ANXIOLYTIC AND ANTICONVULSANT ACTIVITY OF ALCOHOLIC EXTRACT OF HEARTWOOD OF *AQUILARIA AGALLOCHA ROXB,* (THYMELAECEAE) IN MICE

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Summary

The objective of the present study was to assess the anxiolytic and anticonvulsant activity of alcoholic extract of heartwood of *Aquilaria agallocha* (AEAA) in mice. Preliminary phytochemical studies revealed the presence carbohydrates, flavonoids, tannins, saponins and tri terpenoids in AEAA. LD_{50} study was conducted in albino mice as per OECD guidelines no 425 and it was found to be 1098mg/kg. Anxiolytic activity in mice of AEAA was assessed using elevated plus- maze (EPM), Open field test (OFT) and light-dark transition (LDT) test models and anticonvulsant activity in pentylene tetrazole (PTZ), maximal electroshock (MES) induced convulsion models. AEAA at lower dose (30mg/kg, po) did not show any anxiolytic or anti convulsant effect. Pretreatment with AEAA (100 and 300 mg/kg, po) significantly (1) increased the total entries, number of entries into the open and closed arm and the time spent in the open arms in EPA, (2) increase total locomotion, time spent in central compartment and decrease the immobility time in OFT and (3) increase the latency to enter into the dark compartment and the time spent in the light compartment in LDT indicating that AEAA at these dose levels possess anxiolytic activity. Similarly pretreatment with AEAA (100 and 300 mg/kg, po) significantly delayed the onset of PTZ induced clonic convulsions and duration of extensor phase in MES induced convulsions indicating that this extract possess anticonvulsant activity. These results suggest that at doses 100 and 300mg/kg AEAA, exhibited both anxiolytic and anticonvulsant activities. The medium dose (100mg/kg) of AEAA showed more anxiolytic activity and less anticonvulsant activity than higher dose (300mg/kg).

Key words: anxiolytic, anticonvulsant, *Aquilaria agallocha*

Introduction

Anxiety disorders such as generalized anxiety, panic and obsessive-compulsive disorders, phobias or post traumatic stress disorders are common and major cause of disability.[3][1] Anxiety is affecting 1/8th of the total population worldwide and is a very important area of research interest in psychopharmology during this decade.[4][2] Anxiety also is an important component of many psychiatric and medical conditions.[5][3] In recent years there has been high concern over alternative medicines and plant derived medications that affect the "mind." During last few years, there also has been an increase in usage of alternative medicines by the patients for such ailments.[8][4] Self uses of herbal medicines are the most popular means to alternative therapies that include massage therapy, megavitamins, ayurveda and homeopathic medicines. Physicians in Europe and Asia are using these medicines to explore the traditional remedies and to find out a suitable cure for these 'mind affecting diseases'. Agar wood *(Aquilaria agallocha)* is one such plant that has been extensively used in *Ayurvedic* formulations for the treatment of mental illness. In Japanese system of medicine it is used as a sedative and anxiolytic agent and was reported to exhibit CNS depressant activity in mice.[9][5] In our laboratory preliminary pharmacological investigation with alcoholic extract of heart wood of *A. agallocha* had showed sedative and anxiolytic property. Hence the present study is planned to test the anxiolytic and anticonvulsant activity of alcoholic extract of heartwood of *A. agallocha* in mice.

Methods

Preparation of extract and its phytochemical investigation: Dried powdered heart wood was successively extracted with petroleum ether and 95 % ethanol using soxhlet apparatus for 18 hours. The solvents were dried under vaccum and the alcoholic extract of *Aquilaria agallocha* (AEAA) was used for further study. AEAA was subjected to phytochemical investigation[6] to determine the secondary metabolites. AEAA showed presence of carbohydrates, flavonoids and saponins.

Experimental animals: Swiss albino mice weighing between 18-25g were procured from Shri Venkateswara Enterprises, Bangalore for experimental purpose. All the animals were acclimatized for seven days under standard husbandry conditions, i.e.; room temperature of 24 ± 1^0 C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet (Amrut laboratories, Pranava Agro Industries Ltd., Sangli, India), with water supplied *ad libitum* under strict hygienic conditions. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the protocols and the experiments were conducted after prior approval from the Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy, Raichur.

Determination of LD_{50} in mice: Acute oral toxicity was performed in female nulliparous mice (18 – 22 g) as per OECD guidelines 425[7]. The LD_{50} was determined using AOT 425 software provided by Environment Protection Agency, USA. The LD_{50} of AEAA was found out to be 1098 mg/kg (range between 550 – 2000 mg/kg)

Assessment of anxiolytic activity: Male mice (18-22 g) were treated with vehicle (3 % tween 80)/ alcoholic extract (30 or 100 or 300 mg/kg, po)/ diazepam (2 mg/kg, po). One

after oral administration mice were placed in central arm of elevated plus maze (EPA) or central compartment of open field open field chamber or light compartment of light dark apparatus. Anxiolytic activity was measured by observing (1) total entries, number of entries into the open and closed arms and the time spent in the open and closed arms in EPA; (2) total locomotion, time spent in central compartment and immobility time in open field test and (3) latency to enter into the dark compartment in light dark model. Separate sets of mice were used for individual models.

Anticonvulsant activity: For anticonvulsant activity, one hour after oral administration of AEAA, pentylenetetrazole (PTZ) [80 mg/kg, ip) or 60 mA current for 0.2 sec through corneal electrodes (MES) were delivered to produce convulsions. The ability of the extract to prevent clonic, tonic and death of animals in PTZ model and tonic extensor in MES were recorded.

Results

ASSESSMENT OF ANXIOLYTIC ACTIVITY

Elevated Plus-Maze Test: Diazepam has long been reported for its anxiolytic activity in mice with the EPM. In our study also, a significant anxiolytic effect was recorded with diazepam (2 mg/kg, po) as it increased number of entries into open arm and decreased number of entries in to closed arms. It also increased time spent in to open but not in closed arms. Lower dose of AEAA (30mg/kg) had not exhibited any significant effect on number of entries in both open and closed arms and time spent in open and closed arm followed by total number of entries in both the arms. But medium and high doses (100 and 300mg/kg) of AEAA significantly increased open arm entries, time spent in open arm with decrease in closed arm entries and time spent in closed arm (Table 1). However, AEAA and diazepam did not affect the total entries during the test period.

Table 1: Effect of AEAA on Elevated plus maze test in mice
Values are expressed as mean ± SEM from 6 mice. **P*<0.05, ***P*<0.01; ****P*<0.001 as compared to control using one- way followed by Dunnett's test.

Treatment	**Number of entries (Counts/5min)**		**Time spent in (5min)**		**Total number of entries (5min)**
	Open arm	**Closed arm**	**Open arm**	**Closed arm**	
Control (3%tween 80)	1.5± 0.42	19.83± 2.49	8.83± 3.31	250.66± 15.99	21.16± 2.61
Diazepam (2mg/kg, po)	9.83± 1.92***	9.00± 1.71***	117.5± 11.80***	150.00± 12.73***	18.83 ±0.79
AEAA (30mg/kg, po)	1.66± 0.33	16.33± 0.88	11.83± 1.60	229.16± 8.44	17.16± 0.70
AEAA (100mg/kg)	7.83± 1.92**	9.83± 1.77**	118.00± 2.92***	131.16± 6.31***	17.66± 0.98
AEAA (300mg/kg)	6.66± 0.33*	10.33± 0.61**	112.33± 7.15***	130.16± 7.08***	17.00± 0.73

Open-Field Test (OFT): Different doses i.e., 30, 100 and 300mg/kg of AEAA were subjected for anxiolytic activity using open-field test. These doses when administered significantly increased the total locomotion at doses 100 and 300 mg/kg of AEAA. AEAA (100 mg/kg) had exhibited better activity than higher dose. A significant increase in central locomotion was recorded with AEAA (100 mg/kg), but not with low and higher dose levels (Table 2). Numbers of rearing were significantly reduced only with AEAA (100 mg/kg) but not with low and higher doses. Immobility time was significantly reduced only with AEAA (100 and 300 mg/kg). A significant reduction in grooming time was recorded with AEAA (100 and 300 mg/kg) but not with lower dose. Defecation and urination were not significantly altered with different doses of AEAA when compared to control group.

Table 2 : Effect of AEAA on open field test in mice

TREATMENT	Total locomotion	Central locomotion	Immobility time (sec)	Grooming time (sec)	Rearings	Urination	Defecation
Control (3%tween 80)	97.5± 3.31	11.66± 1.47	26.66± 1.80	25.33± 2.60	10.66± 0.88	0.66± 0.21	1.33± 0.42
Diazepam (2mg/kg, po)	199.5± 12.51***	31.00± 1.57***	9.33± 2.31*	15.16± 1.01*	2.50± 0.50***	1.0± 0.36 ns	1.33± 0.71 ns
AEAA (30mg/kg, po)	98.0± 7.35ns	16.33± 2.14 ns	25.66± 7.32 ns	17.5± 2.59 ns	9.16± 0.74 ns	0.33± 0.21 ns	1.33± 0.42 ns
AEAA (100mg/kg)	144..33± 8.17*	19.33± 1.05*	10.33± 0.66*	15.0± 2.25*	06.16± 2.63*	0	0.50± 0.22 ns
AEAA (300mg/kg)	141..33± 15.18*	15.50± 1.80 ns	11.50± 1.40*	13.50± 1.76**	07.83± 2.10 ns	0.66± 0.33 ns	1.16± 0.16 ns

Values are expressed as mean ± SEM from 6 mice. **P*<0.05, ***P*<0.01; ****P*<0.001 as compared to control using one- way followed by Dunnett's test.

Light dark model: AEAA was subjected for anxiolytic activity in LDT test. AEAA (100 and 300 mg/kg, po) had significantly increased the latency time to enter into dark compartment , number of crossings, time spent in light box and number of rearings. No significant effect was observed with lower dose (30mg/kg) of AEAA (Table 3).

Table 3 : Effect of AEAA on Light dark model

Treatment	Latency time to enter into dark compartment (sec)	No. of Crossings	Time (sec) spent in light box	No. of rearings in Light box
Control (3%tween 80)	11.66±1.02	5.50±0.99	28.33±4.37	16.33±0.71
Diazepam (2mg/kg, po)	22.16±2.21**	9.66±1.28*	56.33±4.60***	1.83±0.47***
AEAA (30mg/kg, po)	13.00±1.46	5.50±0.84	30.50±1.23	14.83±1.49
AEAA (100mg/kg)	24.00±3.05**	6.00±0.76$	51.00±5.61**	4.83±1.72 ***
AEAA (300mg/kg)	21.16±0.94*	4.66±0.91	48.66±6.05*	6.50±0.99 ***

Values are expressed as mean ± SEM from 6 mice. **P*<0.05, ***P*<0.01; ****P*<0.001 as compared to control using one- way followed by Dunnett's test.

ASSESSMENT OF ANTI CONVULSANT ACTIVITY

PTZ (Pentylene tetrazole) induced convulsions: AEAA at lower dose (30mg/kg) did not offered any significant anti convulsant effect, medium dose (100mg/kg) had significantly altered onset of clonus but not onset of tonic seizures, But higher dose exhibited a significant anti convulsant effect by increasing latency of clonus, onset of tonic seizures and decreasing the mortality of mice. 33% of animals were survived with AEAA (100 mg/kg) and 67% survived with AEAA (300 mg/kg) (Table 4).

Maximal Electro Shock (MES) induced convulsions AEAA (100 mg/kg, po) had exhibited a significant anti convulsant effect by altering the duration of tonic extensor and latency of clonus with 50% of protection against convulsions. AEAA (300 mg/kg) had not exhibited any significant effect on duration of tonic extensor phase but exhibited significant effect with latency of clonus with 66.66% of protection from convulsions. However, AEAA (30 mg/kg) did not show any anticonvulsant activity (Table 5).

Table 4: Anticonvulsant activity of AEAA against pentylenetetrazole induced convulsions in mice.

TREATEMENT	**LATENCY (Onset of clonus) (Sec)**	**ONSET OF TONIC (Sec)**	**% PROTECTION**
Control (3%tween 80)	49.33±0.88	389.66±24.13	0
Diazepam (5 mg/kg, po)	**No clonus**	**No Tonic**	100
AEAA (30mg/kg, po)	51.00±2.7^{s}	260.2±42.75	0
AEAA (100mg/kg)	59.66±1.54****	451.25±22.67	33.33
AEAA (300mg/kg)	60.20±1.98****	823.66±297.57*	66.66

Values are expressed as mean ± SEM from 6 mice. *P<0.05, **P<0.01; ***P<0.001 as compared to control using one- way followed by Dunnett's test.

Table 5: Anticonvulsant activity of AEAA against maximal electric shock induced convulsions in mice.

TREATEMENT	**DURATION OF TONIC FLEXION (Sec)**	**DURATION OF TONIC EXTENSOR (Sec)**	**LATENCY ONSET OF CLONUS (Sec)**	**% PROTECTION**
Control (3%tween 80)	NO	14.50±0.67	3.5±0.88	16.66
Phenytoin (25 mg/kg po)	7.33±0.61	NO	12.5±1.74***	100
AEAA (30mg/kg, po)	NO	14.83±0.47^{ns}	6.20±0.86^{ns}	16.66
AEAA (100mg/kg)	NO	10.33±1.02*	11.00±1.00*	50
AEAA (300mg/kg)	NO	10.83±1.35^{ns}	9.60±1.20*	66.66

Values are expressed as mean ± SEM from 6 mice. *P<0.05, **P<0.01; ***P<0.001 as compared to control using one- way followed by Dunnett's test.

Discussion

The EPM test is a well-established animal model for testing anxiolytic drugs. [67, 68]8,9 Diazepam, a standard anxiolytic drug used clinically, is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic- like effects.[69]10 The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm.[70]11 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. AEAA, at doses of 100 and 300mg/kg, had increased the time spent and percent of entries in to the open arm with a percent decrease in the closed arm. The medium dose of AEAA (100mg/kg), had increased percent number of entries in to the open arm than higher dose (300mg/kg) and total number of entries in to both arms decreased but not significantly as compared with control group for all doses i.e., 30, 100 and 300mg/kg suggesting that at higher doses of AEAA may have the sedative effect. So, the animals spent more time in open or closed arms. Lower dose of AEAA (30mg/kg) didn't alter the above parameters In the OFT, the confrontation with the situation induces anxiety behavior in rodents. The anxiety behavior is triggered by two factors, i.e., individual testing as the animal was separated from its social group and agoraphobia, as the arena is very large, relative to the animals breeding or the natural environment. In such situations rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation.[63]12Anxiolytic treatment decreases this anxiety-induced inhibition of exploratory behavior. The AEAA medium and high doses 100 and 300mg/kg showed a significant effect on central activity as compared to the effects recorded with 30mg/kg of AEAA. Medium dose of 100mg/kg of AEAA had shown more profound effects on total locomotion, central locomotion and rearings than higher dose (300mg/kg) of AEAA. significantly; suggesting that 30mg/kg of AEAA had not exhibiting anxiolytic effect. Present results suggest that the AEAA (100mg/kg) possess more anxiolytic activity when compared with 300mg/kg of AEAA, which is in agreement with the results obtained in the EPM.

In light-dark transition test (LDT), four behavioral events were observed i.e., latency to enter into the dark compartment, number of crossings between light and dark compartment, time spent in light box and number of rearings in light box. Diazepam 2mg/kg had shown significant effects with all four parameters. AEAA, at the doses of 100 and 300mg/kg, number of crossings only were not significantly increased but other three parameters i.e., latency, time spent in light box and number of rearings significantly altered. But, medium dose (100mg/kg) of AEAA showed better anxiolytic activity than higher dose (300mg/kg) of AEAA and 30mg/kg dose of AEAA had not show any significant effect with all parameters when compared to control group. . The present results suggest that the AEAA medium dose (100mg/kg) possess more anxiolytic activity than higher dose (300mg/kg) of AEAA, which is in agreement with the results obtained in EPM and OFT.

In PTZ induced convulsion model, parameters like latency to produce clonic convulsions, onset of tonic convulsions and percent protection were observed after treatment with

AEAA. Diazepam, 5mg/kg had prevented tonic and clonus convulsions and offered 100% protection. AEAA 30mg/kg dose had not significantly increased the latency of clonus and tonic phase and offered with zero percent protection. But medium and higher (100 and 300mg/kg) were significantly increased the onset of both clonus and tonic phases but 100mg/kg of AEAA didn't increased the latency of tonic phase and percent protection had seen maximum with the dose of 300mg/kg of AEAA. These results suggest that the 100 mg/kg of AEAA has less anticonvulsant effect than 300mg/kg of AEAA, which may be due to the more sedative effect for 300mg/kg as compared to100mg/kg of AEAA.

In MES-induced convulsion model, lower dose (30mg/kg) of AEAA didn't offered any anti convulsant effect against electroshock induced convulsions. In both acute and chronic studies, phenytoin 25mg/kg had prevented clonus convulsions with a 100% protection and produced tonic flexion and extensor convulsions. AEAA with doses of 100 and 300mg/kg had shown a significant anti convulsant effects by altering the duration of extensor phase and onset of clonus and offered with 50%, 66.66% protection without producing flexion convulsions and it was observed that if, the tonic flexion convulsions produced, onset of clonus and percent protection will increase with decrease the duration of tonic extensor. These results suggest that the medium dose (100 mg/kg) of AEAA has less anti convulsant effect than higher dose (300mg/kg) of AEAA; which may be due to the more sedative effect with higher (300mg/kg) dose. From the experimental study it can be concluded that alcoholic extract of heartwood of *Aquilaria agallocha* had exhibited significant anxiolytic and anti- convulsant activity in mice. Phytoconstituents like flavonoids and saponins were reported for their anxiolytic and anticonvulsant effect and these two were present in alcoholic extract. These active principles can be accounted for both anxiolytic and anticonvulsant effect.

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