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Adaptogenic (Anti-Stress) Effect of Aqueous *Musanga cecropioides* (Urticaceae)

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ABSTRACT

Everyone tries to relieve stress by taking anti-stress drugs. However, synthetic anti-stress drugs have unwanted side effects, expensive and not readily accessible. Thus, we aimed at investigating the anti-stress activities of aqueous extract of *Musanga cecropioides* stem bark using forced swimming endurance stress test, anoxia stress tolerance, chronic cold resistance stress test and DPPH radical scavenging capacity. Daily administration of aqueous extract of *M. cecropioides* at doses of 100, 200 and 300mg/kg bodyweight one hour prior to induction of stress, at the lowest dosage (100 mg/kg) significantly increased the anoxia stress and forced swimming endurance stress tolerance times in a dose dependent manner as compared to the control groups (46.48±0.28 and 3.01±0.92 compared 28.80±0.38 and 2.25±0.08 mins), the blood glucose, blood cells, and the urinary ascorbic acid were normalised in all the tested methods including chronic cold stress. The extract also scavenged DPPH significantly in a dose dependent manner. The results showed impressive anti-stress potential and present as far as is known, the first report of the anti-stress (adaptogenic) activities of *M. cecropioides*.

Keywords

Anti-stress, Anti-oxidant, *Musanga cecropioides*

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Introduction

Stress can be termed as the summation of all the reactions of the body, which destabilises the normal physiological condition resulting in a state of threatened homeostasis. Stress is internationally recognized as phenomenon caused by advancement of industrialization in a demanding civilization. Stress represents a reaction of the body to a stimulus that tends to

alter its normal physiological equilibrium or homeostasis (Selye, 1998). Adaptogens (anti-stress) could be chemically or naturally derived active entities which induce non-specific responses that increase the resistance of an organism to a state stress (Ahmad *et al.*, 1998). Most of which are scientifically unproven, yet marketed as a supplement to increase resistance to stress, trauma, anxiety and fatigue, hence it is imperative that such

claim be subjected to scientific studies. This study was aimed at investigating the anti-stress effect of aqueous extract *M. cecropioides* of its many biological activities. Supplements with various natural recipes have been evaluated for their adaptogenic activity during exposure to stressful environmental conditions (Kenjale *et al.*, 2007) but nothing of such on *M. cecropioides*.

M. cecropioides is an evergreen straight stemmed tree up to 18 m tall. The tree's crown is umbrella-like, with very stout and pithy branchlets producing no latex (Orwa *et al.*, 2009). Traditionally, the plant is used to induce labour, reduce elevated blood pressure and also to reduce high blood sugar (Irvine, 1961), anthelmintic and antidysentric (Gill, 1992), treatment of fever, jaundice, acute gastric poisonings and liver diseases (Bunkill, 1985).

Phytochemical studies have reported the presence of kallaic acid stem bark and some other triterpenoid acids in the leaves stem bark and the root wood (Lontsi *et al.*, 1998). Protocatechuic acid (3,4-dihydroxybenzoic acid) and protocatechualdehyde (3,4-dihydroxybenzaldehyde) were obtained from the stem bark of the plant (Ayinde *et al.*, 2007).

Reported biological activities of the plant include; uterotonic (Kamanyi *et al.*, 1992), antidiabetic (Adeneye *et al.*, 2007), hypotensive (Ayinde *et al.*, 2003; Adeneye *et al.*, 2006; Ayinde *et al.*, 2009; Dongmo *et al.*, 1996), Oxytocic effects (Ayinde *et al.*, 2006) etc.

Materials and Methods

Plant materials

The fresh stem bark of the *M. cecropioides* was collected within the locality of Ewu

town, Esan LGA, Edo state, Nigeria in the month of May, 2016. The plant was identified and authenticated by Mr. E.A Emmanuel (of Professor J.C. Okafor Herbarium, Pax Herbal Clinic and Research Laboratories, Ewu, Nigeria) and voucher specimen compared with Herbarium sample in the department of pharmacognosy, University of Benin, Benin city.

Plant processing and preparation of extract

The bark was cut into smaller pieces and air-dried at room temperature for a period of 14 days. Thereafter, they were transferred into an oven maintained at 50°C for another 2 days before pulverised into powder using an electric milling machine (Chris Norris, England).

The powdered stem bark (2kg) *M. cecropioides* was gradually extracted with distilled water (4L) using decoction method for 30 min. The extract obtained was concentrated on electrothermal constant water bath (Model DK-8A) to obtain a brown semisolid paste which was stored in sample bottles kept in refrigerator (4°C) for further use.

Chemicals used

All chemical and reagent used are of analytical grade obtained from Pax herbal clinic and research laboratories.

Experimental animal grouping

The rats were randomly divided into 5 groups (1-5) of 6 rats as follows;

Group 1 Unstressed control group and were given only distilled water

Group 2 Stressed control group and were given only distilled water

Group 3 Rats were given standard drug (diazepam) 1mg/kg

Group 4 Rats were given 200mg/kg of *M. cecropioides* extract

Group 5 Rats were given 400mg/kg of *M. cecropioides* extract.

Forced swimming endurance stress test

Swiss albino mice (18–25g) were used for forced for forced swim endurance stress test. The animals were divided into 5 groups and all were treated as mentioned above in scheduled drug treatments. The drug treatment was given continuously for seven days (same dose daily). On the 8th day the mice were subjected to swimming stress by keeping them in polypropylene tank of dimension 37 × 37 × 30 cm filled with water to a height 25cm. Mice were allowed to swim till complete exhaustion and the end point was taken when animal starting drowning. The mean swimming time for each group was calculated. The blood was collected through retroorbital puncture to estimate biochemical parameters like Serum glucose, Corticosterone and blood cells (Puri *et al.*, 2011).

Chronic cold resistant stress test

Swiss albino mice (18– 25g) were used for forced for chronic cold resistant stress test. The animals were divided into 5 groups and all were treated as mentioned above in scheduled drug treatments. The drug treatment was given continuously for seven days (same dose daily). In Each animals were subjected to cold stress by exposing them to 4 ± 10° C daily for 2 hours. The extracts were administered daily once to respective groups. This procedure was repeated for a period of 7 days. Blood was collected through orbital plexus under light ether anaesthesia to estimate biochemical parameters like blood

glucose, corticosteroid, and blood cell count (RBC and WBC). Animals were sacrificed by giving excess euthanasia (Arif *et al.*, 2013).

Anoxic stress tolerance test

Swiss albino mice (18–25g) were used for forced for anoxic stress tolerance test. The animals were divided into 5 groups and all were treated as mentioned above in scheduled drug treatments. Conical flasks of 250mL capacity were used for the study. These flasks were made airtight using rubber cork before beginning the experiment. Each animal was kept in the airtight vessel and time was noted using a stopwatch. The moment an animal showed first convulsion, it was removed immediately from the vessel and resuscitated if needed. The time duration from the entry of the animal in the hermetic (conical flask) vessel to the appearance of the first convulsion was taken as the time of “anoxic stress tolerance.” The mean time to convulsion was recorded and animal was removed at onset of convulsion. The blood was collected through retroorbital puncture to estimate biochemical parameters like serum glucose and Corticosterone and blood cells (Puri *et al.*, 2011).

Antioxidant activity

The free radical scavenging activity of *M. cecropioides* was determined by the DPPH assay described by Gulcin *et al.*, (2010). 0.1 ml of the sample was added to 2 ml of a 0.1mM solution of DPPH in methanol. The mixture was allowed to stand for 30 min in the dark and the absorbance measured at 517 nm using a UV spectrophotometer. Ascorbic acid was used as the positive control. Free radical scavenging activity from the sample was calculated according to the formula:

$$\% \text{ of DPPH scavenging} = [1 - (A_1/A_0)] \times 100\%$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample.

Results and Discussion

Effect of the extract on forced swimming endurance stress test

The effect of aqueous extract of *M. cecropioides* stem bark on the swimming survival time in forced swimming endurance stress test in rats is as shown in fig. 1, administration of diazepam (1mg/kg) and the oral administration of the three different doses of aqueous extract (100, 200 and 300mg/kg) of *M. cecropioides* is significant at $p < 0.05$ by increasing swimming survival time (3.01 ± 0.92 , 3.34 ± 0.18 , 3.73 ± 0.98 compared to the control group 2.25 ± 0.08 mins. Interestingly, all the doses of *M. cecropioides* are more potent with respect to lower dose of standard drug (1mg/kg) of 2.8 ± 0.37 mins. All the doses were able to increase the swimming endurance when compared with animals who received normal distilled water.

During stress, (chronic cold restraint stress) releases various adrenal hormone such as catecholamines and glucocorticoids which makes the plasma glucose high due to excessive production of cortisol (Mulay, 2004). The treatment with the aqueous extract of stem bark of *M. ceropioides* significantly reduced the cold stress related hyperglycemias as the biochemical parameters on 7th day as presented in table 1 below, revealed higher level of glucose in serum in the stressed control group (1) (78.83 ± 0.28) as compare to unstressed control (73.8 ± 0.24). In the standard group which is treated with diazepam (1mg/kg), the serum glucose level was found to be 68.17 ± 0.08 and the oral administration of the three different doses of aqueous extract (100, 200 and 300mg/kg) of *M. cecropioides* are

67.19 ± 0.29 , 60 ± 0.08 and 61 ± 0.96 . Also, Spleen constricts to release more blood cells (RBC and WBC) during stress, so their weights decrease in stress (Pawar and Hugar, 2012). This stress induced changes were significantly reversed by the test extract at lower and higher doses. The results of the urinary ascorbic acid obtained are in line with several studies which reported that the tissue levels of ascorbic acid decreased on application of stress (Kutlu and Forbes, 1993).

Effect of the extract on chronic cold resistant stress test

Hyper secretion of Corticosterone helps the maintenance of internal homeostasis through the process of gluconeogenesis (Singh *et al.*, 2003). The treatment with the aqueous extract of stem bark of *M. ceropioides* significantly reduced the cold stress related hyperglycemias as the biochemical parameters on 7th day as presented in table 2, revealed higher level of glucose in serum in the stressed control group (2) (106 ± 0.07) as compare to unstressed control (72.6 ± 0.88). In the standard group which is treated with diazepam (1mg/kg), the serum glucose level was found to be 74.33 ± 0.84 and the oral administration of the three different doses of aqueous extract (100, 200 and 300mg/kg) of *M. cecropioides* are 65.5 ± 0.28 , 54.33 ± 0.79 and 61.73 ± 0.95 .

Also, Spleen constricts to release more blood cells (RBC and WBC) during stress, so their weights decrease in stress (Pawar and Hugar, 2012). This stress induced changes were significantly reversed by the test extract at lower and higher doses. The results of the urinary ascorbic acid obtained are in line with several studies which reported that the tissue levels of ascorbic acid decreased on application of stress (Kutlu and Forbes, 1993).

Table.1 The effect of Diazepam and *M. cecropioides* on biological parameters in forced swim test

Groups	Groups Tags	Biochemical/pathological parameters		
		Glucose (mg/dL)	WBC	24hrs Urinary Ascorbic Acid (µg/kg/24hrs)
1	Stressed Control	78.83±0.28	25429.3±0.59	76.17±0.39
2	Unstressed Control	73.8±0.24	22205±0.38	121.67±0.19
3	Diazepam (1mg/kg)	68.17±0.08	16125±0.09	150.83±0.63
4	Test (100mg/kg)	67.19±0.29	18718.33±0.42	122.17±0.28
5	Test (200mg/kg)	60±0.08	18183.83±0.38	140.67±0.47
6	Test (300mg/kg)	61±0.96	15911.69±0.71	148.5±0.08

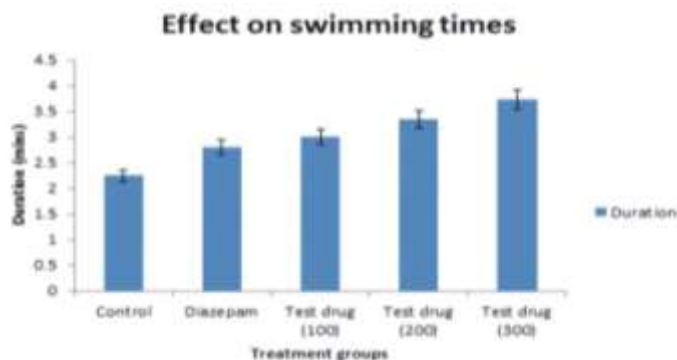
Data represent means ± S.E of 6 rats during the 5minute test session. Comparisons were made by using a one-way ANOVA, followed by T-test $P < 0.05$ was considered to be statistically significant

Table.2 The effect of Diazepam and *M. cecropioides* on biological parameters in Chronic Cold Resistant Stress Test

Group	Groups Tags	Biochemical/haematological parameters		
		Glucose	WBC	24hrs Urinary Ascorbic Acid (µg/kg/24hrs)
1	Unstressed Control	72.6±0.88	17300±0.27	121.38±0.41
2	Stressed Control	106±0.07	19330±0.09	73.26±0.17
3	Diazepam (1mg/kg)	71.33±0.84	15030±0.52	142.11±0.71
4	Test (100mg/kg)	65.5±0.28	16381.6±0.77	110.17±0.82
5	Test (200mg/kg)	54.33±0.79	10451.67±0.28	124.67±0.62
6	Test (300mg/kg)	61.73±0.95	10350±0.82	128.68±0.09

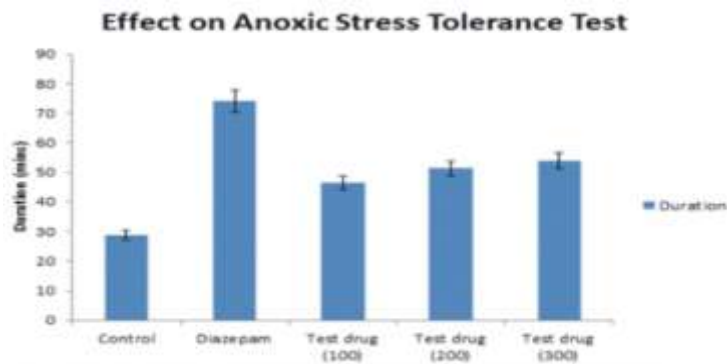
Data represent means ± S.E of 6 rats during the 5minute test session. Comparisons were made by using a one-way ANOVA, followed by T-test $P < 0.05$ was considered to be statistically significant.

Fig.1 The effect of Diazepam and *M. cecropioides* on swimming times in rat using forced swim test



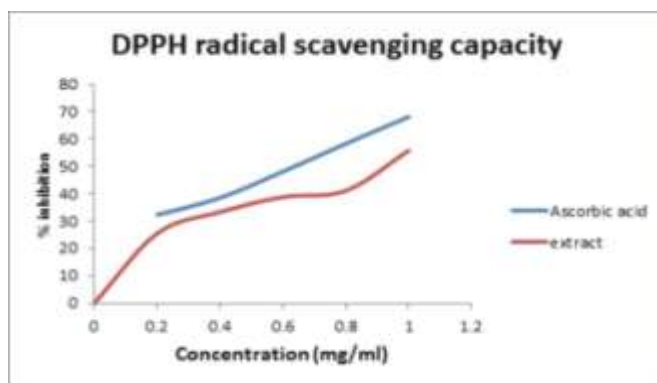
Data represent means \pm S.E of 6 rats during the 5minute test session. Comparisons were made by using a one-way ANOVA, followed by T-test $P < 0.05$ was considered to be statistically significant

Fig.2 The effect of Diazepam and *M. cecropioides* on swimming times in anorexia stress tolerance test



Data represent means \pm S.E of 6 rats during the 2 hrs test session. Comparisons were made by using a one-way ANOVA, followed by T-test $P < 0.05$ was considered to be statistically significant

Fig.3 The antioxidant effect of aqueous extract of *M. cecropioides* using DPPH scavenging activity



Effect of the extract on anoxic stress tolerance test

In anorexia stress tolerance test Group I (control) treated with distilled water (1mL)

was kept in hermetic vessel to for 28.80 ± 0.38 min. Animals treated with diazepam (1mg/kg) standard survived in hermetic vessel for 74.26 min; the test groups animals with varying concentrations (100, 200, and 300mg/kg)

survived in hermetic vessel for 46.48 ± 0.28 , 51.28 ± 0.09 and 53.84 ± 0.63 min respectively.

Effect of the extract DPPH radical scavenging antioxidant activity

DPPH is an oxidizing agent that can be reduced in the presence of an antioxidant, the stable DPPH can thus be used to quantify and compare the free radical scavenging capacities of different antioxidants (Yu, 2001). In this research, the aqueous extract the plant was found to possess impressive antioxidant capacity in a dose dependent manner comparable to that of ascorbic acid as depicted in figure 3.

In conclusion, the aqueous extract showed impressive antistress potential and this present study present as far as is known, is the first report of the antistress (adaptogenic) activities of *M. cecropioides*, this findings have added to the many scientific proven biological activities of *M. cecropioides*.

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