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Evaluation of Anxiolytic Activity of Flower Extracts of *Tagetes Erecta Linn (Asteraceae)* in Rats

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ABSTRACT

Tagetus erecta (Asteraceae) is a plant with diverse medicinal properties hence the present work was selected to evaluate the anxiolytic activity of aqueous and alcoholic extracts of flowers of *T. erecta*. Dried flower powder of *T. erecta* was extracted by maceration process to get alcoholic (AEFTE) and aqueous (AQEFTE) extract. Preliminary phytochemical investigations were carried out to identify various constituents present in AEFTE and AQEFTE. The LD_{50} studies of AEFTE and AQEFTE were conducted in mice till the highest permitted dose level of 2000 mg/Kg following Up and Down method of OECD Guidelines. From the LD_{50} 2 doses of extract as 1/20[th] (low) and 1/5[th] (high) were selected. The anxiolytic activity of AEFTE and AQEFTE was evaluated in laboratory animal models like Elevated plus maze and Light-dark exploration models in rats. Preliminary phytochemical tests revealed that both AEFTE and AQEFTE contained glycosides, phenols, steroids, flavonoids. The LD_{50} studies revealed that both AEFTE and AQETE did not produced any abnormal behaviour or mortality even at the highest permissible dose of 2000 mg/Kg in mice. The Diazepam (2 mg/kg), AQEFTE and AEFTE (100, 400 mg/kg) were tested on Elevated plus maze and Light dark chamber models. After treated with AFETE and AQEFTE shows significant reduction in anxiety activity with the extracts at low and high doses.

INTRODUCTION

It is commonly known as *Genda* and its English name is French *marigold*. Flowering occurs in December and January. It is a common garden plant. *Tagetes is a* genus of herbs commonly known as Marigolds is a native of Mexico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics.

Several species are grown in gardens for ornament. The name Marigold is however, indiscriminately applied to several other genera of Compositae with golden or yellow capitula. Five species have been introduced into the Indian gardens and almost all of them are met with as escapes (Heywood *et al*, 1977). The flower-heads are much used for garlands. Many *Tagetes* species yield strongly aromatic essential oils, all of which are known as *Tagetes* Oil. The oil is obtained from the entire aerial part of the plant by steam distillation for 3-4 hours and absorbing the distillate in petroleum ether or benzene; prolonged distillation spoils the aroma. **(**Husan *et al*, 2002). Anxiety is a feeling of apprehension, diffuse, highly unpleasant (Kessler *et al*, 2005). uncertainty or subjective sense of unease /foreboding, tension stemming from anticipation of an imagined /unreal threat combined with the symptoms of increased sympathetic activity and changes in other psycho physiological indices such as increased heart rate, muscle tone and skin conductance (APA, 2000).

It is also a primary psychiatric condition, approximately 4-6% of the population suffers from anxiety and it disrupts routine life function (Kasper *et al*, 1998). Clinical symptoms of anxiety include panic disorder, agoraphobia, other phobias and generalized anxiety (Morgan *et al*, 1997). The ability to anticipate and prepare is associated with the ability to experience fear and anxiety as we continually strive to adapt to a changing world (Kraig *et al*, 2010).

Anxiety can destroy health and increase vulnerability; it shortens breath, narrows blood vessels and interferes with the functioning of the immune system. Fear is useful energy; it calls to courage, anxiety is useless, it promotes feelings of insecurity, helplessness, and weakness (healthy people.com).

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The distinction between a 'pathological' and a 'normal' state of anxiety is not clear-cut but it represents the point at which the symptoms interfere with normal productive activities.

Despite (or perhaps because of) this loose distinction, anxiolytic drugs are among the most frequently prescribed substances, used regularly by upward of 10% of the population in most developed countries (Rang *et al*, 2003).

The behavioral and physiologic responses that characterize anxiety can take many forms. Typically, the psychic awareness of anxiety is accompanied by enhanced vigilance, motor tension and autonomic hyperactivity. If the patient presents with chronic anxiety as the primary complaint, it may be appropriate to review the diagnostic criteria set forth in the Diagnostic and statistical Manual of Mental Disorders (DSM IV) to determine whether the diagnosis is correct and if treatment should include drug therapy.

For example, excessive or unreasonable anxiety about life circumstances (generalized anxiety disorder), panic disorders and agoraphobia are amenable to drug therapy, usually in conjunction with psychotherapy. In many cases, anxiety is a symptom of psychiatric problems that may warrant the use of antidepressant or antipsychotic drugs **(**Stovdemirea *et al*, 1996).

Impaired concentration or selective attention

Feeling restless and Avoidance, Behavioral problems (especially in children and adolescents), Nervousness and jumpiness, Hypervigilance, Irritability, Confusion, Strong desire to escape , Self- consciousness and insecurity, Fear that you are dying or going crazy **(**Dipro *et al*, 2003).

Physical Symptoms (Dipro *et al*, 2003).

Heart palpitations or racing heartbeat, Chest pain, Hot flashes or chills, Cold and clammy hands, Stomach upset, Frequent urination or diarrhea, Shortness of breath, Sweating, Dizziness, Tremors, twitches, and jitters, Muscle tension or aches, Headaches, Fatigue , Insomnia.

MATERIALS AND METHODS

Drugs and chemicals

Ethanol (Nice,Cochin,India), chloroform(Nice, Cochin, India), Diazepam (Ranbaxy, Hyderabad), Distilled water (Dolphin industries, Bhimavaram).

Collection of plant material

The flowers of the *T. erecta* were collected from Bhimavaram, West godavari Dist. A.P, India, during Nov 2012, and were authenticated at Dr. CSN Degree and P.G College, Bhimavaram.

Preparation of AQEFTE and AEFTE extracts

Preparation of alcoholic extract

The dried seed powder (100 g) was taken in a round bottom flask (2000 ml) and macerated with 300 ml of ethanol (70 %) for 2 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at <50^{o}C to get the alcoholic extract (AEFTE). The extract was then transferred into a previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50^{o}C to get alcoholic (AEFTE) extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated (Mehtha *et al*, 1994; Khandelwal *et al*, 2005; Kokate *et al*, 2007).

Preparation of aqueous extract

The about 100 g of dried seed powder was taken in a round bottom flask (2000 ml) and macerated with 500 ml of distilled water with 10 ml of chloroform (preservative) for 7 days with occasional shaking for every hour in a closed vessel. Then the marc was removed by filtering the extract and then it was concentrated on a water bath maintained at <50 ^{o}C to get the aqueous extract (AQEFTE). Both the extracts were stored in airtight containers in a refrigerator below 10^{o}C. The two extracts were examined for their colour, consistency and percentage yield.

Animals

Albino rats weighing between 150-200 g and each group containing 6 animals will be divided into 6 groups.

Group A-Normal control vehicle treatment only, Group B-Standard Diazepam (2 mg/Kg, i.p.)

Group C -Low dose (100 mg/Kg p.o) AEFTE, Group D-High dose (400 mg/Kg, p.o) AEFTE,

Group E -Low dose (100 mg/Kg p.o) AQEFTE, Group F -High dose (400 mg/Kg, p.o) AQEFTE.

Toxicity studies (OECD, 2001; Vipul *et al*, 2007).

In the present study the AEFTE and AQEFTE were subjected for toxicity studies. For the LD_{50} dose determination AEFTE and AQEFTE were administered upto the dose level of 2000 mg/Kg body weight and both extracts did not produced any mortality. Hence 1/5th, 1/10th and 1/20th doses of maximum dose tested for LD_{50} (2000 mg/Kg) were selected as 100 mg/Kg (Low), 200 mg/Kg (Medium) and 400 mg/Kg (High). For the present study 100 mg/Kg (Low) and 400 mg/Kg (High) doses were selected.

Determination of anxiolytic Activity

Elevated Plus Maze model in rats: (*Bertoglio et al, 2002; Gerhard Vogel et al, 2002; Sanger et al, 1991)*

The test has been proposed for selective identification of anxiolytics and anxiogenic drugs, anxiolytics compounds, by decreasing anxiety, increase the open arm exploration time; anxiogenic compounds have the opposite effect. The primary measure the proportion of entries into the open arms and the time spent on the open arms expressed as a time spent on the both open and closed arms. The elevated plus maze is now widely accepted as an animal model of "anxiety". It features technical simplicity together with a high throughout, thus allowing rapid pharmacological evaluation of drug effects on anxiety. The elevated plus maze test is a rodent model of anxiety that is used extensively in the discovery of novel anxiolytic agents and to investigate the psychological and neurochemicals basis of anxiety.

Parameters recorded

This method measures anxiety response in mice and rats using elevated plus maze test the parameters were recorded and the animals were placed individually at the centre of plus maze with head facing towards open arm at the beginning of the test.

a). Number of entries the animal made in the open and closed arms.
b). Average time each animal spent in open and close arms.

Experimental procedure: (Michael Reibau *et al*, 1993).

Thirty six male wistar rats were housed in a temperature (22 ±2ºc), relative humidity (50%) and photoperiod (12h light/dark cycle 07:00-19:00) controlled room with diet and water was given *ad libitum* to rats. After a short period of acclimatization animals will be randomly divided into six groups of 6 each. The first group (group A) was administered a normal diet. The second group (group B) was administered with a Standard Diazepam (2 mg/Kg, i.p). The third and fourth group (group C and D) were treated with low (100 mg/Kg p.o) and high (400 mg/Kg p.o) dose of AEFTE. The remaining 2 groups E and F were administered with low (100 mg/Kg p.o) and high (400 mg/Kg p.o) dose of AQEFTE, after that each rat was placed individually at the centre of the maze, facing one of the open arms and thereafter the following measures are commonly observed in 5 min duration respectively.

Light-dark exploration model in rats: (Bourin *et al*, 2003; Hascoet *et al*, 1998; Chen *et al*, 2006).

Two compartment exploratory models of Crawley and Goodwin has been validated pharmacologically, behaviorally and physiologically. The two compartment method titrates the natural tendency of rat to explore a novel environment, against the aversive properties of brightly light open field. The time spent in light area and exploratory behavior seemed to be the most reliable parameter for assessing anxiolytic activity. The light and dark box consists of two compartments: one light area (27L×27W×27H cm) was illuminate by 100 W desk lamp was painted white and the other dark area (18L×27W×27H cm) was painted black. The two compartments were separated by partition with tunnel (7.5×7.5 cm) to allow passage from one compartment to other.

1. The number of crossings between the light and dark compartment. (Transitions)
2. Time spent in lit part of compartment
3. Time spent in the dark part of cage

Experimental procedure:

Thirty six male wistar rats were housed in a temperature (22 ±2ºc), relative humidity (50%) and photoperiod (12h light/dark cycle 07:00-19:00) controlled room with diet and water was given *ad libitum* to rats. After a short period of acclimatization animals will be randomly divided into six groups of 6 each. The first group (group A) was administered a normal diet. The second group (group B) wasl administered with a Standard Diazepam (2 mg/Kg, i.p). The third group (group C and D) were treated with low (100 mg/Kg p.o) and high (400 mg/Kg p.o) dose of AEFTE. The remaining 2 groups E and F were administered with low (100 mg/Kg p.o) and high (400 mg/Kg p.o) dose of AQEFTE. The animals can freely move between a brightly- light open field and a dark corner, they show more crossings between the two chambers and more locomotor activity after treatment with anxiolytics. The numbers of crossings between the light and dark sites were recorded (Blkei-Gorzo *et al*, 1998; Vogel *et al*, 2002).

RESULTS AND DISCUSSION

Despite the wide spread traditional use of *T. erecta* for treating various disorders. There are no reports of scientific evaluation of its anxiolytic activity. The present work was performed to evaluate the anxiolytic activity of aqueous extract of *T. erecta* flowers in using elevated plus maze model in rats and stair case model in rats after subjecting to chronic unpredictable mild stress (CUMS) (Willner *et al*, 1986).

Long-term exposure to multiple stressors can cause depression in humans. Induction of depression using CUMS is considered as the most congruent animal model of depressive conditions observed in humans after long term exposure to multiple stressors (Willner *et al*, 1984).

The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABAA-benzodiazepine complex. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances such as the benzodiazepines. In EPM, wistar rats will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytics and the reverse holds true for anxiogenics (Jaiswal *et al*, 1994; Bhattacharya *et al*, 1997).

Present study was aimed at analyzing the anxiolytic activity of aqueous and alcoholic extract of *T. erecta flowers* in rats and to compare this activity. It was conducted in two different animal models for anxiety; elevated-plus maze model and the light-dark chamber model. Results of the study shows that the 100 mg/Kg and 400 mg/Kg *T. erecta* extracts have anxiolytic activity.

In elevated-plus maze, the mean number of entries into open arm in standard group and all the other drug treated groups revealed a statistically significant increase compared to the control group (Table.1 and Fig No.1,2). Among the test drug treated groups, both the groups treated with the 100 mg/Kg of *T. erecta* extract and 400 mg/Kg *T. erecta* flowers extract showed anxiolytic activity which was comparable to 2 mg/kg diazepam treated group. These findings support the evidences from the previously conducted studies, where the same doses were found to have anxiolytic activity that was comparable to 2 mg/kg diazepam (Samad *et al*, 2006).

In case of another parameter, i.e. percentage of total time spent in open arm, the trend was similar. The percentage of total time spent in open arm in standard group and all the other groups showed a statistically significant increase compared to control group. However, the percentage of total time spent by the diazepam treated groups was more than other treated groups. Though the differences between the diazepam group and 100 mg/Kg *T. erecta* extract treated groups were statistically insignificant, the difference between diazepam group and 400 mg/Kg *T. erecta* flowers extract treated group was found to be statistically significant. This may suggest that the anxiolytic activity of 100 mg/Kg *T. erecta* flowers extract treated group may not be comparable to diazepam. Since similar finding was not observed in other parameter and in light-dark chamber model, this observation can be considered as insignificant. In light-dark chamber model, the results were similar to that of the elevated–plus maze model. Compared to distilled water treated control group, all other treatment groups showed statistically significant increase in the number of entries into light arena and percentage of total time spent in the light arena. The differences in the mean number of entries into light arena between diazepam group, 100 mg/Kg *T. erecta flowers* extract treated group and 400 mg/Kg *T. erecta* flowers extract treated groups were statistically significant. Hence, the anxiolytic activity of 100 mg/Kg *T. erecta* extract and 400 mg/Kg *T. erecta* extract can be considered as comparable to 2 mg/Kg diazepam. The anxiolytic activity of *T. erecta* is further supported by the biochemical evidences of changes in brain serotonin levels. Exposure of rats to stress induced anxiety was found to be associated with increase in the synthesis of whole brain and various regional serotonin synthesis. *T. erecta* flowers extract attenuates the anxiety by decreasing the synthesis of serotonin. This was the finding in study by (Samad *et al*, 2006). Overall evidences suggest that the aqueous extract of *T. erecta* at the doses of 100 and 400 mg/Kg has anxiolytic activity and it is comparable to 2 mg/Kg diazepam. The elevated plus maze is currently one of the most widely used model of animal anxiety and it's validated use in rats (Lister *et al*, 1987). 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 GABAA receptor complex, justifying the use of diazepam as a positive control in this study, 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 *T. erecta* flowers had similar effects on these parameters. The (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 (400 mg/kg) of aqueous and ethanolic extracts may have the anxiolytic effects similar to the standard drug as a result, animal spent more time in open arm and less time in closed arm. Therefore behavioural alteration induced by higher dose (400 mg/kg) of aqueous and ethanolic extract and lower dose of (100 mg/kg) of aqueous and ethanolic extract were consistent with dose dependant anxiolytic profile (File *et al*, 1978). Which used a natural form of behaviour as the dependent measure, This opened the way to investigate anxiogenic compounds, and provided a new approach to the neurobiological mechanisms underlying anxiety disorders. From the beginning, attempts were made to validate this test behaviorally and physiologically, as well as pharmacologically and it has proved sensitive to changes anxiety generated by non pharmacological means (Crawley *et al*, 1980).

In present study, aqueous extract of *T. erecta* (400 mg/kg) significantly increased the number of entries and time spent in open suggesting an anxiolytic-like effect. But the effects of aqueous and ethanolic extracts (100 mg/kg) on the above parameter were insignificant as compare with control group (File SE *et al*, 1992). 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. Drugs induced increase in behavior 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 (File *et al*, 2003)

In this study, the time spent in light area, latency to enter dark chamber is an indices of anxiety. The aqueous and ethanolic (400 mg/kg) extract of *T. erecta* significantly increased the time spent in light area, latency to enter dark chamber similar to standard drug, suggesting that anxiolytic activity of extract compared to control group (Takeda *et al*, 1998). But the effects of aqueous and ethanolic extracts (100 mg/kg) on the above parameter were insignificant as compare with control group.

Table. 1: Effect of Diazepam, AEFTE and AQEFTE on Elevated plus maze model in rats.

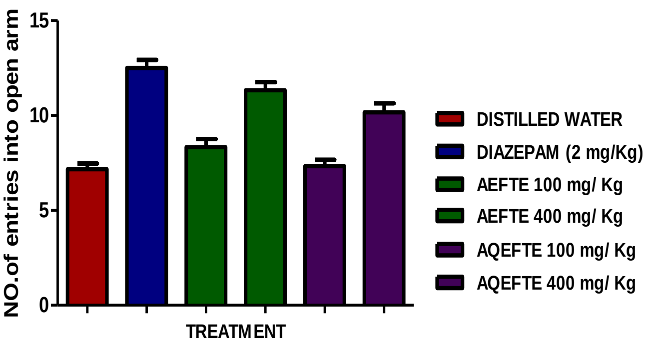
Groups	**NUMBER OF ENTRIES**		**TIME SPENT**	
	OPEN	**CLOSED**	**OPEN**	**CLOSED**
Distilled water	**7.16±0.30**	12.83±0.40	86.06±0.35	176.6±1.17
Diazepam (2 mg/Kg)	12.50±0.42***	6.16±0.47***	185.6±0.78***	69.19±0.55***
AEFTE 100 mg/kg	8.33±0.42	10.67±0.49	137.3±1.28	123.7±1.10
AESPG 400 mg/kg	11.33±0.42***	7.67±0.76***	178.7±1.96***	78.05±1.01***
AQEFTE 100 mg/kg	7.33±0.33	12.50±0.50	111.8±3.23	161.5±0.56
AQEFTE 400 mg/kg	10.17±0.47**	8.50±0.56**	152.6±0.97**	117.1±1.31**

n=6, Significant at **P<0.05,**P<0.01,***P<0.001 & ns = not-significant.*

AEFTE and AQEFTE- Alcohol and Aqueous extract of flowers of *T. erecta*,

Table No: 1 Anxiolytic effects were noted with both the extracts. AEFTE exhibited relatively better anti-anxiety effect and than AQEFTE. The AEFTE and AQEFTE has significantly increased the time spent at open arm of the elevated plus maze model at both high dose levels of 400 mg/Kg p.o. The activity is almost equivalent to that of diazepam which is used as standard anxiolytic agent. The AEFTE and AQEFTE also increase the time spent in open arm of the maze at both dose level of 400 mg/Kg p.o.

The AEFTE and AQEFTE has been observed to decrease the time spent at closed arm of the elevated plus maze model at both high dose levels of 400 mg/Kg p.o.



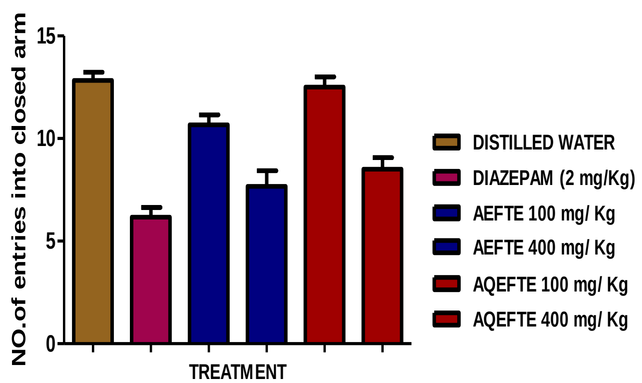


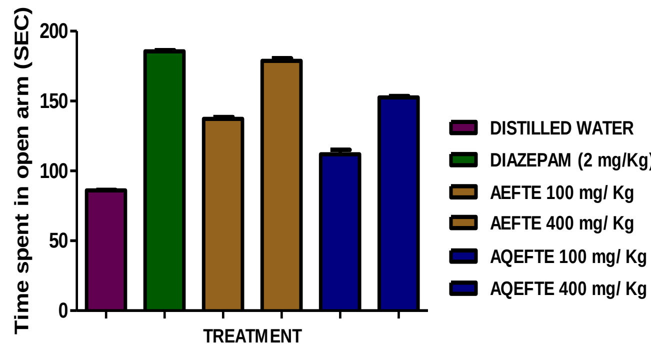
Fig. 1, 2: Effect of Diazepam, AEFTE and AQEFTE rats on number of entries into Elevated plus maze model in rats**.**

Number of Entries into Open Arm

Number of entries into open arm of 6 groups of rats was recorded. In normal treated group (control) average of number of entries into open arm were recorded as (7.16±0.30). With standard Diazepam (12.50±0.42) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (8.33±0.42, 11.33±0.42) and AQEFTE (100, 400 mg/Kg) (7.33±0.33, 10.17±0.47) except with low dose of AQEFTE all other groups were observed increase in number of entries into open arm. Further dose dependent anxiolytic effects were noted with both the extracts. AEFTE exhibited relatively better anti-anxiety effect and then AQEFTE (fig. 1).

Number of Entries into Closed Arm

In normal control average of number of entries into closed arm were recorded as (12.83±0.40). With standard Diazepam (6.16±0.47) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (10.67±0.49, 7.67±0.76) and AQEFTE (100, 400 mg/Kg) (12.50±0.50, 8.50±0.56) except with low dose of AQEFTE all other groups were observed with a significant reduction in number of entries into closed arm (fig. 2).



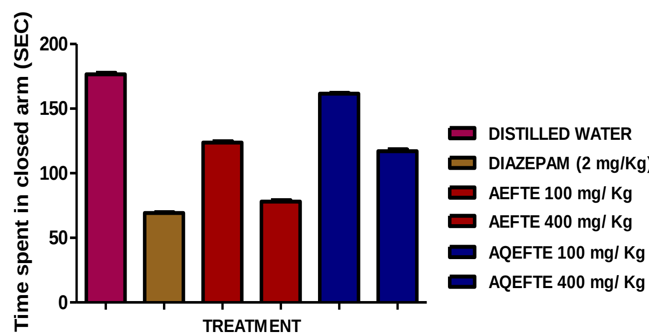


Fig. 3, 4: Effect of Diazepam, AEFTE and AQEFTE rats on time spent into Elevated plus maze model in rats.

Time spent in Open Arm

Time spent in open arm of 6 groups of rats was recorded and the treatment with normal control average of time spent in open arm were recorded as (86.06±0.35). A standard Diazepam (185.6±0.78) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (137.3±1.28, 178.7±1.96) and AQEFTE (100, 400 mg/Kg) (111.8±3.23, 152.6±0.97) except with low dose of AQEFTE all other groups were observed to increase time spent in open arm (Fig. 3).

Further dose dependent anxiolytic effects were noted with both the extracts. AEFTE exhibited relatively better anti-anxiety effect and than AQEFTE. The AEFTE and AQEFTE has significantly increased the time spent at open arm of the elevated plus maze model at both high dose levels of 400 mg/Kg p.o. The activity is almost equivalent to that of diazepam which is used as standard anxiolytic agent. The AEFTE and AQEFTE also increase the time spent in open arm of the maze at both dose level of 400 mg/Kg p.o.

Time spent In Closed Arm

Time spent in open arm of 6 groups of rats were recorded and the group treated with normal control, the average of time spent in closed arm were recorded as (176.6±1.17). With standard Diazepam (69.19±0.55) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (123.7±1.10, 78.05±1.01) and AQEFTE(100, 400 mg/Kg) (161.5±0.56, 117.1±1.31) except with low dose of AQEFTE all other groups were observed to decreased time spent in closed arm (Fig. 4).

The AEFTE and AQEFTE has been observed to decrease the time spent at closed arm of the elevated plus maze model at both high dose levels of 400 mg/Kg p.o.

Table. 2: Effect of Diazepam, AEFTE and AQEFTE on light-dark chamber model in rats.

Groups	Number Of Entries		Time Spent	
	Light	Dark	Light	Dark
Distilled water	1.66±0.33	12.50±0.42	1.49±0.27	8.52±0.35
Diazepam (2 mg/Kg)	6.16±0.60	5.66±0.49	8.45±0.59	3.51±0.24
AEFTE 100 mg/kg	3.00±0.44	8.83±0.70	4.73±0.52	6.03±0.29
AESPG 400 mg/kg	5.333±0.49	6.00±0.68	7.662±0.48	4.89±0.37
AQEFTE 100 mg/kg	1.83±0.30	11.67±0.66	2.73±0.25	7.25±0.54
AQEFTE 400 mg/kg	3.50±0.42	7.83±0.70	6.86±0.45	5.46±0.19

n=6, Significant at ****P<0.05,P<0.01,***P<0.001 & ns = not-significant***

AEFTE and AQEFTE- Alcohol and Aqueous extract of flowers of *T. erecta*,

The groups were observed to increase time spent in light chamber. Further dose dependent anxiolytic effects were noted with both the extracts (Table 2). AEFTE exhibited relatively better anti-anxiety effect and then AQEFTE. The AEFTE and AQEFTE has significantly increased the time spent at light chamber of the light-dark chamber model at both high dose levels of 400 mg/Kg p.o. The activity is almost equivalent to that of diazepam which is used as standard anxiolytic agent. The AEFTE and AQEFTE also increase the time spent in light chamber of the model at both dose level of 400 mg/Kg p.o.

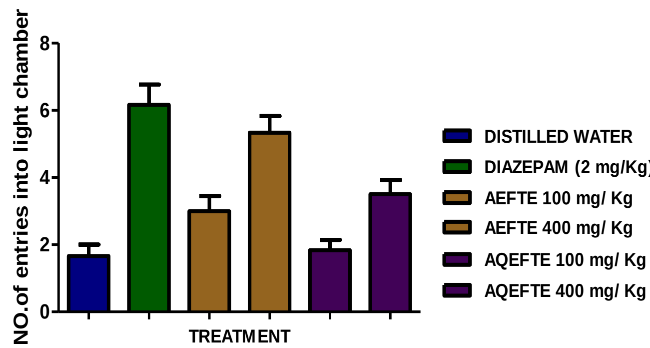
All other groups were observed to decreased time spent in dark chamber. The AEFTE at the dose of 100 mg /kg and 400 mg/Kg significantly reduced the time spent in dark chamber with concomitant increase in time in light chamber indicating that this extract possess anxiolytic properties in this model where as the water extract at 100 and 400 mg/Kg doses failed to increase the time spent in dark chamber indicating that this extract do not exhibit anxiolytic activity. This also confirms that the active principles responsible for anxiolytic property are present in AEFTE extract.

Number of Entries into Light Chamber

Number of entries into light chamber of 6 groups of rats were recorded the treatment with In normal control average of number of entries into open arm were recorded as (1.66±0.33). A standard Diazepam (6.16±0.60) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (3.00±0.44, 5.333±0.49) and AQEFTE(100, 400 mg/Kg) (1.83±0.30, 3.50±0.42) except with low dose of AQEFTE all other groups were observed increase in number of entries into light chamber. Further dose dependent anxiolytic effects were noted with both the extracts. AEFTE exhibited relatively better anti-anxiety effect and then AQEFTE (Fig. 5).

Number of Entries into Dark chamber

In normal control average of number of entries into dark chamber were recorded as (12.50±0.42). A standard Diazepam (5.66±0.49) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (8.83±0.70, 6.00±0.68) and AQEFTE (100, 400 mg/Kg) (11.67±0.66, 87.83±0.70) except with high doses of AQEFTE all other groups were observed with a significant reduction in number of entries into dark chamber (Fig. 6).



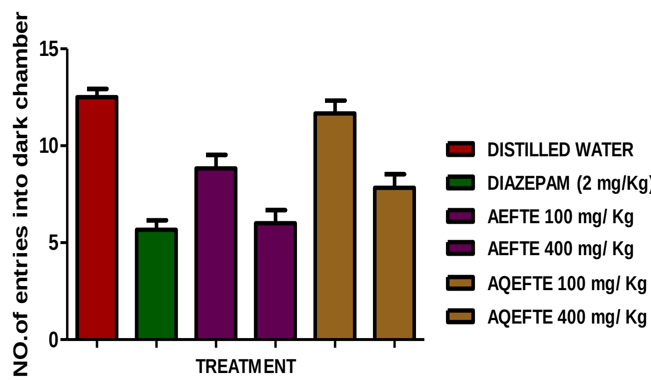


Fig. 5, 6: Effect of Diazepam, AEFTE and AQEFTE rats on number of entries into light-dark chambers.

Time spent In Light chamber

Time spent in light chamber of 6 groups of rats were recorded the treatment with In normal control average of time spent in light chamber were recorded as (1.49±0.27). A standard Diazepam (8.45±0.59) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (4.73±0.52, 7.662±0.48) and AQEFTE (100, 400 mg/Kg) (2.73±0.25, 6.86±0.45) except with low dose of AQEFTE all other groups were observed to increase time spent in light chamber. Further dose dependent anxiolytic effects were noted with both the extracts. AEFTE exhibited relatively better anti-anxiety effect and then AQEFTE. The AEFTE and AQEFTE has significantly increased the time spent at light chamber of the light-dark chamber model at both high dose levels of 400 mg/Kg p.o. The activity is almost equivalent to that of diazepam which is used as standard anxiolytic agent. The AEFTE and AQEFTE also increase the time spent in light chamber of the model at both dose level of 400 mg/Kg p.o (Fig. 7).

Time spent In Dark chamber

Time spent in dark chamber of 6 groups of rats were recorded the treatment with In normal control average of number of entries into open arm were recorded as (8.52±0.35). A standard Diazepam (3.51±0.24) were noted, the groups treated with two different doses of AEFTE (100, 400 mg/Kg) (6.03±0.29, 4.89±0.37) and AQEFTE (100, 400 mg/Kg) (7.25±0.54, 5.46±0.1942) except with high dose of AQEFTE all other groups were observed to decreased time spent in dark chamber (Fig. 8).

The AEFTE at the dose of 100 mg /kg and 400 mg/Kg significantly reduced the time spent in dark chamber with concomitant increase in time in light chamber indicating that this extract possess anxiolytic properties in this model where as the water extract at 100 and 400 mg/Kg doses failed to increase the time spent in dark chamber indicating that this extract do not exhibit anxiolytic activity. This also confirms that the active principles responsible for anxiolytic

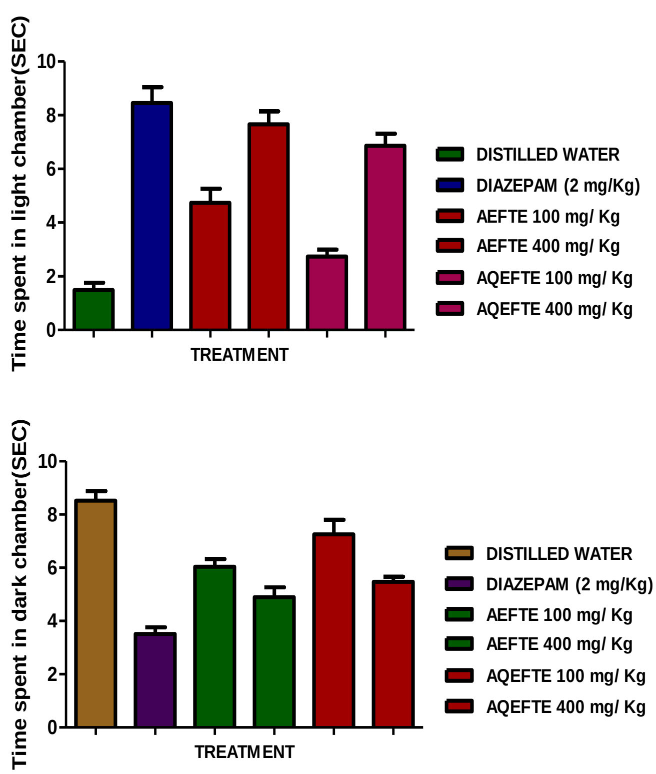


Fig. 7, 8: Effect of Diazepam, AEFTE and AQEFTE on time spent in light-dark chambers in Anxiolytic model in rats.

CONCLUSION

The phytoconstituents like flavonoids were reported for their anxiolytic effect and these constituents were present in aqueous and alcoholic extracts of *T. erecta*, so this active principle might be responsible for anxiolytic effect.

The acute toxicity study conducted for aqueous and alcoholic extracts indicates that they are safe up to 2000 mg/Kg bd wt. *T. erecta* at doses 100 mg/Kg and 400 mg/kg, in the elevated plus maze and light-dark models tested. Antianxiety activity may be due to the large no of chemical constituents present in *T. erecta*. More investigations are necessary to prove the anxiolytic activity of *T. erecta* by other models. However further studies are necessary to identify the exact mechanism of action of *T. ercta* as anxiolytic activity. Standard drug diazepam treated group showed increase in all the parameters and was statistically significant from distilled water treated control group, confirming the anxiolytic activity of diazepam. Among the test drug (AQEFTE and AEFTE) treated groups, both the 100 mg/kg and 400mg/kg *Tagetes Erecta* extract treated groups showed an increase in all the parameters that was statistically significant from control group. This suggests that the *T. erecta* extract at these doses has significant anxiolytic activity. The results obtained from these experimental models clearly confirmed that the anxiolytic activity of aqueous and alcoholic extracts of *T. erecta*. The alcoholic extract possesses good anxiolytic property in both the animal models when compared to aqueous extract.

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