Anxiolytic Activity of Hydro-alcoholic Extract of *Mukia maderaspatana* Linn. Leaves on Experimental Animals

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ABSTRACT

The objective of the present study was to evaluate the anxiolytic activity of the hydro-alcoholic extract of the leaves of *Mukia maderaspatana*. Anxiety is a condition which occurs when exposing animals to unfamiliar conditions and creating fear. More synthetic compounds are available in the market for treating the anxiety, but only few natural compounds are available as anti-anxiety drugs. *Mukia maderaspatana* which belongs to the Cucurbitaceae family is a tropical plant which has the anti-anxiety property. To prove this, the hydro-alcoholic extract of the leaves of *Mukia maderaspatana* was tested on experimental Wister albino rats by using an elevated plus maze test and by using mice in a socio-behavioral deficit test. The drug was given in an oral dose of 150 mg/kg. The result indicates that the hydro-alcoholic extract of *Mukia maderaspatana* exhibited a significant ($p<0.05$) increase in the entry as well as time spent on an open arm in the maze test and reduced the attempts made at escape by the mice in the deficit test when compared with the standard drug diazepam.

Keywords: *Mukia maderaspatana*, Anti-anxiety, Diazepam, Albino rats, Mice, Elevated plus maze, Behavioral deficit.

INTRODUCTION

A lot of medicinal plants are available for the treatment of various diseases. *Mukia maderaspatana* (*M. maderaspatana)* which belongs to the family Cucurbitaceae is traditionally used to cure dysuria, piles, vertigo[1] and indigestion. The plant was scientifically evaluated for its anti-inflammatory[2], hypolipetamic[3], anti-oxidant[4], larvicidal[5], anti-platelet[6], anti-hypertensive[7], and hepato-protective[8] properties as well as its effectiveness against skin diseases.[9] The psychological behavior and physiological response which characterize anxiety can take many forms. Anxiety is often secondary to organic disease states. Another class of secondary state of anxiety is from circumstances, which is known as situational anxiety.[10] In this study, situational anxiety was taken as a point of experiment. Benzodiazepine, barbiturates, alcohol, and tricyclic anti-depressants have been used for a long time to treat anxiety disorders. The serious side effects associated with these drugs, namely rebound insomnia, muscle relaxation, sedation, withdrawl and tolerance, have limited and/or discouraged their use by patients.[11] Due to serious side effects associated with these drugs, the importance of a new anti-anxiety drug has increased.

MATERIALS AND METHODS

Plant Material

M. maderaspatana plant was collected and authenticated by Dr. G. V. S. Murthy, Joint Director of the Botanical Survey of India, Coimbatore, India. The plant material was cleaned from impurities and dried. The plant was then made into coarse powder. One hundred grams of the coarse powder was placed in the soxhlet apparatus

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using 500 mL of 1:1 ratio of water and ethanol. The extraction was carried out for 36 hours. The extract was concentrated and dried in vacuum[12], and then stored in a desiccator until use. The yield of the extract was 22.6 g.

Test Animals

Normally healthy Wister strain rats of 150-200 g and mice of 20-30 g of both sexes were used for the experiment. Animals were maintained under a standard diet and water ad libitum. The experimental protocol has been approved by the Institutional Animal Ethical Committee of The Erode College of Pharmacy, Erode Approval No: ECP/IAEC-A/2010/06/03.

EXPERIMENTAL PROCEDURE

Elevated Plus Maze Test[13]

The plus-maze consisted of two open arms, 50 × 10 × 40 cm, and two enclosed arms, 50 × 10 × 40 cm, with an open roof arranged so that the two open arms are opposite to each other. The maze was elevated to a height of 50 cm. The rats (150–200 g body weight) were housed in pairs for 10 days prior to testing in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. There were three groups that consisted of 6 rats each as follows:

Group I - Control

Group II - Standard – Diazepam (1 mg/kg by intraperitoneal injection)

Group III - Test drug – *M. maderaspatana* extract (150 mg/kg orally)

One hour after oral administration of the test drug and the standard, the rat was placed in the center of the maze, facing one of the enclosed arms. During a 5 min test period, the following measures were taken:

1. The number of entries into and time spent in the open and enclosed arms
2. The total number of arm entries was calculated.

Socio-behavioral Deficit[14-15]

It involved observation of the escape attempts by isolated mice (for 7-9 days) and grouped mice placed under an inverted beaker and tested together. After a 30-sec period of acclimatization, the number of escape attempts for 2 min was counted. Social behavior was calculated using the following formula:

$$\text{Behavioral deficit} = \frac{Eg - Ei}{Eg} \times 100$$

Where

Eg = number of escape attempts by grouped animals

Ei = number of escape attempts by isolated animals

= 94-83*100/94 =11.07

RESULT

M. maderaspatana 150 mg/kg of oral administration significantly increased the number of entries and the time spent in the open arm ($P<0.05$) when compared to the control group. Diazepam (1 mg/kg intraperitoneal) also exhibited the same increase patterns. When the rats were in the apparatus normally, they avoided entering into the open arm due to the fear. However, the groups which received the test substance as well as the standard entered many times and spent more time in the open arm than the normal control rats because the anti-anxiety substances were eliminating the fear. The results are shown in Figures (1) and (2) and Table (1).

Table 1: Effect of Hydro-alcoholic Extract of *M. maderaspatana* on Rats in an Elevated Plus Maze Test

Groups	No of entries in open arm	Time spent in open arm (sec)
Control	1±0.33	1±0.49
Std. (Diazepam 1 mg/kg)	3±0.85	21.66±3.61
M. maderaspatana (150mg/kg)	4±0.95*	14.83±1.68**

*p<0.05, **p<0.001 values are expressed in mean± SEM for 6 rats.

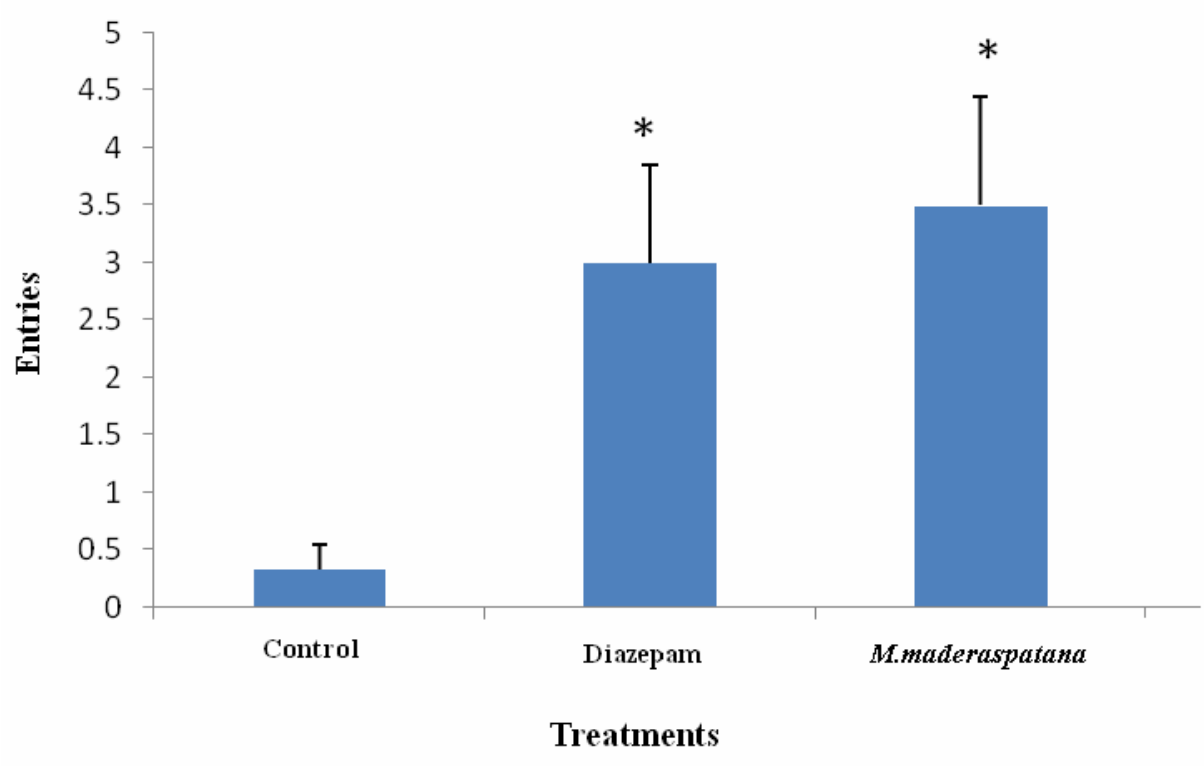

Figure 1: Effect of Hydro-alcoholic Extract of *M.maderaspatana* Leaves on Anxiolytic Activity (Number of Entries in Open Arm) on Rats In an Elevated Plus Maze Test

*p<0.05- Statistically significant when compared to standard.

SEM ±0.95: SEM- Standard Error Mean.

M.maderaspatana =Mukia maderaspatana

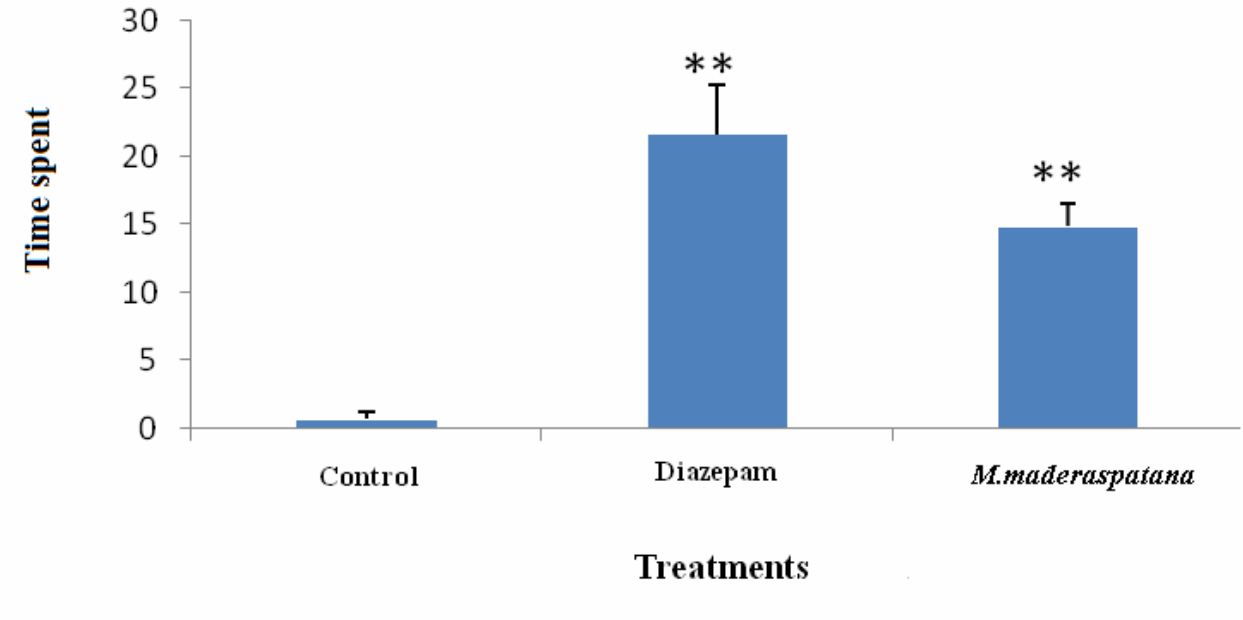

Figure 2: Effect of Hydro-alcoholic Extract of *M. maderaspatana* Leaves on Anxiolytic Activity (Time Spent in Open Arm) of Rats in an Elevated Plus Maze Test

**p<0.001- Statistically significant when compared to standard.

SEM±1.68: SEM- Standard Error Mean.

M.maderaspatana =Mukia maderaspatana

In the socio-behavioral deficit study, *M. maderaspatana* (150 mg/kg orally) significantly (P<0.01) reduced the escape attempts of the mice when compared with the control. The same reduction was observed for diazepam which causes a significant (p<0.01) reduction in behavioral at the dose of (1 mg/kg intraperitoneal). The obtained results show that the extract has significant activity when compared with the standard diazepam as well as the control. The results are shown in Figures (3) and (4) and Table (2).

Table 2: Effect of Hydro-alcoholic Extract of *M. maderaspatana* on Mice in a Socio-behavioral Deficit Test

Groups	No. of attempts (isolated)	No. of attempts (grouped)
Control	23 ± 1.76	41.16±3.36
Std. (Diazepam 1 mg/kg)	13.33±1.02	16.16±5.55
M. maderaspatana (150mg/kg)	16.66±1.53**	15.66±3.01**

**p<0.01, values are expressed in mean± SEM for 6 mice.

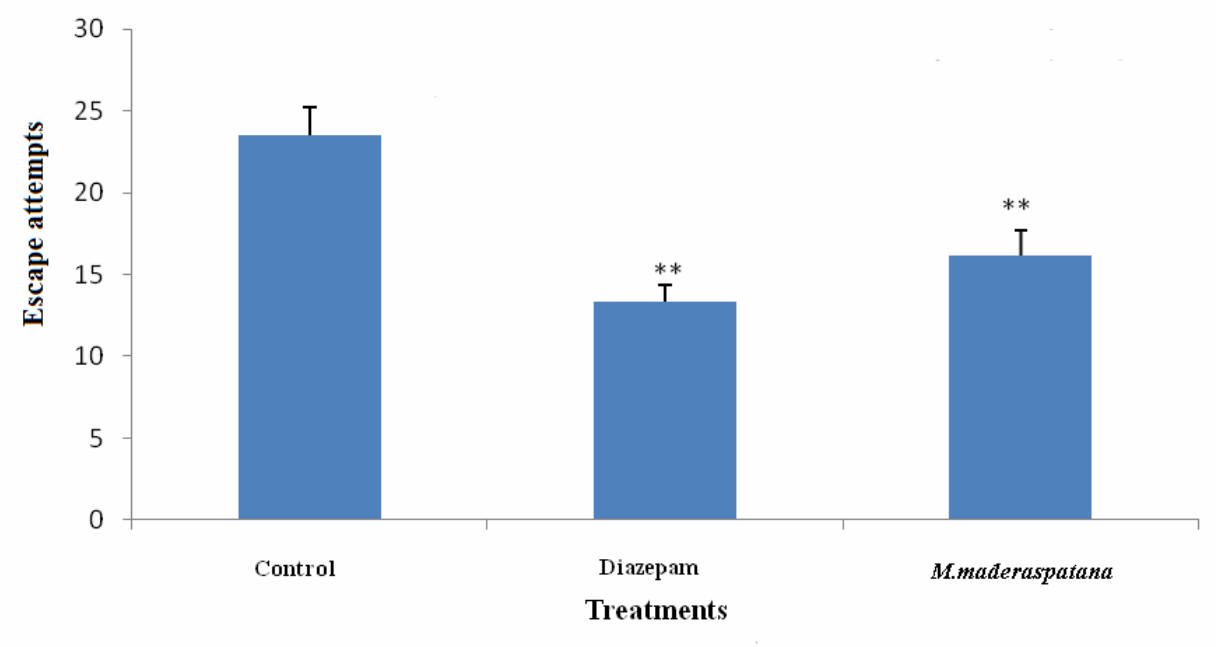

Figure 3: Effect of Hydro-alcoholic Extract of *M. maderaspatana* Leaves in a Socio-behavioral Deficit Test on Isolated Mice

**p<0.01- Statistically significant when compared to control

SEM±1.53: SEM- Standard Error Mean

M.maderaspatana =Mukia maderaspatana

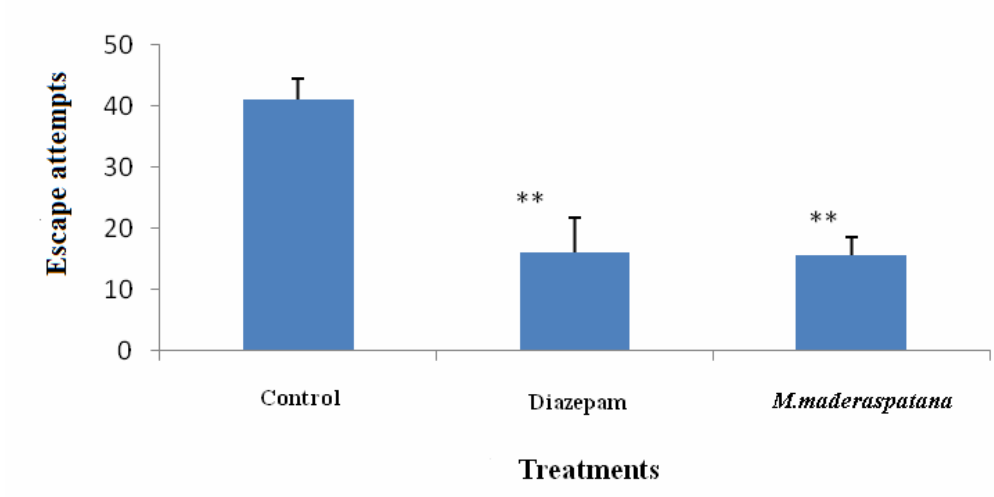

Figure 4: Effect of Hydro-alcoholic Extract of *M. maderaspatana* in a Socio-behavioural Deficit Test on Grouped Mice

**p<0.01- Statistically significant when compared to control

SEM±3.01: SEM- Standard Error Mean

M.madaraspatana =Mukia maderaspatana

DISCUSSION AND CONCLUSION

Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat.[16] Anxiety is both state and trait. Trait anxiety is the persistent and durable feature of the individual personality that reflects the way they interact with their physical and social environment. State anxiety, on other hand, is that seen in a given individual at a given time. It changes over time and in response to the level of stress and to the way that stress is perceived.[17] In this study, the animals were subjected to state anxiety conditions. A majority of the synapses in the mammalian central nervous system (CNS) use amino acids like L-glutamic acid, glycine or γ-amino butyric acid (GABA) for their signaling. GABA possesses two different types of receptors conserved across different species and phyla that conduct both excitation and inhibition. Molecular biological studies of the receptors causing these effects have indicated that GABA's effects on ionic transmission (ionotropic) and metabolism (metabotropic) are mediated by proteins in two different super families. The first super family ($GABA_A$ receptors) is a set of ligand-gated ion channels that convey GABA's effects on fast synaptic transmissions.[18] The second super family ($GABA_B$), which is slower, mediates GABA's action on intracellular effectors through seven trans-membrane spanning receptors.[19] $GABA_A$ receptors are the targets of a number of widely used and prescribed drugs for sleep, anxiety, seizure disorders, and cognitive enhancement; they may also contribute to mediating the effects of ethanol on the body. The conventional plus maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting on the $GABA_A$ benzodiazepine complex.[20]

Plus maze animal models are considered as one of the most widely validated tests for assaying sedative and anxiolytic drugs such as benzodiazepines.[21] A current study on *Sapindus mukorossi* shows that flavonoids, saponins and tannins possess activity against many CNS disorders.[22]

Based on the above references, the anti- anxiety activity of the test plant was evaluated by the elevated plus maze test by using rats and socio-behavioral deficit test was carried out by mice. Both results show significant anxiolytic activity of the hydro-alcoholic extract of the *M.maderaspatana* leaves. The standard used for this study was diazepam, which is a benzodiazepine, so the extract may also have the same mechanism of anxiolysis. The phyto-chemical studies of the *M.maderaspatana* confirmed the presence of flavonoids, saponins and tannins. So, the leaves' CNS activity may be due to these types of chemical constituents as per the reference. Further study should be done to prove this.

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انتزاع الماء والكحول من نبات *Mukia maderaspatana* يؤدي الي الحد من القلق والخوف وتم اختباره علي الحيوانات

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ملخـص

الهدف من هذه الدراسه هوتقليل نشاط القلق والخوف عن طريق دواء يوجدفي نبات يعرف بmaderaspatana Mukia.
القلق هو حاله تظهر عند وجود الحيوانات في بيئة مختلفة عن بيئتها الاصلية وتكون غير مألوفة تجعلها في حالة قلق وخوف.
كما تتوفر كمية قليلة من المركبات الطبيعية عكس التي يتم تحضيرها في المعامل التي تتوفر بصورة اكبر في الصيدليات والمراكز الطبيةمثل نبات الMukia maderaspatana،الذي له عائله تسميCucurbitaceae،وله بعض الميزات حيث انه لا ينمو الا في المناطق الاستوائية.
لاثبات ان انتزاع الماءوالكحول من نبات Mukia maderaspatana يساعد علي تقليل القلق والخوف تم اختباره عمليا علي فأرwister albino rats بواسطه جهاز يسمي Elevated plus maze مع مراعاة استخدام الفئران ذات التعامل الحاد مع بعضها البعض،يعطي هذا الدواء عن طريق الفم بجرعة تساوي150mg/kg.
تؤشر نتيجة انتزاع الماءوالكحول من نباتMukia maderaspatana الي انها ذات اهمية($p>0.05$)وهي تعمل علي زيادة الدخول اليopen arm والبقاء اطول فتره ممكنة علىopen arm كما تعمل ايضا علي تقليل محاولات الاعتداء عند الفأر مقارنة مع اخر تم علاجه بواسطة Diazepam.

الكلمات الدالة: Mukia made aspataa، القلق، الخوف.

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