

Evaluation of Methanolic Extract of Albizia Procera Leaves For Anxiolytic Activity In Rats.

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ABSTRACT:-

Anxiety is an exaggerated feeling of uncertainty and fear. Anxiety is refers to the experience of nervousness,panic restlessness and tension. Neurotransmitters like serotonin, dopamine,noradrenaline and GABA generates anxiety.Nowadays serotonin modulators and benzodiazapines are available to alleviate anxiety. Generally in adults, anxiety disorder are the most common class of mental disorder with a 12- month prevalence rate of 24.9%. The most common disorder were Specific phobias and social anxiety disorder. As compard with adults aged between 18 to 64, the lifetime prevalence was generally less for panic disorder, GAD, and SAD, but specific phobia and agoraphobia without history of panic attacks were most common in adolescents between age of 13 to 17 years. The present paper discusses anti anxiety potential of Albizia procera leaves. Albizia procera leaves contains flavonoids,steroids,tannins, saponins and alkaloids.Flavonoids were the major constituent for the anxiolytic activity.The effect of methanolic extract of Albizia procera 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 albizia procera leaves exhibits significant anxiolytic activity at low dose (200mg/kg) and high dose (400mg/kg). Thus Albizia procera leaves is a promising herbal option in the pharmaceutical world.

Keywords:- Albizia procera,Anxiety, Elevated plus maze apparatus, light and dark box apparatus, Anxiolytic activity

I. INTRODUCTION:

Anxiety is as an exaggerated feeling of uncertainty and fear. It is unpleasant state of stress with an anticipation of imminent danger. (R. S. Adnaik et.al, 2009). Anxiety refers to the experience of fear, nervousness, panic, restlessness, stress and agitation. Symptoms include trembling, faint headaches, and sweating, possibly elevated blood pressure (BP) and changes in other psychophysiological indices such as heart rate, muscle tone, and skin conductance. The neurotransmitters which is involved in anxiety generation includes serotonin, dopamine,noradrenaline, Gama Amino butyric acid(GABA), Corticotropin releasing factor (CRF), Melanocyte stimulating hormone (MSH), neuropeptides and neurosteroids benzodiazepines shows a narrow safety margin between the anxiolytic effect. (J. P. Jhabarmalet.al, 2013).

The physical sign of anxiety includes, dizziness, fatigue, insomnia, headache, palpitationand excessive perspiration. Anxiety is associated with almost all emotional disorder with physical ailment. There is proof that amygdala is liable for expression of anxiety or dread and prefrontal cortex plays a vital role in dread termination by controlling the amygdale – mediated expression of dread although the molecular mechanisms underlying negative and positive regulation of the anxiety are not fully understood, many genes have been reported to affect anxiety or dread. (Mohale D. S et.al, 2012).

Generally in adults, anxiety disorder are the most common class of mental disorder with a 12- month prevalence rate of 24.9%. The most common disorder were Specific phobias and social anxiety disorder. As compard with adults aged between 18 to 64, the lifetime prevalence was generally less for generalised anxiety disorder (GAD), and social anxiety disorder(SAD), panic disorder but agoraphobia and specific phobia without history of panic attacks were most common

in adolescents between age of 13 to 17 years. (Kessler R. C et.al, 2012).

The largest epidemiological studies carried out in the United States, the Epidemiological Catchment Area (ECA) study, observed that the most common psychiatric disorder was specific phobia followed by obsessive-compulsive disorder which ranked four. Another epidemiological studies, including the National Comorbidity Survey, National Comorbidity Survey-Revised (NCS-R), show same prevalence rates. Epidemiological studies was carried out in various countries show identical prevalence rates. From 1990 to 2004, review of 27 epidemiological studies in the European Union (EU) found that the most common psychiatric disorders was anxiety disorders in Europe, 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 (CRF). 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 has 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 yields lesser side effects. Some of important drugs which are obtained from plants are atropine, quinidine, physostigmine, reserpine, tubocurarine, artemisinin, morphine, colchicine, quinine, digoxin, aspirin, pilocarpine, taxol, ephedrine, vinblastine and vincristine. (Olodeji O. et.al, 2016)

Albizia procera (Roxb.) Benth. is rapid-growing subtropical and tropical trees in the subfamily Mimosoideae of the family Fabaceae.

Albizia procera is a traditional herb and it is widely utilised in the Asian traditional medicine as analgesic, antibacterial, antioxidant, antidiabetic, and antidiarrheal drug. (S. Sivakrishnan, 2019)

The color of the *Albizia procera* bark is brown, it has characteristic odour and has slightly bitter in taste. The *Albizia procera* leaf is green in color, has characteristic odour and slightly bitter in taste. (S. Sivakrishnan, 2019)

Literature shows that *Albizia procera* leaves contains tannins, saponins, glycosides, steroids and flavonoids etc. (Asolkar et al, 1992; Rastogi and Mehrotra, 1993).

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

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

II. 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 ($25 \pm 2^\circ\text{C}$), and humidity ($60 \pm 5\%$) 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:-

The leaves of *Albizia procera* belonging to family-Fabaceae were collected in the month of September from the local area of Yavatmal district, Maharashtra, India. The plant material was identified and authenticated by Prof. Mrs. A. M. Gaharwar and Asso. Dean of Vasantrao Naik College Of Agriculture Biotechnology, Yavatmal (Ref no. VNCABT/Ytl/Hort/1030/2019).

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

For the extraction of *Albizia procera* leaves methanol and water were used as solvents.

Methanol and water were used in the proportion of 7:3. Glass bottle was used for the process of extraction. Dried leaves of Albizia procera and water and methanol poured in glass bottle for extraction. In maceration procedure, powdered leaves were macerated ; it was occasionally stirred at regular intervals of time. It was then filtered and concentrated. Then it was dried by evaporation. (Mst Mahfuza Khatoun 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 indicate 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 Albizia procera orally for 21 days.

Group IV (High dose group)- Rats were subjected for restraint stress and treated with 400mg/kg methanolic extract of Albizia procera 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 Albizia procera 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 400mg/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 commonly 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).

III. RESULTS:-

Elevated Plus Maze Apparatus Results:-

Table 1 : Effect of methanolic extract of Albizia procera leaves on Elevated plus maze test closed arm and open arm in anxious rats.

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	66.66±3.59	60.52±6.63***	35.35±2.70	41.25±0.38***
4.	High dose group	62.77±3.41	54.75±8.85***	39.70±1.90	47.20±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 entries was 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 Albizia procera leaves. on time spent in closed arm and open arm in anxious rats.

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	48.50±8.90	39.10±9.65***	44.22±2.70	44.98±0.88*
4.	High dose group	52.21±2.35	38.40±14.70***	51.18±1.93	49.89±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 increased in time spent in open arm was observed compared to negative control group.
 **P<0.001. Significant increased 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 the low dose, high dose treated group there was significant (P<0.0001) decreased time spent in closed arm entries.
 Table 2 shows that 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 Albizia procera 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)	21.7±1.12**
4	High Dose(400kg/mg)	19.5±1.45**
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 Albizia procera linn. on transfer latency (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, Albizia procera 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 Albizia procera 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	36.25±4.70 ^a
3.	Low dose group	63.12±1.02	64.18±0.92**	42.62±2.10	41.55±1.48**
4.	High dose group	64.22±0.88	62.12±2.19***	41.21±1.95	43.15±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 increased in light box entries was observed compared to negative control group.

Table 4 shows 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. Table 4 shows that there 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 Albizia procera 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	91.76±40.06*	228.19±3.11	191.23±36.95***
4.	High dose group	73.00±2.35	79.13±5.20***	239.50±4.70	209.12±31.02***
5.	Diazepam std	41.27±3.22	61.52±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.

IV. 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 days 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 *Albizia procera* leaves contains saponins, steroids, tannins, glycosides and flavonoids etc. (Asolkar et al, 1992; Rastogi and Mehrotra, 1993).

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 *Albizia procera* 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 *Albizia procera* leaves exists anxiolytic activity due to the presence of flavonoids as a major constituent.

V. CONCLUSION:-

The present findings indicates that the methanolic extract of *Albizia procera* leaves exhibits significant anxiolytic activity at low dose (200mg/kg) and high dose (400mg/kg). Thus *Albizia procera* leaves is a promising herbal option in the pharmaceutical world.

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