Original Research Article

New insight in the Sedative and Anxiolytic Activities of *Amaranthus tricolor L.* Leaves Extract in mice

ABSTRACT

Aims: Recent decades have witnessed a resurgence of interest in Amaranthus spp. as nutraceuticals and natural protector against chronic ailments. But still there is no scientific report on neuropharmacological activities of Amaranthaus tricolor L. For this reason, the present study was designed to analyze in vivo sedative and anxiolytic potential of methanolic extract of Amaranthaus tricolor L. leaves in experimental mice model.
Place and Duration of Study: Department of Pharmacy, between January 2018 and August 2018.
Methodology: In this experiment, the crude extract of Amaranthus tricolor L. was evaluated for its CNS depressant effect using rodent behavioral models, such as open field, hole cross and rota rod tests for its sedative properties and an elevated plus maze test for its anxiolytic potential, respectively.
Results: In sedative assay, a dose-dependent and statistically significant ($p<0.05$) suppression of locomotors activity of the mice in both open field and hole cross test was exhibited by the extract at a dose of 200 and 400 mg/kg body weight. The extract also displayed increased percentage of entry into open arms at both doses in anxiolytic potential study. At a dose of 400 mg/kg body weight significant anxiolytic activity ($p<0.05$) was found compared to the standard diazepam.
Conclusion: This research unfolded the pivotal CNS depressant and anxiolytic effect of the methanolic extract of Amaranthus tricolor L leaves. Further studies on biologically active phytochemicals of the extract can provide access to therapeutic involvement.

Keywords: Amaranthus tricolor L., anxiolytic, elevated plus maze, sedative

1. INTRODUCTION

Anxiety and depressive disorders are the most common psychiatric conditions now-a-days. In many reports, it's stated that, more than 20% of the adult populations suffer from this condition at any stage during their life [1]. It has become an important area of research interest in psychopharmacology during the decade [2]. Due to presence of some adverse effects of antidepressant drugs searching for new pharmacotherapy from remedial plants for psychiatric illnesses has proceeded appreciably in the past decades [3]. According to WHO estimated, 121 million people suffer from clinical depression [4]. It occurs usually in the early adult life of patient with decrease in monoamine neurotransmitter [5]. Anxiolytic substances (mostly benzodiazepine drugs) are the most utilized drugs for man. Benzodiazepines act via the benzodiazepine receptors which are present on the GABA pentameric complex [6].Different side effects like psychomotor impairment, sedation, myorelaxation, ataxia, amnesia, potentiation of other central depressant drug and dependence liability limit the clinical uses of benzodiazepine [7-9]. Drugs with greater efficacy, less undesirable effects, low tolerance are the utmost need of human [10]. Medicinal plants therapies can effectively substitute the treatment of depression as they have fewer side effects than the synthetic medicine [11]. It has contributed appreciably towards the development of modern medicine. Recently, traditional medicine is being re-evaluated by extensive research on different plant species and their active therapeutic principles in worldwide [12].

Amaranthus tricolor; Common name: Lal-Shak, Chinese Spinach, Lal-Marsa [13] is spread all over the world including India, Assam, Kerala, Maharashtra, Bangladesh. The whole plant is astringent. A decoction of old plants is taken internally to improve vision and strengthen the liver. Amaranth has compounds with various health benefits, which are mostly present in the oil extracted from the seeds. Most pronounced compounds are: unsaturated fatty acids, tannin, saponins, lectins,tocopherols,tocotrienols,phytosterols,squalene,isoprenoid compounds, aliphatic alcohols, terpene alcohols and polyphenols, which have properties related to enhancing the immunity system, protection against cancer, prevention against oxidation, control serum lipid levels, decrease pain [14]. This plant acts as astringent in menorrhagia, leucorrhoea, dysentery, diarrhoea, hemorrhagic colitis, also used in cough, bronchitis and externally used as emollient [15]. So far there has been no scientific report in literature about the sedative and anxiolytic activity of methanolic extract of A. tricolor leaves. Therefore, in the present research, methanolic extract of A. tricolor leaves was evaluated to investigate its in vivo sedative and anxiolytic potential on some experimental animal models.

2. EXPERIMENTAL DETAILS

2.1 Plant material

The plant was collected from a field area in Halishahar upazila, on the outskirts of Chittagong city in 2018 and authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong. A voucher specimen has been deposited at the Department of Pharmacy, University of Science and Technology Chittagong (USTC).

2.2 Preparation of Plant Extract

The plant leaves were thoroughly washed with water and dried under subdues sunlight with air flow. After drying the leaves were then coarsely powdered using a suitable grinding mill. About 500g of powdered material was macerated with methanol (1:10) at room temperature for a period of 7 days with occasional shaking and stirring. After that the plant extract was filtered with clean cotton filter followed by Whitman filter paper(No. 1). The solvent was evaporated by Rotary evaporator (Lab Tech EV311) at 40 ºC under reduced pressure. The extract was then preserved in a refrigerator (2-8ºC) till further use.

2.3 Experimental animals

Swiss albino mice of either sex, weighing between 18-28g, were collected from Animal Research Branches of BCSIR, Chittagong. Animals were maintained under standard environmental conditions [(24.0±1.0)ºC, relative humidity: (55-70)% and 12 h light/dark cycle]. The animals were provided with standard laboratory food and water ad libitum. Before conducting tests, the animals were adapted to laboratory condition for one week. All experiments were carried under isolated and sound attenuated room.

2.4 Sedative activity/Exploratory activity

2.4.1 Open field test

The method was adopted as described by Barua A et al. [16] was slightly modified and used for the screening of depressive action of the extract on CNS in mice. The animals were divided into control, positive control, and test groups. The test groups received Amaranthus tricolor L. methanolic extract at a dose of 200 and 400mg/kg body weight p.o. and the control group received vehicle (1% Tween 80 in water) at a dose of 10ml/kg p.o. and positive control received standard drug Diazepam at a dose of 1mg/kg body weight p.o. The floor of an OFT of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a 40cm height wall. The number of squares traveled by the animals was counted for 3minutes, at 0, 30, 60, 90, and 120 minute during the study period after oral administration of both extract and standard [17].

2.4.2 Hole cross test

The method was carried out as described by Yadav G et al.[18],the apparatus was a cage of 30cm×20cm×14cm with a steel partition fixed in the middle, dividing the cage into two chambers. A hole of 3.5cm diameter was made at a height of 7.5cm in the center of the cage. The animals were divided into control, positive control, and test groups with 5 animals in each group. The test groups received Amaranthus tricolor L. methanolic extract at a dose of 200 and 400mg/kg body weight p.o. whereas the control group received vehicle (1% Tween 80 in water) at a dose of 10ml/kg p.o. and positive control received standard drug Diazepam at a dose of 1mg/kg body weight p.o. The number of passages of the animals through the hole from one chamber to the other was counted for 3min at 0, 30, 60, 90, 120min after oral administration of the extract as well as diazepam and vehicle. The apparatus was thoroughly cleaned after each test.

2.4.3 Rota rod test

By using a rota rod apparatus, the effect of drug on motor coordination was studied. This test is performed using rota rod apparatus, and it consists of four-compartment model (V. J, Instruments, India Inc.).Twenty four mice were divided into 4 groups (n=6) and served as group I for control (1% Tween-80 in saline, 10 ml/kg; p.o.) and groups III to IV were served as test (200 mg/kg and 400 mg/kg), while the positive control group (group II) received 1 mg/kg, i.p. of diazepam 30 minutes after i.p., and 60 min after oral administration of standard and test extract, animals were placed on rota rod rotating at a speed of 24 rpm. Mice of all the groups were kept on rota rod test, and the time each animal falls off from the rod was noted at 30 min, 60 min, and 90 min,120 min respectively. The distinction in fall of time observed with a negative control group and extract treated group was an index of muscle relaxant activity [19].

2.5 Anxiolytic activity

2.5.1 Elevated plus maze test

The method was employed according to Barua CC et al.with minor modification [20]. The apparatus consists of two open arms(5 × 10 cm) and two close arms (5 × 10 × 15 cm) radiating from a platform (5 × 5 cm) to form a plus –sign figure situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The animals were divided into control, positive control, and test groups with 5 animals in each group. The test groups received Amaranthus tricolor L. methanolic extract at a dose of 200 and 400mg/kg body weight p.o. whereas the control group received vehicle (1% Tween 80 in water) at a dose of 10ml/kg p.o. and positive control received standard drug Diazepam at a dose of 1mg/kg body weight p.o. Sixty minutes after administration of the test samples, each animal was individually placed in the center of the EPM and were allowed 5 min for free exploration. Next, the number of open and close arms entries, and time spent on open and close arms were manually registered. The whole test was carried out in a sound attenuated room. Entry into an arm was defined as the point when the animal placed all four paws onto the arm.

% of time spent in open arm=(Time spent in open arm)/(Time spent in open arm+Time spent in close arm)

% of entry in open arm=(No.of entry in open arm)/(No.of entry in open arm+No.of entry in close arm)

2.6 Statistical analysis

The data were expressed as mean ± Standard deviation (SD). One way ANNOVA followed by Dunnett's multiple comparison tests was used to perform statistical comparisons. The values were obtained were compared with the vehicle control group and were considered statistically significant when $p<0.05$.

3. RESULTS

3.1 Open field test

In the OFT, Amaranthus tricolor L. treated groups (200 and 400 mg/kg body weight) showed considerable and dose-dependent reduction of movement from its initial value at 0 to 120 min (Figure 1). The number of squares traveled by the mice was decreased significantly from its initial value at 0 to 120 min at a dose level of 400 mg/kg body weight ($p<0.05$) of the methanolic extract of Amaranthus tricolor L.

3.2 Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 30 to 120 min (Figure 2). Hole cross test of Amaranthus tricolor L. treated groups produced a significant ($p<0.05$) decrease of locomotion from its initial value during the period of the experiment at a dose of 400 mg/kg body weight, which was comparable to the reference diazepam ($p<0.05$) .

3.3 Rota rod method

The methanolic extract of Amaranthus tricolor was subjected to screening for muscle coordination activity by Rota rod method. The test was performed by taking methanolic extract at doses of 200 mg/kg and 400 mg/kg body weight. In this method mice falling from the rota rod in both diazepam (1 mg/kg) and ATMEx-treated groups exhibited a mild reduction in time spent by the mice in the rota rod test when compared with the reference drug diazepam group.

3.4 Elevated Plus Maze test

In the EPM, the behavior of mice model, as observed, provided an assurance of the anxiolytic activity of standard diazepam ($p<0.05$) as reported previously. Significant and dose-dependent increment of percentage of entries of mice in open arms, and the percentage of time spent in open arms of the EPM was showed by Amaranthus tricolor L. treated groups (200 and 400 mg/kg body weight) as shown in table 1. At a dose of 400 mg/kg body weight maximum anxiolytic activity was found as the maximum percentage of entries in open arms was displayed($p<0.05$) which was comparable to the standard diazepam.

4. DISCUSSION

The incidence of anxiety and depression in the community is very high and is associated with lot of morbidity. Hence, addressing these problems and finding effective remedies are very important. Several drugs are available but all are associated with some limitations and there is an urgent need for alternative medications for these disorders [21]. The first step toward the understanding of the effects in the central nervous system of the crude extract obtained from the leaves of A. tricolor on mice is represented by the present study and established that it has sedative and anxiolytic like activities.

The locomotors activity is a measure of the level of the excitability of the CNS and sedation resulting from depression of the central nervous system [22]. The study on locomotors activity, as measured by open field and hole cross tests, showed that the frequency and amplitude of movements was decreased by both doses of methanolic extract from the leaves of A. tricolor. Since locomotors activity is a measure of the level of excitability of the CNS [23], this decrease in spontaneous motor activity could be attributed to the sedative effect of the plant extracts [24]. The locomotors activity lowering effect was evident at the 3rd observation (60 min) and continued up to the 5th observation period (120 min). The result is also dose dependent and statistically significant (Figure 1 and Figure 2).

The elevated plus maze (EPM) is one of the most widely validated tests and is highly sensitive to the influence of anxiolytic drugs acting the gamma amino butyric acid type A (GABA A)-benzodiazepine complex [25]. In EPM, normally spending much of their allotted time in the closed arms will be preferred by normal mice. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drug like diazepam that increases open arms exploration are considered as anxiolytic [26]. An increase in open arm exploration (anxiolytic activity) was showed by the extract of A. tricolor, reflected by an increase in the percentage of entries into and time spent on the open arms. Although the A. tricolor methanolic extract at 200 mg/kg body weight, in mice, did not display a significant increase in the percentage of entries into open arms, the same extract at a 400 mg/kg body weight showed a significant increase in the percentage of time spent in the open arms of the maze. This was slightly minor than the effects observed

following treatment with the reference anxiolytic drug diazepam, in a dose dependent manner. An anxiolytic-like activity of the methanolic extract from the stems of A. tricolor could be indicated by these results.

Rota rode test a classical animal model used to evaluate peripheral neuromuscular blockade and the motor coordination [27], a deficit in motor coordination would very likely affect performance in the behavioral tests. This work showed that A. tricolor at both doses (200 and 400 mg/kg), unlike diazepam (1 mg/kg), had no significant effect on motor coordination, is additional evidence of centrally mediated actions and not blockade of neuromuscular system [28, 29]. Promising anxiolytic effects was showed by the methanolic extract of A. tricolor without causing any neuromuscular side effects.

GABAA-benzodiazepine receptors are the most abundant inhibitory receptor system in the CNS and binding of a benzodiazepine agonist to its recognizing site results in increased chloride ion flux [30] which in turn hyperpolarizes the postsynaptic membrane at a level below that at which spike generation is possible and for this reason some GABAA agonists are frequently used for their sedative effects. The compounds identified from the Amaranthus tricolor L. act as GABAA agonists and this agonistic property could be attributed to the CNS depressant effect of Amaranthus tricolor L. although there is no consensus about which substances are exactly responsible for these effects. However, further studies are necessary to address the contribution of other substances that are isolated for the activity observed, because it still remains to be determined which components exactly were responsible for these effects.

The presence of flavonoids, phenolic compounds, alkaloids, glycosides, saponins, steroids and tannins was revealed by phytochemical screening of A. tricolor [33,34]. Further studies of A. tricolor leaves showed the presence of betacyanins, betaxanthins with isoquercetin and rutin flavonoids. The investigators also found the common phenolic acids like salicylic, syringic, gallic, vanilic, ferulic, p-coumaric, ellagic and sinapic acid [31]. In addition to that, known betalains, red-violet amaranthin, a novel betaxanthin, arginine betaxanthin and betalamic acid were also reported in A. tricolor leaves [32].

It is suggested by substantial scientific report that saponins are known to show amphetamine antagonism, sedative property and decrease spontaneous motor activity in the experimental animal model [35]. It has also been reported that the presence of flavonoids, alkaloids and glycosides in plant extract possesses sedative and anxiolytic effect through the interaction with GABA-A receptors [36-38].Considering our results and previously published reports, it is possible that the above mentioned phytochemicals in the extract contribute at least in part to the observed CNS activities.

5. CONCLUSION

It is suggested by our preliminary pharmacological studies that the methanolic extract of Amaranthus tricolor leaves possesses CNS effect in experimental animals, which authorize the possible sedative and anxiolytic potential of the extract. Therefore, we advance the suggestion that the therapeutic need for the treatment of anxiety and related neuropsychiatric disorders may be achieved by the extract. However, further studies would be necessary to identify, isolate, characterize and estimate the active compounds for the activity showed as it still remains to be determined which components were exactly responsible for these effects. This bioactive-guided phytopharmacological research will give us the opportunity to identify pharmaceutical lead(s) with better tolerability and lesser side effects in new drug development.

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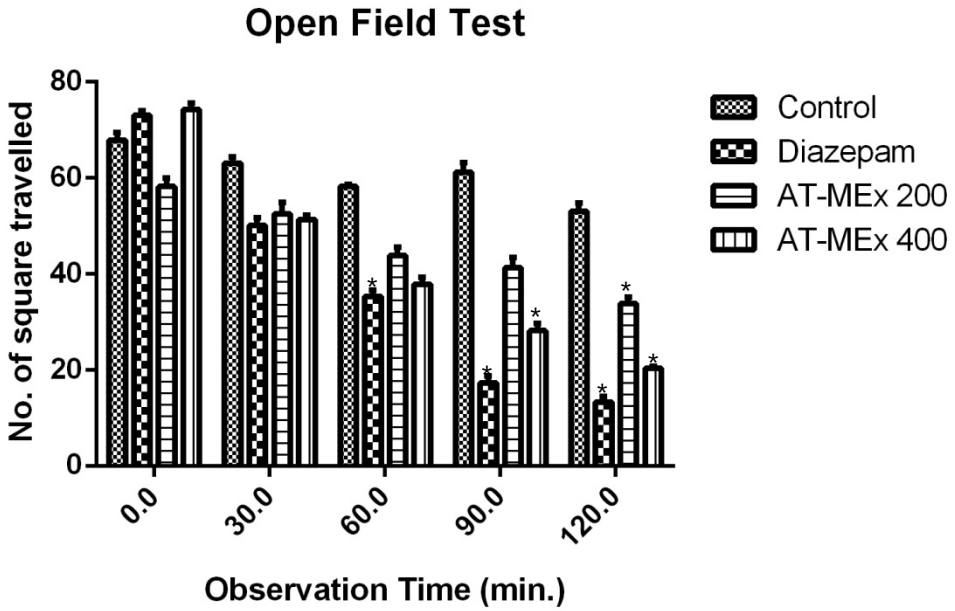

Figure 1: Effects of *A. tricolor* leaves extract on the open field test in mice. Values are mean±SEM., (n=6); * $p<0.05$, Dunnett test as compared to control (vehicle=10ml/kg)

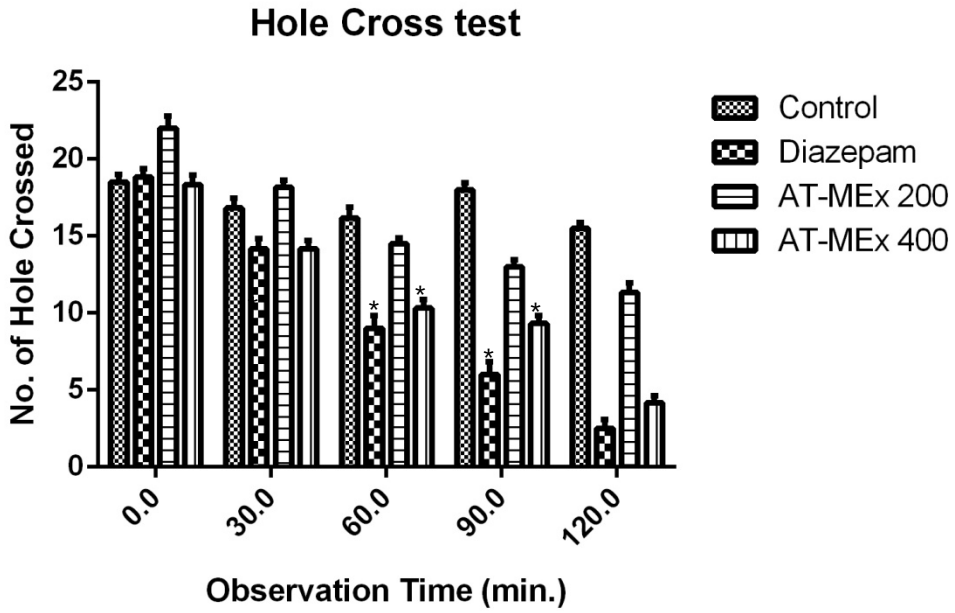

Figure 2: Effects of *A. tricolor* leaves extract on the Hole cross test in mice. Values are mean±SEM., (n=6); * $p<0.05$, Dunnett test as compared to control (vehicle=10ml/kg)

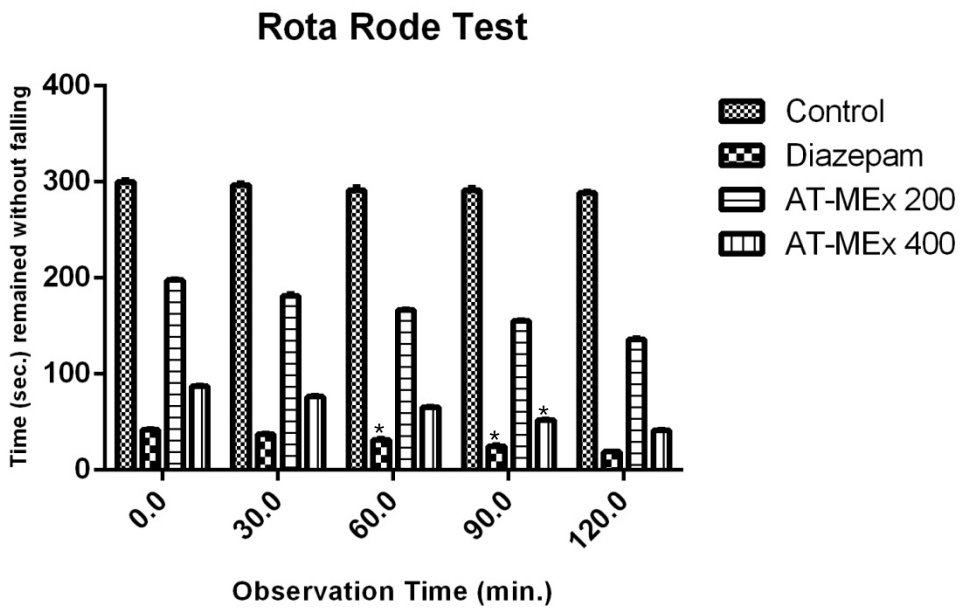

Figure 3: Effects of *A. tricolor* leaves extract on the Rota rod test in mice. Values are mean±SEM., (n=6); * $p<0.05$, Dunnett test as compared to control (vehicle=10ml/kg)

Table 1: Effect of methanolic extract of *A. tricolor* on EPM test during 5 min test period.

Animal Group	Dose	% of number of entry into open arm	% of Time (in seconds) spent in open arm
Control	10ml/kg	44.18±1.16	15.96±1.16
Diazepam	1mg/kg	63.35±1.47*	65.06±0.95*
AT-MEx 200	200mg/kg	44.63±2.56	24.08±0.72
AT-MEx 400	400mg/kg	48.59±3.12	48.95±1.06*

Values are mean±SD., (n=5); * $p<0.05$, Dunnet test as compared to control (vehicle=10ml/kg)