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A study on Anxiolytic activity on *Solanum Nigrum* Linn Leaf Extraction in Experimental models

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ABSTRACT

Anxiety disorders are marked by excessive fear, often in response to specific objects or situations and in the absence of true danger and they are extremely common in the general population. According to a recent epidemiological study, the lifetime prevalence of any anxiety disorder is 28.8%. Anxiety disorders are associated with impaired workplace performance and hefty economic costs. The clinical applications of benzodiazepines as anxiolytic are limited by their side effects. The aim of the present study is to investigate the effects of aqueous and ethanolic extracts of *Solanum Nigrum* leaves. Elevated plus maze test, light/dark test used. In the elevated plus maze, aqueous and ethanolic extracts (400mg/kg; p.o) showed an anxiolytic effect by increasing the percentage of time spent in open arms and the percentage of open arm entries as compared to control group.. In the light/dark transition test, aqueous extracts (400mg/kg; p.o) had increased the time spent in light area, latency to enter dark chamber and tunnel crossing. Whereas, ethanolic extract of (400mg/kg; p.o) has showed significant result. These results suggested that the extracts of *Solanum Nigrum* leaves possessed anxiolytic effect in mice, in contrast to diazepam, had no inhibitory effect on locomotion in these tests, its side effect profile might be superior to the benzodiazepines.

Keywords: *Solanum Nigrum* leaves; Anxiolytic property; Elevated plus maze; light/dark test.

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INTRODUCTION

Anxiety disorders are marked by excessive fear (and avoidance), often in response to specific objects or situations and in the absence of true danger, and they are extremely common in the general population. According to a recent epidemiological study, the lifetime prevalence of any anxiety disorder is 28.8%¹. Anxiety disorders are associated with impaired workplace performance and hefty economic costs².

Anxiety disorders as recognized clinically include, generalized anxiety –GAD (an ongoing state of excessive anxiety lacking any clear reason or focus), panic disorders-PD (attacks of overwhelming fear occurring in association with marked somatic symptoms, such as sweating, tachycardia, chest pain, trembling, choking, etc), phobias (strong fears of specific things or situations e.g. snakes, open spaces, flying, social interaction) post traumatic stress disorder-PTSD (anxiety triggered by insistent recall of past stressful experiences)³.

Anxiety is also an important component of many other psychiatric or medical conditions. Effective treatments such as anxiolytic drug therapy or cognitive behavioural therapy exist but many patients remain untreated, experience adverse effects of benzodiazepines, or do not benefit from full symptom control. It has been estimated that 43% of anxiety sufferers use some form of complementary therapy. The most popular treatments include herbal medicines. Similarly, anxiety disorders are amongst the most common reason for people to try with herbal medicines⁴.

Medicinal plants are now becoming more widely used by people all over the world. People understand the gentle strength of these natural remedies. Doctors and scientist are validating the wisdom of traditional use. Although a few of the remedies are strong, all can be used safely and without the side effects of drugs. Furthermore, these natural remedies can be used to cure problems rather than just mask symptoms. Much of the information about medicinal plant is in the form of actions. Rather than saying a plant is used for insomnia, many references will state that it has the action “sedative” or “hypnotic” properties and actions of medicinal plants describes the action of the plants on the body⁵.

The increasing awareness of herbal medicine is acknowledged by WHO. WHO estimate about three-quarters of the world’s population currently use herbs and other forms of traditional medicines to treat their diseases. WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, almost for several hundred years, before the development and spread of modern medicine and are still in use today. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. herbal dugs

constitute only those traditional medicines, which are primarily use medicinal plants preparations for therapy ⁶.

The development of new anxiolytic drug has been an area of interest. There is growing interest in the various types of herbs and herbal medicines being used as anxiolytic drugs. Ethno pharmacological studies and ethno botanical surveys points to growing interest in the screening and understanding role of various herbs in CNS related disorders - especially on neuro and psychopharmacological aspects. There is a considerable popular interest in the use of natural remedies or herbal products.

Various traditional herbal medicines have also been suggested to possess anxiolytic activity. Interestingly, several indigenous herbs have been screened for such activity as like *Scutellaria later flora* (Blue skullcap), *Centella asiatica* (Centella asiatica), *Paullinia cupana* (Guarana), *Piper methysticum* (Kava), *Bacopa monniera* (Keenmins), *Cymbopogon citratus* (Lemon grass), *Passiflora incarnate* (Passion flower), *Valeriana officinalis* (Valerian) ⁴.

The *Solanum Nigrum Linn* Family-Solanaceae, has been used traditionally to treat various ailments such as pain, inflammation fever^{8,9} and enteric diseases.¹⁰ It possess many activities like antitumorigenic, antioxidant,¹¹ anti-inflammatory,⁹ hepatoprotective,¹² diuretic,^{7,9} and antipyretic agent,^{7,9} antibacterial,¹⁰ mycotic infection¹⁷, cytotoxicity, anti-convulsant, antiulcerogenic.^{16,17} . It is also used against sexually transmitted diseases.¹⁵

The flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. To date about 3000 varieties of flavonoids are known. Flavonoids exhibit several biological effects such as anti-inflammatory, hepatoprotective and anti-ulcer actions. They also inhibit enzyme such as aldose reductase and zanthine oxidase. They are potent antioxidants and have free radical scavenging abilities. Many have anti-allergic, antiviral actions and some of them provide protection against cardiovascular mortality. They have been shown to inhibit the growth of various cancer cell lines in vitro, and reduce tumour development in experimental animals ¹⁰.

The phytoconstituent like flavones like apigenin specifically recognise the central BDZ Receptors and it has been found that flavones like apigenin bind with high affinity BDZ site of the GABA _A receptor ⁹⁵

S. nigrum possesses active components are flavanoids (apigenin) glycoalkaloids, glycoproteins, and polysaccharides, polyphenolic compounds such as Gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin¹⁵

In view of the above stated apigenin having anxiolytic effect⁹⁵ and solanum nigrum posses active constituent apigenin¹⁵ there are no reports of scientific evaluation of its anxiolytic activity, there for the present study has been under taken to explore anxiolytic potential of the plant into two different models in mice.

Objective:

The literature reveals that anxiolytic activity on *Solanum Nigrum* has not been reported. In view of this, leaves of *Solanum Nigrum* has selected for anxiolytic activity on experimental animals.

The present study is therefore plan with the following objective

1. Extraction of *Solanum Nigrum* leaves with suitable solvents.
2. Preliminary phytochemical screening of crude extract.
3. Toxicity study
4. Evaluation of various solvent extracts of *Solanum Nigrum* leaves for its anxiolytic activity by following models-
 - a) Elevated plus maze model.
 - b) Light and dark box test

REVIEW OF LITERATURE:

Review of literature was made from various sources, including e-sources, to access various pharmacological and chemical natures of herbs. For the sake of convenience, it is divided into three parts.

In part I, preliminary details of herbs viz., taxonomical data, vernacular names, geographical distribution, chemical nature, constituents, traditional uses, folk lore uses and reported pharmacological activities of plant.

In part II contains detail information of anxiety disorders from various journals and web sources (abstract data bases) etc., relevant for current study have been reviewed.

In part III, animal models for screening and evaluating anxiolytic potential of plant with including rodent models of anxiety.

**Figure: 1** Whole plant**Figure: 2** leaves & fruits**About plant:**¹⁸**Botanical name:** *Solanum Nigrum Linn***Family** : Solanaceae**Varieties** : Black & white**Vernacular Names:**¹⁸**Tamil** : Manatakali

Milagutakali

Telugu : Block night shade

Hounds berry

Petty morel

Wonder berry

Taxonomical Classification:¹⁸Kingdom: [Planate](#)

Unranked: Angiosperms

Unranked: Eudicots

Unranked: Asterides

Order : Solanaces

Family : Solanaceae

Genus: *Solanum*

Species: *Solanum nigrum* Linn

Morphology¹⁸

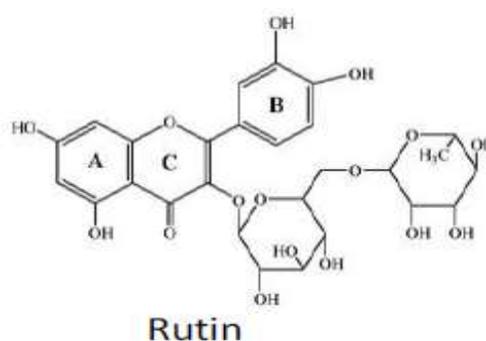
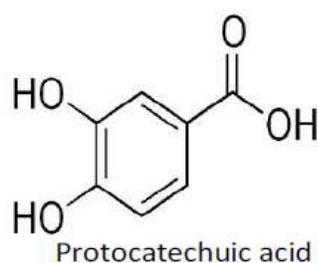
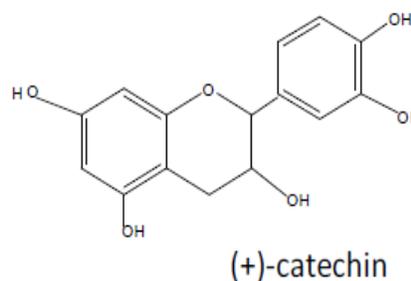
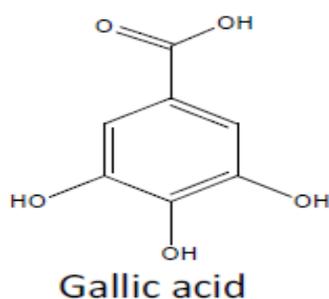
Solanum nigrum is 25-100 cm tall, erect annual herb, pubescent with simple hairs. Stems are often angular, sparsely-pubescent. The fruits are dull black, globose, 8-10 mm in diameter. The leaves are ovate, the bases are cuneate, 4-10 and 3-7 cm wide, pubescent, coarsely dentate, the apex is obtuse. Inflorescences are extraaxillary, umbels, the calyx cup-shaped, the corolla is white, the lobes ovate-oblong, pubescent abaxially, ciliate spreading. Filaments are 1-1.5 mm long; anthers are 2.5- 3.5 mm long.

Traditional Uses

S. nigrum has been used traditionally to treat various ailments such as pain, inflammation fever^{8,9} and enteric diseases.¹⁰ It possess many activities like antitumorigenic, antioxidant,¹¹ anti-inflammatory,⁹ hepatoprotective,¹² diuretic,^{7,9} and antipyretic agent,^{7,9} antibacterial,¹⁰ mycotic infectio¹⁴, cytotoxicity, anti-convulsant, antiulcerogenic.^{16,17} . It is also used against sexually transmitted diseases.¹⁵

Chemical constituents

S. nigrum possesses numerous compounds that are responsible for pharmacological activities. Its active components are flavanoids(apigenin) glycoalkaloids, glycoproteins, and polysaccharides, polyphenolic compounds such as Gallic acid, catechin, protocatechuic acid (PCA), caffeic acid, epicatechin, rutin, and naringenin¹⁵



REPORTED ACTIVITIES ON SOLANUM NIGRUM:

1. **Ravi V, et al** Anti-Inflammatory Effect of Methanolic Extract of *Solanum nigrum* Linn Berries
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2. **Muhammad Zubair et al** Antimicrobial potential of various extract and fractions of leaves of *Solanum Nigrum*²⁰
3. **Noel N Wannang et al** Anti-seizure activity of the aqueous leaf extract of *Solanum nigrum* Linn (Solanaceae) in experimental animals²¹
4. **Rui-lian Sun et al** Cadmium accumulation in relation to organic acids in leaves of *Solanum nigrum* L. as a newly found cadmium hyperaccumulator Plant Soil²²
5. **Tariq Javed et al** In-vitro antiviral activity of *Solanum nigrum* against Hepatitis C Virus
Javed et al. Virology Journal 2011, 8:26²³
6. **Sanjay patel et al** In-vitro cytotoxicity activity of *solanum nigrum* extract against *hela* cell line and *Vero* cell line.²⁴
7. **A. Geo vigila et al** Nephroprotective activity of aqueous extract of *Solanum nigrum* in Amphotericin B induced Wister rats²⁵
8. **Sudhanshu et al** Antioxidant activity of *Solanum surattense* and *Solanum nigrum* Methanolic extract an *in vitro* evaluation.²⁶
9. **Syed Kashif Zaidia et al** Protective effect of *Solanum nigrum* leaves extract on immobilization stress induced changes in rat's brain²⁷
10. **Kusum Singh et al** Effect of *solanum nigrum* on protein content of liver and kidney Of albino rats *Pharmacie Globale (IJCP)* 2011, 4 (08)²⁸
11. **Maddala Rajeswari et al** Anti gastritic and antiulcerogenic effects of *Solanum nigrum* in laboratory animals²⁹
12. **Nazoora Saleh Aali et al** Protective effect of ethanolic extract of *solanum nigrum* on the blood sugar of albino rats *IJPSR*³⁰
13. **Vipin Kumar Tiwari et al** Hypoglycemic Activity of Ethanolic Extract of *Solanum nigrum* Linn. Leaves on Alloxan Induced Diabetes Mellitus in Rats³¹
14. **Abd-Elraheim Ali Elshater et al** The hepato-ameliorating effect of *Solanum nigrum* against CCl₄ induced liver toxicity in Albino rats³²
15. **Serem, Erick Kibichiy et al** Effect of Long Term Administration of *Solanum Nigrum* Extracts in Female SwissWhite Mice Infected with *Trypanosoma Brucei Rhodesiense*³³

16. Parameswari.K, Sudheer et al In Vitro Antibacterial Activity In The Extracts Of *Solanum Nigrum* ³⁴

17. Jasmin Šutković et al In vitro production of solasodine alkaloid in *Solanum nigrum* under salinity stress ³⁵

The other plants having Anxiolytic activities :

There have been several reports of natural drugs which possessing anxiolytic activities ³⁶. Plant extracts, teas and food provide an ever increasing number of constituents and ingredients which seem to interact functionally with different organ systems of body including brain ³⁷.

Following research work of plant extracts reveals that their constituent's posse's anxiolytic activity and this has lead us to investigate anxiolytic activity of *Solanum nigrum* leaf extracts.

1. Anxiolytic effect of *Equisetum arvense* Linn. Extract in mice ⁹⁵
2. Effect of the active constituent of *Crocus sativus L.*, crocins, in light/dark animal model of anxiety in mice shows significant results at 50mg/kg dose ³⁸.
3. Anxiolytic effect of saponins from *Panax quinquefolium* on EPM, L/D, HB, in mice shows significant results at 25 – 100mg/kg dose ³⁹.
4. Evaluation of anxiolytic properties of Gotukola- (*Centella asiatica*) extracts and asiaticoside- is active triterpenes found in this plant in rat behavioral models ⁴⁰.
5. Anxiolytic effect of *Stachys lavandulifolia Vahl* on the elevated plus maze model at 100mg/kg dose on mice ⁴¹.
6. Lemon grass (*Cymbopogon citratus*) A placebo-controlled, double-blind RCT of Lemon grass extract included 18 patients suffering from trait anxiety. The extract was administered as a single dose of abafado (Brazilian lemon grass tea), and its effects were quantified 30 min later under the stress of a cognitive test. The results revealed no significant inter-group differences to suggest an anxiolytic effect ⁴².
7. Passion flowers (*Passiflora incarnata*) in a double-blind RCT, 32 patients with generalized anxiety disorder were randomized to receive 45 drops of a passionflower tincture or 30 mg oxazepam per day. After 4 days of treatment, no significant differences in terms of anxiety levels were noted. Patients treated with passionflower reported fewer adverse effects than those receiving the synthetic anxiolytic ⁴³.
8. Aqueous extract of dried Ginkgo leaves have been reported to inhibit MAO-A and MAO-B. *Ginkgo biloba* (*Ginkgoaceae*) leaves contain active constituent flavonol glycosides and it shows effect on behavior, learning and memory. It also shows anxiolytic effect at 0.01-10 mg/kg dose range ⁴⁴.

ANXIETY:**Introduction:**

Anxiety disorders are conditions in which extreme, often disabling, anxiety or fear is the shared primary symptom. Normal anxiety may be defined as “a diffuse, unpleasant, vague sense of apprehension, often accompanied by autonomic symptoms—such as headaches, palpitations, tightness in the chest, restlessness, mild stomach discomfort that can be an appropriate response to a threatening situation or stimulus”⁴⁵.

Whereas fear is considered specific and targeted, anxiety is considered more diffuse and unfocused. Pathological anxiety and fear, as compared to normal symptoms, are diagnosable conditions when the anxiety, fear, or both cause significant distress, interfere with functioning, or are marked by time consumption⁴⁶.

Epidemiology:

Several large, methodologically rigorous epidemiological studies have indicated that anxiety disorders are one of the most prevalent categories of childhood and adolescent psychopathology⁴⁷. The most recent prevalence estimates from a paediatric primary care sample including more than 700 families suggest that approximately 20% of children (ages 8–17 years) were above the clinical cut off on a brief anxiety screen measure by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)⁴⁸.

Although comorbidity rates vary depending upon the primary diagnosis, there exists a strong comorbidity among anxiety disorders in youth⁴⁹. For example, an epidemiological study of paediatric OCD revealed that 84% of youth diagnosed with OCD had comorbid disorders, including major depression (62%), social phobia (38%), alcohol dependence (24%), and dysthymia (22%)⁵⁰. The most common comorbid diagnoses include other anxiety disorders and depressive disorders⁵¹. Additionally, children with anxiety disorders frequently experience other psychiatric conditions, including attention deficit/hyperactivity disorder and the disruptive disorders⁵².

Etiology:

The etiology of child and adolescent anxiety may be of a biological and/or learned nature. Indeed, researchers posit that anxiety arises from a complex interaction of specific characteristics related to the child (e.g., biological, psychological, and genetic factors) and his or her environment (e.g., conditioning, observational learning, family relations, traumatic events⁵³). There are an abundance of theoretical models that would define child and adolescent anxiety, some are as follows.

Biological Model:

Within a biological model of etiology, researchers have investigated genetic influences as well as neurobiological structures and circuits. A recent meta-analysis of the genetic epidemiology of anxiety disorders demonstrated that PD, phobias, OCD, and GAD aggregate in families and concluded that genetic factors have a moderate influence on the development of anxiety disorders⁵⁴. Researchers have suggested that, although clearly not the only contributing influences, genetic factors may help us understand why certain individuals exposed to similar experiences have different responses and outcomes concerning the development of pathological anxiety⁵⁵.

Research aimed at identifying specific brain areas and circuits underlying anxiety disorders has provided support for neurobiological influences in anxiety. The most support for neuroanatomical influences has come from research investigating the amygdala's role in fear conditioning. Research in this area has implicated the amygdala in the pathophysiology of anxiety disorders⁵⁶. Neurochemical factors have also been implicated in the development of anxiety symptoms. Abnormal functions of serotonin, norepinephrine, dopamine, and γ -aminobutyric acid systems as well as abnormal chemoreceptor reactivity have all been implicated in anxiety⁵⁷.

Cognitive–Behavioral Model:

Within a cognitive–behavioural model, abnormal thoughts, feelings, and behaviours are described as reactions that have been learned as a result of conditioning and observation⁵⁸. A behavioural theorist highlighted behavioural conditioning as an important etiological factor in the development and maintenance of anxiety and posited that an individual associates a threatening stimulus with a non threatening stimulus so that the latter by itself triggers anxiety. Once the fearful or anxious reaction has been learned through classical conditioning, the fear or anxiety is maintained through the operant mechanism of negative reinforcement. Negative reinforcement is manifested by avoidance learning, escape learning, or both. Escape learning involves terminating an aversive situation, whereas avoidance learning involves avoiding fear- or anxiety-provoking situations. Consequently, without opportunities for new learning provided by exposure, the fear or anxiety does not extinguish. This process of acquisition and maintenance of fears is known as Mowrer's two factor theory⁵⁹.

In addition to the two-factor theory, observational learning influences the development of anxiety. Children learn about anxiety-provoking situations by observing others experience such situations or by acquiring information through activities like reading or watching the news on television⁶⁰. Furthermore, they are capable of retaining and reproducing event memories acquired via observational learning⁶¹.

Ecological Models:

Ecological models focus on the impact of the family system and other environmental influences on the development of anxiety disorders and particularly highlight the bidirectional relationships among child, family, and other environmental contributions to anxiety. For example, research has revealed relationships among levels of child temperamental characteristics (i.e., behavioural inhibition), insecure parent–child attachment, and anxious and controlling parenting styles⁶². Parental modelling of fearful and anxious expressions and behaviours has also been found to contribute to the development of anxiety in children⁶³.

Types of Anxiety Disorders:

The core symptoms for six anxiety disorders are listed in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR)⁴⁶, are described below.

Separation Anxiety Disorder (SAD):

SAD is characterized by excessive worry about separation from another person who represents safety for the affected child, typically a parent. In new, unfamiliar, or feared situations, youth with SAD are often dependent on their safety figure. Common features of the disorder include excessive demonstration of distress upon real or threatened separation (e.g., tantrums, crying, somatic complaints), fear of harm or permanent separation from caretaker, and fear of getting lost, kidnapped, or dying. School refusal is a common symptom of SAD, occurring in approximately 75% of children with the diagnosis⁶⁴.

Within the clinical setting, children with SAD may present with sleep problems, such as nightmares. Furthermore, these children may experience a number of somatic complaints (e.g., stomach-ache) related to the distress associated with SAD. The presence of clingy and whiny behaviour within the clinical setting may also be an indicator of SAD. The clinical presentation of SAD may vary with age, with younger children exhibiting excessive crying and temper tantrums upon separation from the attachment figure and older children displaying social withdrawal and manipulative behaviour to avoid school or separation⁶⁵.

Panic Disorder (PD):

PD is characterized by both the actual occurrence of panic attacks and persistent worry and vigilance about prospective symptoms of another panic attack. Panic attacks involve an overwhelming fear of being in danger for no apparent reason as well as physiological symptoms such as pounding heart or chest pain, sweating, trembling or shaking, shortness of breath or choking sensation, nausea, dizziness, feelings of unreality or depersonalization, and fear of going crazy or dying⁴⁶.

The most common symptoms reported are palpitations, shortness of breath, sweating, faintness, and weakness. In adolescence, chest pain, flushes, trembling, headache, and vertigo are also commonly reported symptoms. In youth, cognitive symptoms are less common, with the most frequent cognitive symptoms being a fear of losing control. As with adults, there is a strong association between PD and agoraphobia in youth ⁶⁴.

The presenting problem for youth with PD will pertain to one or more of the many physiological symptoms of panic attacks. Parents of youth with PD may also report agoraphobic symptoms related to their child's panic attacks. Unlike in adulthood, catastrophic interpretations of physiological symptoms may not be part of the clinical presentation ⁶⁶. PD is less common in childhood than in adolescence, and the clinical presentation of PD varies across the developmental span ⁶⁷. Specifically, younger children's panic attacks are often related to particular triggering events whereas adolescent's panic attacks are more often reported as unexpected and not linked to a particular antecedent event ⁶⁶.

Social Phobia:

Social phobia, or the fear of embarrassment or negative evaluation in social or performance situations, is manifested by the avoidance of situations in which the child fears acting in a humiliating or embarrassing manner ⁴⁶. Three main factors in the development and maintenance of social phobia are highlighted: (a) cognitive biases (e.g., beliefs that individuals will predictably interact with others in a manner that will elicit rejection and/or negative evaluation from others), (b) deficits in social skills, and (c) operant conditioning (e.g., negative reinforcement for avoidance behaviours ⁶⁸.

Within the clinical setting, youth with social phobia may present as shy and socially withdrawn and may exhibit noticeable anxious–somatic symptoms, including blushing, sweating, and shaking, when interacting with unfamiliar people. Limited eye contact is also quite common. In extreme presentations, youth may have difficulty with articulation or may become mute. Interpersonal deficits may be evident when interacting with socially phobic youth, who often report having few close friendships with their peers. Whereas younger children with social phobia tend to hide behind adults or attempt to physically escape from a social situation, elder children tend to remain in the social situation but with few efforts to engage or participate ⁶⁹.

Obsessive–Compulsive Disorder (OCD):

OCD is characterized by recurring intrusive thoughts or excessive worries (obsessions) and/or activities or habits the person feels driven to perform to reduce anxiety (compulsions). The

obsessions and/or compulsions are distressing, time-consuming (more than one hour per day), or debilitating (interfere with normal functioning)⁴⁶.

The most common obsessive themes in the paediatric population include fears of contamination (e.g., dirt, germs, toxins); preoccupations about harm to self or others; the need for symmetry, exactness, and order; concerns with religious or moral conduct (e.g., being concerned with committing a sin); lucky or unlucky numbers; and preoccupations concerning forbidden sexual or aggressive thoughts. The most common compulsive themes include cleaning or decontamination rituals (e.g., excessive washing, bathing, or grooming); checking, counting, repeating, straightening, and routinized behaviours (e.g., doors, locks, homework, appliances); confessing, praying, and reassurance seeking; touching, tapping, and rubbing; measures to prevent harm to self or others; and hoarding and collecting⁷⁰.

Youth with OCD may present to health professionals with a number of physical or behavioural complaints that are consequences of obsessive-compulsive symptoms. For example, dermatological problems may arise secondary to compulsive hand washing or skin picking. Weight loss may occur due to refusal to eat certain foods that are perceived as contaminated. Compulsive avoidance of bathrooms due to contamination fears may lead to the development of secondary encopresis or enuresis. Additionally, youth may present to their dentists with bleeding gums as a result of excessive teeth cleaning⁷¹.

Research has supported a distinction between early- and late-onset OCD, such that early-onset (i.e., prepubertal) OCD is more likely to occur in males, to be characterized by symptom presentations characteristic of compulsions without obsessions and more primitive compulsions (i.e., touching, tapping, rubbing), to have co morbiditic symptomatology, and to involve family members in their rituals⁷².

Posttraumatic Stress Disorder (PTSD):

PTSD is characterized by recurrent symptoms of anxiety related to past trauma, such as physical abuse or natural disasters⁴⁶. Cognitive, autonomic, and behavioural symptoms of anxiety are typically involved. The main manifestations of traumatic reactions include repetitive and intrusive thoughts about the trauma, flashbacks or nightmares in which the child reexperiences the trauma, heightened arousal, avoidance of stimuli associated with the trauma, sleep disturbances, and separation difficulties. Cognitive changes, such as difficulties in concentration and memory problems, are also common. Additionally, a child may report a sense of foreshortened future or a premature awareness of his or her own mortality⁷³. This disorder always involves significant distress and can result in marked interference with functioning⁴⁶.

Primary complaints of youth with PTSD in the clinical setting may involve physiological arousal symptoms such as difficulty sleeping or exaggerated startle response. Parents of youth with PTSD may report a temporal association between a particular traumatic event and the onset of atypical behaviour such as sexual acting out or aggression. It is common for youth with PTSD to be reluctant about discussing the traumatic event, and their descriptions of the traumatic event often lack a discussion of their associated emotional experience ⁶⁹.

Generalized Anxiety Disorder (GAD):

GAD involves diffuse excessive worry over a wide variety of routine daily activities such as school performance, social concerns, or family interaction. It is characterized by 6 months or more of chronic, exaggerated worry and tension that are unfounded or much more severe than the anxiety that most people experience. The excessively anxious thoughts generally involve thoughts related to negative, uncontrollable, or catastrophic outcomes. Studies of youth with GAD have demonstrated that youth selectively attend to negative and the threat-related information ⁷⁴.

Avoidant behaviour is common for situations that provoke anxiety. GAD may be accompanied by physiological or somatic symptoms, including trembling, twitching, muscle tension, irritability, hot flashes, nausea, frequent urination and fatigue ⁷⁵. Symptoms must interfere with some aspect of daily functioning to meet the diagnostic criteria of GAD ⁴⁶. Within the clinical setting, nurses may observe children with GAD engage in excessive attempts to seek approval from their parents or other adults. Whereas younger children report anxiety pertaining to specific situations, older children increasingly report “generalized” anxiety about a number of different situations ⁶⁹.

Symptoms of anxiety:

According to Lang's multiple-systems theory of emotion, symptoms are of a cognitive (e.g., worry thoughts), physiological (e.g., racing heart rate), or behavioural (e.g., avoidance) nature. The cognitive component of anxiety is related to the anxious thoughts that develop in response to cognitive distortions in the attention, interpretation, and memory components of information processing ⁷⁶.

The physiological component of anxiety disorders consists of the associated autonomic or somatic sensations. Although individuals experience physiological arousal symptoms in response to feared situations, individuals with anxiety disorders experience physiological symptoms that are excessive in duration or intensity for the particular situation or stimulus ⁷⁷.

Sleep-related problems are more prevalent among clinically anxious youth and are associated with increased anxiety severity and interference in family functioning. In a recent study of sleep-related problems in children with generalized anxiety disorder (GAD), separation anxiety disorder (SAD),

and/or social phobia. Alfano reported that the most common sleep-related problems were insomnia, nightmares, and refusal/reluctance to sleep alone ⁷⁸.

The following table presents a complete list of the most common physiological symptoms associated with anxiety disorders ⁴⁶.

Table: 1 Common physiological symptoms associated with anxiety disorders

Systems	Symptoms
Cardiac	Accelerated heart rate, Heart palpitations, Chest pain Shortness of breath, Heart pounding
Gastrointestinal	Difficulty swallowing, Nausea, Diarrhoea, Gastrointestinal discomfort, Frequent urination
Respiratory	Shortness of breath, Smothering sensation Choking sensation, Dry mouth.
Neurological	Numbness, Tingling Trembling/Shaking
Temperature regulation	Sweating, Hot flashes Chills, Cold, clammy hands
Vestibular system	Dizziness, Light-headedness, Faintness, Feeling unsteady
Sleep related problem	Insomnia, Reluctance/Refusal to sleep alone Nightmares, Talks/Walks in sleep, Excessive tiredness
Other	Exaggerated startle response, Muscle tension

The behavioural component of anxiety refers to the action that individuals take to prevent exposure to feared stimuli or to reduce anxiety associated with exposure to the feared stimuli. Among the most common behavioural symptoms associated with the anxiety disorders is avoidance, in which individuals avoid specific stimuli (e.g., bridges) or situations (e.g., public speaking) to prevent anticipated harm. Avoidance often leads to impairment in maintaining normal routines or in family, academic and/or social domains of functioning. Another behavioural symptom associated primarily with obsessive-compulsive disorder (OCD) is the engagement of rituals (e.g., hand washing) that serve to reduce anxiety. These rituals are either excessive or unrealistic strategies for preventing the feared situation from occurring ⁷⁹.

Treatment ⁸⁰:

Antianxiety drugs include the benzodiazepines and the nonbenzodiazepines

. Benzodiazepines:

1. Alprazolam.
2. Chlordiazepoxide.
3. Clorazepate.

4. Diazepam.
5. Lorazepam.
6. Oxazepam
7. Flurazepam

All benzodiazepines are classified as Schedule IV in the Controlled Substances Act by the Drug Enforcement Agency (DEA) regulations.

Nonbenzodiazepines:

1. Zolpidem
2. Zolpiclone
3. Zoleplon

Atypical Anxiolytics:

1. Buspirone
2. Ipsapirone
3. Gepirone

Mechanism of Action:

Benzodiazepines (once thought to be acting as 'non-specific depressants') act selectively on GABA_A receptors, which mediate fast inhibitory synaptic transmission throughout the central nervous system (CNS). Benzodiazepines enhance the response to GABA by facilitating the opening of GABA-activated chloride channels. They bind specifically to a regulatory site of the receptor, distinct from the GABA-binding site, and act allosterically to increase the affinity of GABA for the receptor. Single-channel recordings show an increase in the frequency of channel opening by a given concentration of GABA, but no change in the conductance or mean open time, consistent with an effect on GABA binding rather than the channel-gating mechanism. Benzodiazepines do not affect receptors for other amino acids, such as glycine or glutamate³.

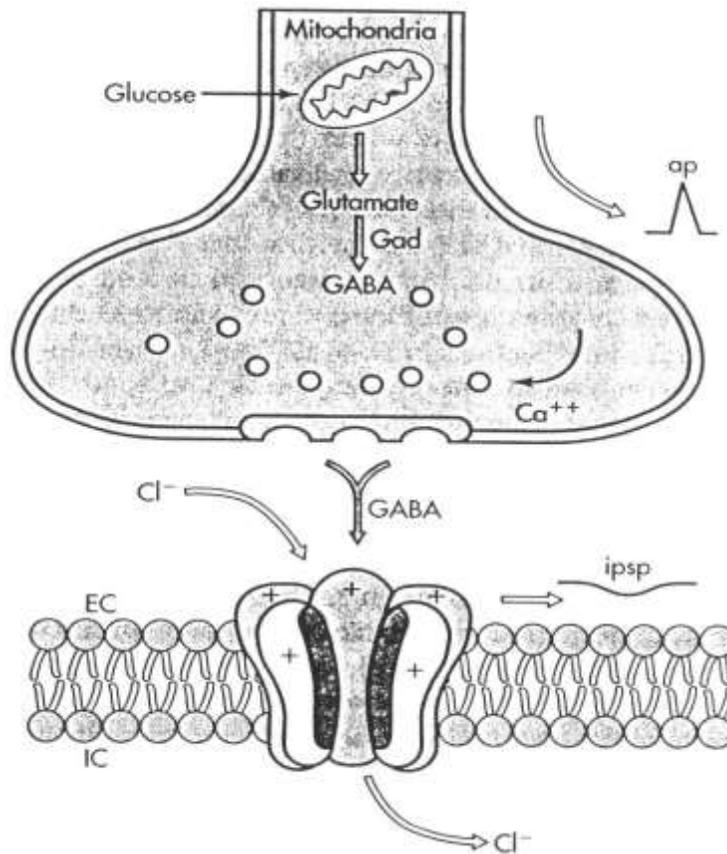


Figure: 3 Mechanisms of Action Benzodiazepines

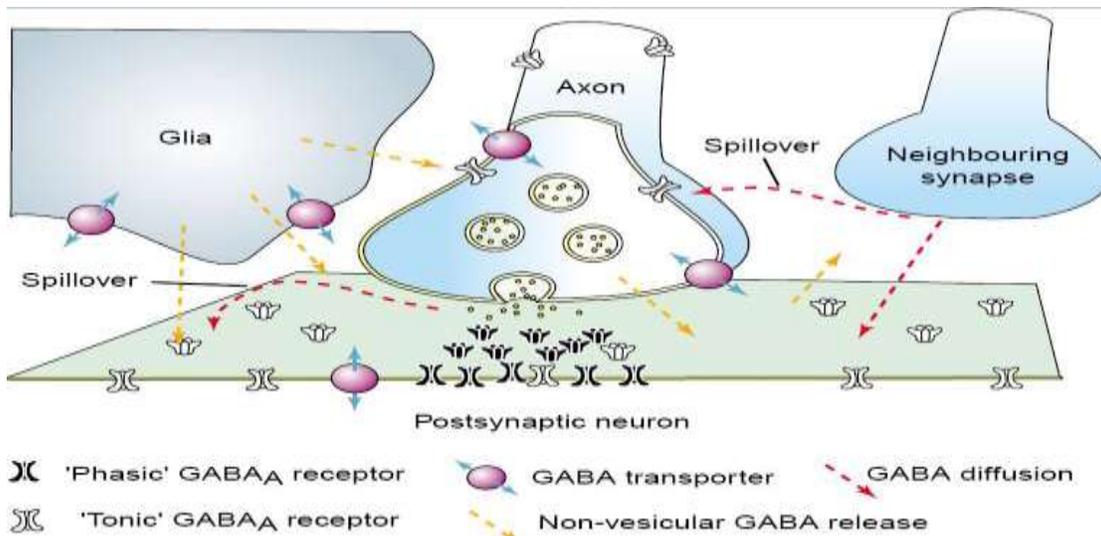


Figure:4 GABA transportin

Uses:

Antianxiety drugs are used in the management of anxiety disorders and short-term treatment of the symptoms of anxiety. Long-term use of these drugs is usually not recommended because

prolonged therapy can result in drug dependence and serious withdrawal symptoms. Some of these drugs may have additional uses as sedatives, muscle relaxants, anticonvulsants, and in the treatment of alcohol withdrawal. For example, clorazepate and diazepam are used as anticonvulsants.

Adverse reactions:

Transient, mild drowsiness is commonly seen during the first few days of treatment with antianxiety drugs. Discontinuation of therapy because of the undesirable effects of the antianxiety agent is rare. Depending on The severity of anxiety or other circumstances, it may be desirable to allow some degree of sedation to occur during early therapy. Other adverse reactions include lethargy, apathy, fatigue, disorientation, anger, restlessness, constipation, diarrhoea, dry mouth, nausea, visual disturbances, and incontinence. Some adverse Reactions may be seen only when higher dosages are used.

Dependence:

Long-term use of antianxiety drugs may result in physical drug dependence (addiction) and tolerance (increasingly larger dosages required to obtain the desired effect). Withdrawal syndrome has occurred after as little as 4 to 6 weeks of therapy with a benzodiazepine. Withdrawal syndrome is more likely to occur when the benzodiazepine is taken for 3 months or more and is abruptly discontinued. The antianxiety drugs must never be discontinued abruptly because withdrawal symptoms, which can be extremely severe, may occur. The onset of withdrawal symptoms is usually within 1 to 10 days after discontinuing the drug, with the duration of withdrawal symptoms from 5 days to 1 month.

Symptoms of Withdrawal:

Increased anxiety	Fatigue
Hypersomnia	Metallic taste
Concentration difficulties	Fatigue
Headache	Tremors
Numbness in the extremities	Nausea
Sweating	Muscle tension and cramps
Psychoses	Hallucinations
Memory impairment	Convulsions (possible)

Contraindications:

The antianxiety drugs are contraindicated in patients with known hypersensitivity, psychoses, acute narrow-angle glaucoma, and shock. These drugs are also contraindicated in patients in a

coma or with acute alcoholic intoxication with depression of vital signs. The benzodiazepines are Pregnancy Category D drugs, and the drug metabolite freely crosses the placenta. Use of these drugs during pregnancy is contraindicated because of the risk of birth defects or neonatal withdrawal syndrome manifested by irritability tremors and respiratory problems. The benzodiazepines are contraindicated during labor because of reports of floppy infant syndrome manifested by sucking difficulties, lethargy, and hypotonic. Lactating women should also avoid the benzodiazepines because of the effect on the infant, who becomes lethargic and loses weight.

Precautions:

Antianxiety drugs are used cautiously in patients with impaired liver or kidney function and in elderly and debilitated patients. The metabolism of the benzodiazepines is slowed in the liver, increasing the risk of benzodiazepine toxicity. Lorazepam and oxazepam are the only benzodiazepines whose elimination is not significantly affected by liver metabolism. Two nonbenzodiazepines are Pregnancy Category B drugs (buspirone and zolpidem); hydroxyzine is a Pregnancy Category C drug. No adequate studies have been performed in pregnant women. These drugs should be used during pregnancy only when clearly needed and when the potential good would outweigh any harm to the fetus.

Interactions

Central nervous system (CNS) depressants such as alcohol, narcotic analgesics, tricyclic antidepressants, and the antipsychotic drugs, increase the sedative effects of the antianxiety drugs. Combination of any of these drugs with the antianxiety drugs is dangerous and can cause serious respiratory depression and profound sedation. Ingestion of alcohol with the antianxiety drugs can cause convulsions and coma. Buspirone causes fewer additives CNS depression than do the other antianxiety drugs. However, it is recommended that concurrent use with a CNS depressant be avoided. Buspirone may increase serum digoxin levels, which increases the risk of digitalis toxicity.

The use of animal behavioural models to assess anxiety

Introduction:

Animal models of psychiatric diseases attempt to capture various feature of the human condition, from behavioral and physiological changes that are indicative of the emotional state to the disease and the effects of therapeutic intervention. According to Mc.Kinney, animal models are “experimental preparation developed in one species for the purpose of studying phenomena occurring in another species. In the case of animal models in human psychopathology one seeks to

develop syndromes in animals which resemble those of human in certain ways in order to study selected aspects of human psychopathology". Currently, the third criteria is regarded as having heuristic value because the central nervous processes that lead to anxiety still have to be elucidated; therefore this criterion is regarded as desirable, but not essential. Thus, in an ideal and perfect model one would like to have causative conditions, symptom profiles and treatment response identical to those seen in the human disease state ⁸¹.

The anti-anxiety and antipsychotic indicate a qualitative distinction in the clinical use and mode of action of the drug. Pathological anxiety in man has been defined by its interference with normal functions, by manifestations of somatic disorders, emotional discomfort, interference with productivity at work, etc. This complex characterization of anxiety in man already indicates the difficulties to find appropriate pharmacological models. Therefore, several tests have to be performed to find a spectrum of activities which can be considered to be predictive for therapeutic efficacy in patients.

For *in vivo* studies, most investigators use a battery of anticonvulsive tests, anti-aggressive tests and evaluation of conditioned behaviour. Most of the actions of benzodiazepines are thought to be mediated by potentiation of g-amino-butyric acid (GABA). Two subtypes of GABA receptors (GABA_A and GABA_B) have been described. Moreover, specific binding sites for benzodiazepines have been discovered near these GABA receptors in various areas of the brain. These sites occur in a macromolecular complex that includes GABA-receptors, benzodiazepine receptors and receptors for other drugs, and a chloride channel. The benzodiazepines potentiate the neurophysiological actions of GABA at the chloride ion channel by increasing the binding of GABA to GABA_A receptors. This implies that the GABA_A receptor is involved in anxiety and that its direct activation would have an anxiolytic effect. Based in these findings various *in vitro* tests have been developed ⁸².

Anxiety enables the individual to recognize danger and to deal with an unknown or vague internal or external threat. Fear is a similar alerting signal, but differs from anxiety in that it is regarded as response to a known, definite, non-conflictual threat. Clinicians assessing anxiety distinguish between "normal" and "pathological" anxiety. Normal anxiety is an advantageous response to a threatening situation that accompanies many aspects of daily life. By contrast, pathological anxiety is an inappropriate response to an external or internal stimulus. In light of the high complexity of anxiety disorders and the comorbidity with major depressive disorder, the chance of succeeding in developing comprehensive animal models that accurately reflect the relative influences of contributing factors in human is probably quite poor ⁸³.

Animal behavioural models of anxiety:

Animal behavioural models of anxiety-disorders are unlikely to contribute significantly to our understanding of the neuroanatomical basis of the more subjective elements of normal and pathological anxiety. In contrast, brain imaging studies in the living human brain are more likely to provide important information in this area. Nevertheless animal behavioural models have yielded much information on the neuroanatomical structures and pathways involved in the expressive and evaluative components of anxiety⁸⁴.

Major animal behavioural tests currently used in anxiolytic drug research⁸⁵ –**Conflict-punishment tests**

Punished instrumental behaviour -

Response-contingent shock

Fear of punishment

Punishment of spontaneous behaviour-

Punished drinking (consumption)

Four-plate test (exploration)

Conflict without punishment-

Dark-light chamber

Elevated plus maze

Food consumption (novel environment, unfamiliar food)

Social interaction

Frustration (non-reward) and aversive stimulation-

Defensive burying.

Aversive PAG stimulation.

Anxiety induced by anxiogenic chemicals.**Withdrawal anxiety.****Ultrasonic distress cries.**

Animal tests of anxiety can be divided into several categories⁸⁶. These include those based on conditioned fear or conflict, those in which anxiety is generated by novel environments and those in which anxiety is generated by pharmacological agents or by electrical stimulation of discrete brain areas.

Conflict tests have been widely used. The paradigm involves repeated alternating exposure to two different experimental schedules. In the Geller-Seifter test rats are trained to press a lever to obtain a food reward. In the punished schedule the rats also receive an electric shock signalled by a light

whereas in the unpunished schedule pressing for the food reward is not punished by electric shock. A similar protocol exists in the Vogel test but in this case thirsty rats are trained to drink from a spout. In both these tests it is presumed that the behavioural suppression (reduced response rate) that occurs in the punished 'conflict' component of the schedule is a result of the animal anticipating the punishment. Anxiolytic drugs such as the benzodiazepines increase rates of punished but not unpunished responding⁸⁴.

In contrast to conflict tests, tests involving exposure to novel environments do not depend on the rat being conditioned to fear a stimulus that is linked to primary drive behaviour such as eating or drinking. Instead, these ethologically based tests rely on the rodent's innate fear of novel environments and measure natural behaviours. For example, the social interaction test⁸⁷ relies on the social behaviour of rats to decrease under aversive situations. In this test the factors that modify behaviour are the lighting level and the familiarity with the environment. Another test, the elevated plus or X maze⁸⁸, is based upon the relative degree of exploration into the open and enclosed arms of the elevated maze. The rationale being that the open arms are more fear-provoking than the enclosed ones. The light-dark box exploits the rodent's natural aversion to bright areas compared to darker ones. In this model animals make fewer crossings to the light side than the dark one⁸⁹.

Drug discrimination procedures have been employed to model chemically-induced anxiety⁹⁰. This model uses a two-choice situation in which pressing on one lever will produce a food reward after a saline injection and pressing on the other will result in a food reward after injection of an anxiogenic drug. This model is considered to be a good model for revealing something about the subjective drug experience.

It is clear from the aforementioned discussion that the different models measure different aspects of anxiety. Moreover, the components of anxiety (i.e. the subjective experience, the evaluation procedure and the expression of anxiety) are likely to differ amongst the models. For example, in some, such as the conflict tests, conditioning is an important component whereas in others, such as the plus-maze, animals have to make a decision about exploring areas with different levels of aversion. These different components will no doubt involve recruitment of different neuronal systems in the different models, although there are also likely to be common neuroanatomical structures and pathways involved. Given that the different models measure different forms of anxiety with a different neuronal basis. The clinically effective anxiolytics and those under investigations as potential anxiolytics, displays different behavioural profiles in the different models.

It is also importance comment that many animal models are influenced to model state anxiety and as such may be questioned for their usefulness in the development of drugs for the treatment of generalized anxiety-disorders. However, it is not easy to devise models for trait anxiety. One possibility is the use of genetically 'anxious' strains of animals, although as yet there has been little validation of the use of such strains ⁸⁴.

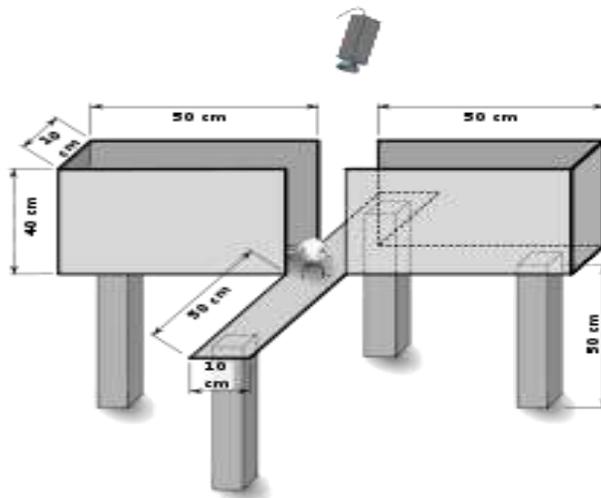


Figure:5 Plus maze apparatus



Figure: 6 Dark night apparatus

METHODOLOGY

Materials:

Plant material:

The plant *Solanum nigrum* belongs to the family Solanaceae. The description, history, cultivation and constituents of which have been already described. The plant is largely found in the Tamilnadu. The leaves of the plant are collected from the local area of Guntur district in the winter. The plant was authenticated by Dr.Satyanarayana Raju (M.Sc., M.Phil., Ph.D.) , plant

taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna nagar, Guntur-522510,A.P

Drugs:

Diazepam I.P

Reagents:

Benedict's reagent.

Barfoed's reagent.

Millon's reagent.

Dragendroff's reagent.

Hagers reagent.

Mayer's reagent.

Wagner's reagent.

Chemicals:

All chemicals used were of analytical grade.

Petroleum ether.

Chloroform.

Ethanol.

Instrument:

- Elevated plus maze apparatus.
- Social interaction test box.
- Light/dark test box.
- Soxhlet apparatus.
- Double distillatory.
- Electronic weighing balance.
- Oral feeding needle.

Animals:

Albino male Wister rats and albino mice weighing between 150 to 200g and 18 to 22 gm respectively were procured from registered breeders (1242/PO/BC/08/CPCSEA/GENTOX BIO SERVICES PVT LIMITED, Hyderabad.). The animals were housed under standard conditions of temperature $25 \pm 20^{\circ}\text{C}$ and relative humidity 30- 70% with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (Nutrivet life sciences, sinhad road, Pune-411051) and water

ad libitum. Approval of CPCSEA for the Institutional Animal Ethics Committee (IAEC) of AM Reddy Memorial College of Pharmacy, Narasaraopet was taken for conducting anxiolytic activity.

Methodology:

Extraction ⁹¹:

The plants collected were carefully protected and leaves were separated. The leaves were carefully washed with tap water and left to dry for 15 days in the shade at room temperature. Then they were stored in well sealed cellophane bags, so as to prevent from the environmental effects. The shade dried powdered leaves were used for the extraction with Petroleum ether, Chloroform, Ethanol and Distilled water. In each case, the powder weighing approximately 225-250 gm was extracted by adding 1000 ml of the solvents. The duration of extraction varies with the solvent and was found to be between 08-12 h with all the solvents. The extract was filtered and the filtrate evaporated to dryness under reduced pressure using a rotary evaporator.

Qualitative chemical test ^{92,93}:

Preliminary phytochemical investigation of extract:

Qualitative chemical tests were conducted for petroleum ether, chloroform, ethanolic and aqueous extracts of leaves of *Solanum nigrum* to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded and tabulated in results (Table 2).

Tests for Carbohydrates:

Molisch's test (General test): To 2-3 ml aqueous extract, few drops of α -naphthol solution in alcohol was added, shaken and concentrated H₂SO₄ was added from the sides of the test tube. It was observed for violet ring at the junction of two liquids.

For Reducing Sugars:

a) **Fehling's test:** 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one min. Equal volume of test solution was added. Heated in boiling water bath for 5-10 min and observed for a yellow, then brick red precipitate.

b) **Benedict's test:** Equal volume of Benedict's reagent and test solution (T.S.) in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in test solution.

Test for Monosaccharides:

Barfoed's test: Equal volumes of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Observed for red precipitate.

Test for Hexose Sugars: Cobalt-chloride test: 3 ml of test solution was mixed with 2ml cobalt chloride, boiled and cooled, added few drops of FeCl_3 and NaOH solution. Solution was observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

Tests for Non-Reducing Sugars:

- a) Test solution does not give response to Fehling's and Benedict's test.
- b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

Tests for Proteins:

- a) **Biuret test:** (General test): To 3 ml T.S. added 4% NaOH and few drops of 1% CuSO_4 solution and observed for violet or pink colour.
- b) **Millon's test:** (for proteins): Mixed 3 ml T.S. with 5 ml Millon's reagent, white precipitate obtained. Precipitate warmed turns brick red or precipitate dissolves giving red color.
- c) **Xanthoprotein test:** (For protein containing tyrosine or tryptophan): Mixed 3ml T.S. with 1 ml concentrated H_2SO_4 , observed for white precipitate.
- d) **Test for protein containing sulphur:** Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled, turns black or brownish due to lead sulfide formation.

Tests for Steroids:

- a) **Salkowski Reaction:** To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4 was added. Shook well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.
- b) **Liebermann-Burchard Reaction:** Mixed 2ml extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops concentrated H_2SO_4 from the side of test tube, observed for first red, then blue and finally green colour.
- c) **Libermann's reaction:** Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 , observed for blue colour.

Tests for Amino Acids:

- a) **Ninhydrin test** (General test): 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min and observed for purple or bluish colour.
- b) **Test for Tyrosine:** Heated 3 ml T.S. and 3 drops Millon's reagent. Solution was observed for dark red colour.
- c) **Test for Tryptophan:** To 3 ml T.S. added few drops glycoxalic acid and concentrated H_2SO_4 observed for reddish violet ring at junction of the two layers.

Tests for Flavonoids:

- a) **Shinoda test:** To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.
- b) To small quantity of residue, added lead acetate solution observed for Yellow colored precipitate.
- c) Addition of increasing amount of sodium hydroxide to the residue was observed as to whether it showed yellow colouration, which was decolourised after addition of acid.
- d) Ferric chloride test: To test solution, added few drops of ferric chloride solution observed for intense green color.

Tests for Alkaloids:

- a) **Dragendroff's test:** To 2-3 ml filtrate added few drops Dragendroff's reagent and was observed for orange brown precipitate.
- b) **Mayer's test:** 2-3 ml filtrate with few drops Mayer's reagent was observed for precipitate.
- c) **Wagner's test:** 2-3 ml filtrate with few drops of Wagner's reagent was observed for reddish brown precipitate.

Tests for Tannins and Phenolic Compounds:-

To 2-3 ml test solution, added few drops of following solutions and was looked for respective coloration or precipitate:

- a) 5% Ferric chloride solution: - Deep blue-black colored.
- b) Lead acetate solution: - White precipitate.
- c) Bromine water: - Decoloration of bromine water.
- d) Acetic acid solution: - Red color solution.
- e) Potassium dichromate: - Red precipitate.
- f) Dilute iodine solution: - Transient red color.
- g) Dilute Nitric acid: - Reddish to yellow color.

Tests for Glycosides:

General test for Glycosides:

Part A: To 2-3 ml of extract dil H_2SO_4 was added and heated on a water bath for 1- 2 min. Neutralise with 10% NaOH, check with litmus paper and to resulting solution add Fehling's A and B. Increased red precipitate in this case shows glycosides are present.

Part B: To 2-3 ml of extract, water was added and heated. According to need, NaOH was added for neutralization and also added equal quantity of water. To the resulting solution added Fehling's A and B. Increased red precipitate in this case showed glycosides are absent. Compare Part A and B.

Tests for Cardiac Glycosides:

a) Baljet's test: The test solution was observed for yellow to orange color with sodium picrate.

b) Legal's test (For cardenoloids): To aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside, observed for pink to red color.

c) Test for deoxysugars (Kellar Killani test): To 2 ml extract added glacial acetic acid, one drop of 5% FeCl₃ and concentrated H₂SO₄, observed for reddish brown color at junction of the two liquid and upper layers bluish green.

d) Libermann's test (For bufadenolids): Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H₂SO₄ observed for blue color.

Tests for Saponin Glycosides:-

a) Foam test: The drug extract or dry powder was shaken vigorously with water. Persistent foam was observed.

Methods:**Acute oral toxicity study ⁹⁴:**

Acute oral toxicity study for the formulation was carried out using **OECD guideline 420 (modified, adopted 23rd march 2006)**. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD₅₀, confidence intervals. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Principle of the FDP:

The fixed dose procedure is method for assessing acute oral toxicity that involve the identification of a dose level that cause evidence of non-lethal toxicity (termed evident toxicity) rather than a dose level that cause lethality. Evident toxicity is terms describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would result in the development of sever toxic signs and probably mortality.

Procedure: As suggested, after acclimatization of animals for 4-5 days, study was carried out as follows:

- Healthy, young adult Albino Swiss female mice (18-25gm), nulliporous and non pregnant were used for this study Food, but not water was with held for 3-4 hours and further 1-2 hours post administration of sample under study.
- Fixed dose level of 5, 50, 500 mg/kg were initially chosen as dose level that would be expected to allow the identification of dose producing evident toxicity.

- During the validation procedure, a fixed dose of 2000mg/kg was added to provide more information on substance of low acute toxicity.
- Dosed one animal at the test dose by oral route.
- Since, this first test animal survived, four other animals were dosed (orally) as subsequent days, so that a total of five animals were tested.

Observation:

After the administration of aqueous and ethanolic extracts, animals were observed individually during the first 30 min and periodically during 24 hours with special attention during the first four hours and daily thereafter for a period of 14 days. Once daily animals were observed principally in relation to changes in skin, fur, eyes and mucous membrane (nasal) and also autonomic symptoms like sedation, lacrimation, perspiration, piloerection, urinary incontinence and control nervous system (ptosis, drawsiness, gait tremors and convulsion).

Elevated plus maze model:

Elevated plus maze (EPM) one of the commonly used animal model (exteroceptive aversion stimulus model) for testing anti anxiety drugs was employed to assess anxiolytic activity of sample under study. EPM is based on the apparent natural aversion of rodents to open and high spaces animals have tendency to spend more time in enclosed arms than in the open arms⁷⁵.

Procedure:

Albino mice of either sex weighing between 18-25 gm were dividing into six experimental groups of six animals each.

- Group I - Control (2% gum acacia).
- Group II- Standard drug (Diazepam 2mg/kg i.p.)
- Group III- Ethanolic extracts dose EESN1 (200 mg/kg p.o).
- Group IV- Ethanolic extracts dose EESN2 (400 mg/kg p.o).
- Group V- Aqueous extracts dose AESN1 (200 mg/kg p.o).
- Group VI- Aqueous extracts dose AESN2 (400 mg/kg p.o).

Standard drug diazepam was administered 45 min prior to testing and extracts were administered p.o 45 min prior to testing. Anxiolytic activity was measured using the elevated plus maze test⁷⁶. The maze consisted of two open (28 cm x 5 cm) and two closed (28 cm x 5 cm x 14 cm) arms, extending from the central platform (5 cm x 5 cm) and elevated up to the height of 40 cm above the floor. The entire maze was made of clear Plexiglass. Mice were individually placed on the centre of the maze facing an open arm, and the number of entries and the time spend in

closed and open arm were recorded during a 5 min observation period. Arm entries were defined as entry of all four paws in to the arm. The percentage of open arm entries ($100 \times \text{open}/\text{total}$ entries) was calculated for each animal.

The light/dark transition test:

The light/dark transition test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodent in response to mild stressors, that is, novel environment and light ⁷⁸. A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in novel situations. Thus, in the light/dark test, drug induced increase in behaviour in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity ⁷⁹.

Procedure:

Albino mice of either sex weighing between 18-25 gm were dividing into four groups of six animals each. Standard drug (Diazepam) was administered 30 min prior to testing and extracts were administered p.o 45 min prior to testing.

- Group I - Control- (2% gum acacia).
- Group II- Standard drug (Diazepam 2mg/kg i.p.)
- Group III- Aqueous and ethanolic extracts dose EESN1 (200 mg/kg p.o).
- Group IV- Aqueous and ethanolic extracts dose EESN2 (400 mg/kg p.o).
- Group V- Aqueous and ethanolic extracts dose AESN1 (200 mg/kg p.o).
- Group VI- Aqueous and ethanolic extracts dose AESN2 (400 mg/kg p.o).

The apparatus for light/dark transition test consist of two compartments: one light area (27 L x 27 W x 27 H cm), illuminated by 100 W desk lamp was painted white, and the other dark area (18 L x 27 W x 27 H cm) was painted black. The two compartments were separated by a partition with a tunnel (7.5 x 7.5 cm) to allow passage from one compartment to the other. The experiments were performed between 09:00 and 14:00. Animal was placed in the centre of the light area with its back to the opening. The following parameter were recorded during 5 min: Latency time for the first crossing to the dark compartment, the number of transition between the light and the dark compartment (tunnel crossing), the total time spent in the light compartment. The apparatus was cleaned thoroughly between trials.

RESULTS AND DISCUSSION

Preliminary Phytochemical testing of *Solanum nigrum* leaves extracts:

The investigation of the preliminary phytochemical qualitative examination of various extracts of *Solanum nigrum* shows the presence of different constituents in Table. 2

Table.2. Preliminary Phytochemical testing of *Solanum nigrum* leaves extracts:

S. No	Chemical Test	Petroleum ether extract	Chloroform extract	Alcohol extract	Aqueous extract
1	Tests for steroids				
	a. Salkowski test	-	-	+	+
2	b. Liebermann Burchard test .	-	-	+	+
	Tests for saponin				
3	a. Foam test	-	+	-	-
	Tests for steroidal saponin				
4	a. Salkowski test	-	+	+	-
	b. Liebermann-Burchard test	-	-	+	+
5	Tests for Alkaloids				
	(a) Mayer's test	-	-	+	+
6	(b) Dragendroff's test	-	-	-	-
	(c) Wagner's test	-	-	+	+
7	(d) Hager's test	-	-	-	-
	Tests for carbohydrates				
8	(a) Molisch's test	-	-	+	-
	Test of reducing sugar				
9	(a) Fehling's test	-	-	-	-
	(b) Benedict's test	-	-	+	+
10	Test for Monosaccharides				
	(a) Barford's test	-	+	+	+
11	Tests for Flavonoides				
	(a) Shinoda test	-	-	+	-
12	(b) Ferric chloride test	-	-	+	+
	(c) Lead acetate test	-	-	+	+
13	(d) Zinc hydrochloride reduction test	-	+	-	+
	Tests for Tannins				
14	(a) Ferric chloride test	-	+	+	+
	(b) Gelatin test	-	-	+	+
15	(c) Bromine water test	-	-	+	+
	(d)Vanillin hydrochloride test	-	-	+	+
16	Tests for Cardiac Glycosides				
	(a) Baljet test	-	+	+	+
17	(b) Legal's test	-	-	+	+
	(c) Kellar-Killiani test	-	-	+	+
18	(d) Lugal's test	-	-	+	+
	Tests for Proteins				
19	(a) Ninhydrin reagent test	-	-	+	+
	(b) Xanthoprotein test	-	-	+	+

9	(c) Biuret test	-	-	+	-
	(d) Millon's test	-	+	+	-
	Test for Amino acids	-	-	-	-
	(a) Ninhydrin test	-	-	-	-
	(b) Test for tyrosine	-	-	-	-
	(c) Test for tryptophan	-	-	-	-

'+' = Present

'-' = Absent

Acute toxicity study:

Acute toxicity studies for ethanolic, chloroform, aqueous extracts of *Solanum nigrum* (*Solanaceae*) were conducted as per OECD guidelines 420 using albino Swiss mice. Each animal was administered aqueous and ethanolic extracts by oral route. The animals were observed for any changes continuously for the first 2 h and up to 24 h for mortality, there were no mortality and noticeable behavioural changes in all the groups tested. The extracts were found to be safe up to 2000 mg/kg body weight.

Elevated plus maze test:

Ethanol extracts of SN on EPM:

The behavioural effects of aqueous extract E ESN1, EESN2, and diazepam, on the behaviour of mice in the elevated plus maze test were summarized in Table 3 and Figure. - 7 to 9.

Diazepam has increased the percentage of time spent and of arm entries in open arms significantly ($P < 0.001$, Figure.ure.7 and 9), whereas in closed arm it has decreased significantly ($P < 0.001$, Figure.8) as compare to control group. It was seen that the EESN1 has increased percentage of time spent in open arm significantly ($P < 0.05$, Figure.ure.7) with no entry in to open arm as compare to control.

Table 3: Effects of ethanolic extracts of SN from elevated plus maze test in mice

Treatment	% open arm time	% closed arm time	% open arm entry
Control	52.6 ± 3.04	216 ± 3.94	4.03 ± 3.02
Diazepam(2 mg/kg)	145.3 ± 0.52 ***	52 ± 1.571 ***	8.8 ± 3.04 ***
EESN1(200 mg/kg)	113.6 ± 3.853*	76 ± 1.22 *	6.9 ± 3.76
EESN 2(400 mg/kg)	134 ± 4.587***	54 ± 3.040 ***	7.0 ± 3.16 ***

The studies with that of EESN2 shows significant results as compare to control group.EESN2 and AESN2 has significantly increased the percentage time spent and arm entry in open arms ($P < 0.001$, Figure.ure.7 and 9) whereas in closed arm it has decreased ($P < 0.001$, Figure. 7 and 9) as compare to control group.

All values are mean ± SEM, (n=6), one way ANOVA, followed by Dunnet's test.

* P < 0.05, **P < 0.01, *** P < 0.001, when compared to vehicle treated group

Effects of aqueous extracts of SN leaves from elevated plus maze test after acute treatment with 200 mg/kg and 400 mg/kg in mice

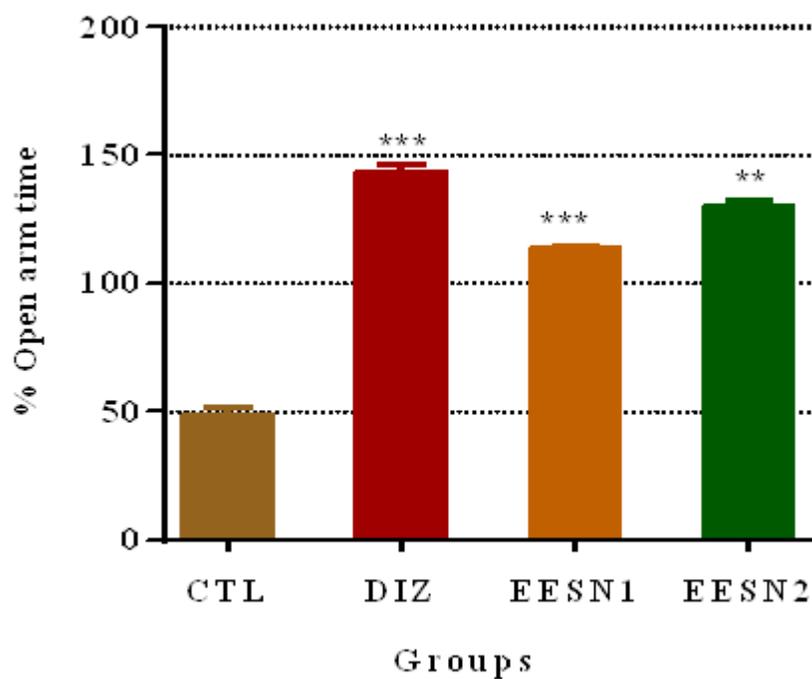


Figure- 7 Percentage of open arm time in 5-min EPM.

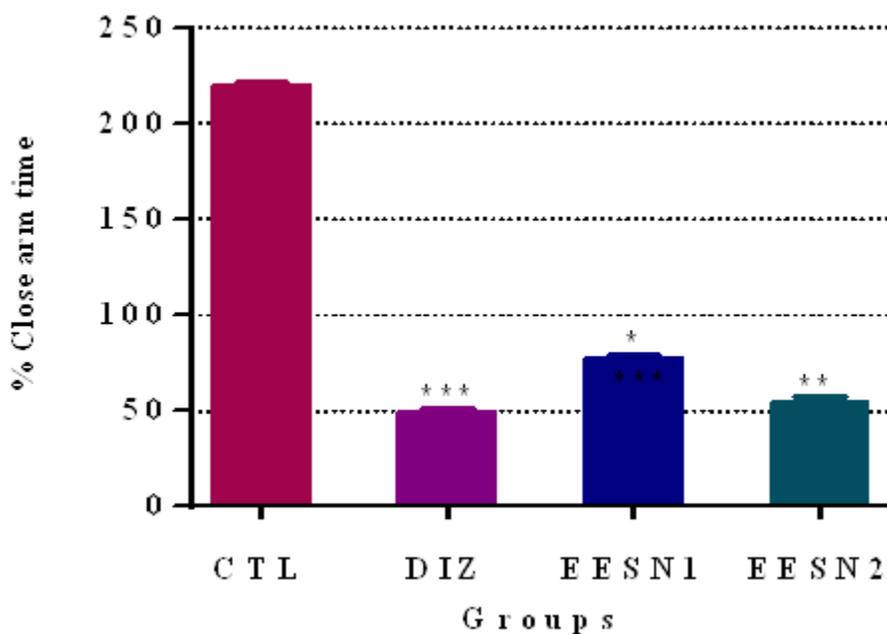


Figure 8: Percentage of closed arm time in 5-min EPM.

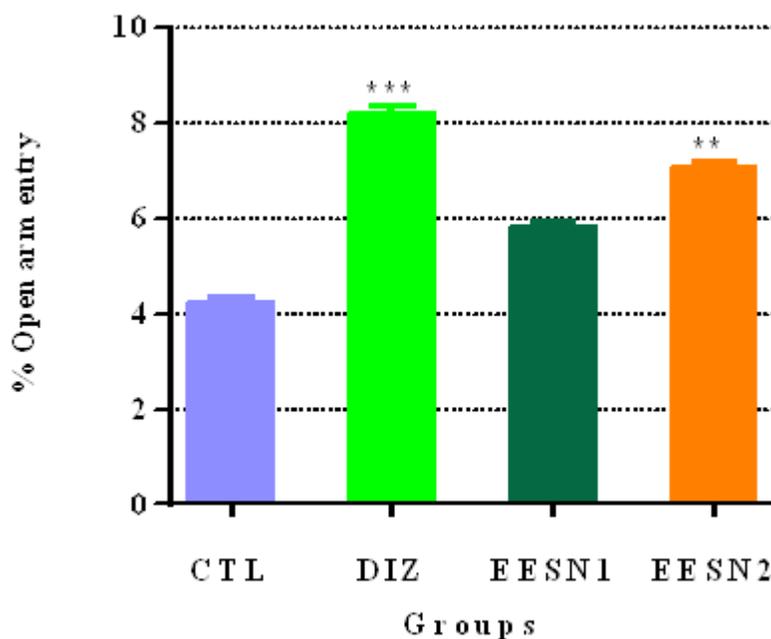


Figure 9: Percentage of open arm entry in 5-min EPM.

Aqueous extracts of SN on EPM:

The effects of ethanolic extract of AESN1, AESN2 and diazepam on the behaviour of mice that were summarized in Table 4 and Figure.10 to12.

Diazepam has increased the percentage of time spent and of arm entries in open arms is significant ($P < 0.001$, Figure. 10and 12), whereas in closed arm it has decreased significantly ($P < 0.001$, Figure 11) as compare to control group.

The AESN1 has shown insignificant in percentage of time spent and arm entry in open and closed. The AESN2 has shown increase in percentage of time spent and of arm entry in open arms significantly ($P < 0.01$ and 0.05 respectively, Figure.ure.10 and 12) and decreased in percentage of time spend and of arm entries in the closed arms ($P < 0.01$ and 0.05 respectively, Figure.ure.11) as compare to control group.

Table 4: Effects of Aqueous extracts of AESN from elevated plus maze test in mice.

Treatment	% open arm time	% closed arm time	% open arm entry
Control	52.6 ± 3.04	216 ± 3.94	4.03 ± 3.02
Diazepam(2 mg/kg)	145.3 ± 3.044 ***	52 ± 6.094 ***	8.8 ± 0.126 ***
AESN1(200 mg/kg)	108.33 ± 3.736*	77 ± 2.418 *	4.75 ± 0.27
AESN 2(400 mg/kg)	116 ± 2.275**	73.66 ± 3.813 **	5.50 ± 0.178**

All values are mean ± SEM, (n =6), one way ANOVA, followed by Dunnet's test.

* $P < 0.05$, *** $P < 0.0001$ when compared to vehicle treated group.

Effects of aqueous of AESN from elevated plus maze test after acute treatment with 200 mg/kg and 400 mg/kg extract in mice.

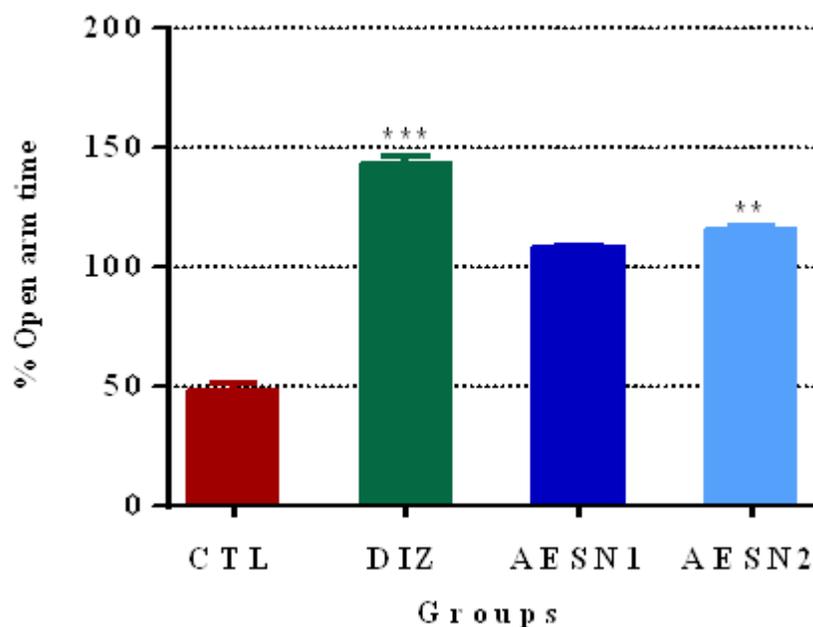


Figure 10: Percentage of open arm time in 5-min EPM.

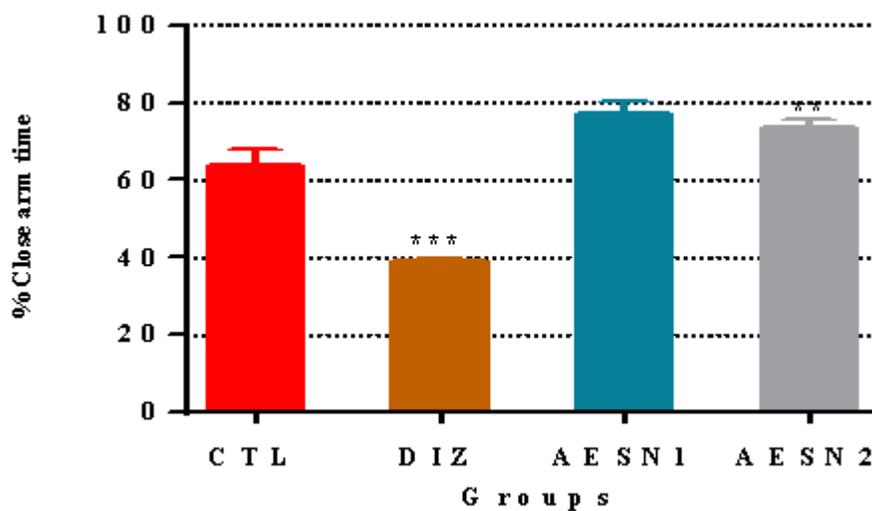


Figure 11: Percentage of closed arm time in 5-min EPM.

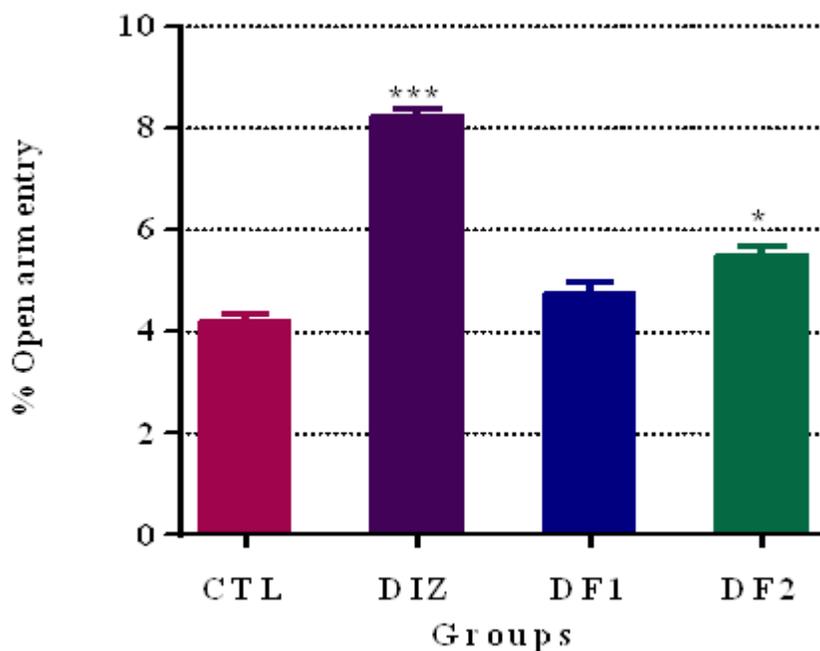


Figure 12: Percentage of open arm entry in 5-min EPM.

The Light/Dark transition test:

Aqueous extract of AESN on L/D test:

Results of the light/dark test of shows in table 5 and figure 13 to 15. Diazepam treatment group showed significantly increased time in light area ($P < 0.001$, Figure 13), latency in to enter dark compartment ($P < 0.01$, Figure 14) and number of tunnel crossing ($P < 0.01$, Figure 15).

Table 5 - Effect of aqueous extract of AESN from light/dark transition test in mice.

Treatment	Social interaction. time (Sec)	Locomotion
Control	58.78 ± 22.05	145 ± 10.74
Diazepam(2 mg/kg)	190.1 ± 23.21 *	111.0 ± 8.73 *
AESN 1(200 mg/kg)	78.51 ± 30.18	133.7 ± 4.41
AESN 2(400 mg/kg)	169.0 ± 24.80 *	134 ± 8.32

All values are mean ± SEM, (n=6), one way ANOVA, followed by Dunnet's test.

* $P < 0.05$, * $P < 0.001$ when compared to vehicle treated group.

The DF1 has not significant in above paradigms as compare to control group, whereas DF2 showed significantly increase the time in light area ($P < 0.01$, Figure 13), latency in to enter dark compartment ($P < 0.05$, Figure 14) and number of tunnel crossing ($P < 0.05$, Figure 15).

Table 6 : Effect of Aqueous extract of DF from light/dark transition test in mice.

Treatment	Time in light area(sec)	Latency to enter dark (sec)	Tunnel crossing
Control	100.0 ± 7.55	23.67 ± 3.28	11.17 ± 0.87

Diazepam 2 mg/kg	155.0 ± 9.89 ***	44.33 ± 3.19**	16.67 ± 0.88 **
AESN 1 200 mg/kg	126.3 ± 9.97	26.00 ± 2.33	10.67 ± 0.88
AESN2 400 mg/kg	142.0 ± 7.13 **	40.00 ± 5.00 *	15.00 ± 1.29 *

All values are mean ± SEM, (n =6), one way ANOVA, followed by Dunnet's test.

* P < 0.05, **P < 0.01, *** P < 0.001 when compared to vehicle treated group.

Effects of aqueous extracts of AESN from Light/dark transition test after acute treatment with 200 mg/kg and 400 mg/kg extract in mice.

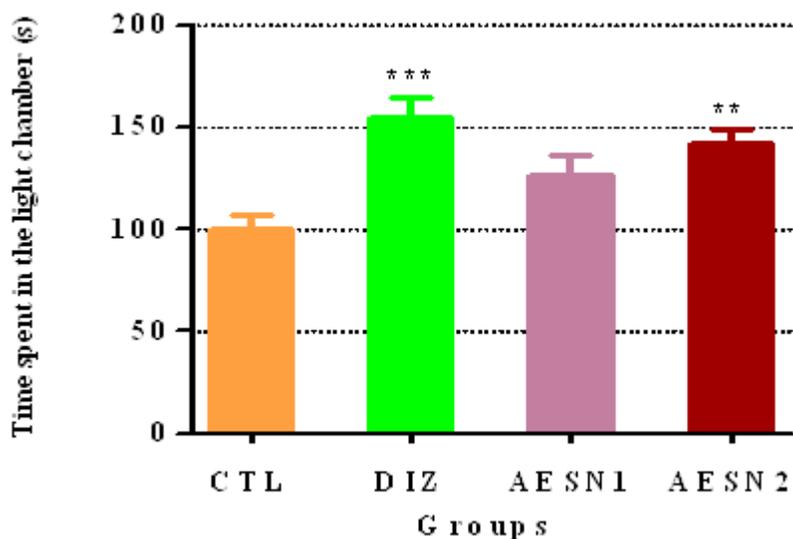


Figure 13- Time spent in the light chamber (sec) in 5-min Light/Dark test.

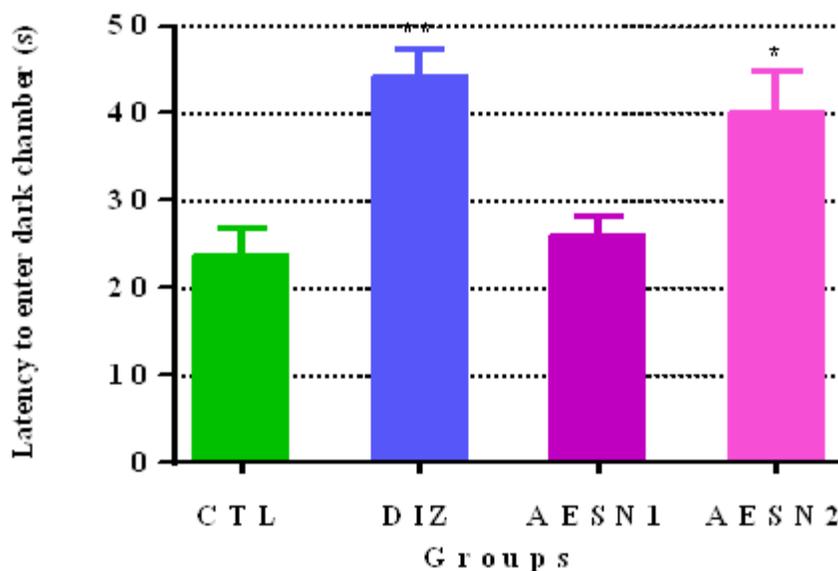


Figure 14- Latency to enter dark chamber (sec) in 5-min Light/Dark test.

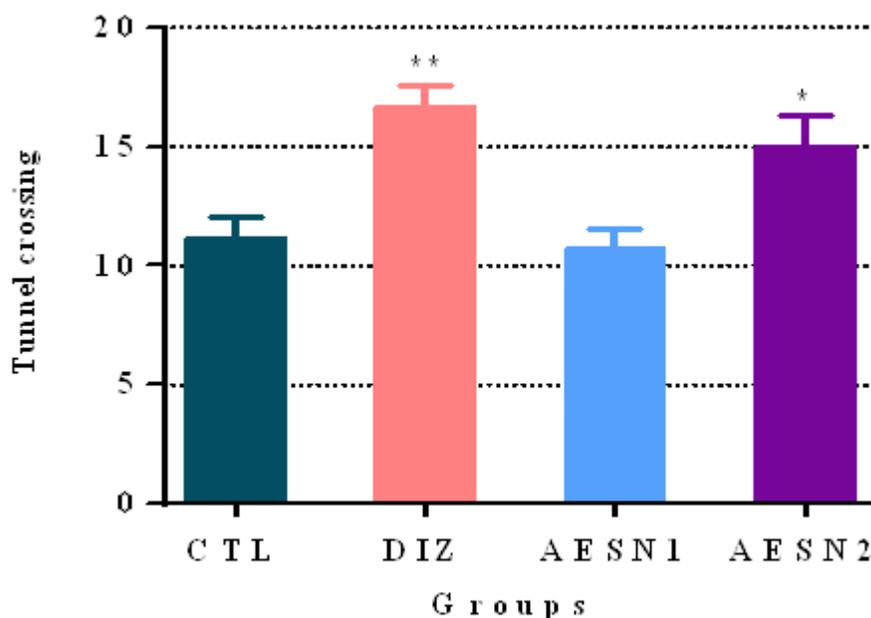


Figure 15- Tunnel crossing in 5-min Light and Dark test.

Ethanollic extract of EESN on light/dark transition test:

Result of the light/dark transition test shown in table 8 and figure 16 to 18. Diazepam treatment group showed significantly increase time in light area ($P < 0.01$, Figure 16), latency to enter dark compartment ($P < 0.01$, Figure 17) and number of tunnel crossing ($P < 0.01$, Figure 18).

The EESN2 was showed significantly increase the time in light area ($P < 0.05$, Figure 16), latency to enter the dark chamber ($P < 0.05$, Figure 17) and the number of tunnel crossing ($P < 0.05$, Figure 18) as compare to control group whereas EESN2 has not significant in above paradigms.

Table 7 - Effect of ethanollic extract of EESN from light/dark transition test in mice.

Treatment	Time in light area(sec)	Latency to enter dark (sec)	Tunnel crossing
Control	70.17 ± 18.15	20.00 ± 4.42	7.66 ± 2.06
Diazepam 2 mg/kg	155.0 ± 9.89 **	47.67 ± 2.97 **	16.77 ± 0.88 **
EESN1 200 mg/kg	104.3±22.92	34.83±4.22	34.83 ± 4.22
EESN2 200 mg/kg	143.8 ± 10.22 *	42.00 ± 6.88 *	14.50 ± 1.64 *

All Values are mean ± SEM, (n =6), one way ANOVA, followed by Dunnet's test. * $P < 0.05$, ** $P < 0.01$, when compared to vehicle treated group.

Effects of ethanollic extracts of EESN from Light/dark transition test after acute treatment with 200 mg/kg and 400 mg/kg extract in mice.

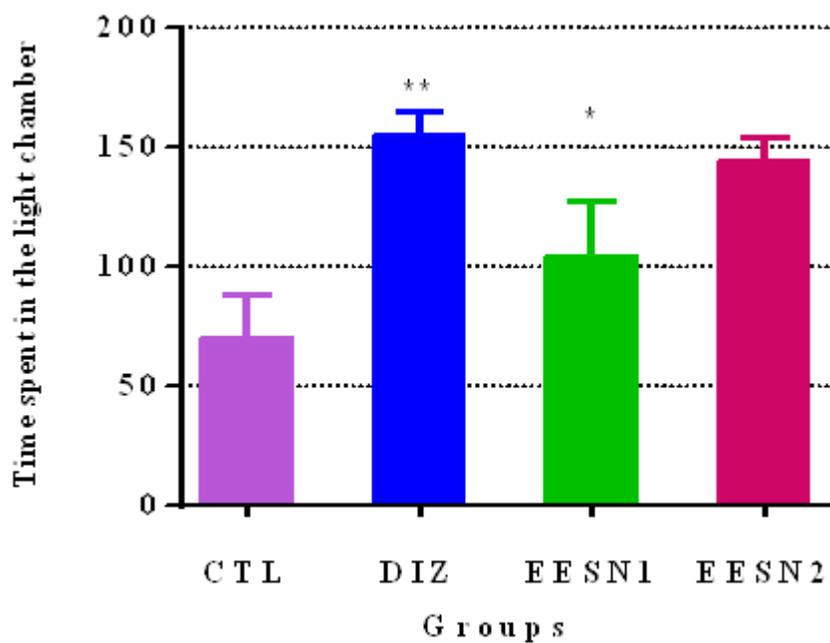


Figure 16- Time spent in the light chamber (sec) in 5-min Light/Dark test.

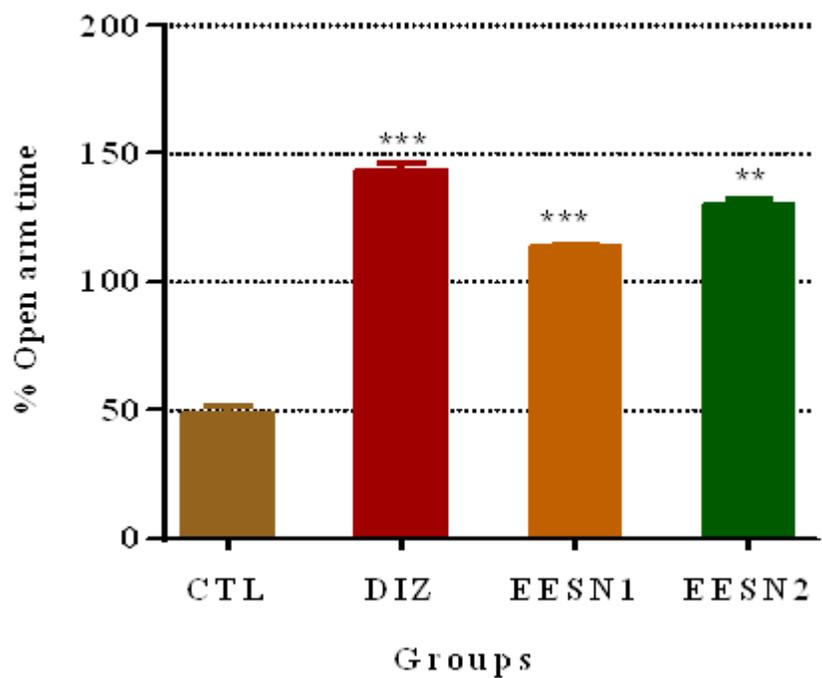


Figure 17: Latency to enter dark chamber (sec) in 5-min Light and Dark test.

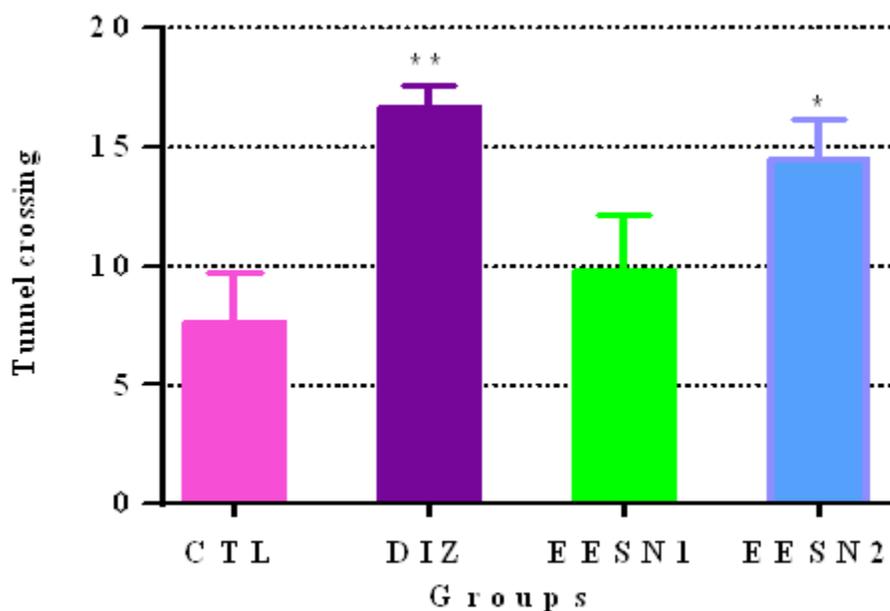


Figure 18: Tunnel crossing in 5-min Light and Dark test.

DISCUSSION

The study was to evaluate the anxiolytic effect of ethanolic and aqueous extracts of *Solanum nigrum* leaves by using behavioural animal models of anxiety. The major finding of present investigation propose the anxiolytic activity on elevated plus maze test, light/dark transition test in mice

The elevated plus maze is currently one of the most widely used model of animal anxiety and it's validated and use in rats and mice ⁷⁶. The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than evoked by the exposure to an enclosed arm ³. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in to the open arm are sensitive to agents thought to act via the GABA_A receptor complex, justifying the use of diazepam as a positive control in this study ⁶², even when the compound being screened does not act via benzodiazepine receptors. Diazepam increases the percentage of entries and the time spent in the open arm confirms its anxiolytic effects. The aqueous and ethanolic extract of *Solanum nigrum* leaves had similar effects on these parameters. The aqueous extract dose AESN1 shows increase percentage of time spent in open arm but not entry whereas EESN1 ethanolic extract is insignificant as compare to control. The AESN2 (400 mg/kg) of aqueous and ethanolic extracts had increased the percentage in time spent and entry in to open arm with decreased in closed arm.

It can be suggested that SN (400 mg/kg) of aqueous and ethanolic extracts may have the anxiolytic effects similar to the standard drug as result animal spent more time in open arm and less time in closed arm. EESN1 (200 mg/kg) of ethanolic extract did not alter the above parameter significantly therefore it does not exhibit anxiolytic effect. There for behavioural alteration induced by higher dose SN (400 mg/kg) of aqueous(AESN2) and ethanolic extract(EESN2) and lower dose of EESN1 (200 mg/kg) of aqueous extract were consistent with dose dependant anxiolytic profile.

Light/dark box is another widely used rodent anxiety model for screening anxiolytic or anxiogenic drugs. It is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors that is novel environment and light ⁷⁸. Drugs induced increase in behaviour in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of anxiolytic activity ⁷⁹. In this study, the time spent in light area, latency to enter dark chamber and tunnel crossing is an indices of anxiety. The aqueous extract of AESN2 (400mg/kg) and ethanolic extract of EESN2 (400mg/kg) had significantly increased the time spent in light area, latency to enter dark chamber and tunnel crossing, similar to standard drug, suggesting that anxiolytic activity of EESN leaves extract as compare to control group..

The phytoconstituent like flavonoids (Apigenin) were reported for their anxiolytic effect and these constituents were present in aqueous and ethanolic extracts of *Solanum nigrum* leaves, so this active principle might be responsible for anxiolytic effect. The mechanism of anxiolytic activity of *Solanum nigrum* leaves extracts may be due to flavones like apigenin specifically recognise the central BDZ Receptors and it has been found that flavones like apigenin bind with high affinity BDZ site of the GABA_A receptor ⁹⁶ all these reports support the fact that apigenin act as ligand for control BDZ receptors exerting anxiolytic effects . There for the anxiolytic effect of aqueous and ethanolic extract of *Solanum nigrum* could be related atleast in part to flavanoids or specifically to apigenin.

CONCLUSION

The results obtained from these experimental models clearly confirmed that the anxiolytic activity of aqueous and ethanolic extracts of *Solanum nigrum* leaves. The acute toxicity study conducted for aqueous and ethanolic extracts indicates that they are safe up to 2000mg/kg body weight.

The acute treatment with aqueous extract of AESN2 (400mg/kg) clearly demonstrate a dose dependant anxiolytic effect comparable to standard diazepam (2mg/kg; i.p) in all experimental

models of anxiety, while EESN2 (400mg/kg) of ethanolic extract had significant anxiolytic activity comparable to standard drug diazepam (2 mg/kg; i.p) in all models.

The phytoconstituent like flavonoids (Apigenin) were reported for their anxiolytic effect and these constituents were present in aqueous and ethanolic extracts of *Solanum nigrum* leaves, so this active principle might be responsible for anxiolytic effect.

SUMMARY

The study was complete to investigate the anxiolytic property of aqueous and ethanolic extracts of *Solanum nigrum* leaves on elevated plus maze test, light/dark test as behavioural models of anxiety. In this study the 200mg/kg and 400mg/kg dose of aqueous and ethanolic extracts has selected after end of fixed dose procedure of acute oral toxicity study and the dose 2000mg/kg; p.o is safe. In phytochemical investigation of aqueous and ethanolic extracts of *Solanum nigrum* leaves, showed the presence of flavonoids(apigenin) and tannins. The phytoconstituents like flavonoids (apigenin) were reported for their anxiolytic effects⁹⁵, so this active principle might be responsible for anxiolytic effects. The results of both extracts by mentioned above models are summarised that in elevated plus maze test of aqueous and ethanolic extracts of 400mg/kg has shown significantly increase percentage of time spent and of entry in open arms.

In light/dark transition test 400mg/kg dose of aqueous extract significantly increase the time spent in light chamber, latency to enter dark chamber and tunnel crossings whereas in ethanolic extract 400mg/kg dose is significant. It is now clear that the aqueous and ethanolic extracts have significant anxiolytic activity which was comparable to diazepam.

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