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Evaluation of Anti-Anxiety Activity of *Withania somnifera* (Leaves) in Albino Mice

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ABSTRACT:

Anxiety disorders are the most prevalent form of mental illness characterized by excessive worry, fear, and physiological disturbances. Traditional therapies, including benzodiazepines and SSRIs, are often limited by side effects and dependency issues. Therefore, interest in natural alternatives such as *Withania somnifera* (Ashwagandha) has grown. The present study investigates the anxiolytic properties of ethanolic and aqueous extracts of *Withania somnifera* leaves in Wistar albino rats using behavioral assessment models including the Elevated Plus Maze (EPM) and Open Field Test (OFT). Rats treated with *Withania somnifera* showed increased exploration of open arms in the EPM and central zones in the OFT, indicating significant anxiolytic effects. HPLC analysis confirmed the presence of active constituents such as Withaferin A and Withanone, which may contribute to the plant's therapeutic action.

Keywords: Anxiety disorders, *Withania somnifera*, Elevated Plus Maze, Open Field Test.

1. INTRODUCTION:

The anxiety disorders are the most common, of frequently occurring mental disorders They ease my trip a group of conditions that share extreme in pathological anxiety as the principal disturbance of mood or emotional tone. Anxiety, which may be understood as a pathological counterpart of normal fear, is manifested by disturbances of mood, as well as thinking, behavior, nail physiological activity. The anxiety disorders include panic disorder (with and without a history of agoraphobia), agoraphobia (with and without a history of panic disorder), generalized anxiety disorder, specific phobia, social phobia, obsessive-compulsive disorder, acute stress disorder, and post traumatic stress disorder. In addition there are adjustment disorders with anxiety features, and disorders due to general medical conditions and substance-induced anxiety disorders.

There are two main forms of anxiety These are;

- Normal Anxiety
- Pathological (or Neurotic) Anxiety

Normal Anxiety: This is a normal response to an observable threat, which some call fear Fear is a reaction to danger from a specific external source.

Pathological Neurotic Anxiety: This is an affective cognitive/behavioural/physiological response to an internal or external threat, real or imagined, during which the person experiences a "felt" unpleasant emotional state. Thus, it is an inappropriate reaction in a given stimulus;, it is the form of anxiety experienced by those with anxiety disorders and is different from fear.

Several types of anxiety disorders exist

- Agoraphobia
- Anxiety disorder due to medical condition
- Generalized anxiety disorder

- Panic disorder
- Selective mutism
- Separation anxiety
- Substance-induced anxiety disorder
- Other specified anxiety disorder and unspecified anxiety disorder

1.1 Characteristics Of Anxiety Disorders:

1. Excessive worry.

2. Fear of illness or impending doom.

3. Multiple somatic complaints

4. Avoid behaviour of anxiety-provoking stimuli.

1.2 Causes Of Anxiety Disorder:

Some uses of anxiety disorders are:

- Genetics.
- Brain chemistry.
- Environmental stress.
- Drug withdrawal or misuse .
- Medical conditions.

1.3 Signs & Symptoms:

- Feeling nervous, restless or tense
- Having a sense of impending danger, panic or doom
- Breathing rapidly
- Sweating
- Trembling
- Feeling weak or tired.

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1.4 Pathophysiology:

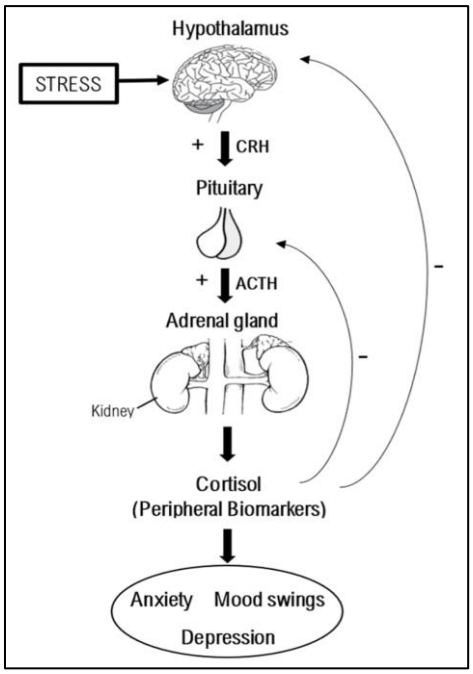

Fig: 1 Anxiety

1.5 Natural Herbs That Are Used In The Treatment Of Anxiety:

Natural alternatives are derived from plants and have demonstrated anxiolytic effects in both humans and animals. They have been used as a traditional food and medicines for a change of es for centuries. A systematic review of the published literature revealed trial data for several plant species (Ginkgo biloba, Lavandula angustifolia. Hypericum perforatum, Valeriana officinalis, Crataegus oxyacantha and Eschscholzia californica as well as Matricaria recutita,Melissa officinalis, Passiflora incarnata and Piper methysticum) demonstrating high level of evidence for the treatment of anxiety disorders.

2. PLANT PROFILE:

Fig: 2 *Withania somnifera*

- **Name:** Withania Somnifera
- **Synonym:** Withania, winter cheney, Indian winter, Indian ginseng, Ashwagandha
- **Family:** Solanaceae
- **Tamil name:** Amukkara

- **Malayalam:** Amukkuram Bengali: Ashwagandha
- **Telugu:** Ashwagandha

2.1 Collection & Cultivation:

Withania somnifera is cultivated in many of the drier regions of India, such as Mandsaur, district of Madhya Pradesh, Punjab Gujarat, and Rajasthan. It is also found in Nepal. Withania somnifera is grown on sub-marginal waste lands and low fertility areas. Plants grow well in red, sandy, black and loamy soil with PH 6.5-8.0 with good water drainage. It can he cultivated an altitudes of 1000 meter.

2.2 Distribution:

It is found throughout the drier parts in sub-tropical regions and upper Gangetic plain. The distribution area of Withania somnifera extends from the Canary Island and the Mediterranean region through Africa, the Middle East, India and Sri Lanka to China It also occurs in Australia.

2.3 Morphological Characteristics:

It is a dense, hairy, erect, greyish-tomentose here under shrub, and grows up to a height of 1.5 metre. Its all parts are covered with whitish, stellate trichomes. Branching extensive: leaves are simple, alternate or sub-opposite, ovate, entire, basis cunate. 10 cm long. The roots are stout, long tuberous, fleshy, whitish brown.

2.4 Chemical Constituents:

The leaves of the plant contain 12 variants of withanolides and 5 unidentified alkaloids. withaferin A is the main withanolide and in addition to that is the presence of free amino acids. Mand tanzuns, chlorogenic acid, flavonoids, glucose, and glycosides also present in leaves well the green cherries contain flavonoids, condensed tannins and proteolytic enzymes thermore, the shoots specifically tender shoots are ample source of crude protein, calcium, flavonoids, phosphorous, condensed tannins and scopoletin.

2.5 Therapeutic Use:

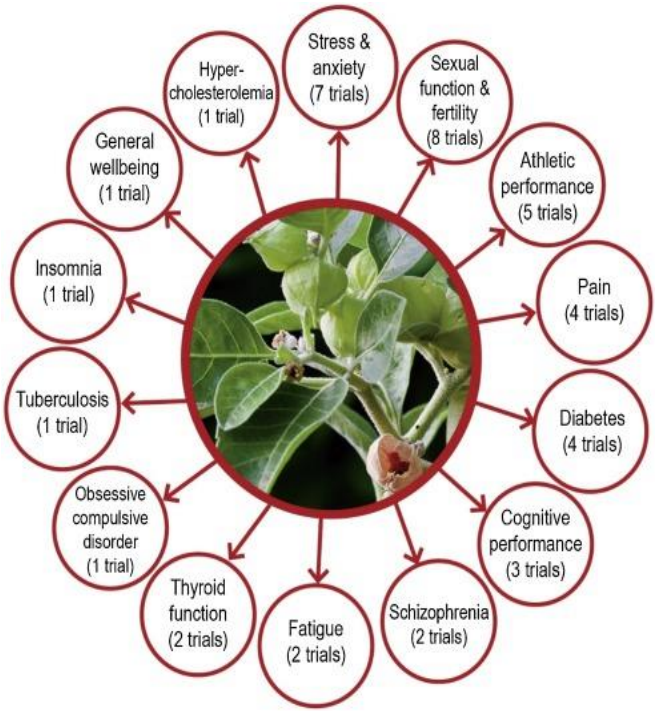

Fig: 3 Therapeutic Use

MATERIALS AND METHODS:

3.1 Selection Of Plant:

The commercially available powdered leaf of withania somnifera was obtained from indian Medical Practitioners Co-operative society, Adyar, Madras, India and its aqueous suspension was used at 100 mg/kg dosage, (derived from recommended human dosage on body weight basis) in this study.

3.2 Selection Of Animals:

Adult wistar strain albino rats of either sex (weighing 150-180g) were used for the study. All the animals were maintained under standard laboratory conditions and fed with rat feel pellets and water and libitum. Adult male wistar albino rats weighing 120-140g purchased from the animal house colony of the National Research Center (Dokki, Giza, Egypt) and were kept in the animal house under conventional laboratory conditions. Experiments were performed according to the National Regulations of Animal Welfare and Institutional Animal Ethical Committee (IAEC).

3.3 Experimental Design:

The rate weighing around 200g housed in pairs for 10 days prior to testing 6 animals selected for each group.

- Group 1: Normal Saline
- Group 2: Diazepam
- Group 3: Test treated with herbal extract at lower dose
- Group 4: Test treated with herbal extract at higher dose

3.4 Preparation Of Ethanolic Extract:

The leaves of withania somnifera were dried under shade and powdered with a mechanical grinder. The powder was passed through sieve no: 40 and stored in an airtight container for further use.

3.4.1 Method of extraction:

Preparation of water extract of Withania somnifera leaves: The Withania somnifera leaf extract was prepared by suspending 10g of dry leaf powder in 100ml of double distilled water. The suspension was stirred at 45+ 5 covernight and filtered. The filtrate so obtained was treated as 100% water extract.

Preparation of crude alcoholic extract of Withania somnifera leaves: Crude alcoholic extracts of roots and leaves were prepared for chemical analysis. Leaf powder was suspended in 85% ethanol in a ratio of 1:30 and incubated at 85 e for 2hrs in a reflux system. The collected extract was filtered and concentrated by evaporation at 60 e. The filtrate was lyophilized, by freeze drying for overnight HPLC analysis of the extract was performed using shimadzu HPLC system using YMC-Pack ODS-A column. Purified and well characterized Withaferin A and Withanone were used as standards.

2.5 Preliminary Phytochemical Analysis:

- Test for carbohydrates
- Test for Alkaloids
- Test for Steroids and Sterols
- Test for Glycosides
- Test for Saponins
- Test for Tri-terpenoids (Triterpene lactone)
- Tests for Tannins and Phenolic Compounds
- Test for Fixed Oils and Fatty acids
- Test for Gums and Mucilage
- Test for Proteins and Amino acids

3.6 Estimation Of Behavioural Assessment Parameters:

3.6.1 Elevated Plus Maze:

This is a common and most simplified behavioural test which is used to evaluate behaviour related to anxiety disorders in rodents. In early time, an elevated y-shaped apparatus was offered by Montgomery in 1955 to evaluate the anxiolytic effect but later on, it was modified by Handley and Mithani in 1984 and pellow and its cool leagues modified it in 1985 into an elevated plus maze with two open and two closed arms which were arranged to form a plus shape maze. Afterwards, the same apparatus was offered by Lister in 1987 for mice. This test was investigated to evaluate fear-induced behaviour. The elevated plus maze helps in rapid screening of drugs which modulate anxiety or mouse genotype CCK2 KO without involvement of complex schedules or training. It has an advantage over other models or behaviour tests which use food or water deprivation or shock administration to assess anxiety.

A day before experiment animals should be placed in each arm of the elevated plus maze so that the animal gets familiar with the apparatus and will not take time to respond while actual activity will perform. Dimensions are specific for the elevated plus maze apparatus It consists of plus shape maze elevated above the ground with four arms ie., two closed arms with an open roof and two open arms positioned oppositely. Measurement of the apparatus can be changed according to the type of study and to get the precise result. There are different measures for different species Lefor the rats, mate should be approximately 45 cm and 10cm respectively and for the mice name should be approximately 30cm above the ground with the length and breadth of the arm in approximately 30 cm and Sem respectively. It also consists of a centre point where the animal will be placed during the experiment. A video camera also mounted above the maze for reconling and subject movement and analysed by a video tracking system. As rodents prefer to live in dark places and avoid light or bright places to live plus, they also have anxiety effects with height. Therefore time spent in the open or closed arm is calculated to measure anxiety-like behaviour or the anti anxiety effect of a drug. Ethanol of 70% (v/v) ethanol should be used to clean the maze after each and every trail and at the end of the day it should be cleaned with 10% bleach. After administration of the drug experiment should be started according to dose and their peak plasma level. The animal should be kept in the centre with the head facing towards the open arm and the animal will start moving in different arms as per the conditions.

The behaviour which is typically recorded is the time spent and entries made on the closed and open arms. In this task. behaviour reflects a conflict between their living preference for protected areas and their innate motivation to explore novel environments with open arms. In elevated plus maze, there is no such evidence of age-related restriction in behavioural testing There is various data available which support that all age group rodents can be used to evaluate anti-anxiety activity of a drug. But later on behavioural study was conducted which introduced that the animals having age less than 3 months experienced less anxiety on elevated plus maze also encouraged the exploration of open sides of maze Anxiolytics would he expected to increase the proportion of entries and time spent in open arms. As the number and time spent in open arms is increased it cannot be concluded that it enhances the impulsivity as this test may not be an optimum measure of motor activity. If a researcher believes that the new drug is increasing the impulsivity in animals then the impulsivity test shall be performed to correlate the result.

3.6.2 Open Field Test:

The open field test was first developed by Hall in 1934 which was initially used to measure emotional behaviour in rodents. The open field test provides easy and fair evaluation of behaviours which does not require pre-training. This test can include other animal species such as pigs, primates, calves, honey bees, rabbits and lobsters. In 1957 and 1958 Broadhurst Studied that the movement in the open-field model was mainly determined by the exploratory drive which was concluded by the detailed study of some environmental variables. First it was used to measure emotional behaviour while later on it was used to measure behavioural changes induced by psychotropic drugs and electro-convulsive shock.

Later on, Royce included monitoring of central versus oblique movement around the field in 1977. Animals which spent most of the time of experiment near the central part, considered as less fearful or less anxious rather than those which prefer perimeter areas. This consideration was the informative measure of the open-field test and it was acknowledged that the other behavioural measures could be distorted by the locomotor's activity of animals. As per the previous studies Rodger in 1997 found that the difference in locomotor activities of individuals confound the measure of emotion which follows that the difference in emotionality of individuals will confound the locomotor activity. Hence, this complication seems to be largely being ignored these days.

To perform the experiment, it would be desirable to perform the test as animals are in their diurnal phase. Open-fields test consists of four chambers which are used for the analysis. measurements and lightning effects vary from lab to lab. one example of measurement of open field test is 50 (length) ×50 (width) x 38 (height) of each chamber and it is made up of high density and non-porous plastic. it can be of any shape and size such as a rectangular square. circular corridor And round. Rearing, time spent moving, disiara moved, and change in activity over time are the various means which can be reported and tabulated. Walls have been made Just to prevent escape. It can be visualized by installing, video camera during experiment or by photo-cells based sensors. Equal

mini squares can be drawn on the floor of the apparatus to count no of lines or squares crossed by animals during experiment which shows the locomotor activity precisely. Various outcomes such as centre time, activity and defecation within 5 minutes of experiment, gauge various aspects of emotionality including anxiety.

Each animal should be placed in the centre of the apparatus. The person who is placing the mice should maintain no sudden motion of noise, because it cao severely affect the results Duration of test session should be 5 minutes for oval sesame but to examine the habituation an increasingly Familiar environment, a session of 30 minutes is recommended Number fines or square caressed will be recorded automatically and at the end of the experiment result will be marked to check exploratory behaviour. It was originally introduced for the ratshut sensually if it was found equally successful for the mice. In scientific research, ile test is used to evaluate anxiety and locomotor activity Levels in rodents. Many compounds like Viazolobenzodiapenes (such as alprazolam, SSRI and adinazolam) which has various spectrum of is pharmacological effect as an anxiolytic in various anxiety (like panic disorder Generalized anxiety disorder and OCD) have shown very less potency as an anxiolytic when pasted in spen field seat . However, this study suggests that this paradigm option for exploratory avoir and locomotory measurement rather than anxiety-like behaviour. The open field sesi qualitatively and quantitatively used to measure willingness to explore and general locomotor activity in rodents. However, the extent to which rodents behaviour in the test correlates with the general locomotor activity in various other situations such as in a brune cage or an activity on wheel, is still controversial.

3. RESULTS AND DISCUSSION:

The anxiolytic potential of Withania somnifera leaf extract was evaluated using two behavioral models: the Elevated Plus Maze (EPM) and the Open Field Test (OFT). The results are presented as mean ± SEM. Statistical comparisons were made between the control, standard (Diazepam), and test (low and high dose) groups.

4.1 Nature & Colour Of Extract:

Table: 1 Nature and Colour Of Extract

S.No.	Name of extract	Colour	Consistency	Yield%W/W
1	Ethanolic extract	Light greenish	Sticky mass	12.3
2	Aqueous extract	Light yellow	Sticky mass	17.4

4.2 Preliminary Phytochemical Studies:

Table: 2 Preliminary Phytochemical Studies

Phyto-Constituents	Ethanol Extract	Aqueous extract
Alkaloids	+	+
Saponins	-	-
Glycosides	+	+
Carbohydrates	+	+
Tannins	-	-
Flavanoids	+	+
Terpenoids (Triterpene lactone)	+	+
Steroids	+	+
Phenolic compounds	-	-
Proteins and amino acids	-	+
Fixed oils and fatty acids	-	+
Gums and mucilage	-	-

4.3 Elevated Plus Maze (EPM):

- Control group (saline-treated) rats spent significantly more time in the closed arms, reflecting high anxiety levels.

- Diazepam-treated rats showed a significant increase ($p < 0.01$) in both time spent and entries in the open arms, confirming its anxiolytic action.

- Rats treated with Withania somnifera extract (low dose) showed a moderate increase in time spent and entries into the open arms ($p < 0.05$).

- High dose of Withania somnifera extract produced a significant anxiolytic effect ($p < 0.01$), comparable to Diazepam.

Table: 3 Elevated Plus Maze

Group	Open Arm Time (sec)	Open Arm Entries	Closed Arm Time (sec)
Control	32.1 ± 4.1	4.2 ± 1.2	227.8 ± 5.3
Diazepam (2 mg/kg)	95.2 ± 6.8**	9.4 ± 1.5**	164.6 ± 4.7**
W. somnifera (100 mg/kg)	59.7 ± 5.2*	6.1 ± 1.1*	196.2 ± 6.2*
W. somnifera (200 mg/kg)	87.9 ± 5.9**	8.3 ± 1.3**	172.1 ± 5.1**

4.4 Open Field Test (OFT):

- Control group rats displayed low locomotion, mostly staying in the periphery, indicating anxiety.
- Diazepam increased central zone activity and total crossings.
- Low dose *Withania somnifera* showed a moderate anxiolytic effect.
- High dose group showed increased central zone time, more rearing, and locomotor activity, similar to Diazepam.

Table: 4 Open Field Test

Group	Center Time (sec)	Squares Crossed	Rearing
Control	17.9 ± 2.4	41.3 ± 3.1	6.1 ± 1.0
Diazepam (2 mg/kg)	56.4 ± 4.3**	78.2 ± 5.5**	12.3 ± 1.2**
W. somnifera (100 mg/kg)	38.2 ± 3.1*	58.9 ± 4.7*	8.9 ± 1.3*
W. somnifera (200 mg/kg)	51.8 ± 3.9**	70.6 ± 4.9**	11.1 ± 1.4**

DISCUSSION: The behavioral models used in this study effectively demonstrated the anxiolytic potential of *Withania somnifera*. Rats administered with the ethanolic extract at higher doses exhibited a statistically significant increase in the time spent and the number of entries into the open arms of the Elevated Plus Maze compared to the control group. This suggests a reduction in anxiety-related behavior. Similarly, in the Open Field Test, treated rats showed increased time spent in the central zone and greater locomotor activity, further supporting the anti-anxiety effect of the extract. Phytochemical analysis using HPLC identified Withaferin A and Withanone as major active components, both of which are known for their neuroprotective and adaptogenic properties. The findings align with existing literature that supports the anxiolytic efficacy of various plant species including *Valeriana officinalis*, *Passiflora incarnata*, and *Lavandula angustifolia*. Notably, *Withania somnifera* demonstrated anxiolytic effects comparable to Diazepam but without observable sedative or motor impairments. These results underline the therapeutic potential of *Withania somnifera* as a natural alternative to conventional anxiolytics, especially in populations susceptible to drug dependence or side effects.

4. CONCLUSION:

The current investigation confirms the anxiolytic effects of *Withania somnifera* in rodent models through established behavioral paradigms. The observed increase in exploratory behavior and decrease in anxiety indicators suggest that *Withania somnifera* may be an effective, natural therapeutic agent for anxiety disorders. Further studies, including clinical trials and mechanistic investigations, are necessary to validate its efficacy and safety in humans.

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