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Anti stress activity of *Mikania micrantha* Kunth roots in Wistar albino rats

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Abstract

Objective: The present study was designed to evaluate adaptogenic activity of methanolic and aqueous extract of roots of *Mikania micrantha* Kunth in Wistar albino rats using different experimental models such as Anoxia stress tolerance, swimming endurance and immobilisation stress. **Methods:** The plant was subjected to preliminary phytochemical screening. The parameters like anoxia stress tolerance and swimming endurance time were recorded. The estimation of biochemical marker levels and determination of organs weight were carried out in immobilisation stress model. These activities are tested at oral doses of extract at 250 and 500 mg/kg and Diazepam 2mg/kg was used for comparison. **Results:** Preliminary phytochemical screening revealed the presence of flavonoids, steroids and tannins. Pretreatment with alcoholic extract showed increase in anoxia stress tolerance time and swimming endurance time. There was dose dependant significant reduction in biochemical parameters like serum glucose, cholesterol and BUN levels exhibited by alcoholic extract. The stress induced increase in liver, adrenal gland weight and decrease in weight of spleen were significantly reversed by the methanolic extract at higher dose. **Conclusion:** The results from the study indicated that methanolic extract of *Mikania micrantha* roots possessed significant antistress activity.

Keywords: *Mikania micrantha* Kunth, Plant extract, Phytochemical screening, Antistress activity, Acute toxicity studies (OECD Guidelines 423).

Introduction

Stress can be defined as the sum total of all the reactions of the body, which disturb the normal physiological condition and results in a state of threatened homeostasis. Normally stress induced changes are compensatory, self limiting and adaptive. However in higher animals when stress events of any nature (Physical, Chemical, Biological and Emotional) over certain 'threshold' limits, the changes become rather irreversible. It leads to altered homeostasis and exhaustion, manifesting itself in the pathologic form of stress induced disease and maladjustment. There is no treatment in modern drug therapy for stress related diseases. The available techniques for increasing endurance performance include physical training for endurance work, yogic and meditation practices, supplementation of nutraceuticals and intervention by adaptogens. Present study will provide a scientific base for experimental research on Indian herb for stress related diseases. The term 'Adaptogen' denotes an agent that improves adaptation capacity of the organism during stress and "Antistress" agent is a pharmacological word for the same, meaning an agent, which nullifies or prevents ill effects of stress and improves adaptation.[1]

Mikania micrantha Kunth (Asteraceae) is found in the tropics of America and Asia, and is widely known as guaco, the plant is a branched, extensively scrambling and twining slender-stemmed vine. Mikania comprises about 300 identified species, but only 20 of them have been studied. It is used to treat fever, rheumatism, influenza and respiratory diseases. Terpenes such as mikanolide are the major constituents isolated from plants of this genus. In the present study, an attempt has been made to investigate the adaptogenic activity using aqueous and methanolic extract of roots of the plant *Mikania micrantha* in view of reported adaptogenic activity of other species of Mikania namely Mikania cordata.[2]

Materials and methods

Collection and identification of the plant materials

Mikania micrantha roots were collected from Kottayam, Kerala, India during the month of March, 2011 and were authenticated by Mr. Joby Paul, Botanist, School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala (Voucher No. 1461).

Preparation of extracts

Extraction of roots of *M. micrantha* was carried out using methanol by hot continuous extraction method using soxhlet apparatus. 500 g of dried roots were taken, size reduced, extracted with 2 L of methanol in the round bottom flask and extraction was continued for few hours. The extract obtained was collected and concentrated by gentle heating. The concentrated extract was then weighed and stored. Thus total methanolic extract is obtained. Aqueous extraction was carried out with the remaining marc by reflex method. The marc was packed in a round bottom flask and refluxed for 2 h using a reflex condenser. The extract was then concentrated to dry residue by heating. Percentage yield of methanolic and aqueous extracts were found to be 7.7 and 3.8 % w/w.

Preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical constituents present in methanolic and aqueous extracts using standard methods.[3,4]

Selection of animals

Healthy adult Wistar albino rats, weighing about 150-220g obtained from the registered Animal house of University College of Pharmacy, MG University, Kottayam were used for the study. The study protocol was approved by the Institutional Animal Ethical Committee, University College of Pharmacy, Cheruvandoor Campus [001/MPH/UCP/CVR/12]. All the animals were housed individually in polypropylene cages, maintained under standard husbandry conditions (12 h light and dark cycles, room temperature and 45-55% relative humidity). They had been given standard pellet diet and water ad libitum throughout the course of the study.

Acute toxicity studies (OECD Guidelines 423)

The acute toxicity studied was carried out in female albino rats by "acute toxic class method" (OECD guidelines 423). The animals were fasted overnight and extract of the herb *M. micrantha* suspended in 0.5% Na CMC was administered starting at 2000 mg/kg; food was withheld for next 3-4 h. The animals were observed continuously for Body weight, any changes in skin and fur, eyes , behavior pattern and also signs of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma every 30 min for next 3 h and finally death after 24 h.

Evaluation of antistress activity

Anoxia stress tolerance test-

Albino Wistar rats of either sex weighing 150-220 g were selected and divided into 5 groups of six each as Group I Control (Received only vehicle CMC 0.5%w/v p.o.), Group II Methanolic extract of *M. micrantha* root (MEMMR 250mg/kg p.o.) Group III MEMMR (500 mg/kg p.o.) Group IV Aqueous extract of *M. micrantha* root (AEMMR 500 mg/kg p.o.) and Group V diazepam (2 mg/kg p.o.). Animals were treated as shown above for the 3 weeks. At the end of 1st, 2nd and 3rd week i.e. on 7th, 14th and 21st day 1 h after the treatment stress was induced by placing each animal individually in the hermetic vessel of 1 L capacity to record anoxia tolerance time. The time duration of entry of the animal into the hermetic vessel and the appearance of the first convulsion was taken as time of anoxia.

Swimming endurance test-

Albino Wistar rats of either sex weighing 150-220 g were selected and divided into five groups of six each as mentioned above. The rats were subjected to swimming stress by keeping them in propylene tank of dimension (37X37X30 cm), filled with water to a height of 25cm. Extracts were given to rats, once daily for period of 7 days. On 8th day the rats were allowed to swim till complete exhaustion and the endpoint was taken when the animal

started drowning. The mean swimming time for each group was calculated.[5]

Immobilisation stress-

Adult male albino rats of 150 -220 g were selected and divided into 6 groups of 6 animals each as Group I Negative control (Unstressed, untreated), Group II Positive control (Stressed, received vehicle), Group III MEMMR (250 mg/kg p.o.), Group IV MEMMR (500 mg/kg p.o.), Group V AEMMR (500 mg/kg p.o.), Group VI diazepam (2 mg/kg p.o.). The treatment was made as stated above for 10 days 1 h before the exposure of stress. Stress was induced by immobilizing rats with head down, supine position by fixing the forelimbs and hind limbs to a wooden board inclined at an angle of 600, daily 2 h for a period of 10 days. The animals were sacrificed at the end of specified period and blood was collected by retro-orbital for estimation of biochemical parameters such as, serum glucose, cholesterol and Blood Urea Nitrogen. The weight of organs, such as liver, spleen and adrenal glands after washing with alcohol was recorded per 100 g body weight of animal.[6]

Statistical analysis

The statistical analysis was performed using 'Graph pad prism 6' software by one way ANOVA followed by Dunnett's multiple comparison tests. All data were expressed as Mean ±SEM, P <0.05 was considered as statistically significant.

Results

Preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different phytochemical constituents present in methanolic and aqueous extracts of *M. micrantha*. The observations showed the presence of alkaloids, flavonoids, steroids, tannins and phenolics, which were found to be more in methanolic extract.

Acute toxicity studies

The methanolic and aqueous extracts of the plant *M. micrantha* was found to be safe up to 2000 mg/kg body weight by oral route. After 24 h animals were found well tolerated, there was no mortality and no signs of toxicity. The extracts were found to be safe, so the two dose levels i.e. 250 and 500 mg/kg body weight were selected for the present study.

Adaptogenic (antistress) activity

Anoxia stress tolerance time-

The results obtained from the anoxia stress tolerance test was expressed as Mean ±SEM. Anoxia stress tolerance time was significantly (P< 0.05) enhanced on 7th, 14th and 21st day in MEMMR (500 mg/kg) and Diazepam (2mg/kg) treated groups. There was increased anoxia tolerance time also seen after 2nd and 3rd week of MEMMR (250 mg/kg) treated group but not statistically significant result was obtained on 7th day. However the effect of AEMMR (500 mg/kg) on anoxia stress tolerance time in rats was not statistically significant at the end of 1st, 2nd and 3rd week of treatment (Table 1 and Figure 1).

Table 1: Effect of *M. micrantha* on anoxia stress tolerance time in rats

Treatment groups	Duration of anoxia stress tolerance in minutes		
	First week	Second week	Third Week
Positive control CMC (0.5%W/V) p.o	29±1.80	29.16±3.49	30.33±2.49
MEMMR(250mg/kg) p.o	38.66±4.62	41.33±3.42*	44±4.81*
MEMMR(500mg/kg) p.o	46.5±3.27**	46.33±4.34*	48.66±3.60**
AEMMR(500mg/kg) p.o	31.33±1.94	32.66±5.20	31.33±2.23
Diazepam (2 mg/kg) p.o	49.16±3.68***	48±3.91**	48.83±2.70**

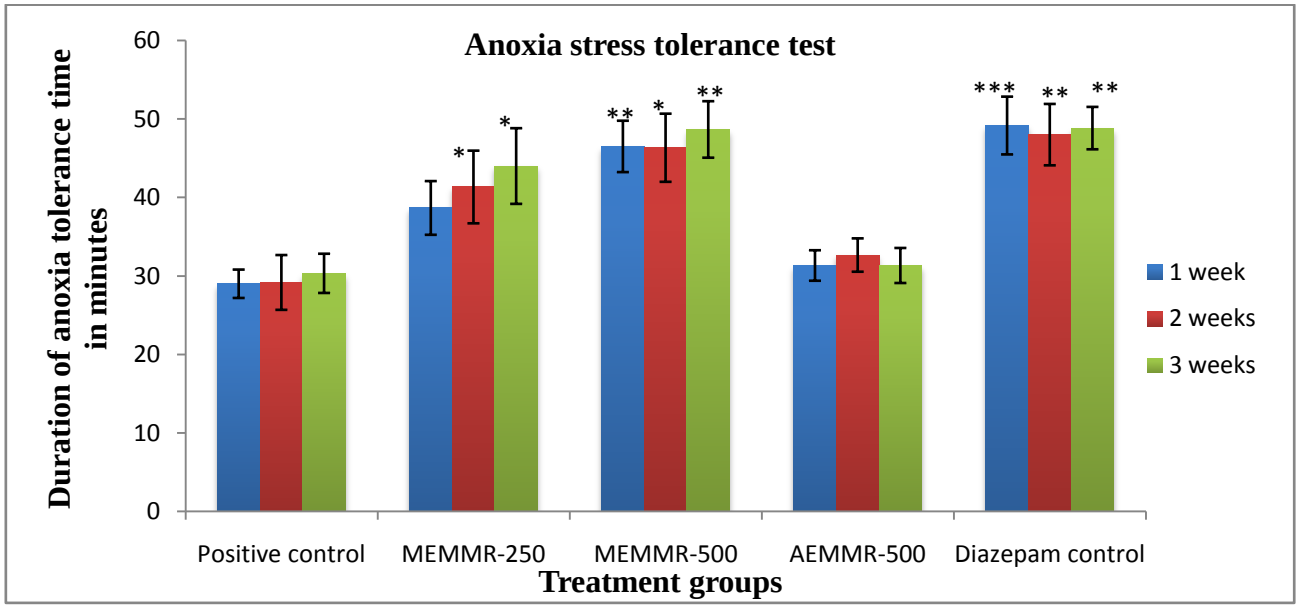

Figure 1: Effect of M.micrantha on anoxia stress tolerance time in rats

Values are expressed as Mean ± SEM (n=6), analysed by one-way ANOVA followed by Dunnett's post hoc test, *Represents statistical significance vs. control ($p<0.05$)

Swimming endurance test

The swimming endurance time was significantly ($P< 0.05$) enhanced on 8th day in MEMMR (250 mg/kg), MEMMR (500 mg/kg) and diazepam (2mg/kg) treated groups as compared to the stressed groups.

Table 2: Effect of *M. micrantha* on swimming endurance time

Treatment	**Swimming endurance time in minutes**
Positive control CMC (0.5%W/V) p.o	24.83±2.21
MEMMR (250mg/kg) p.o	35±1.98*
MEMMR (500mg/kg) p.o	38.5±2.47**
AEMMR (500mg/kg) p.o	31.5±3.27
Diazepam (2 mg/kg) p.o	39.66±3.11**

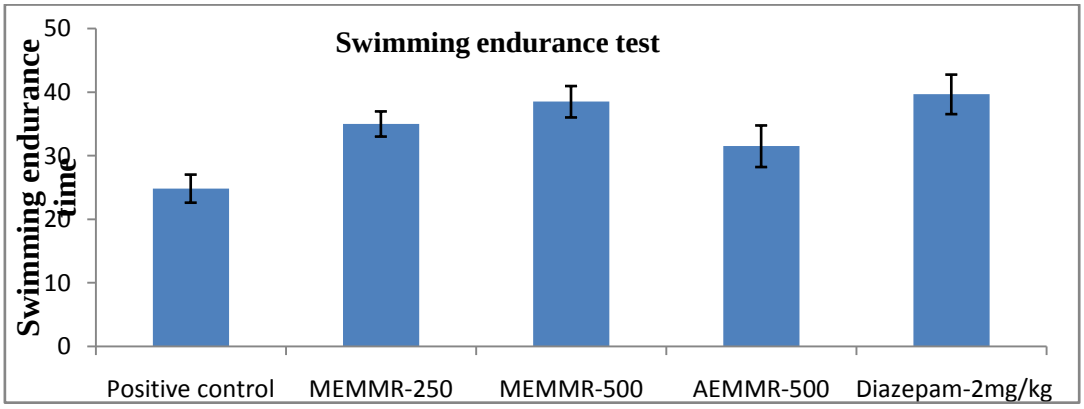

Figure 2: Effect *M. micrantha* on swimming endurance time in rats

Values are expressed as Mean ± SEM (n=6), analyzed by one-way ANOVA followed by Dunnett's post hoc test, *Represents statistical significance vs. control (p<0.05)

Immobilisation stress

Effect on biochemical parameters- The immobilisation stress caused marked increase in biochemical parameters such as glucose, cholesterol and blood urea nitrogen in stressed group when compared to the control group. This stress induced elevated level of biochemical parameters were significantly reversed in MEMMR 500 (mg/kg) treated groups. Pretreatment with AEMMR was found to be failed to reverse the elevated levels of biochemical parameters significantly.

Effect on organ weight- Weight of liver and adrenal gland was increased, while weight of spleen was reduced in stressed group compared to unstressed group. Pretreatment with MEMMR extract at high dose significantly (P< 0.01) reduced the weight of the liver, adrenal gland and increased the weight of the spleen. However the MEMMR extract at low dose (250 mg/kg) and AEMMR (500 mg/kg) were failed to protect the immobilisation stress induced changes in organ weight such as liver, adrenal gland and spleen.

Table 3: Effect of *M. micrantha* on immobilization stress induced changes in biochemical parameters

Treatment groups	Biochemical estimation (mg/dl)		
	Glucose	**Cholesterol**	**Blood urea nitrogen**
Negative Control	78.66±2.41	75.66±2.12	24.16±1.25
Positive control CMC (0.5%W/V) p.o	110.33±4.16	95.16±3.07	36±1.36
MEMMR (250mg/kg) p.o	102.5±3.58	86.33±3.45	32±1.75
MEMMR (500mg/kg) p.o	96.66±3.71*	83.33±3.82*	28.83±1.62*
AEMMR (500mg/kg) p.o	104.5±4.45	90.5±2.14	35.33±2.26
Diazepam (2 mg/kg) p.o	97±2.17*	84±2.68*	28.66±1.76*

Values are expressed as Mean ± SEM (n=6), analyzed by one-way ANOVA followed by Dunnett's post hoc test, * P<0.05, ** P<0.001, *** P<0.0001

Table 4: Effect of *M. micrantha* on immobilization stress induced changes in organ weight

Treatment groups	Organ weight (gm/100gm B.W)		
	Liver	**Adrenal gland**	**Spleen**
Negative Control	3.71±0.08	0.015±.001	0.410±0.018
Positive control CMC (0.5%W/V) p.o	4.95±0.14	0.032±.002	0.287±0.025
MEMMR (250mg/kg) p.o	4.50±0.24	0.025±.002*	0.348±0.032
MEMMR (500mg/kg) p.o	4.41±0.024*	0.02±.0016**	0.384±0.016*
AEMMR (500mg/kg) p.o	4.90±0.21	0.03±.0025	0.301±0.017
Diazepam (2 mg/kg) p.o	4.37±0.22*	0.026±.008*	0.312±0.028

Values are expressed as Mean ± SEM (n=6), analyzed by one-way ANOVA followed by Dunnett's post hoc test, * P<0.05, ** P<0.001, *** P<0.0001

Discussion

In the present investigation methanolic and aqueous extracts of *M. micrantha* has been evaluated for the antistress (adaptogenic) activity against different types of stresses viz. Anoxia, swimming endurance and immobilisation models. Diazepam, benzodiazepine anxiolytics was used for the comparison. Diazepam is reported to possess a non-specific anti-stress activity involving the mesocortical dopamine system and the norepinephrine and 5HT levels of whole brain and hypothalamus. It is proposed that this effect is produced

through an enhancement of GABAergic neurotransmission.[7]

In anoxia stress tolerance model, depletion of oxygen in hermetic vessel leads to convulsions in animals and pretreatment with methanolic extract of *M. micrantha* had increased the duration of stress tolerance indicating their adaptogenic/ anti-stress activity (Table 1). This effect may be due to that during stress, the methanolic extract of *M. micrantha* was capable of increasing succinate dehydrogenase (SDH) in the brain. This enzyme is responsible for utilization and conservation of energy in the cellular system of the organism, which helps adaptive processes during stress. Adaptogens producing beneficial effects in stress are believed to act by increasing non-specific resistance.

In case of swimming endurance test MEMMR exhibited significant antistress activity as indicated by increase in swimming endurance time (Table 2). There are reports that plasma levels of adrenaline and noradrenaline are enhanced during stress induced by swimming endurance test. In addition, monoamine oxidase (MAO) levels in the brain are reportedly decreased during stress.[8] The swim endurance test results indicate clearly that the methanolic extract of *M. micrantha* has the properties whereby it increases the physical endurance as well as the overall performance in rats and possessed significant anti-stress activity. It may be possibly normalizing the plasma level of catecholamine and MAO.

The immobilisation stress caused marked increase in biochemical parameters such as glucose, cholesterol and blood urea nitrogen in stressed group when compared to the control group. In the present study, a significant hyperglycemia was observed with immobilization stress model. Under stressful conditions, cortisol in human and corticosterone in rats will be secreted by adrenal cortex. Hyper secretion of cortisol helps the maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis.[8] Methanolic extract of *M. micrantha* significantly reduced the hyperglycemia may be by reducing the hyperactivity of adrenal cortex and also by maintenance of homeostatic mechanism in immobilization stress animals.

The mechanism by which stress rises serum cholesterol is likely to be related to the enhanced activity of hypothalamo-hypophyseal axis (HPA) resulting in liberation of catecholamines and corticosteroids. This could lead to increase in blood cholesterol level since epinephrine is known to mobilise lipids from adipose tissues. The increase in release of catecholamines leads to elevated levels of glucose and BUN.[9] This stress induced elevated level of biochemical parameters were significantly reversed in MEMMR 500 mg/kg treated groups (Table 3).

Adrenal glands and liver weights were significantly increased in immobilization stress models. Stress induces adreno-medullary response in man to release adrenaline which in turn stimulates β2 receptors on the pituitary gland. It leads to greater release of ACTH that can stimulate the adrenal medulla as well as cortex resulting in further release of adrenaline and increase in weight of adrenal gland to a greater extent. Cortisol increases mRNA levels in liver cells, this lead to increase in weight of liver. Spleen constricts to release more blood cells (RBC) during stress. So its weight decreases during stress.[6] This stress induced changes of organs weight were significantly reversed by the methanolic extract at higher dose 500 mg/kg (Table 4).

Literature survey indicates that flavonoids, triterpenes and tannins were reported to possess variety of pharmacological activities including antistress activity. In the present investigation also preliminary phytochemical screening on MEMMR gave positive tests for flavonoids, steroids and tannins, this might be the reason for significant adaptogenic property of test extract.

Conclusion

The result from the study showed an increase in duration of anoxia tolerance and swimming endurance time in rats treated with methanolic extract. The reversal of immobilization stress induced changes in biochemical parameters and organs weight were also exhibited in alcoholic extract treated groups. So the results suggest the adaptogenic activity of the plant *M. micrantha*, hence it can be categorized as plant adaptogen. The results are encouraging to pursue further studies on the bioactivity guided fractionation of these extracts to isolate and characterize probable bioactive molecule responsible for ant-stress activity.

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