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Research Article

Anxiolytic Activity of Grewia Tiliaefolia in Stress Induced Albino Rats

V.R. Tagalpallewar, A.V. Chandewar

P. Wadhwani College of Pharmacy, Yavtmal

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Corresponding author: V.R. Tagalpallewar

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Abstract:

Anxiety usually refers to the experience of fear, apprehensiveness, nervousness, panic, restlessness, tension, and agitation. The percentage of anxiety among people increasing day by day, hence need to focus on it. Anxiety disorders are the most common class of mental disorder in adult. Specific phobias and social anxiety disorder are very common among this disorder. Some Neurotransmitters including serotonin, dopamine, noradrenaline and GABA are responsible for generation anxiety. Serotonin modulators and benzodiazapines are mostly used to improve anxiety. The present paper discusses anti-anxiety potential of *Grewia tiliaefolia* leaves. *Grewia tiliaefolia* leaves contains flavonoids, steroids, tannins, saponins and alkaloids. Flavonoids were the major constituent for the anxiolytic activity. The effect of methanolic extract of *Grewia tiliaefolia* leaves(200mg/kg and 400mg/kg, orally, daily, 21 days) on anxiolytic activity was assessed by using Modified elevated plus maze apparatus and Light-dark box apparatus. Anxiety activity in rats was induced by restraint stress. The results shows that Open arm entries were increased and closed arm entries were decreased in modified elevated plus maze apparatus(MEPMA). Light box entries were increased and dark box entries were decreased in light and dark box model(LDB). Thus we can conclude that methanolic extract of *Grewia tiliaefolia* leaves exhibits significant anxiolytic activity at low dose (200mg/kg) and high dose (400mg/kg). Thus *Grewia tiliaefolia* leaves is a promising herbal option in the pharmaceutical world.

Keywords: *Grewia tiliaefolia,* Anxiety, Elevated plus maze apparatus, light and dark box apparatus, Anxiolytic activity

Introduction

Typically, when we talk about anxiety, we mean feelings of fear, apprehension, nervousness, panic, restlessness, tension, and agitation. Since anxiety is becoming more prevalent among people, attention must be paid to it. The most prevalent category of mental diseases in adults is anxiety disorders. This disorder is fairly frequent and includes specific phobias and social anxiety disorder (J.P.Jhabarmal et.al, 2013). Trembling, light headaches, and sweating are among the symptoms. Elevated blood pressure (BP) and alterations in the heart rate, muscular tone, and skin conductance

are all possible. Serotonin, dopamine, noradrenaline, gamma amino butyric acid (GABA), corticotropin releasing factor (CRF), melanocyte stimulating hormone (MSH), neuropeptides, and neurosteroids benzodiazepines show a narrow safety margin between the anxiolytic effects. These neurotransmitters are involved in the generation of anxiety (J. P. Jhabarmalet.al, 2013).

Anxiety can manifest physically in the form of headaches, palpitations, increased sweating, exhaustion, and dizziness. Nearly every emotional disorder and physical ailment are linked by anxiety. There is evidence that the amygdala is responsible for the manifestation of fear or dread, and that the prefrontal cortex is essential in ending dread by regulating the amygdala-mediated expression of dread Numerous genes have been identified to influence anxiety or dread, despite the fact that the molecular mechanisms underpinning negative and positive control of the condition are not entirely known (Mohale D. S et.al, 2012).

Anxiety can manifest physically in the form of headaches, palpitations, increased sweating, exhaustion, and dizziness. Nearly every emotional disorder and physical ailment are linked by anxiety. There is evidence that the amygdala is responsible for the manifestation of fear or dread, and that the prefrontal cortex is essential in ending dread by regulating the amygdala-mediated expression of dread Numerous genes have been identified to influence anxiety or dread, despite the fact that the molecular mechanisms underpinning negative and positive control of the condition are not entirely known (Kessler R. C et.al, 2012).

The Epidemiological Catchment Area (ECA) study, one of the largest epidemiological investigations ever conducted in the United States, found that particular phobia was the most prevalent mental disease, followed by obsessive-compulsive disorder, which came in at number four. The National Comorbidity Survey, National Comorbidity Survey-Revised (NCS-R), and other epidemiological research reveal same prevalence rates. Similar incidence rates were found in epidemiological investigations conducted in other nations. An analysis of 27 epidemiological studies conducted in the European Union (EU) between 1990 and 2004 revealed that anxiety disorders were the most prevalent psychiatric disorders there, with a median 12-month prevalence of 12% (M. Miyazaki et.al, 2017).

In Central Nervous System (CNS), the main mediators of the anxiety disorders symptoms are gamma-aminobutyric acid (GABA), serotonin, norepinephrine, dopamine, other peptides and neurotransmitters such as corticotropin-releasing factor(CTRF).The Autonomic Nervous System (ANS), mainly the adrenergic nervous system mediates most of the symptoms. Elevated flow in the right parahippocampal region and decrease serotonin type 1A receptor binding in the anterior and posterior cingulate and raphe of patients are the detection factors for prevalence of anxiety disorder. Central to the processing of fear and anxiety is amygdala, and amygdala function disarranged in anxiety disorders. The amygdaloid neurons with dendritic arborization has been implicated for the anxiety processing in the basolateral amygdala. Inhibitory influence on action potentials and reduce arborization mediated by SK2 potassium channels. (Shelton et.al, 2004).

Plant is the main source of medicine and it plays a vital role in world health. Medicinal herbs or plants have been known to be potential source of therapeutics. Medicinal plants are widely used and have got a major role in health system throughout the whole world. The main reason for utilising the plant is due to their, better compatibility,

adaptability and better cultural acceptability with the human body and yeilds lesser side effects.Some of important drugs which are obtained from plants are atropine, quinidine, physostigmine, reserpine, tubocurarine, artimesinin, morphine, colchicine, quinine, digoxin, aspirin, pilocarpine, taxol, ephedrine, vinblastine and vincristine.(Olodeji O. et.al,2016)

Grewia tiliaefolia is a moderate-sized to large tree, attaining a bole length of about 30 ft. and a girth of 7 ft. or more. Bark grey or dark brown; leaves stipulate, ovate with oblique base, acuminate, crenate-dentate; flowers small, borne on thick axillary peduncles; drupe globose and of the size of a pea, 2-4 lobed, black and edible. *G. tiliaefolia* is a very close cousin of Phalsa. (Vahl V. Rajesh Kumar and V. V. Venkatachalam2016)

Literature shows that Grewia genus contains terpenoid, Alkaloid, flavones etc. (Wali Ullah et al 2012).

Flavonoids were the major constituents for the anxiolytic activity. (Mst Mahfuza Khatoon et.al, 2014).

Thus the present study aims to investigate the anxiolytic activity in rat model by the administration of methanolic extract of *G. tiliaefolia* leaves by administration of methanolic extract of *G. tiliaefolia* leaves.

Material and Method:

Materials:

Animals: 8 weeks of healthy female Sprague-dawley rats (weighing 150-250 gm) were used for this study. Animals were housed in polyethylene cages with wire mesh top and husk breeding was maintained under controlled condition of light (12h-light, 12h-dark), temperature (25±2ºC), and humidity(60±5%) and fed with a standard pellet diet and water ad libitum, were used for the entire animal study. The experiments were performed during the day time (8.00-16.00 hrs). The rats were housed and treated according to the rules and regulations of CPCSEA and IAEC. The protocol for all the animal study was approved by Institutional Animal Ethics Committee (IAEC).

Method:

Grewia tiliafolia plant were collected from local area of Kalamnuri, Hingoli, District Maharashtra (India). The plant material was identified and authenticated By Dr. Punjabrao Deshmukh Krishi Vidyapeeth Akola Vasantrao Naik Collage Of Agriculture Biotechnology, Yavatmal. (Reference No. VNCABT/Ytl/ Hort/ 1087 /2022).

Leaves were dried in a shade and then powdered to get a course powder. This powder was stored in air tight container and used for extraction.

For the extraction of *Grewia tiliafolia* leaves methanol was used as a solvents. The extraction needs a glass bottle. The dried powder *Grewia tiliafolia* leaves were placed into a glass bottle for extraction. Powdered leaves were macerated during the maceration process; they were occasionally stirred at regular intervals of time. It was filtered. The extract was concentrated and dried at a controlled temperature of 40 °C on a water bath, and the percentage yield was found to be 8.7%. A dried extract of the leaves was used for further investigation. (Mst Mahfuza Khatoon et.al, 2014).

Phytochemical Screening

Test for Alkaloid:

1 ml of filtrate with 2 ml of Dragendroff's reagent gives turbid orange colour. (S. Sivakrishnan et.al, 2014).

Test for Tanins:

1 ml of filtrate with 2ml of ferric chloride gives dark green colour. (S. Sivakrishnan et.al, 2014).

Test for saponin:
1 ml of filtrate with 2ml distilled water is taken vigorously and allowed to stand for 10 minutes. Development of foam on surface of the mixture, lasting for 10 minutes indicates the presence of saponin (S. Sivakrishnan et.al, 2014).

Test for Phenolic Flavonide:

1 ml of filtrate with 2ml of 10% lead acetate gives brown precipitate (S. Sivakrishnan et.al, 2014).

Test for Flavonoids:

1 ml of filtrate with 2ml of dilute NaOH show development of golden yellow colour (S. Sivakrishnan et.al, 2014).

Experimental Design:
For this study animals were divided into five groups,

Group I (Vehicle control group)- Rats received only saline solution.

Group II (Negative control group)- Rats were subjected for restraint stress for 21 days using saline bottle.

Group III (Low dose group)- Rats were subjected for restraint stress and treated with 200mg/kg methanolic extract of *Grewia tiliafolia* orally for 21 days.

Group IV (High dose group) - Rats were subjected for restraint stress and treated with 400mg/kg methanolic extract of *Grewia tiliafolia* orally for 21 days.

Group V (Standard group)- Rats were subjected for restraint stress and treated with 2mg/kg Diazepam for 21 days.

Induction of anxious state:

All groups were subjected for 21 days for restraint stress except normal control group which was placed in normal condition in animal house. For the induction of anxiety rats was packed in saline bottle for 6 hrs daily for 21 days.

Drugs and dosing:

Diazepam (2mg/kg) was used as standard drug. Diazepam was diluted to 1.5 mg/10ml with distilled water. Two different concentrations (200 and 400 mg/kg) of the *Grewia tiliafolia* leaves extract were prepared by dissolving the extract in distilled water. All solutions were prepared freshly on test days and administered orally according to body weight of rats. Low dose group extract were calculated 200mg/kg and high dose group extract were calculated 400 mg/kg of rats. Then dosing were given to rats in the concentrations like 0.1ml, 0.2ml, 0.4ml….etc.

Anxious behavioral state of animals after 21 days were checked by using elevated plus maze apparatus and light and dark box model.

Elevated plus maze apparatus:

Elevated plus Maze Apparatus (EPMA) was widely used for the assessment of anxiolytic activity.30 min before the experiment, mice were individually placed in the center of the apparatus facing one of the open arms.The number of entries and time spent on the open and enclosed arms was observed during a period of 5 min. An arm entry was counted when all four paws were in the arm. The percentage of open arm entries and the time spent in open arm were measured using the following formula:

% of open arms time spent = [open arms time/(open arms time + closed arms time)] ×100% of open arms entries = [open arms entries/(open arms entries + closed arms entries)]×100 (Vijendar Kumar et.al, 2013).

Light and Dark apparatus:

Light and dark apparatus was commony used model for the assessment of anxiolytic activity. This apparatus was consist of two compartment, one-third for the dark compartment while two-third for the light

compartment. The light compartment was brightly illuminated with a light source of 400 lux which is placed 35cm above the box. 30 minutes after treatment with the vehicle, methanol extract or diazepam, each mouse was individually placed in the corner of the light compartment, facing away from the entry to the dark compartment. The mice were monitored for a period of 5 min and the following parameters were observed and quantified:

(a) latency of the first crossing from one compartment to the other, (b) time spent into light and dark compartment, (c) the number of transition between the light and dark compartment (Michel Bourin et.al, 2003).

Results:

Elevated Plus Maze Apparatus Results:

Table 1: Effect of methanolic extract of *Grewia tiliafolia* leaves in anxious rats using Elevated plus maze test.

Sr No.	Groups	Number entries in closed arm (%) (0day)	Number entries in closed arm (%) (21day)	Number entries in open arm (%) (0day)	Number entries in open arm (%) (21day)
1.	Normal control	70.84±0.76	69.92±0.68	31.12±0.85	32.04±0.98
2.	Negative control	64.27±2.22	73.90±9.80 @	37.53±2.08	28.14±3.16@
3.	Low dose group	65.66±3.46	61.35±6.52***	35.12±2.65	39.11±0.38***
4.	High dose group	60.27±3.41	53.45±8.85***	40.22±1.90	46.17±1.02***
5.	Diazepam Std	68.05±0.70	52.51±15.54***	33.57±1.70	49.35±4.70***

Values are expressed in Mean±SEM (n=6)

@P<0.0001 Significant increase in closed arm and decrease in open arm entrieswas observed compared to normal control group.

***P<0.0001.Significant decreased in closed arm entries and increased in open arm entries was observed compared to negative control group.

#P>0.05 when compared with negative control.

Table 1 shows that there was a significant (P<0.0001) increased in the closed arm entries of negative control as compared to normal control and the low dose, high dose treated group there was significant (P<0.0001) decreased closed arm entries.

Table 1 shows that there was a significant (P<0.0001) decreased in the open arm entries of negative control as compared to normal control and the low dose, high dose treated group there was significant (P<0.0001) increased open arm entries.

Table 2: Effect of methanolic extract of Grewia tiliafolia leaves in anxious rats on time spent in closed arm and open arm of.

Sr No.	Groups	Time spent in closed arm (sec) (0day)	Time spent in closed arm (sec) (21day)	Time spent in open arm (sec) (0day)	Time spent in open arm (sec) (21day)
1.	Normal control	43.25±0.98	40.25±3.20	53.10±9.87	54.40±7.52
2.	Negative control	45.30±1.30	49.05±4.90 a	51.33±2.10	39.85±11.98 a
3.	Low dose group	47.40±7.87	38.10±9.65***	42.22±2.70	46.98±0.88*
4.	High dose group	52.21±2.35	39.47±14.70***	51.18±1.93	51.89±1.27**
5.	Diazepam std	52.19±1.98	35.52±16.80***	43.07±3.53	50.58±7.56***

Values are expressed in Mean±SEM (n=6)
@P<0.0001 Significant increase in time spent in closed arm and decreased in time spent in open arm was observed compared to normal control group.
*P<0.05.Significant increase in time spent in open arm was observed compared to negative control group.
**P<0.001.Significant increase in time spent in open arm was observed compared to negative control group.
***P<0.0001.Significant decreased in time spent in closed arm was observed compared to negative control group.
#P>0.05 when compared with negative control.

Table 2 shows that there was a significant (P<0.0001) increased in the time spent in closed arm of negative control as compared to normal control and in the low dose, high dose treated group there was significant (P<0.0001) decreased time spent in closed arm entries.

There was a significant (P<0.001) decreased in the time spent in open arm of negative control as compared to normal control and the low dose, high dose treated group there was significant (P<0.05,P<0.001) increased time spent in open arm entries.

Table 3: Effect of Methanolic extract of Grewia tiliafolia on transfer latency of anxious rats on EPM.

Sr no.	Groups	Transfer latency in secs
1	Positive Control	28.0±0.93
2	Negative control	49.5±0.85@
3	Low Dose(200kg/mg)	22.1±1.12**
4	High Dose(400kg/mg)	19.4±1.25**
5	Standard (Diazepam)	37.0±0.99**

All values are Mean ± SD @ p<0.01 compared with control group,**p<0.01 compared with negative control group.

Table 3 shows the effect of *Grewia tiliafolia* linn. on transfer latancy (TL) in Elevated plus maze (EPM) in anxious rats. There was significant (p<0.01) increased TL in negative control group as compare to control group. Whereas, *Grewia tiliafolia* linn. Low dose, high dose, standard treated groups there was significant (p<0.01) decreased in TL as compared to negative control group.

Light and Dark Box Apparatus Results:

Table 4: Effect of Methanolic extract of *Grewia tiliafolia* leaves on Light and dark box model in dark box and light box in anxious rats.

Sr No.	Groups	Number entries in darkbox (%) (0day)	Number entries in darkbox (%) (21day)	Number entries in lightbox (%) (0day)	Number entries in lightbox (%) (21day)
1.	Normal control	61.25±0.86	62.05±1.47	44.22±2.90	43.11±0.90
2.	Negative control	64.15±1.05	69.35±4.95 [a]	41.18±3.80	31.25±4.70 [a]
3.	Low dose group	63.12±1.02	61.18±0.92**	42.62±2.10	38.55±1.48**
4.	High dose group	64.22±0.88	59.12±2.19***	41.21±1.95	42.15±0.08***
5.	Diazepam std	63.53±0.95	59.82±4.15***	42.37±3.95	46.35±4.12***

Values are expressed in Mean±SEM (n=6)
[a] P<0.0001 Significant increase in dark box and decreased in light box entries was observed compared to normal control group.
**P<0.0001.Significant decreased in dark box and increased in light box entries was observed compared to negative control group.
***P<0.001.Significant increase in light box entries was observed compared to negative control group.

Table 4shows that there was a significant (P<0.0001) increased in the dark box entries of negative control as compared to normal control, the low dose, and high dose treated group there was significant (P<0.0001) decreased dark box entries. **T**here was a significant (P<0.001) decreased in the light box entries of negative control as compared to normal control the low dose, and high dose treated group there was significant (P<0.001) increased light box entries.

Table 5: Effect of methanolic extract of *Grewia tiliafolia* leaves on time spent in dark box and light box in anxious rats.

Sr No.	Groups	Time spent in dark box (sec) (0day)	Time spent in dark box (sec) (21day)	Time spent in light box (sec) (0day)	Time spent in light box (sec) (21day)
1.	Normal control	52.75±3.10	60.83±9.44	248.36±5.90	242.00±6.17
2.	Negative control	58.39±11.90	125±65.10 [a]	258.66±2.87	159.60±97.93 [a]
3.	Low dose group	51.62±3.88	90.76±40.06*	228.19±3.11	192.23±36.95***
4.	High dose group	73.00±2.35	78.13±5.20***	239.50±4.70	211.12±31.02***
5.	Diazepam std	41.27±3.22	60.52±19.95***	257.43±2.72	235.19±21.75***

Values are expressed in Mean±SEM (n=6) a P<0.0001 Significant increase in time spent in dark box and decreased in time spent in light box was observed compared to normal control group. *P<0.05.Significant decrease in time spent in dark box was observed compared to negative control group. ***P<0.0001.Significant decreased in time spent in dark box and increased in time spent in light box was observed compared to negative control group. Table 5 shows that there was a significant (P<0.0001) increased in the time spent in dark box of negative control as compared to normal control, the low dose, and high dose treated group there was significant (P<0.05, P<0.0001) decreased time spent in dark box. Table 5 shows that there was a significant (P<0.0001) decreased in the time spent in light box of negative control as compared to normal control, the low dose, and high dose treated group there was significant (P<0.0001) increased time spent in light box entries.

Discussion:

Anxiety is defined as an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger. It may be regarded as a particular form of behavioral inhibition that occurs in response to environmental events that are novel. Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade (R. S. Adnaik et.al, 2009).

GABAA receptors are involved in anxiety and their direct activation would have an anxiolytic effect. It is well documented that pentylenetetrazole-induced convulsions are produced due to diminution of GABA level in brain (R.S Adnaik et.al, 2009).

Anxiety can be produced by different methods such as using chemicals, social model and stress evoked, sensory models, transitory model, restraint stress etc. One of the commonly used model is restraint stress, which is a modified form of immobilization stress, restraint stress has been widely used as a model of chronic psychoemotional stress to induce depressive and anxiety like behaviours, learning and memory deficits, and hippocampal neuronal damage in mice. Restraint stress method is very economic, easy availability, easy induction of depression and anxiety and it also shows good results. Hence we choose restraint stress for generation of anxiety in this work (Hanwoong Woo et.al, 2018).

During this procedure inescapable physical and mental stress is induced by placing the animals in a plastic bottle in order to block their movements. This is a validated experimental stressor involving both physical and psychological effects at the same time (Pitman et al., 1988; Jaggi et al., 2011).

Now a day's herbal medicine are used to treat intense and constant sicknesses. Herbal remedies have been used for huge number of years. In fact, herbal medicine is the establishment of modern medicine. This medicine also has very less side effects.(Olodeji O. et.al,2016).

It has been reported that many flavonoids and neuroactive steroids were found to be ligands for the GABA receptors in the central nervous system; that can act as benzodiazepine-like molecules (Mst Mahfuza Khatoon et.al, 2014).

Literature shows that Grewia genus contains terpenoid, Alkaloid, flavones etc. (Wali Ullah et al 2012)

Flavonoids were the major constituents for the anxiolytic activity (Mst Mahfuza Khatoon et.al, 2014).

Our study confirms the presence of glycosides, saponins, tannins, steroids and flavonoids etc. in methanolic extract of *Grewia tiliafolia* leaves.

Flavonoids present in the extracts may be responsible for its CNS depressant activity.(Mst. Mahfuza Khatoon et al, 2014).

There are many models for the screening of anxiety like modified elevated plus maze apparatus, light and dark apparatus, elevated T maze, elevated zero maze, open field test, and white black box. In this study for the assesment of anxiolytic activity we have used modified elevated plus maze apparatus (MEPA) and light and dark model (LDB) due to their economic, easily availability, popularity, accuracy, specificity and shows good results.

In modified elevated plus maze apparatus, closed arm entries of control vehicle, low dose, high dose and standard group after 21 days were decreased as compared to 0 days entries. Only negative control group had more no.of entries in closed arm as compared to 0 day entries. In closed arm, time spent by rats in control vehicle, low dose, high dose and standard group after 21 days were decreased as compared to 0 day readings time spent by rats. Only negative control group had more time spent in closed arm as compared to 0 day readings time spent by rats.

In open arm, entries of control vehicle, low dose, high dose and standard group after 21 days were increased as compared to 0 days entries. Only negative control group had less no.of entries in open arm as compared to 0 day entries. In open arm, time spent by rats

in control vehicle, low dose, high dose and standard group after 21 days were increased as compared to 0 days readings time spent by rats. Only negative control group had less time spent in open arm as compared to 0 day readings time spent by rats.

In light and dark model, no.of entries and time spent by rats in dark box were decreased by treated groups of rats compared with negative control group. No.of entries and time spent by the rats in light box were increased by Treated Groups of rats Compared with negative control.

From this result we can say that methanolic extract of *Grewia tiliafolia* leaves exists anxiolytic activity due to the presence of flavonoids as a major constituent.

Conclusion:

The present findings indicates that the methanolic extract of *Grewia tiliafolia* leaves exhibits significant anxiolytic activity at low dose (200mg/kg) and high dose (400mg/kg).

Thus *Grewia tiliaefolia* leaves are a promising herbal option in the pharmaceutical world.

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