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PHARMACOLOGICAL EVALUATION OF *RUELLIA TUBEROSA* STEM BARK ON ANTIANXIETY ACTIVITY

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ABSTRACT

Anxiety is one of the most common mental disorders, which affects more than 10-15% of the population. It is a psychological and physiological state characterised by somatic, emotional, cognitive and behavioural components associated with significant disability which has a negative impact on the quality of life. The objective of the study was to investigate the antianxiety activity of Methanolic and Ethyl acetate extracts of *Ruellia tuberosa* stem bark. The stem bark of *Ruellia tuberosa* belongs to the family Acanthaceae and used as diuretic, anti diabetic, antipyretic, analgesic, antihypertensive, gastro protective, used to treat gonorrhoea, thirst quenching, antinociceptive and anti-inflammatory. The potential antianxiety activity of *Ruellia tuberosa* was studied in Wistar albino rats. The evaluation of in-vitro antianxiety activity in rats was done using various experimental models Elevated plus Maze test and Light and dark test. The extract administered orally in two doses (200mg/kg and 100mg/kg p.o.) during a 5min period for elevated plus maze test and 10 min for light and dark test evaluation parameters were recorded. Diazepam 2mg/kg i.p. was used as a standard drug. Evaluation of Elevated plus includes No. of entries in open and closed arm, time spent in open and closed arm and time spent in central zone whereas Light and dark test evaluates time spent in light and dark zone, No. of entries in light and dark zone. The Methanolic test rats showed the increased locomotor activity and behavioural changes at a concentration of 200mg/kg and 100mg/kg as compared to ethyl acetate.

Keywords: *Ruellia tuberosa* stem bark, Antianxiety activity, Elevated plus maze test, Light and dark test, Methanol, Ethyl acetate.

I. INTRODUCTION:

Anxiety is an emotion characterized by feelings of tension, worried thoughts and physical changes like increased blood pressure. People with anxiety disorders usually have recurring intrusive thoughts or concerns. A feeling of apprehension and fear characterized by physical symptoms such as palpitations, sweating and stress. One of the most common symptoms is excessive and intrusive worrying that disrupts daily functioning. Other signs or physical symptoms include agitation, restlessness, and fatigue, difficulty in concentrating, irritability, tense muscles and trouble sleeping. According to the WHO study, there are about 10% of people in the world are suffering from acute or chronic anxiety and has become an important area

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of research interest in psychopharmacology during this decade. Anxiety is a universal human experience, it is not a disease, and it is associated with several psychiatric disorders.
There are various types of Anxiety: Generalized anxiety or acute anxiety (It is a simple anxiety, which can be easily diagnosed and treated by some changes in behaviour), Panic or chronic anxiety (It is a result of untreated acute anxiety), Post-traumatic anxiety (It is the fear of any incident, which had already been happened), Obsessive-compulsive disorder (Obsession and compulsions are the important component of OCD) [1].

Drugs used to treat anxiety by maintaining the normal calm level of body and brain is known as antianxiety drugs. Antianxiety agent was formerly called minor tranquilizer they are chemical agent, which are used to control the effect of stress, discomfort, fearful anticipation, and dysphasia in patients with neuroses and mild depressive state. These drugs are used in special condition only because they have tendency to cause addiction and habituation, the patient may become addicted from their regular dose [2].
Several parts of the brain are key factors in the production of fear and anxiety, the amygdala and the hippocampus play significant roles in most anxiety disorders.

The amygdala is an almond-shaped structure deep in the brain that is believed to be a communications hub between the parts of the brain that process incoming sensory signals and the parts that interpret these signals. It can alert the rest of the brain that a threat is present and trigger a fear or anxiety response. The hippocampus is another part of the brain that encodes threatening events into memories.

In addition to the activity of each brain region, it also is important to consider the neurotransmitters providing communication between these regions. Increased activity in emotion-processing brain regions in patients who have an anxiety disorder could result from decreased inhibitory signalling by γ-amino-butyric-acid (GABA) or increased excitatory neurotransmission by glutamate. Any change in the normal level of neurotransmitters like serotonin having function of regulation of mood, aggression, impulses, sleep, appetite and pain, nor-epinephrine having functions of regulation of sleep, mood, B.P and fight or flight response and GABA plays a role in inducing sleep and relaxation also prevent over excitation ,may responsible for anxiety [3].

Anxiety and stress both are different things anxiety can look like stress but the reality isn't so simple. Anxiety can arise as a result of stress, but stress can manifest in other ways. Stressors can make a person sad, angry, worried or anxious, while anxiety is specifically that feeling of fear, dread and apprehension. Stress is often caused by external influences while anxiety is an internal response. That's part of what makes anxiety intrinsically different than stress, and also what makes it so difficult to manage [4].
Benzodiazepine anti-anxiety agents are the most widely prescribed psychotherapeutic drugs. It is believed that benzodiazepines affect the gamma-amino butyric acid (GABA) receptors of the brain. This action results in slowing of the central nervous system (CNS), inducing a state of relaxation. Benzodiazepines are fairly quick-acting, relieving symptoms in a short amount of time. Numerous plants were used as medicines for regulation of anxiety. Various psychological treatments were used to treat anxiety and anxiety like symptoms but none of them accomplish satisfactory response because almost all showed side effects [5].

In the present study *Ruellia tuberosa* L. belongs to the family acanthaceae was used to evaluate antianxiety activity, it is a tropical plant and widely distributed in South East Asia, in folk medicine, it has been used as diuretic, anti diabetic, antipyretic, analgesic, antihypertensive, gastro protective, used to treat gonorrhoea, thirst quenching, antinociceptive and anti-inflammatory agent. It has also recently been incorporated as a component in an herbal drink in Taiwan [6].

A survey of literature revealed that there is no systemic & scientific study on antianxiety activity of *Ruellia tuberosa* stem barks extracts are available. Therefore it was thought worthwhile to explore this indigenous plant for its antianxiety activity.

II. MATERIALS AND METHODS

1. Animals

Albino rats (Wistar Strain) weighing between (150-250 g) were used, for this study. Animals were kept under 12hr light and dark cycles and controlled temperature (24± 2^0 c) and fed with commercial pellet diet and water ad libitum. All the experiments [1613/PO/Re/S/12/CPCSEA] were carried out, reviewed and approved by the institutional animal ethics committee (IAEC).

2. Chemical and Drugs

All the chemicals used in this study were of analytical grade. Diazepam (2mg/kg) was used as a standard drug.

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3. Grouping

Rats were divided into six groups (n = 6 for each group) and were housed in separate cages under controlled conditions of temperature (24 ± 2°c) and humidity (30-70). Grouping of Animals is given below

Group I- Animals will be receive DMSO (Dimethyl sulfoxide 0.5% dissolve in water)

Group II - standard drug (Diazepam 2.0 mg/kg I.P)

Group III- Lower dose of Ethyl acetate extract of *Ruellia tuberosa L.* stem bark (RTEAE 100mg/kg oral)

Group IV - Higher dose of Ethyl acetate extract of *Ruellia tuberosa L.* stem bark (RTEAE 200mg/kg oral)

Group V - Lower dose of Methanolic extract of *Ruellia tuberosa L. stem bark* (RTEE 100mg/kg oral)

Group VI- Higher dose of Methanolic extract of *Ruellia tuberosa L. stem bark* (RTEE 200mg/kg oral)

III. BEHAVIOURAL MODELS FOR EXPERIMENTAL ANTIANXIETY ACTIVITY

1. Elevated plus maze test [7]

Principle:

This model is based on natural behaviour of rodents for open spaces and fear of height. Rodents always tend to avoid the open areas and stay in darker areas, more enclosed spaces. When animal is placed on EPM anxious animals spend more time in enclosed arms and non-anxious animals explore and spend more time on open arms. Anxiolytic compounds by decreasing anxiety, increases the open arm exploration time.

Procedure:

The elevated plus maze consist of two open arms, 35x15x15 cm & two enclosed arms, 35x15x15 cm, that extend from a common central platform; with an open arm roof, arranged so that the two open arms are opposite to each other. The entire maze was elevated to a height of 50 cm above the ground level. The rats were housed in group of six in cages prior to testing in apparatus. During this time the rats were handled by investigator on alternate days to reduce stress. The animals were divided in six groups. Four groups test drug (p.o.) & standard drug (i.p.) were administered 1 hour before testing. After 1 hr rat was placed in centre of maze, facing one of enclosed arms. During a period of 5 min the following parameters were observed by using video tracking system; Number of entries in open arm & enclosed arms, time spent in open arm, enclosed arm & centre, total number of arm entries.

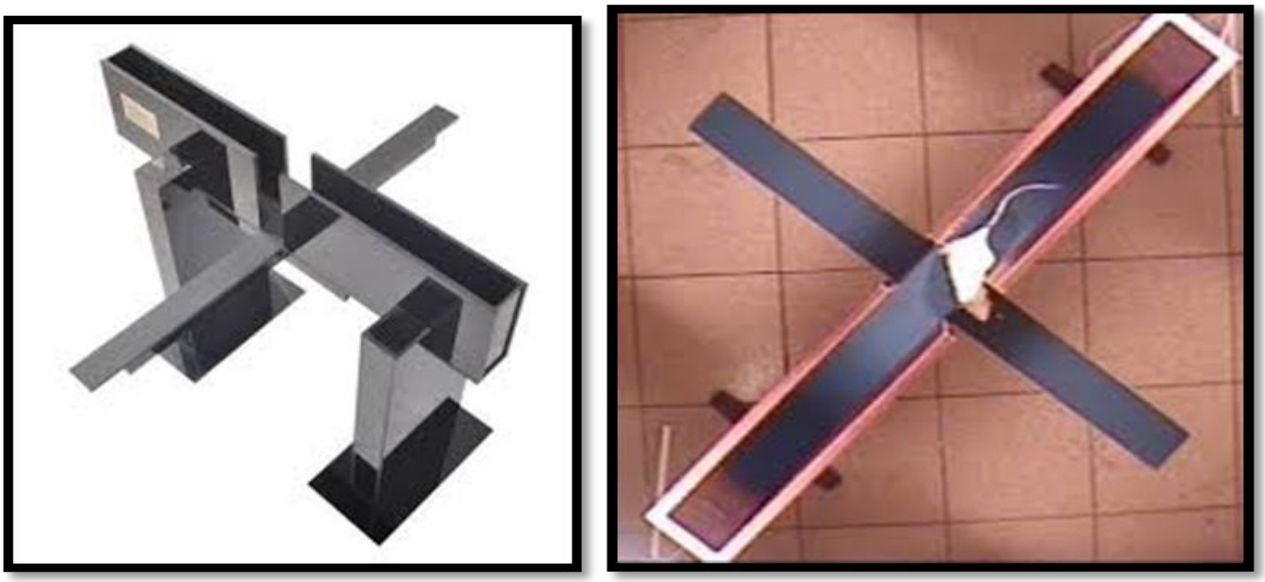

Figure 1 & 2: Elevated plus maze apparatus

Evaluation:

Evaluation of antianxiety activity was done by observing the parameters like number of entries in open & enclosed arm, time spent by the rats in open & enclosed arms & comparing these parameters with that of control group, anxiolytic agents increases the motor activity thereby increase in open arm exploratory time.

2. Light-dark method

Principle:

In a two chambered system, where the animals can freely move between a brightly-lit open field and a dark corner, they show more crossings between the two chambers and more loco motor activity after treatment with anxiolytics. The numbers of crossings between the light and dark sites are recorded.

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Procedure:

The testing apparatus consists of two compartment chamber (47X27X27 cm) comprising of two-third brightly illuminated area & one-third dark area separated by a wall with a round hole (13 cm long X 5 cm high). A partition containing an opening separate the dark one third from the bright two third of the cage. The animals were treated with test drug (p.o.) & standard drug (i.p.) half hour prior testing. The rats were placed individually in the illuminated part of the cage & the electronic video tracking system was used to automatically count movements through the partitions & clocked the time spent in light & dark compartments [8].

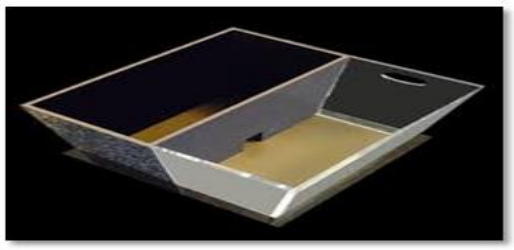

Figure 3: Light and Dark model

Evaluation:

The parameters like time spent in light compartment, time spent in dark compartment, number of crossings between these two compartments & transfer latency of rats were evaluated.

IV. RESULTS:

***Table 1: Ruellia tuberosa* stem bark extracts average readings from Elevated plus maze model**

Treatment	Time spent in open arm (sec)	Time spent in enclosed arm (sec)	No. of entries in open arm	No. of entries in enclosed arm	Time spent in central zone (sec)
DIAZEPAM (mg/kg)	204 ± 4.5	61.6 ± 9.3	28.8 ± 6.3	4.6 ± 1.03	26 ± 3.6
Control	104.8 ± 3.12	178.6 ± 5.6	6.6 ± 1.6	10.5 ± 1.5	17.1 ± 1.9
RT-ME (200mg/kg)	196 ± 3.7*#	103.8 ± 5.2	25.6 ± 2.6*#	5.8 ± 0.7	21.16 ± 2.7
RT-ME (100mg/kg)	191.8 ± 3.4**	140.1± 7.4	21.3 ± 1.8*	7.8 ±1.16	14.83 ± 1.6
RT-EAE (200mg/kg)	158.5 ± 4.3	152.3± 5.3	17 ± 4.05	8.5 ± 1.09	20.8 ± 2.1
RT-EAE (100mg/kg)	131 ± 7.8	169.3 ± 3.9	14.8 ± 2.7	9.1 ± 3.06	14.8 ± 1.4

Values are expressed as mean ± SEM (n = 6).
*#p <0.05, **p<0.01 v/s Control (vehicle) (One-way ANOVA followed by Tukey's test.)

The vehicle treated rat spent less time in open arm (104.8 ± 3.12 s) and more time in enclosed arm (178.6 ± 5.6 s) with 6.6 ± 1.6 entries in open arm and 10.5 ± 1.5 entries in enclosed arm. The RT-EAE (200mg/kg) & RT-ME (200mg/kg) show highly significant decrease in time spent in enclosed arm. Administration of RT-EAE (100mg/kg), RT-EAE (200mg/kg), RT-ME (100mg/kg) & RT-ME (200mg/kg) & Diazepam (2mg/kg) show significant (p <0.01 & p<0.0001) increase in number of entries in open arm than the control (vehicle).

The RT-ME (200mg/kg), RT-ME (100mg/kg) extract and Diazepam (2mg/kg) show significant (p<0.05, p <0.01) increase in the occupancy in open arm indicating antianxiety activity of RT-ME (200mg/kg) and RT-ME (100mg/kg) extract as compared to RT-EAE (200mg/kg) RT-EAE (100mg/kg) extracts.

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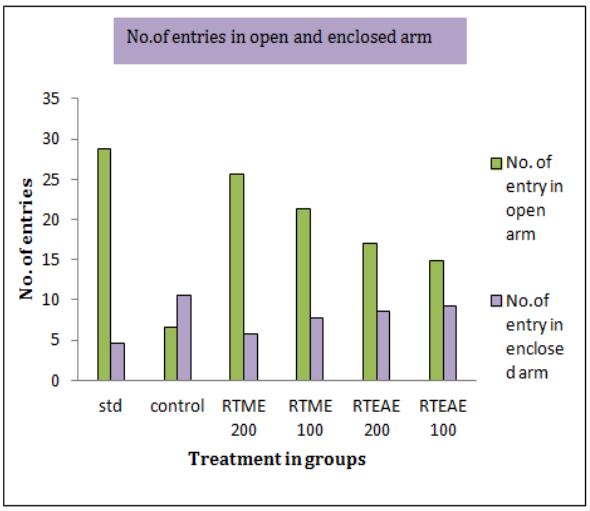

Chart 1: No. of entries in open and No. of entries in enclosed arm in elevated plus maze test.

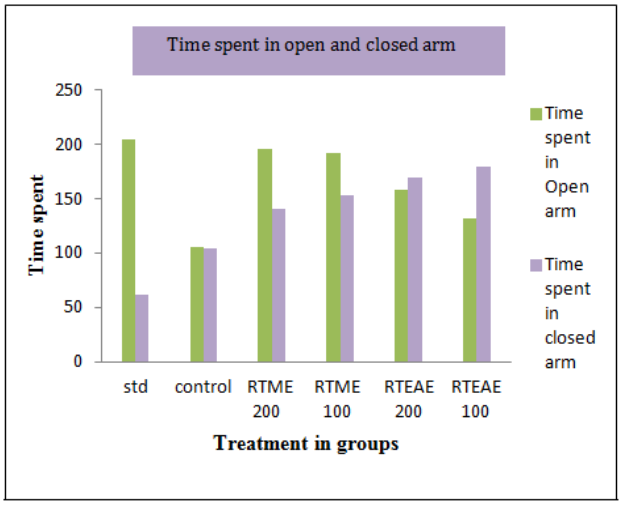

Chart 2: (a) Time spent in open and (b) Time spent in closed arm (in sec) in elevated plus maze test.

***Table 2: Ruellia tuberosa* Linn stem bark extracts average readings from Light & Dark test**

Treatment	No. of crossings	Time spent in dark zone (sec)	Time spent in light zone (sec)	Transfer latency
Diazepam (2mg/kg)	32.33 ± 2.8	87.5 ± 5.6	203.1 ± 3.6	26.5 ± 1.5
Control	13 ± 2.2	191.6 ± 4.17	104.8 ± 4.2	16.8 ± 3.3
RT-ME (200mg/kg)	30.1 ± 3.1	133.1 ± 5.5	197.1 ± 5.3	25 ± 1.4
RT-ME (100mg/kg)	26 ± 3.3	170.6 ± 3.3	192.6 ± 4.2	20.1 ± 1.1
RT-EAA (200mg/kg)	21.8 ± 1.4	159.5 ± 4.8	143.16 ± 4	21 ± 3.5
RT-EAA (100mg/kg)	20 ± 1.4	160.3 ± 5.5	132.5 ± 4	19.3 ± 2.3

Values are expressed as mean ± SEM (n = 6).
**p <0.001,*p<0.05 v/s Vehicle (One-way ANOVA followed by Tukey's test.)

The animal treated with RT-ME (200mg/kg) & Diazepam (2mg/kg) show highly significant (p <0.001) and RT-EAA (200mg/kg) show significant increase in time spent in light zone & decrease in time spent in dark zone. Administration of RT-ME (200mg/kg) & RT-EAE (200mg/kg) show significant decrease in time spent in dark zone as compared to the vehicle group.

Animal treated with RT-ME (200mg/kg) show increase in no. of crossing & transfer latency as compared to vehicle group & RT-EAE (200mg/kg) in light & dark test indicating the antianxiety activity of RT-ME (200mg/kg) & RT-ME (100mg/kg) extract as compared to RT-EAE (200mg/kg) & RT-EAE (100mg/kg) extract.

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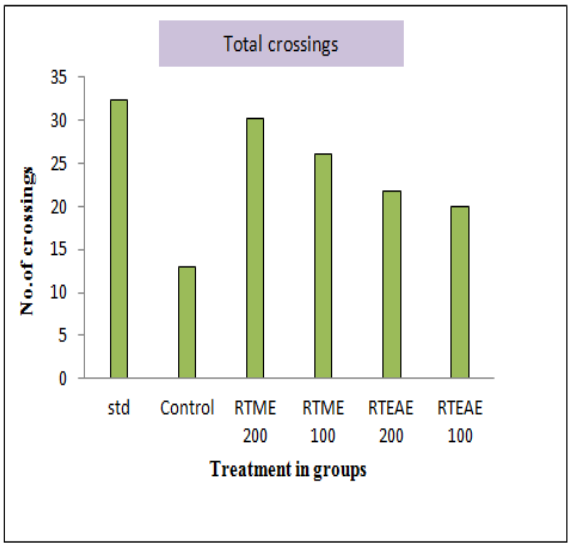

Chart 3: Total crossings in Light and Dark test

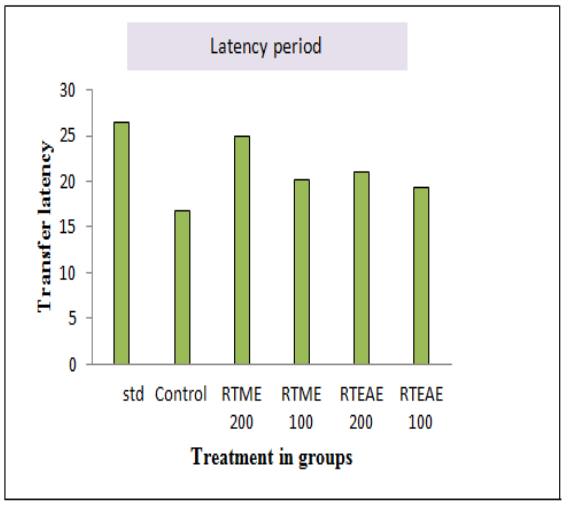

Chart 4: Latency period in light and dark test

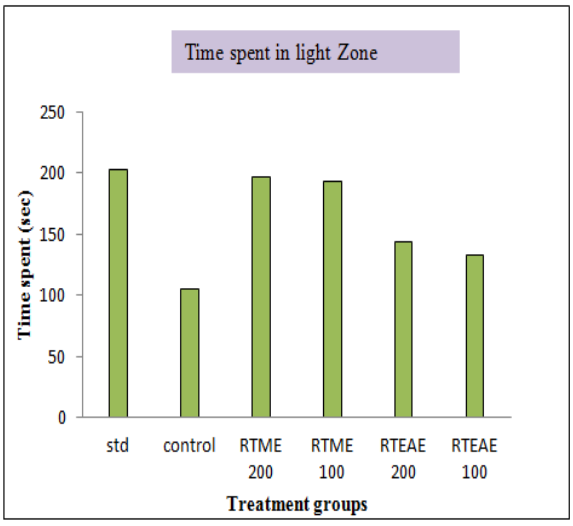

Chart 5: Time spent in light zone

V. DISCUSSION:

Anxiety is a negative emotion that occurs in response to perceived threats that can come from internal or external sources and can be real or imagined. According to the World Health report, approximately 450 million people suffer from serious brain/ mental or behavioural disorder, yet only a few of them receive even the most basic treatment. In the search for new therapeutic agents for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [9]. For centuries, plant and plant products have been used for treating various illnesses. Today, several medicinal plants and their products are still being employed as house remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market.

Ruellia tuberosa L. belongs to family Acanthaceae, a native of Central America, introduced into Indian garden as ornament. *Ruellia tuberosa* is a low-growing perennial herb with tuberous roots, growing to a height of a foot or more. In folk medicine it is considered as diuretic, antidiabetic, antipyretic & analgesic. Studies have suggested that it has anti-inflammatory, antioxidant, antinociceptive, gastro protective properties. It is well known in Marathi as ruwel. After subjecting to phytochemical screening of extracts it showed the presence of different compounds like carbohydrates, proteins, flavonoids, alkaloids, fixed oils, steroids.

For studying *in-vivo* antianxiety activity, two animal models were used, Elevated plus maze model and Light and dark model. 72 rats were required, animals were divided in six groups; control, standard & four test groups (100, 200 mg/kg) each group containing six animals.

The antianxiety activity of *Ruellia tuberosa* stem bark extracts was evaluated by exposing rats in selected models i.e. Elevated plus maze model and Light and Dark model.

In elevated plus maze, rats were allowed to move in apparatus which consist of two open arms & two closed arms for period of 5 min. The fear due to height induces anxiety in animals when placed on EPM. The parameters like number of entries in open arm & enclosed arms, time spent in open arm, enclosed arm & central arm were considered as evaluation parameters of antianxiety activity. Methanolic and Ethyl acetate extract at 200 mg/kg concentration showed increased open exploratory time in rats as compared to control group. Whereas the Methanolic extract show highly non significant

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effect in rats when compared with the standard drug Diazepam.

In light and dark model, rats were allowed to move in light and dark region for 10 min, the fear due to less space in dark area induces anxiety in animals. Total time spent and no. of entries were considered as evaluation parameters. Methanolic and Ethyl acetate extracts at 200 mg/kg concentration showed increased light region exploratory time and no. of entries in light area in rats as compared to control group and amongst them Methanolic extract show highly no significant difference when compared with standard drug Diazepam.

VI. CONCLUSION:

From the results it was revealed that both extract i.e. Ethyl acetate and Methanolic showed effective antianxiety activity. Although Methanolic extract at 200 mg/kg improved significantly antianxiety like behavior by using Elevated plus maze & Light and dark models in rats.

VII. ACKNOWLEDGEMENT:

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