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STUDY OF ANTI ANXIETY ACTIVITY OF PIPER NIGRUM FRUIT IN SWISS ALBINO MICE

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ABSTRACT

Anxiety is a feeling of fear. Anxiety is may be due to biochemical changes in brain. It is natural to be anxious about our upcoming test or to worry about our financial condition. In animal model study, it has been seen that height, hunger, loneliness, disease, thrust, threatening condition, violence, pain, darkness, and different stimulus such as current, heat, cold, pinch, and self-image are used for the development of anxiety. Thus, different models that have been made by researchers are based on different principle and methodology so we cannot judge the efficacy of any reference or experimental drug. One common principle among all of these models is that they are based on the behaviour of experimental animal. The anti-anxiety activity of ethanolic extract of piprer nigrum L fruits was tested by Elevated Plus maze test model in swiss albino mice. Animals were divided into 4 groups, each group contain 5 animals and orally administered with Normal Saline, Standard Drug, Test 1 and Test 2 drug. Duration of immobility was observed for 5 minutes. It has been observed that both the doses Test 1 (200 mg/kg) and Test 2 (400 mg/kg) showed significant ($P<0.05$) anti-anxiety activity in test models. In these animal model, the anxiety-related behaviors in mice were significantly decreased indicates that anxiety in mice was relieved after treatment with extract. The ethanolic extract of fruit has significant anti-anxiety activity in animal models.

Keywords:-*Anti-anxiety, Plus maze model, piprer nigrum L, Fruit, Animal study, Black pepper*

1. INTRODUCTION

Anxiety is a fearful feeling expressed in a single word. The object of fear may be real or imagined, but common reasons of worry include exam anxiety, financial planning for family survival, a previous occurrence, the fear of standing in front of a crowd, and speaking in front of them. Worries, doubts, and fears are all part of the human experience. It's reasonable to be concerned about a forthcoming exam or our financial situation. Generalized anxiety disorder differs from regular worrying in that it is excessive, intrusive, chronic, and debilitating.[1] The complexity of daily life in modern society frequently leads to varying degrees of anxiety. Anxiety disorders have been found to be associated with chronic pain among hospitalized patients in both developed and developing countries.1 Anxiety disorders, the most prevalent psychiatric illnesses in the general community, are present in 15-20% of hospitalized patients.[2]

Anxiety states are controlled by both inhibitory and facilitatory mechanisms that either counter or favor anxiety states. These neurochemical and neuropeptide systems have been shown to have effects on distinct cortical and sub cortical brain areas that are relevant to the mediation of the symptoms associated with anxiety disorders. Regional brain networks involved in such stress, anxiety, and anxious behaviors may be appropriate targets for actions of anxiolytics. broad range of pharmacologic agents are available to treat anxiety disorders namely Selective Serotonin Reuptake Inhibitors, Selective Nor epinephrine Reuptake Inhibitors, Tricyclic Antidepressants, MonoAmine Oxidase Inhibitors, Buspirone, Benzodiazepines, Hydroxyzine, Antipsychotic, Anticonvulsants and Adrenergic agents.[3]

Sleep disturbance is amongst the most frequent health complaints, which the Physicians encounter. It is popularly known as insomnia. It is defined as persistent difficulty in falling or staying asleep. Sleep is a physiologic recuperative state that can be disturbed by many factors such as illness, stress and noise. Chronic sleep disorder leads to some health repercussions such as slower reactions, poor memorizing, emotional disturbances, and changes in the immune response [4]. Today, sleep disorders have a relatively high prevalence and are a growing public health problem. It is estimated that more than 27% of people worldwide suffer from sleep disorders with difficulty in initiating or maintaining sleep. In addition, it is expected that by the middle of the 21 century, about 31% of all people will be chronic and frequent users of sleep medications [5].

Drugs used to treat anxiety by maintaining the normal calm level of body and brain is known as anti-anxiety drugs. Anti-anxiety agent was formerly called minor tranquilizer and known as anxiolytics tensiolytics. They are chemical agent, which are used to control the effect of stress, discomfort, fearful anticipation, and dysphonia in patients with neuroses and mild depressive state [6].

Anti-anxiety medications are categorized as benzodiazepines, barbiturates, benzodiazepine antagonists, and other hypnotic medicines. Alprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, lorazepam, flumazenil, antidepressant, amobarbital, pentobarbital, phenobarbital, antihistaminic, chlorhydrate, and eszopiclone are some of the anti- anxiety medications. Anti-anxiety drugs act on the GABA receptor, opening the chloride channel and extending its penetration through it. Chloride channels are responsible for the negative charge inside the cell, and after some time the negativity was balanced due to the presence of potassium ion, and thus the cell was no longer anxious [7-8].

Medicinal plants and plant derived products are known to play significant role in primary healthcare and in the management of various ailments since long times. Bioactive plant extracts are the integral part of traditional system of medicine and often exhibit multiple biological activities including antioxidant activities [9]. This has led the researchers for the systemic evaluation of various biological properties of medicinal plants. *Piper nigrum* is a widely used plant in traditional remedies and known for its numerous biological properties. As a result, the current study was designed to access anti-anxiety effect to that of *piprer nigrum L.* in mice model. Many herbal medications have been tried to treat anxiety issues in people over the years. Patients are increasingly contacting herbalists, neuropaths, and other healers in addition to physicians, due to the growing popularity of herbal treatments [10-11].

2. MATERIAL AND METHOD

2.1 Procurement of the plant parts

The dried seed of piper nigrum were collected from the shop of Anant Jain beej bhandar, Chhavani chouraha, Indore, (M.P.).

2.2 Preparation of Extract

The seed will washes with the water and keep it for drying for 2 hours. The collected plant seed has been dried by fan aeration in shade. The air dried plant material will then grinded to reduce them into coarse powder with the help of a suitable grinder. The powder will then subject to extraction with ethanol. The seed of piper nigrum was extracted with the help of soxhlet apparatus [12-13].

2.3 Preliminary phytochemical screening

The phytochemical evaluation has been carried out as per standard methods. The obtained stock solutions were subjected to phytochemical analysis for determination of phytoconstitutes alkaloids, tannins, saponins, steroids, phenols, glycosides, terpenoids, flavonoids [14-16].

2.3.1 Detection of alkaloids:

The extracts were mixed with ammonia and then extracted with chloroform solution. To this dilute hydrochloride acid was added. The acid layer was used for chemical tests for alkaloids.

a) **Mayer's test** (Potassium Mercuric Iodide) the acid layer with few drops of Mayer'sreagent it was observed a creamy white precipitate.

b) **Hager's Test** (Saturated solution of picric acid) the acid layer with Hager's reagent it was observed yellow precipitate.

c) **Dragendroff's test** (Solution of Potassium Bismuth Iodide) Acid layer with few drops of Dragendroff's reagent it was observed reddish brown precipitate.

2.3.2 Detection of Phenols: The extract was dissolved in water and ethanol and was then used for chemical test for phenol.

a) **Ferric Chloride Test:** Extracts was treated with 3-4 drops of 5% ferric chloride solution. The deep blue black colour indicated the presence of phenols.

b) **Lead Acetate Test:** Extract was treated with 3ml of 10% lead acetate solution. A bulky white precipitate indicated the presence of phenolic compounds.

2.3.3 Detection of Flavonoids:

a) **Ferric chloride test**: Alcoholic solution of the extracts was mixed with few drops of neutral ferric chloride solution. The green colour indicated flavonoids.

b) **Lead acetate tests**: Alcoholic solution of the extracts was mixed with few drops of 10 % lead acetate observed yellow precipitate.

2.3.4 Detection of Saponins:

a) **Froth Test:** Extracts was diluted with distilled water to 20ml and was shaken in graduated cylinder for 15 minutes. Formed 1cm layer of foam indicated the presence of the saponins.

b) **Foam Test**: 0.5 gm of extract was shaken with 2ml of water. If foam produced persists for ten minutes, it indicated the presence of saponin.

2.3.5 Detection of tannins:

a) **Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formed white precipitate indicated the presence of tannins.

2.3.6 Detection of Glycosides: Extracts was hydrolyzed with dil. HCL, and then subjected to test for glycosides.

a) **Legal's Test**: To 2 ml of extract with dilute HCl and 2 drops of Sodium nitropruside in few ml of pyridine and 20% sodium hydroxide solution was added. Formed pink to blood red color indicated the presence of Cardiac glycoside.

2.3.7 Detection of Carbohydrates:

a) **Fehling's test: -** Few drops of extract are heated with Fehling's A and B solution. Appearance of orange red precipitate indicates presence of carbohydrates.

2.4 Pharmacological Methods [17-20]

2.4.1 Experimental Animal:

Swiss albino mice, weighing 25-30 gm, were obtained from the Animal house of the Department of Pharmacology of the Swami Vivekanand College of Pharmacy, Indore, India. They are kept in polypropylene cages under 12 hr light/dark cycles, with food and water ad libitum. The experiments will perform between 09:00 am to 04:00 pm. The experimental protocols will approve by the Institutional Animal Ethics Committee and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Animals were housed at four per cage, allow free access to water and food, and maintain under constant temperature (23±1 0C) and humidity (60±10%).

2.4.2 Chemicals;

Diazepam was used as reference standards for anti-anxiety activity.

2.4.3 Toxicological Studies:

Piper nigrum shows toxic effect in the dose more than 2000 mg, so the LD_{50} of piper nigrum is found less than 2000 mg.

2.4.4 Experimental design:

Overnight fasted animals were selected randomly on the day of experiment for administration of vehicle, standard drug and study drug. The animals were acclimatized one hour before for behavioural tests. 30 minutes and 1 hour time interval between drug administration and behavioural tests were maintained in case of intraperitoneally and oral administrations respectively [21-24].

A total number of 24 mice were divided into four groups of six mice each:

Group I: Control (Normal Saline, 1ml/kg)

Group II: Standard (Diazepam, 0.5mg/kg)

Group III: Test (Ethanolic extract of piper nigrum, 200 mg/kg)

Group IV: Test (Ethanolic extract of piper nigrum, 400 mg/kg)

2.4.5 Procedure:

1) The male swiss albino mice, weighing between 25-30 gm were selected for the experiment.
2) Prior to experiment the mice were divided randomly in to four groups. Each group contains 5 mice.
3) First group was treated as Control (Normal Saline) and second group was treated with the Diazepam (0.5mg/kg).
4) Third and Fourth group were treated with ethanolic extract of piper nigrum (200 mg/kg and 400 mg/kg).

3. RESULTS

3.1 Phytochemical Analysis:

The phytochemical analysis of various extracts was performed and presence of flavonoids, phytosterol, carbohydrate, terpenoids, Proteins and amino acids were significant (Table 1).

Table 1: Phytochemical constituents of ethanolic extract of piper nigrum seeds

Sr. No.	Phytochemical Constitutes	Ethanolic extract of piper nigrum
1	Alkaloids	+ve
2	Alcohols	+ve
3	Flavonoids	+ve
4	Phenols	+ve
5	Glycosides	+ve
6	Saponins	-ve
7	Steroids	+ve
8	Carbohydrates	+ve
9	Terpenoids	+ve
10	Proteins and Amino acid	+ve

Note: +ve (Positive), -ve (Negative)

3.2 Invivo Studies

3.2.1 Elevated plus Maze Test:

The elevated plus maze test is most popular test for evaluation of anxiolytic compounds. The elevated plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drug acting at the GABAA benzodiazepine complex. The EPM test is used to evaluate the psychomotor performance and emotional aspects of mice. EPM is considered as one of the well-established model for unconditioned anxiety to detect anxiolytic/anxiogenic like activity by investigating aspects of physiological and pharmacological behavior. In EPM, mice will normally prefer to spend much of their allotted time in enclosed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. In the EPM test increased number of entries and time spent into the open arm are taken as the index/reliable indicators of decreased anxiety or indicating the anxiolytic-like activity of a compound.

The percentage open arms entry with control group was 2.5±0.35 and the percentage time spent in open arms was 28.73±1.14 seconds. With diazepam in group II, the percentage open arms entries was 8.6±0.45 and the percentage time spent in open arm was 56.65±6.13 seconds when compared to control groups. With test group III i.e. extract of seeds 200mg/kg, the percentage of open arm entry was 5.35±0.56 and percentage time spent in open arm was 48.57±2.12 seconds. With test group IV i.e. extract of seeds 400mg/kg, the percentage of open arm entry was 4.73±0.68 and percentage time spent in open arm was 41.23±2.31 seconds.

Table 2: Effect of administration of piper nigrum seeds on mice behavior in elevated plus maze

Drugs	Dose/Treatment	Number of open arm entries	Time spent in open arm (Sec.)
Control	**(1ml/kg)**	2.5±0.35	28.73±1.14

Diazepam	**(0.5mg/kg)**	8.6±0.45	56.65±6.13
Test-I	**(200mg/kg)**	5.35±0.56	48.57±2.12
Test-II	**(400mg/kg)**	4.73±0.68	41.23±2.31

All reading are expressed as mean± S.E.M, Values obtained was compared with Tukeys test and found to be statistically significant *p<0.05.

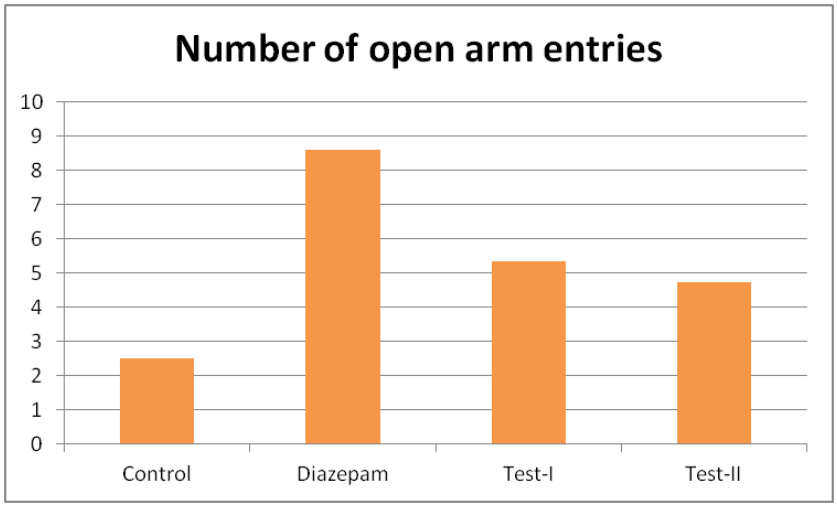

Figure 1: Number of open arm entries in EPM

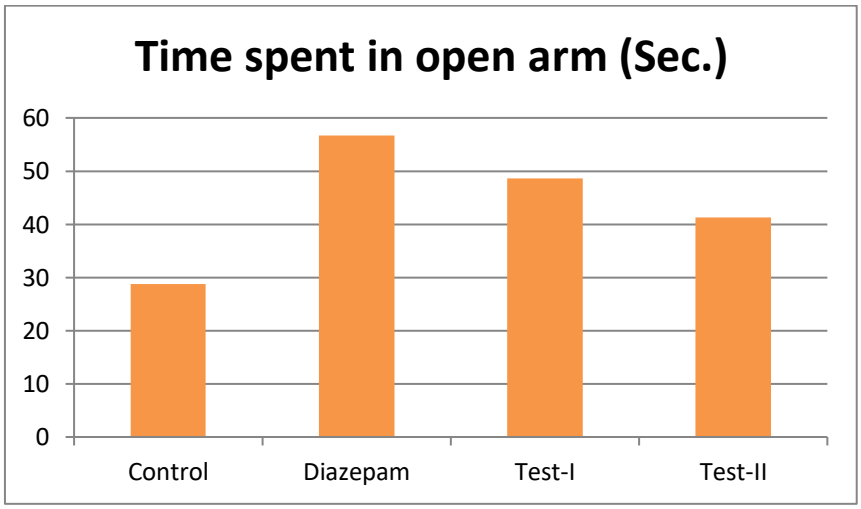

Figure 2: Time spent in open arms

Table 3: Effect of administration of *piper nigrum* seeds on mice behavior in elevated plus maze

Drugs	Dose/Treatment	Number of close arm entries	Time spent in close arm (Sec.)
Control	**(1ml/kg)**	**4.83±1.72**	**37.83±3.34**

Diazepam	**(0.5mg/kg)**	**6.89±1.40**	**16.83±2.65**
Test-I	**(200mg/kg)**	**4.87±1.41**	**24.33±1.56**
Test-II	**(400mg/kg)**	**4.17±1.15**	**26.0±2.63**

All reading are expressed as mean± S.E.M, Values obtained was compared with Tukeys test and found to be statistically significant *p<0.05.

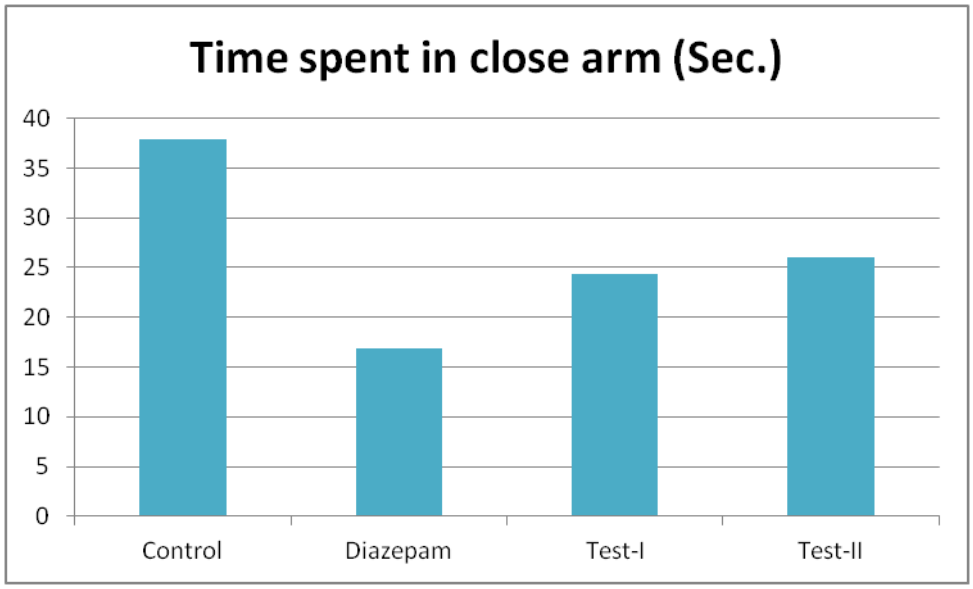

Figure 2: Time spent in closed arms

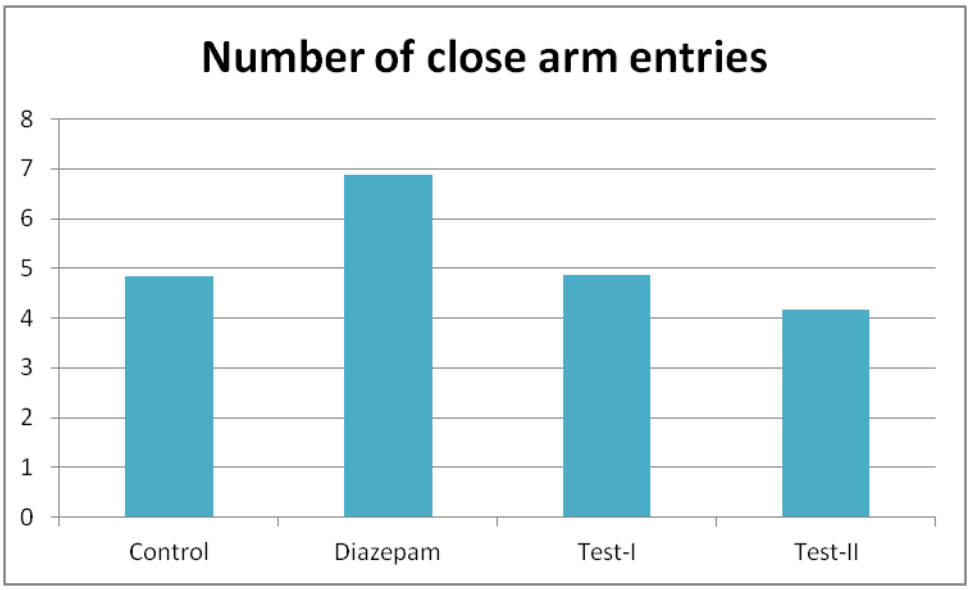

Figure 4: Number of closed arm entries in EPM

3.2.2 Light and Dark Model Test

Table 4: Effect of administration of piper nigrum seeds extract on time spent in light and dark area

Drugs	Treatment	Time spent in Light area(Sec.)	Time spent in Dark area (Sec.)
Control	**(1ml/kg)**	89.49±13.41	241.20±13.51
Diazepam	**(0.5mg/kg)**	188.20±16.32	101.63±14.62
Test-I	**(200mg/kg)**	153.06±11.43	126.02±12.13
Test-II	**(400mg/kg)**	148.68±10.49	130.28±11.39

All reading are expressed as mean± S.E.M, Values obtained was compared with Tukeys test and found to be statistically significant *p<0.05.

Table 5: Effect of administration of piper nigrum seeds extract on transitions and latency in light and dark area

Drugs	Treatment	Latency (Sec.)	Transitions
Control	**(1ml/kg)**	20.62±0.56	21±0.33
Diazepam	**(0.5mg/kg)**	15.33±0.73	13.01±0.34
Test-I	**(200mg/kg)**	18.14±0.41	15±0.24
Test-II	**(400mg/kg)**	16.16±0.54	14±0.32

All reading are expressed as mean± S.E.M, Values obtained was compared with Tukeys test and found to be statistically significant *p<0.05.

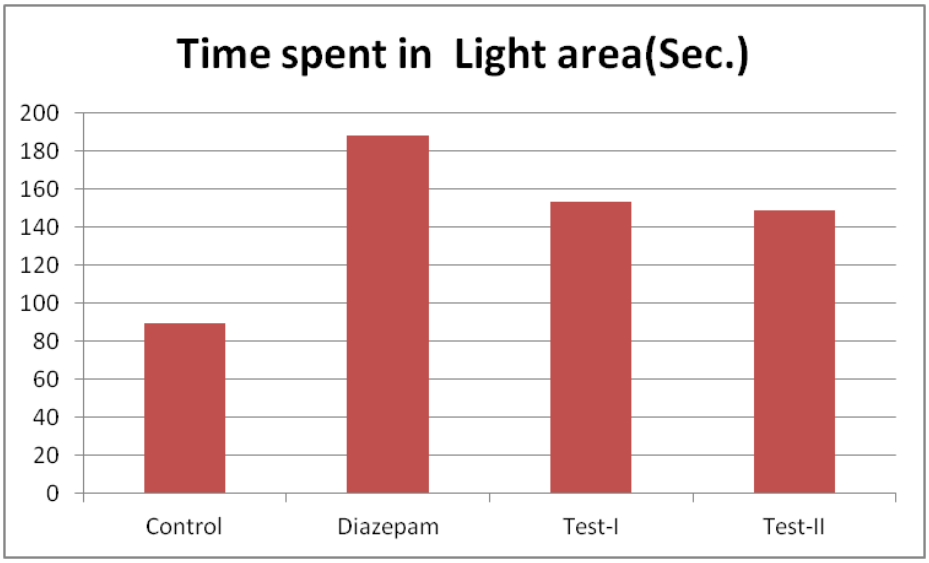

Figure 5- Time spent in Light area (Sec.)

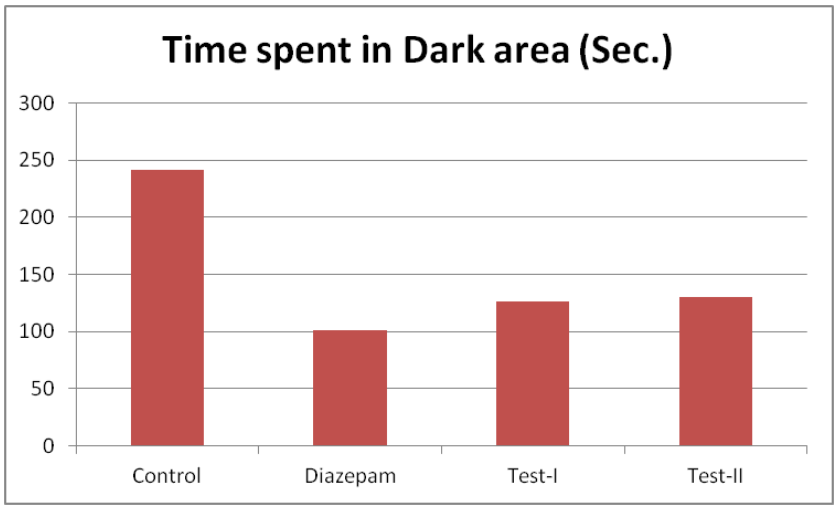

Figure 6- Time spent in Dark area (Sec.)

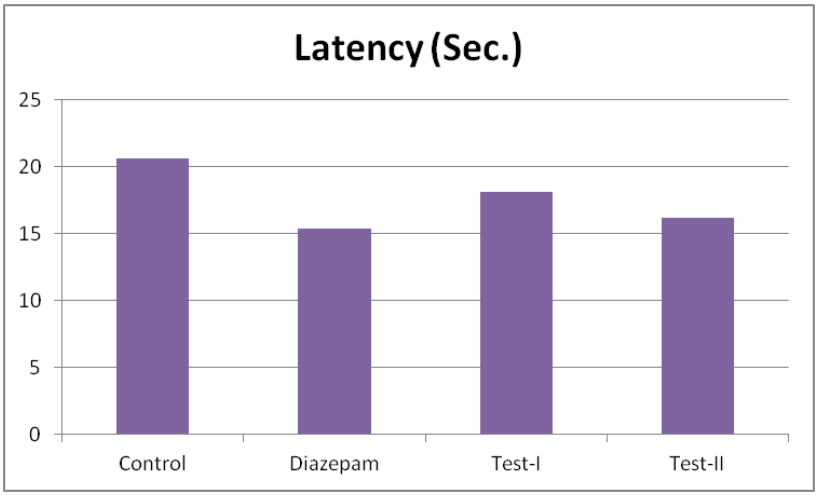

Figure 7- Latency (Sec.)

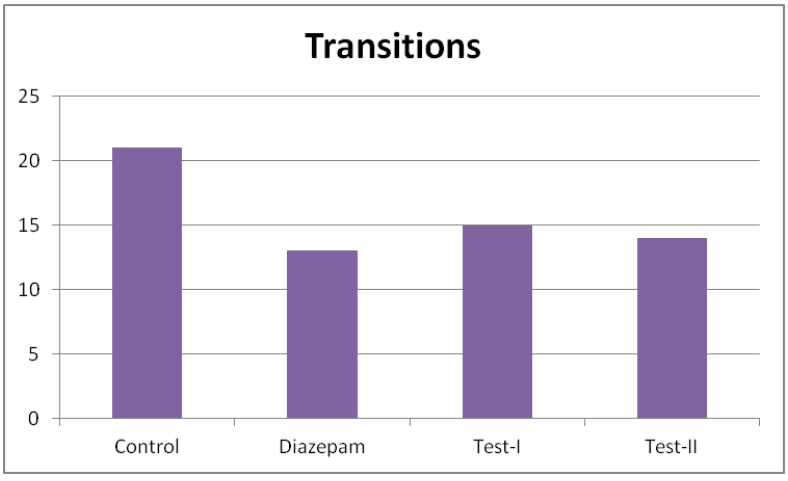

Figure 8- Transitions

4. DISCUSSION

When a person is confronted with a danger or threat, they have an anxiety reaction. Anxiety triggers physical and mental responses that equip each person to respond correctly in a given situation. The anxiolytic efficacy of extract in mice was assessed using the EPM test and the light and dark test in this study. One of the most commonly used models of animal anxiety is EPM test. Anxiolytics enhance the number of times a person enters the EPM's open arms and the amount of time they spend there and it is hypothesized to work through the GABAa receptor complex. According to preliminary phytochemistry, alkaloids, glycosides, flavonoids, tannins, carbohydrate, were found in extract.

The fruits of P. nigrum have been widely used for thousands of years in household spices as condiment, as well as for the treatment of various disorders. Piperine, a major alkaloid of black pepper (P. nigrum L.) used extensively as condiment and flavoring for all types of savory dishes, also presents analgesic, anti-inflammatory, anticonvulsant, antioxidant, antidepressant and cognitive-enhancing effects.

The anxiety indicators in the elevated plus-maze showed up being sensitive to the drugs which were thought to act via the GABAA receptor complex. The therapeutic action of the benzodiazepines and other pharmacological compounds used to treat anxiety, panic, insomnia, and epilepsy is mediated by an enhancement of GABAergic neuronal inhibition through GABAA receptors. Moreover, it is reported that piperine modulates GABA type A (GABAA) receptors. In light with these reports, piperine containing extract have increased anxiolytic-like behavior and exploratory activity in treated animals.

The anti-anxiety efficacy was investigated in two classic animal models i.e. EPM test and the light and dark test. The light and dark test is also commonly used to screen anxiolytic medications in rodents. It has been observed that merely measuring the time spent in the light region, rather than the number of transfers is the most constant and helpful statistic for evaluating an adolescent's performance. Test doses of 200 and 400 mg/kg were employed for pharmacological activity. The animal with test dose of 200 mg spent more time in the light arm and the number of entries are also more as compare to control group in EPM test, while test dose of 400 mg does not show any superior effect than 200 mg dose. The time spent in the light area by the animals is more in case of 200 mg dose as compared to control group and the transition for 200 mg dose was less as compared to control group which shows the less anxiety in treated groups.

5. CONCLUSIONS

In conclusion, findings imply that the plant has anti-anxiety capabilities. Because medicinal plants have been used as a source of medicine since ancient times, and because of the above useful animal study, it can be concluded that the plant extract has strong anti-anxiety action in the Light and Dark Test and Plus Maze Test models of anxiety. Further detailed investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions. It will help in the development of novel and safe drugs for the treatment of disorder.

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