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Research Article

CNS ACTIVITY OF METHANOLIC EXTRACT OF *ANOGEISSUS LATIFOLAWALL* LEAVES ON VARIOUS EXPERIMENTAL RAT MODELS

VEERESH. K[1],VIJUSHA. M*, N.PRUTHVI RAJU[1], RAJANI. A[2], HEMAMALINI. K[3]

***Assistant Professor,Teegala Ram Reddy College of Pharmacy, Meerpet, [1]M. Pharm student, [2]Sree Dattha Institute of Pharmacy, Sheriguda, Ibrahimpatnam, Hyderabad. [3]H.O.D, Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad, Andhra Pradesh, India.**
Email:rkhemamalini@gmail.com

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ABSTRACT

The aim of this study was to evaluate the muscle coordination and anxiolytic activities of methanolic extracts of *Anogeissus latifolaWall* (combretaceae) in mice. The muscle coordination and anxiolytic activities were evaluated by using various models of using mice. Diazepam was used as standard drug for muscle coordination and anxiolytic studies. The extracts were administered orally at 300, 400 mg/kg. The results of the present study indicates that the methanolic extract of *Anogeissus latifola* leaves are effective in inducing a significant protection against muscle coordination and anxiolytic activities, as evidenced by various CNS models with respect to control. This study confirmed the muscle coordination and anxiolytic activities of this plant as it is used in traditional medicine.

Keywords: *Anogeissus latifola,* muscle coordination and anxiolytic activities.

INTRODUCTION

Anxiety and related disorders will become the second leading cause of disability in both developed and developing countries by the year 2020.[1]Benzodiazepines have been extensively used for the last 40 years to treat several forms of anxiety.[2]Anxiety and musculoskeletal disorders are extremely dramatic and debilitating disorders and it is now becoming clear that without knowledge of clinical and biological aspects of anxiety and musculoskeletal disorders, it is impossible to offer effective treatment strategies for the patients. Various herbal remedies are present that possess lesser side effects than the conventional drugs and thus are safer to use.

MATERIALS AND METHODS

Anogeissus latifolia DC belonging to combretaceae family is a large or moderate sized tree which is available in dry deciduous forests and available throughout India. The tree has been studied for antioxidant activity, hydrogen donating ability, nitric oxide, super oxide scavenging activity and hydrogen peroxide decomposition activity [3]. Leaves are opposite or sub-opposite. Bark is smooth with grey-white colour and exfoliating in irregular thin scales. A variety of substance which contributes to hepatoprotective activity has been identified in the extracts of *Anogeissus latifolia* which includes tannins [4], gallic acid, ellagic acid and flavonoids such as leutin, quercetin which are known as potential antioxidants. The bark of the plant has also reported to have several biological activities such as anti ulcer, anti microbial and wound healing activities [5]. The hydro alcoholic extract of *Anogeissus latifolia* has reported to have chemoprotective activity in paracetamol induced toxicity in rat model. Thus, the present study was undertaken for the investigation of analgesic activity of methanolic extract of *Anogeissus latifolia* [6-7].

Collection and authentication of plant materials: The plant material was collected in the month of June 2011 from srichalam hills and a specimen was dropped in the herbarium and the leaves was authenticated by Dr. Madhavachetty. The collected powdered material was shade dried and pulverized.

Solvent for extraction: Petroleum ether and methanol

Preparation of the extract: The dried powders of leaf of anogeissus latifolia **were** defatted with petroleum ether (60-80ºc) in a Soxhlet Apparatus by continuous hot- percolation. The defatted powder material (marc) thus obtained was further extracted with methanol with same method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Phytochemical Screening: The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

Test for reducing sugars (Fehling's test): The aqueous ethanol extract (0.5 g in 5 ml of water) of individual plants was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for anthraquinones: The individual plant extract (0.5 g) was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for terpenoids (Salkowski test): To 0.5 g each of the individual extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration was confirmed for the presence of terpenoids.

Test for flavonoids: A portion of the individual plant extract (0.5 g) was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration indica test the presence of flavonoids.

Test for saponins: To 0.5 g of each plant extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins: About 0.5 g of the individual extract was boiledin 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride (FeCl3) was added and observed for brownish green or a blue-black coloration

Test for alkaloids: 0.5 g of each extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was

extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test): To 0.5 g of individual plant extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

EXPERIMENTAL ANIMALS

Healthy albino rats of either sex between the age of 2-3 months and weighing 150-200 gms and Albino mice weighing 18-25 gm were used for the present study. The animals were kept in well aerated laboratory cages in the animal house and were allowed to acclimatize to the laboratory environment for a period of 2 weeks before the commencement of experiment. They were maintained on standard animal feed and drinking water ad libitum during the stabilization period. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, Ethical committee IAEC reg.no (1447/po/a/11/ CPCSEA)

EXPERIMENTAL PROTOCOL

The anti-anxiety activity was evaluated by using the following models

- Staircase model
- Elevated plus maze model
- Dark and light model

Treatment

The extract of anogeissus latifoliawas freshly dissolved in a suitable amount of distilled water to be acutely administered per os (p.o.) by an intra-gastric cannula in mice, or intraperitoneally (i.p.) in rats. One hour after p.o. or 30 min after i.p. administration, the animals were submitted to the various CNS tests. Doses of the extract and the time intervals were determined in preliminary tests. Diazepam (2 mg/kg) was dissolved in 40% propylene glycol and distilled water, respectively, immediately prior to use and given intraperitoneally. All administrations were performed in a dose volume of 1 ml/kg body weight. Control groups received only distilled water in the same volume by the same route. The Animals were fasted 18hrs prior to the experiment; treatment group was done as follows,

Group-1: Control administered with 1 ml of distilled water orally.

Group-2: 300mg/kg b.w of Anogeissus latifolia orally.

Group-3: 400 mg/kg b.w of anogeissus latifolia orally.

Group-3: Standard drug diazepam 2mg/kg

Staircase model: Staircase consists of five identical steps of 2.5cm high, 10cm wide and 7.5cm deep. The internal height of the walls is constant along whole length of the staircase. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears are counted over a 3 min period. A step is considered to be climbed only if the mouse had placed all four paws on the step.

Elevated plus maze model: The plus maze apparatus consisting of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, with the plus maze elevated (25cm) from the floor, was used to observe anxiolytic behavior in animals. All the animals in the different groups were administered the normal water, extract and standard drug orally using a tuberculin syringe fitted with oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the elevated plus maze apparatus 45min after the administration of the dose. Each mouse was placed at the centre of the elevated plus maze with its head facing the open arm. During this 5min experiment the behavior of the mouse was recorded as

- Preference of the mouse for its first entry into the open arms
- The number of entries into the open arms or closed arms
- Average time spent by the mouse in the open arms (Average time= total duration in the arms/number of entries)

During the entire experiment, the animals were allowed to socialize. Every precaution was taken to ensure that no external stimuli could invoke anxiety in the animals. Similar observations were recorded for the standard group (Diazepam 2mg/kg) as well as the control group (vehicle 1ml).[8]

Dark and light model

The light/dark box consist of a light, open topped, opaque, plexiglass box connected to a dark, closed topped, plexiglass box. Each compartment measuring (30×40×40cm). The boxes were connected by a small opening that allows the rat to cross between chambers. Each rat was placed individually in the center of the light compartment and observed for the next 5 minutes for the number of crossing between two compartments and time spent in the light and dark compartments. All the animals were placed in this and the observation was noted.

Skeletal muscle relaxant activity or Muscle co-ordination Test:

- Rota rod apparatus
- Traction test

Rotarod apparatus: The Rotarod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by metallic discs, allowing the simultaneous testing of 6 mice. The animals were trained to remain for 3min on the rod rotating at a speed of 25rpm. On the next day either vehicle or methanolic extract of Anogeissus latifolia (300mg/kg and 400mg/kg) was administered orally and their ability to remain on the rotating rod was assessed before and 30min after the oral administration. The fall-off time from the rod was noted for each animal.[9]

Traction test: The fore paws of the mice were placed in a small twisted wire rigidly supported above the bench top. The screening of animal was performed by traction test. Normally the mice grasp the wire with the forepaws and place at least one hind foot on the wire for 5 sec when allowed to hang free. The test was conducted on four groups of animals (n=6) that were previously screened, on the 15th day 30min after the administration of methanolic extract of Anogeissus latifolia (300mg/kg and 400mg/kg), vehicle and diazepam (5mg/kg) i.p the test was carried out. Inability to put up at least one hind foot is considered as failure in the traction test.[10]

STATISTICAL ANALYSIS

The results of study were subjected to one way Analysis of Variance (ANOVA) followed by Dunnett's t test. Values with $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemical screening of the extracts of Anogeissus latifolia showed the presence of various chemical constituents, mainly alkaloids, flavonoids, tannins, saponins, phenols, terpinoids, glycosides and sugars.

Staircase test: The statistical summary of the rearing and number of steps climbed is presented in Table 1. After 60 and 90 min of treatment, a reduction in anxiety linked behavior was indicated by a reduction in number of rearing and sedation that was evaluated by number of steps climbed. The dose of methanolic extract of *Anogeissus latifolia* leaves (300 mg/kg/p.o) and standard drug (Diazepam, 2mg/kg/i.p) significantly reduced the number of rearings as well as Number of steps climbed. Other dose of methanolic extract of *Anogeissus latifolia* leaves (400 mg/kg/p.o) did

not produced a significant decrease in the number of rearing or the number of steps climbed.

Table 1: Effect of methanolic extract of *Anogeissus latifolia* leaves on stair case model in rats

Treatment group	Dose	No of climbing's in 3min	No of rearing in 3 min
Control	1ml/kg/p.o	21.15±1.34	9.45±0.63
Diazepam	2mg/kg/i.p	5.00±0.64**	4.8±0.58**
MEAL	300 mg/kg/p.o	7.12±0.60*	6.00±0.52**
MEAL	400 mg/kg/p.o	11.42±0.72*	8.45±0.44*

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test.*p<0.01, **p<0.001.

Elevated plus maze test: The dose of methanolic extract of *Anogeissus latifolia* (300 mg/kg/p.o) significantly increased the time spent and no of entries into open arms when compared with control showed in Table 2.

Table 2: Effect of methanolic extract of *Anogeissus latifolia* leaves on Elevated plus maze apparatus in rats

Treatment group	Dose	No of entries		Time spent
		Closed arm(sec)	Open arm(sec)	Open arm(sec)
Control	1ml/kg/p.o	15.83±1.35	10.66±0.80	97.16±5.89
Diazepam	2mg/kg/i.p	7.16±0.95**	21.83±1.35*	193.51±15.84**
MEAL	300 mg/kg/p.o	9.34±0.95*	18.83±1.35*	143.51±15.84*
MEAL	400 mg/kg/p.o	12.16±0.95	15.83±1.35*	132.51±13.84*

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test.*p<0.01, **p<0.001.

Dark and light model: Table 3 indicate the significant increase in the time spent in light compartment with administration of Anogeissus latifolia at a dose of 300mg/kg when compared to control. Other dose of methanolic extract of *Anogeissus latifolia* leaves (400 mg/kg/p.o) did not produce a significant increase in the time spent and no of entries in the light compartment.

Table 3: Effect of methanolic extract of *Anogeissus latifolia* leaves on Dark/light box test in rats

Treatment group	Dose	No of entries in the light compartment	Average Time spent in light compartment
Control	1ml/kg/p.o	3.8±0.37	25.17±0.60
Diazepam	2mg/kg/i.p	7.2±0.37**	35.17±0.95**
MEAL	300 mg/kg/p.o	7.4±0.40*	35.83±0.95*
MEAL	400 mg/kg/p.o	4±0.45*	25.85±0.60

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test.*p<0.01, **p<0.001.

Rotarod apparatus: Represented in Table 4 In this test, methanolic extract of *Anogeissus latifolia* (300 mg/kg/p.o) significantly reduced the time spent by the animals on revolving rod when compared to control. Other dose of methanolic extract of *Anogeissus latifolia* leaves (400 mg/kg/p.o) did not produce a significant effect.

Table 4: Effect of methanolic extract of *Anogeissus latifolia* leaves on Rota rod apparatus test in mice

Treatment group	Dose	0 min	30 min
Control	1ml/kg/p.o	328.17±1.62	322.7±24.85
Diazepam	2mg/kg/i.p	340.50±18.93	104.8±2.85**
MEAL	300 mg/kg/p.o	342.23±18.60	189.5±41.57*
MEAL	400 mg/kg/p.o	342.50±12.23	201.0±32.45*

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test.*p<0.01, **p<0.001.

Traction test: Represented in Table 5 In this test, methanolic extract of *Anogeissus latifolia* (300 mg/kg/p.o) significantly reduced the motor co-ordination of tested animals.

Table 5: Effect of methanolic extract of *Anogeissus latifolia* leaves on Traction test in mice

Treatment group	Dose	% of animals showing negative test
Control	1ml/kg/p.o	0
Diazepam	2mg/kg/i.p	100
MEAL	300 mg/kg/p.o	78.3
MEAL	400 mg/kg/p.o	67.2

The values are Mean±SEM (n=6). Statistical significant test for comparison was done by one way Analysis of Variance (ANOVA) followed by Dunnett's test.*p<0.01, **p<0.001.

DISCUSSION

The present study investigated the putative central effects of the methanolic extract of the leaves of anogeissus latifolia a plant generally used in folk medicine as a sedative and antihypertensive remedy. Thus, given acutely at single doses of 100mg/kg, the extract of anogeissus latifoliaproduced significant dose-related decreases rearing and grooming behavior. The evaluation of the putative anxiolytic activity of anogeissus latifoliawas performed with the EPM, hole-boardand Stair case. In the EPM, rats with i.p. treatment showed a significant increase in both the number of entries and the percentage of time spent in the open arms of the maze, similar to the effects observed after administration of the reference anxiolytic drug diazepam. The oral treatment of mice with the extract promoted a slight enhancement of the percentage of entries in open arms at 300 mg/kg and 400mg/kg. Moreover, this treatment produced a decrease on head-dipping and stretch-attend postures along with an increase on rearing. Altogether, these results could indicate a mild anxiolytic-like activity of the extract of anogeissus latifolialeaves. However, the same treatment was unable to induce any significant effect in mice evaluated in the hole-board tests, which could be attributed to different kinds of anxiety. The results obtained after i.p. administration of the extract in rats demonstrate the high potency of the CNS effects of this plant. Chemical studies have reported the presence of several compounds on different parts of the plant. It is well known that the Methanolic extractof leaves of anogeissus latifoliais, which is proposed as its hypotensive principle. In conclusion, our results provide evidence that the Methanolic extract of the leaves of anogeissus latifoliapossesses CNS properties. Besides, anxiolytic-like effects are suggested by the EPM experiments, stair case and hole board and the motor coordination is through rota rod and traction test. However, further studies are necessary to confirm and extend these results. The findings presented here are relevant because they validate, for the first time, the folk uses of anogeissus latifolia, an important medicinal plant.

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