicates the presence of flavonoids.

• Test with sulphuric acid:

When a small amount of h2so4 is added to the mixture, the yellow colour disappears.

Experiments for Glycosides.

• Foam test:

Approximately 1 ml of both alcoholic and aqueous extracts were mixed with distilled water to reach a volume of 10 ml, and then shaken in a graduated cylinder for 15 minutes before being set aside. A 1 cm layer of foam that forms after standing for 30 minutes suggests the presence of saponins.

Tests for tannins and phenolic compounds.

• Test with lead acetate:

Tannins form a solid substance when mixed with lead acetate.

• Test with ferric chloride:

Typically, phenols are separated using a 5% w/v solution of ferric chloride in 90% alcohol, allowing for their detection.

• Test with gelatine solution:

To a solution containing tannins (0.5 - 1%), a gelatine solution (1%) and sodium chloride (10%) were added. The presence of a white buff precipitate confirms the compounds.

Assessment of anxiolytic effects and CNS depressant properties:

Anxiolytic and CNS depressant activity of eichhornia crassipes whole plants extracts were studied by using following methods-

1. The elevated plus maze method was used to assess the anxiety levels of the mice.

The elevated plus maze was initially created to assess the effectiveness of anti-anxiety medications. The elevated plus maze apparatus is made up of two open arms and two closed arms that branch out from a central platform. The two pairs of identical arms were positioned in opposite directions. The entire setup was raised to a height of 50cm above the ground. When the animals are exposed to a new maze alley, they experience a conflict between approaching and avoiding it, and this conflict is more intense in the open arm compared to the enclosed arm. Rats and mice have a strong dislike for open and high spaces, and they tend to spend more time in enclosed areas. When animals encounter open spaces, they freeze, become motionless, release waste, and exhibit fearful behaviours -like movements. The plasma cortisol level is also reported to be elevated, which is considered a true reflection of anxiety.

Major advantages of this test procedure are:

a) it is simple, fast and less time consuming, b) no prior training, or noxious stimuli (sound or light) is required and c) it is predictable and reliable procedure for studying anxiety response as well as anxiolytic action of drugs.

Twelve Wister albino rats of both sexes, weighing appropriately, were divided into six separate groups (n=6). At the start of the session, each animal was placed in the center of the maze, with its head

facing the open arm. It was given 5 minutes to freely explore the maze. Then, various medications were given to the different groups of animals orally, following the prescribed schedule.

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Table- 4.1

GROUP	DRUG	DOSE (mg/Kg)	NATURE
I	Distilled Water	10ML/Kg	Control
II	Diazepam	1	Standard
III	MEPP-200	200	Test-1

After thirty minutes, the drug administration positioned the animal in the center of the maze, with its head facing towards the open arm for ten minutes. The administration then measured the drug's acute anxiolytic effects based on specific parameters.

• Number of entries in open and closed arm

• Average time spent in each arm

1. Light and dark model:

The testing apparatus comprises a chamber with a light and a dark section, separated by a designated area. A polypropylene animal cage, measuring 44 × 21 × 21 cm, is covered with black spray to create a darkened area on one-third of its surface. A partition measuring 13 cm in length and 5 cm in height divides the cage into a dark one third and a bright two thirds. Albino rats were individually placed into the cage for duration of 10 minutes. The animals were given the test drugs or the standard drugs or the vehicle orally 30 minutes before the experiment and were then observed for 10 minutes. The following parameters were recorded:

• Number of entries in to light and dark chamber.

• Average time spent in each chamber

After the elevated plus maze test, the animals from each group were positioned at the center of the illuminated area. The number of people entering and the duration of their stay in each room were recorded. The effects of the drug were examined 30 minutes after it was given to the participants.

2: Actophotometer:

When the dosage of a drug is increased or decreased, it will result in either an increase or decrease in spontaneous motor activity (SMA) in the animals. The Actophotometer is built on this principle. The locomotor activity can be accurately measured using an actophotometer, which utilizes photoelectric cells connected in a circuit with a counter. It has a iron rods at the bottom, 6 light and 6 photo cells placed in the outer periphery of the bottom in such a way that a mice or rat can block only one beam. Technically its principle is that a photocell is activated when the rays of light falling on photocells are cut off by animal crossing the beam of light. The photocells are linked to an electronic automatic counting device that keeps track of the number of times the 'cut off' occurs. The actophotometer is primarily utilized to investigate the depressant effects of the drug on the central nervous system.

Following the elevated plus maze test and light and dark method tests, the animal of each group was placed at the centre of the actophotometer 1 hour after the drug administration and study the locomotors activity. The animal was placed in the centre of the actophotometer and the light and dark method was used to measure the locomotors activity.

Statistical analysis:

The data collected from the study are presented as the mean SEM (standard error of mean) for six animals. The variation among means was examined using a student's t test, employing prism software. Differences were deemed statistically significant at a probability level of less than 5% ($p<0.05$).

Stress is a state of mind and body that involves various aspects such as thoughts, physical sensations, emotions, and actions. These elements come together to form an unpleasant sensation that is commonly linked to feelings of unease, anxiety, fear, or worry. Anxiety is a broad emotional state that frequently arises without a specific cause or trigger. As a result, it is different from fear, which arises when one encounters a real or perceived danger. Furthermore, fear is connected to the actions of escaping and avoiding, while anxiety is the outcome of threats that are believed to be uncontrollable or unavoidable.3.

Central nervous system depression refers to physiological depression of the central nervous system that can result in decreased rate of breathing, decreased heart rate, and loss of consciousness possibly leading to coma or death. CNS depression most often results from the use of depressant drugs such as alcohol, opioids, barbiturate, benzodiazepines, general anaesthetics, and anticonvulsants such as valproate used to treat epilepsy. They produce their action by increasing the activity of a particular neurotransmitter known as gamma-amino butyric acid (GABA), although other targets such as the n-methyl d-aspartate (NMDA) receptor, μ opioid receptor and cb1 cannabinoid receptor can also be important, depending on which drug is involved.

In the elevated plus maze, open arms are more fear provoking than the closed arms and the ratio of entries, time spent and rearing behaviour in open arms/closed arms reflects the animals perception of safety towards closed arms and fearfulness towards open arms. Typical anxiolytic drugs increase the proportion of entries, time spent, rearing in the open arms, and the ratio of open arm to closed arm entries. In the present study standard and control group animals showed increased in the time spent and rear in the open arms, but reduction the time spent in closed arms as compared to normal control animals in the elevated plus maze. In the bright and dark arena test, the brightly lit area represents a noxious environmental stressor that inhibits the normal exploratory behaviour of rodents and reduction in the number of entries, time spent, and rearing in the bright chamber are considered to be a markers of anxiety. In this models also, the standard and control group animals demonstrated a marked increase in the time spent and rearing behaviour in the bright chamber as compared to normal control animals. The animals in the methanolic and aqueous groups exhibited notable reductions in anxiety compared to the control group. Behavioural changes in both these models suggest that hydro alcoholic extracts of eichhornia crassipes plants have anxiolytic effects and on repeated uses the effects are increases. The methanolic and aqueous extracts of eichhornia crassipes also shows CNS depressant activity as it significantly reduces the locomotor activity in actophotometer.

From the above findings it is suggested that methanolic and aqueous extracts of eichhornia crassipes have significant anxiolytic and CNS depressant effect at 200mg/kg. Additional research is anticipated to identify and analyse the active components of the substance, examining their effects on anxiety and the CNS depressant activity.

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