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EVALUATION OF THE NEUROPHARMACOLOGICAL ACTIVITIES OF HYDROETHANOLIC STEM EXTRACT OF *Massularia acuminata* (G. DON) BULLOCK (RUBIACEAE) ON MICE

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ABSTRACT

In traditional South-Western Nigerian medicine, the stem of *Massularia acuminata* (G. Don) Bullock (Rubiaceae) is used as chewing stick to improve oral health and for management of mental disorders. The aim of the study was to evaluate the neuropharmacological effects of *M. acuminata* hydroethanolic stem extract on mice. Anxiolytic activity of *M. acuminata* (50, 100 and 200 mg/kg p.o.) was evaluated using the elevated plus maze, hole board and open field tests. Forced swim and tail suspension tests were used to investigate antidepressant activity, while inclined plane and traction tests were used to evaluate muscle relaxant activity. *M. acuminata* extract (100 and 200 mg/kg p.o.) significantly ($p < 0.05$) increased number of open arms entries and time spent in open arms compared to control, thereby showing anxiolytic activity. This was comparable to that of diazepam (1 mg/kg). However, the extract at all doses tested did not show significant effect relative to control in hole board and open field tests. Moreover, *M. acuminata* extract did not show significant decrease in duration of immobility compared to control in forced swim and tail suspension models for depression. Also, in the inclined plane and traction tests, there was no observed muscle relaxant activity for *M. acuminata* compared to control. The results of this study showed that hydroethanolic stem extract of *M. acuminata* possesses anxiolytic activity, while it lacks significant antidepressant and muscle relaxant properties in mice. One or more of the phytochemicals present in the extract may be responsible for its anxiolytic activity. This finding gives some scientific evidence behind the folkloric use of the plant.

Key words: *Massularia acuminata, Elevated plus maze, Diazepam, Open field test, Traction test*

INTRODUCTION

Mood disorders are severe and chronic diseases, most of which could be classified as lifelong disorders (Marneros 2006). These disorders rank among the major health problems worldwide because they are highly prevalent in the general population, and also because they cause significant loss of quality of life and social functioning of an affected individual (Jacobi et al. 2005). They were shown to account for 21.2% of the years lived with disabilities worldwide (Vos et al. 2013).

The plant *Massularia acuminata* (G. Don) Bullock belongs to the family Rubiaceae. It is known to have a high inhibitory activity against some bacteria species (Barnabas and Nagarajan 1998). In Sierra Leone, the juice obtained from the fruit of the plant is used as antibiotics for treating eye infections (Yakubu et al. 2011). In traditional South-Western Nigerian

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medicine, the stems are used as chewing stick for oral hygiene and for the management of mental disorders (Ndukwe et al. 2004; Odugbemi and Akinsulire 2008). The decoction or infusion of the stem has also been claimed to be used as aphrodisiac and anticarcinogenic (Gill 1992; Odugbemi and Akinsulire 2008; Yakubu et al. 2011). The aim of the study was to evaluate the neuropharmacological effects of hydroethanolic stem extract of *M. acuminata* on mice.

MATERIALS AND METHODS

Collection and Identification of Plant

The stems of *M. acuminata* were purchased in Mushin market, Lagos State. The stems were identified at the Department of Botany, University of Lagos by Dr. Nodzer George Isaac, with voucher specimen number LUH 8249 assigned.

Extract Preparation

Dried stems of *M. acuminata* were crushed into smaller sizes to increase the surface area. *M. acuminata* (598 g) was macerated in 6 L of a 70:30 mixture of ethanol and distilled water for three days at room temperature. The hydroethanolic solution was decanted and filtered using Whatman filter paper, and the filtrate was oven-dried at a temperature of 40°C. The percentage yield was calculated to be 4%.

Preliminary Phytochemical Screening

Qualitative screening of the phytochemical constituents of the hydroethanolic stem extract of *M. acuminata* was carried out using the methods outlined by Sofowora (1993) and Egwaikhide and Gimba (2007).

Experimental Animals

Albino mice of either sex weighing between 20-25 g were used. The mice were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The mice were fed growers marsh (Animal Care, Ogere Remo, Ogun State, Nigeria) and had free access to drinking water. The animals were housed in plastic cages at room temperature (23-29°C) under standard environmental conditions and in accordance to guidelines for care of laboratory animals. Ethical approval for the mice experiments was obtained from College of Medicine, University of Lagos, Nigeria.

Acute Toxicity Test

The acute toxicity of hydroethanolic stem extract of *M. acuminata* was evaluated using 6 mice fasted overnight prior to the experiment and given a single dose of 2000 mg/kg. Signs of toxicity and mortality were observed for 24 hours.

Elevated Plus Maze Test

The elevated plus maze is an apparatus with two open arms and two closed arms (50 × 10 × 40 cm) placed at a height of 50 cm. Five groups of mice, six animals each were pretreated as follows: Group 1 - distilled water (10 ml/kg *per os*, p.o.); Group 2 – diazepam (1 mg/kg p.o.) and Groups 3-5 *M. acuminata* (50, 100 and 200 mg/kg p.o., respectively). One hour after the treatment, each mouse was placed in the centre of the apparatus and observed for five minutes. The number of entries of mice into the open arms and time spent in open arms within a duration of five minutes were observed (Vogel and Vogel 1997; Akindele and Adeyemi 2010). These measurements served as index of anxiety-like behaviour.

Hole Board Test

This experimental method is used to measure anxiety, stress, neophilia and emotionality in laboratory animals (Brown and Nemes 2008). Five groups of mice, six animals each, were pretreated as follows: Group 1 – distilled water (10 ml/kg p.o.); Group 2 – diazepam (1 mg/kg p.o.) and groups 3-5 *M. acuminata* (50, 100 and 200 mg/kg p.o.). An hour post-treatment, the pretreated mice were individually placed on the hole board and observed for head dipping and locomotion (Akindele and Adeyemi 2010; Adebesin et al. 2015).

Open Field Test

This test utilizes behavioural changes in rodents exposed to a novel environment and is used to confirm anxiolytic effect, based on the conflicting innate tendencies of avoidance of bright light, open spaces and exploration of new environments (Seibenhener and Wooten 2015). Five groups of mice, six animals each, were pretreated as follows: Group 1 – distilled water (10 ml/kg p.o.); Group 2 - diazepam (1 mg/kg p.o.) and groups 3-5 *M. acuminata* (50, 100 and 200 mg/kg p.o.). One hour post-treatment, each mouse was placed in the centre of the open field box (60 cm × 60 cm × 15 cm) which is divided to a grid of 16 equally sized squares divided by lines and a central square. The mouse was allowed to explore for five minutes, then the number of lines crossed by the mouse, its centre square entries and number of rearing were measured and used as indices for the degree of anxiety (Brown et al. 1999; Adebesin et al. 2015).

Forced Swim Test

Five groups of mice, six animals each, were pretreated as follows: Group 1 - distilled water (10 ml/kg p.o.); Group 2 - imipramine (20 mg/kg p.o.) and groups 3-5 *M. acuminata* (50, 100 and 200 mg/kg p.o.). After one hour of administration, each mouse was placed in a cylindrical apparatus (50 cm x 20 cm) filled with water three quarter from the bottom. Each session was 6 minutes long, divided into pre-test (the

first 2 minutes) and test (the last 4 minutes). The duration of immobility was recorded. The mouse was judged to be immobile when it stopped struggling and just remained floating (Porsolt et al. 1977; Yankelevitch-Yahav et al. 2015).

Table 1: Effect of *M. acuminata* Stem Extract on Mice in Elevated Plus Maze Test

Treatment Groups	Dose (mg/kg)	Number of Open Arm Entries	Time Spent in Open Arms (s)
Distilled water	10 ml/kg	0.67 ± 0.33	1.62 ± 1.11
M. acuminata	50	4.00 ± 0.37*	9.48 ± 2.14
M. acuminata	100	5.50 ± 0.89***	24.94 ± 5.02**
M. acuminata	200	1.50 ± 0.34	25.62 ± 6.01***
Diazepam	1	5.33 ± 1.36**	12.70 ± 5.62

Values are expressed as mean ± S.E.M. (n=5). *p < 0.05, **p < 0.01 and ***p < 0.001 versus distilled water.

Tail Suspension Test

Five groups of mice, six animals each, were pretreated as follows: Group 1 – distilled water (10 ml/kg p.o.); Group 2 – fluoxetine (20 mg/kg p.o.) and Groups 3-5 *M. acuminata* (50, 100 and 200 mg/kg p.o.). One hour post oral administration, adhesive tape was wrapped around the tail of each mouse around three quarters of the distance from the base of the tail. Animals were suspended in turns and duration of immobility was measured in seconds within 6 minutes (Steru et al. 1985; Salako et al. 2018).

Traction Test

Mice were screened for their ability to grasp the wire with their fore limbs plus at least one of the hind limbs within 5 seconds. Five groups of the selected mice, six animals each, were pretreated as follows: Group 1 – distilled water (10 ml/kg p.o.); Group 2- diazepam (1 mg/kg i.p.) and groups 3-5 *M. acuminata* (50, 100 and 200 mg/kg p.o.). One hour after, the time taken for selected mice to grasp the wire with their fore limbs plus at least one of the hind limbs was recorded (Adebesin et al. 2015).

Inclined Screen Test

The inclined plane test is used to determine skeletal muscle relaxant activity. The plane consists of transparent glass inclined at an angle of 30º. Each mouse was placed on the plane and then it tries to move on the glass without sliding off. Five groups of mice, six animals each were pretreated as follows: Group 1 - distilled water (10 ml/kg p.o.); Group 2 – diazepam (1 mg/kg i.p.) and groups 3-5 *M. acuminata* (50, 100 and 200 mg/kg p.o.). One hour after oral administration, the mice were kept in the superior part of the inclined plane to hang on or slide off within 5 minutes (300 s) (Adebesin et al. 2015).

Statistical Analysis

Results are expressed as mean ± standard error of mean. The data were analysed using one-way analysis if variance followed by Tukey's multiple comparison tests using Graphpad Prism (Graphpad software version 5.0). Results were considered significant with P-values of < 0.05.

RESULTS

Preliminary Phytochemicals Screening

Hydroethanolic stem extract of *M. acuminata* contains saponins, flavonoids, terpenoids, tannins, alkaloids and anthraquinones.

Acute Toxicity Test

No mortality was recorded within twenty-four hours of *M. acuminata* stem extract administration in mice at 2000 mg/kg.

Elevated Plus Maze Test

M. acuminata stem extract at doses 50 mg/kg (4.00 ± 0.37) and 100 mg/kg (5.50 ± 0.89) exhibited significant increase ($p < 0.05$) in the number of entries into open arms, while the time spent in open arms was significantly increased ($p < 0.01$) at doses 100 mg/kg (24.94 ± 5.02 s) and 200 mg/kg (25.62 ± 6.01 s) relative to the control (1.62 ± 1.11 s). The effect of the extract was comparable with that of

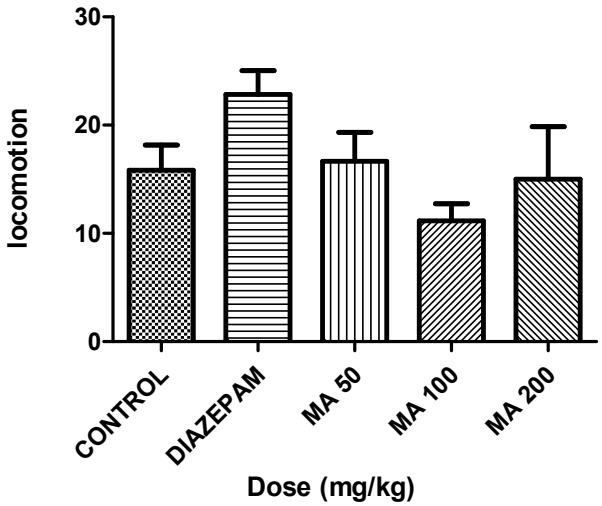

Figure 1: Bar chart Showing Effect of *M. acuminata* Stem Extract in Hole Board on Locomotion. Bars are expressed as mean ± S.E.M. (n=5). No significant difference between groups (P > 0.05). MA= M. acuminata stem extract.

diazepam (Table 1).

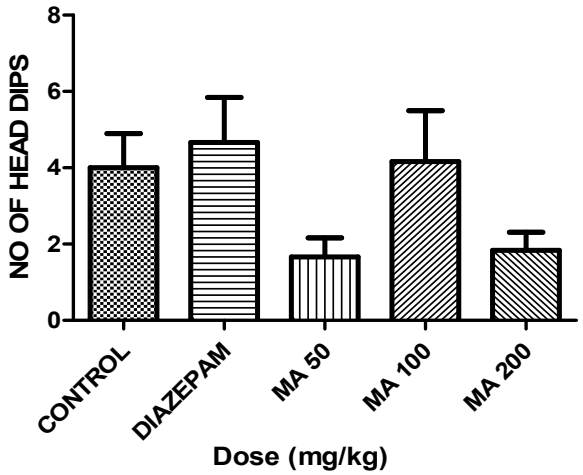

Figure 2: Bar Chart Showing Effect *M. acuminata* Stem Extract in Hole Board on Head Dipping. Bars are expressed as mean ± S.E.M. (n=5). No significant difference between groups (P > 0.05). MA= *M. acuminata* stem extract.

Hole Board Test

In respect of line crossing, *M. acuminata* stem extract did not increase number of line crossings relative to the control. Diazepam 1 mg/kg (22.83 ± 2.21) increased the number of line crossing in comparison to control (15.83 ± 2.33). However, the increase was not statistically significant (Figure 1). Similarly, diazepam (4.67 ± 1.17) increased the number of head dips relative to control (4.0 ± 0.89) but the increase was not statistically significant. On the other hand, *M. acuminata* stem extract at 50 mg/kg (1.67 ± 0.49) and 200 mg/kg (1.83 ± 0.47) reduced number of head dips relative to the control but not significantly (Figure 2).

Table 2: Effect of *M. acuminata* Stem Extract on Mice in Open Field Test

Treatment Groups	Dose (mg/kg)	Locomotion (Number of sectional crosses)	Centre Square Entries	Rearing
Distilled water	10 ml/kg	51.0 ± 2.44	0.66 ± 0.41	0.61 ± 0.42
M. acuminata	50	67.0 ± 2.16	2.0 ± 0.58	1.17 ± 0.83
M. acuminata	100	76.63 ± 4.10	1.17 ± 0.75	2.67 ± 1.09
M. acuminata	200	74.33 ± 4.33	2.00 ± 0.57	3.30 ± 1.63
Diazepam	1	73.5 ± 7.14	1.0 ± 0.52	0 ± 0

Values are expressed as mean ± S.E.M. (n=5). No significant difference between groups (p > 0.05).

Open Field Test

The number of lines crossed and number of centre square entries were increased in all treatment groups relative to control. However, the increase was not statistically significant. With respect to rearing, *M. acuminata* stem extract increased number of rearing at all doses with peak effect at 200 mg/kg (3.30 ± 1.63) relative to control (0.61 ± 0.42). Also, the increase was not statistically significant (Table 2).

Forced Swim Test

M. acuminata stem extract did not produce any significant decrease in duration of immobility at all doses compared to control (152.7 ± 27.53 s). Imipramine (IMP) at dose 20 mg/kg, decreased duration of immobility (79.40 ± 21.06 s) compared to control but it was not significant (Figure 3).

Tail Suspension

M. acuminata stem extract did not produce any significant decrease in duration of immobility at all doses compared to control (202.2 ± 25.12 sec). Fluoxetine at dose 20 mg/kg, decreased duration of immobility (142.1 ± 36.39 sec) compared to control but it was not significant (Figure 4).

Traction Test

M. acuminata stem extract showed no significant difference at all doses compared to control (0.2 ± 0.1 s) in the duration for at least one of the hind limbs of the mice to grasp the wire. On the other hand, diazepam (1 mg/kg) showed a significant increase ($p < 0.05$) in the duration for at least one of the hind limbs of the mice to grasp the wire (11.8 ± 5.8 s) compared to control (Figure 5).

Inclined Screen Test

There was no significant reduction in time spent on the inclined plane by *M. acuminata* stem extract at all doses tested compared to control (300.0 ± 0.0 sec). However, *M. acuminata* stem extract at dose 200 mg/kg slightly reduced the time spent on the inclined plane (241.5 ± 58.5 sec) though not significant when compared to the control. Diazepam (8.2 ± 2.9 sec) on the other hand significantly ($p < 0.001$) reduced the time spent on the inclined plane by mice (Figure 6).

DISCUSSION

The present study evaluated the anxiolytic, antidepressant and muscle relaxant effects of various doses of the hydroethanolic stem extract of *M. acuminata* in mice. The elevated plus maze, open field and hole board tests are standard models for screening central nervous system activity providing information about anxiety (Soussa et al. 2004).

The results obtained in the elevated plus maze test showed that oral treatment with *M. acuminata* stem

extract at doses 50 and 100 mg/kg significantly increased ($p < 0.05$) the number of entries into the open arms. The extract at doses 100 and 200 mg/kg significantly increased ($p < 0.05$) the time spent in the open arms relative to control. Agents that increase entries and duration spent in the open arms relative to control are regarded to have anxiolytic activity

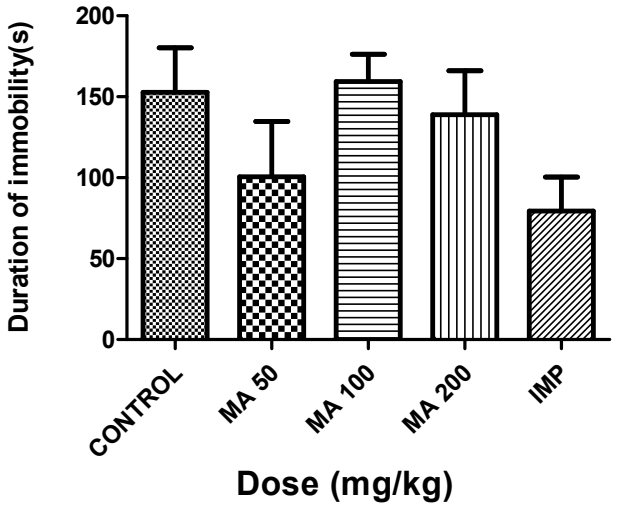

Figure 3: Effects of *M. acuminata* Stem Extract on Forced Swim Test Showing Duration of Immobility. Bars represent mean ± S.E.M. (n=5); P-values are not significant compared to control (distilled water) group ($p > 0.05$). IMP= imipramine; MA= *M. acuminat*a stem extract.

(Akindele and Adeyemi 2010). Diazepam as expected significantly increased ($p < 0.05$) the activity in the open arms of the elevated plus maze apparatus, confirming its anxiolytic activity.

With respect to locomotion in the hole board test, results obtained revealed that *M. acuminata* stem extract at all doses tested did not increase locomotion relative to control. Diazepam, the standard drug, did not significantly increase locomotion in comparison to control mice as expected. It has been proposed that the expression of an anxiolytic state in animals might be reflected by an increase in head-dipping behaviour (Takeda et al. 1998), while a decrease in the number of head dips was found to be correlated with depressant effect (File and Pellow 1986). Results obtained revealed that *M. acuminata* stem extract at doses 50 and 200 mg/kg reduced the number of head dips relative to control. It is not clearly understood why there was no increase in the head dips with the extract treatment as expected. Diazepam gave an increase in number of head dips in comparison to control as expected. However, results from the hole board test were not statistically significant.

The open field model is a useful model that measures the general activity of rodents and for elimination of the possibility of obtaining false positive results from neurobehavioural models (Karl et al. 2003). In the study, locomotor activity was not significantly different between the extract treated groups, diazepam treated group and the control group, which had the lowest locomotor activity. This also further confirms the absence of false positive results in the observed anxiolytic activity of the extract, especially at 100 mg/kg and diazepam. Furthermore, there was also no significant increase in the amount of rearing relative to the control group.

Forced swim and tail suspension tests, as antidepressant models, measure the duration of immobility when mice are exposed to an inescapable situation. Both paradigms are widely accepted behavioural models for assessing pharmacological antidepressant activity (Porsolt et al. 1977; Salako et al. 2018). Results obtained in the forced swim and tail suspension tests revealed that *M. acuminata* stem extract did not significantly reduce duration of immobility in the treated mice relative to control. This implies that the extract does not possess significant antidepressant activity.

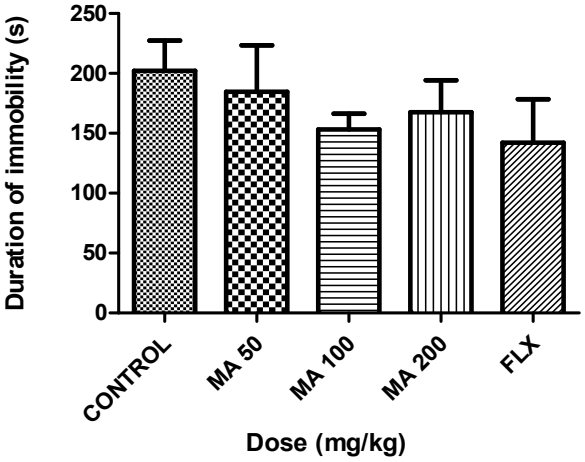

Figure 4: Effects of *M. acuminata* stem extract on tail suspension test showing duration of immobility. Bars represent mean ± S.E.M. (n=5); P-values are not significant compared to control (distilled water) group ($P > 0.05$). FLX= fluoxetine; MA= *M. acuminata* stem extract.

Traction and inclined screen tests are models for evaluating muscle relaxant activity of an agent. In the traction and inclined screen models, all mice treated with extract at various doses showed no muscle relaxant effect compared to control. On the other hand, diazepam showed significant muscle relaxant activity as expected.

Hydroethanolic stem extract of *M. acuminata* has an oral median lethal dose greater than 2,000 mg/kg in mice. This implies that the extract is relatively safe for humans in accordance to the Organisation for Economic Co-operation and Development (OECD) guideline (2001). Preliminary phytochemicals screening showed that hydroethanolic stem extract of *M. acuminata* contains saponins, flavonoids, terpenoids, tannins, alkaloids and anthraquinones.

One or combination of these phytochemical constituents might be responsible for the anxiolytic activity shown by the extract. For instance, saponins from plant extract have been shown to have significant anxiolytic activity in mice (Wei 2007).

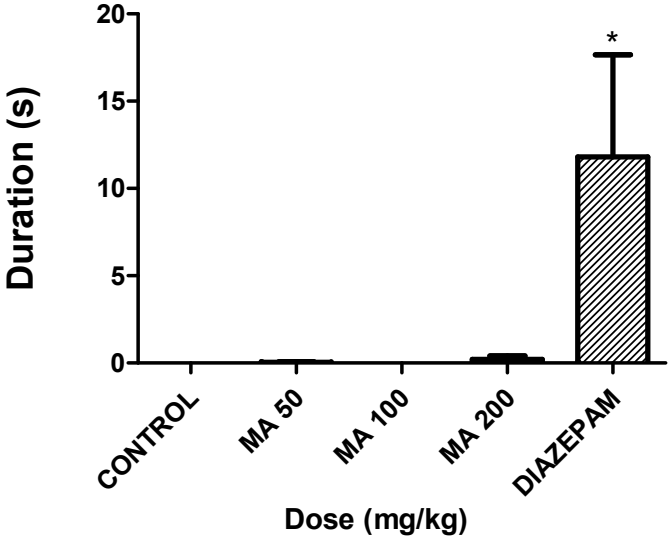

Figure 5: Effects of *M. acuminata* stem extract on traction test in mice. Bars represent mean ± S.E.M.; *P<0.05 vs control (distilled water) group (n=5) (one-way ANOVA followed by Tukey's multiple comparison test). MA= *M. acuminata* stem extract.

Conclusion

The results of this study showed that the hydroethanolic stem extract of *M. acuminata* possesses anxiolytic activity while it lacks significant antidepressant and muscle relaxant properties in mice. One or more of the phytochemicals present in the extract may be responsible for its anxiolytic activity. This finding gives some scientific evidence behind the folkloric use of the plant as one of the medicinal plants claimed to be used for the management of mental disorders.

Conflict of Interest

None declared.

Acknowledgement

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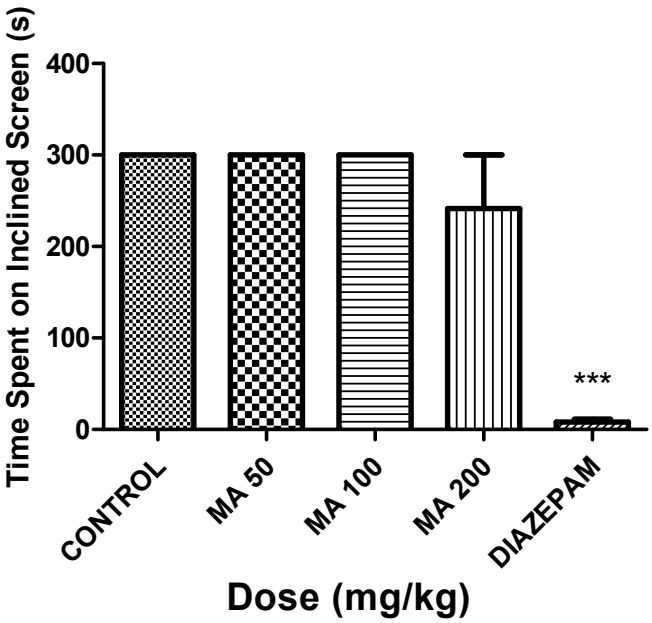

Figure 6: Effects of M. acuminata stem extract on inclined screen test in mice. Bars represent mean ± S.E.M.; ***P<0.001 versus control (distilled water) treated, (n=5) (one-way ANOVA followed by Tukey's multiple comparison test). MA= *M. acuminata* stem extract.

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