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Diuretic Effects of Aqueous Crude Extract of *Musanga Cecropioides* in Normotensive Sprague Dawely Rat

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Abstract
The diuretic effects of the water extract of *Musanga cecropioides* was investigated to see the mechanism of its reported blood lowering effect. Sprague dawley rats were divided into four groups and giving water, 1.33mg/100ml, 5.3mg/100ml and furosemide (0.6mg/100ml). The water intake and the urine volume were measured and their cation content was determined by flame photometry. *Musanga cecropioides extract* (1.33mg/100ml) increases urinary output, sodium excretion significantly ($p< 0.001$) compared to control. There was no significant difference in these parameters between *Musanga cecropioides* and furosemide. This suggests a diuretic effect. Chemical analysis of the plant shows a flavonoid, tannin, alkaloid, anthraquinone and reducing sugar which alone or in combination may account for this diuretic effect and thus the antihypertensive mechanism.
Keywords: *Musanga cecropioides,* Diueresis, Hypertension, Urinary output.

1.0 INTRODUCTION

Hypertension, for several decades has been globally recognised as the most prevalent cardiovascular disease. It is reported to be the next to malaria as most serious health problem in developing tropical countries (Gilford, 1975, Agunwa, 1998, Ajagbonna, 2000).

Presently there are several classes of agents in current use in the control of hypertension. They are classified based on their primary site of action. These includes (i) diuretics like furosemide which lower blood pressure by increasing urinary water and sodium excretion (Aguiyi and Obi, 1998). (ii) sympatholytic drug such as guanethidine, which lower blood pressure by reducing the peripheral resistance and cardiac output (Falase and Balako, 1979, Oduntola, 1989). (iii) Angiotensin converting enzyme inhibitors such as captopil which lower blood pressure by blunting the normal aldosterone response to sodium loss, thus,the normal role of aldosterone to oppose diuretic induced natriuresis is diminished.

The uses of these conventional antihypertensive agents have been associated with arrays of side effects apart from the high cost some of these drugs.

If therefore becomes imperative to search for a novel drug with better cost effectiveness and lesser side effects.

In some part of the world and indeed Nigeria, medicinal plants and plant products are employed in the management of several diseases especially hypertension (Ajagbonna and Adegunloye, 1998, Ajagbonna and Onyeyili, 2002, Ajagbonna et al, 2002) one of these plant is *Musanga cecropioides.*

Musanga cecropioides is a rapidly growing plant belonging to the cecropiaceae family ubiquitous to the tropical rain forests particularly of West Africa. It i known as the 'umbrella tree' (from its shapeor cork word English parasolier (for its close resemblance to a parasol).

Traditionally, preparation from various part of the plant such as the leaves, latex, stem bark and roots are used as remedies for various illness especially hypertension, trypanosomiasis, leprosy, chest infection and rheumatism (Bouquest, 1969).

More specifically, water that exude from the cut stem of the plants is reported to have a diuretic effect and thereby lowering blood pressure. While several workers have demonstrated the scientific basis for some of the reported traditional use of the plant very little or no work has been done to validate the diuretic claim of this plant. Thus, this study was design to investigate the mechanism involved in the reported blood lowering effect of the water extract of *Musanga cecropioides* with the aimof demonstrating and reevaluate the diuretic effect of graded doses of the aqueous extract of the plant stem bark in the normotensive rats.

2.0 METHODOLOGY

2.1 COLLECTION OF WATER EXTRACT OF *MUSANGA CECROPIOIDES*

The liquid extract of *Musanga cecropioides* was collected from the deciduous forest of Ijebu Igbo in Ijecu North Local government of Ogun State, Nigeria. This was obtained by a cut of the stem of the plant with a cutlass. The exuded liquid from the cut stem is then collected into a container. The collected water extract used in this study is colourless and odourless.

2.2 EXPERIMENTAL ANIMALS

Healthy Sprague Dawley rats of both sexes weighing between 170-230gms were obtained from Veterinary animal unitof Amadu Bello University Zaria. These animals were allowed to acclimatise to laboratory condition for two weeks. Water and feed (vitafeed® ltd Nigeria) were provided adlibitum throughout the course of the experiment.

2.3 EXPERIMENTAL GROUPING

The rats were randomly divided into 4 groups (1-4) of 5 rats as follows
Group 1 control group and were given only distilled water
Group 2 Rats were given 5.3mg/100ml of *Musanga cecropioides* water
Group 3 Rats were given 1.33mg/100ml of *Musanga cecropioides*
Group 4 Rats were given standard drug (Furosemide at a dose rate of 0.6mg/kg)

2.4 PREPARATION FOR FUROSEMIDE SOLUTION

Furosemide tablets (Fidson Nig Ltd) were ground into powdered and dissolved in distilled water. Drug dose was determined based on the volume of solution consumed per average weight of rats.

2.5 DETERMINATION OF DAILY FLUID INTAKE

Usually a known volume of water, *Musanga cecropioides water extract* or furosemide solution was placed in the appropriate cage in the morning. The following morning, 24 hours after, the volume of the fluid remaining was measured. Fluid intake was determined by subtractingthe volume of the fluid left from the initial volume of the fluid placed in the cage.

2.6 METABOLIC CAGE AND URINE COLLECTION

Daily urine volume from the rats were determined using metabolic cage. Usually two rats were randomly picked from each group and placed in the metabolic cage. The rats were left in the cages overnight and urine was collected, measured and stored in a freezer until required for analysis. The process was repeated for three weeks. At the end of the experiment, the cation (Na^+ and k^+) contents of the urine samples were determined by flame photometry.

2.7 ACUTE TOXICITY EFFECT OF MUSANGA CECROPIOIDES WATER

A group of 5 rats were administered with 5.3mg/100ml of *Musange* cecropioides water . Each of the animals was placed in a metabolic cageand observed for 48hours and for any short-term behaviour signs or death. They were later observed for 12 days for a long-term possible lethal outcome.

2.8 STATISTICAL ANALYSIS

Results are presented as mean ± standard error of mean (SEM). Statistical analysis was done using oneway analysis of variance (ANOVA). $P<0.05$ and $P< 0.001$ was regarded as statistically significant.

3.0 RESULTS

3.1 PYTOCHEMICAL STUDIES

Phytochemical analysis from this study shows that the extract contains alkaloids, tannins, cardiac glycosides, flavonoids, anthraquinones,phlobatannins, anthocyaaaaaanosides, saponins, cyanogenic glycosides and reducing sugar

3.2 ACUTE TOXICITY STUDY

Administration of the water of *Musanga cecropiodes* for short term of 48 hours and long term of 12 days did not produce death nor any observable lethat behavioural signs.

3.3 EFFECT OF *MUSANGA CECROPIODES* ON FLUID INTAKE

The daily fluid intake in the four groups. There was no significant difference in daily fluid intake of all the groups of rats at the beginning of the experiment (week 0).
The fluid intake was however significant lower ($p<0.001$) in the group that was given 5.3mg/100ml *Musanga cecropiodes* compared to the other three groupds. The fluid intake in the test group (group with 1.3mg/100ml and that of furosemide) was also significantly lower ($p<0.001$) than the control group.

3.4 EFFECT OF *MUSANGA CECROPIODES* ON URINARY SODIUM

Represent the daily sodium excretion of control rats and the test groups with the *Musanga cecropiodes* water and those that consume furosemide solution. These was no significant difference in urinary sodium excretion

between all the groups at the beginning of the experiment (week 0)
The urinary sodium excretion was however signifantly higher ($p<0.05$) in all the test groups compare to control for the three week period. The highest sodium excretion was however observed in the group that took 1.33mg/100ml *Musanga cecropiodes*

3.5 EFFECT OF *MUSANGA CECROPIODES* ON POTASSIUM EXCRETION

Shows the daily k^+ excretion in all the four groups
At the beginning of the experiment, there was no significant difference between the groups. However, by the second and third week, the k^+ excretion of *Musanga cecropiodes* (1.33mg/100ml) rats and that of furosemide was significantly higher ($p<0.001$) than the control rats.

3.6 EFFECT OF *MUSANGA CECROPIODES* AND FUROSEMIDE ON URINE OUTPUT

Represent the daily urinary output of the control rats and the test group with *Musanga cecropiodes* and furosemide solution.

At the beginning (week 0) there was no significant difference ($p<0.05$) in the urine output among the groups, however, by week 1 through week 3, urine output of the rats given (1.33mg/100ml) *Musanga cecropiodes* was significantly higher ($p<0.001$) than control rats and rats consuming 5.3mg/100ml *Musanga cecropiodes.* The significant increase is comparable to that shown in rats taking furosemide.

Table 3.1: PHYTOCHEMICAL RESULT FOR *Musanga cecropiodes* WATER EXTRACT

S/NO	TEST	RESULTS
A	ALKALOIDS	
1	Dragen Doff's Reagent	+
2	Mayer's Reagent	+
3	Wagner's Reagent	+
B	TANNINS	
1	Feric Chloride Reagent	+
2	Bromine Water	+
C	CARDIAC- GLYCOSIDE	
1	Legal Test	+
2	Keddle Test	+
3	Liebermans's Test	+
4	Salkowski Test	+
5	Keller- Kiliani Test	+
D	FLAVONOID'S TEST	
1	Ferric Chloride Test	+
2	Lead Acetate Test	+
3	Sodium Hydroxide Test	+
E	ANTHRAQUINONES	
1	Borntragers Test	+
F	PHLOBATANNINS TEST	+
G	ANTHOCYANOSIDES TEST	-
H	SAPONINS TEST	
1	Benedict's Test	+
2	Frothing Test	+
3	Emulsion Test	+
I	CYANOGENIC GLYCOSIDES	-
J	REDUCING SUGAR COMPOUND	
1	Fehling Test	+
2	Barfoed Test	+
3	Resorcinol Test	+
4	Phloroqlucinol Test	+

\+ Represent presence of the chemical constituent in the plant
\- Represent absence of the chemical constituent in the plant

Table 3.2: The daily urinary volume of control rats and rats given extract (5.3mg/100ml), extract (1.33mg/100ml) and furosemide.

	WEEK 0	WEEK 1	WEEK 2	WEEK 3
Group 1 (control)	1.5 ± 0.12	1.3 ± 0.19	1.69 ± 0.19	1.5 ± 0.12
Group 2 (extract 5.3mg/100ml)	136.5 ± 8.56	208.0 ± 17.24**	203.33 ± 10.48*	213 ± 16.56**
Group 3 (extract 1.33mg/100ml)	153.0 ±4.32	321.33 ± 15.76***	347.33 ± 45.11***	336.33 ± 24.27***
Group 4 (furosimide)	160 ± 6.9	290.67 ±14.7***	240.67 ± 29.45***	289.33 ± 25***

n = 5, values are represented as Mean ± SEM
** represents values are significant at P < 0.01
*** represents values are significant at P < 0.001.
NS. Represents values are not significant at P > 0.05 compared with the control.

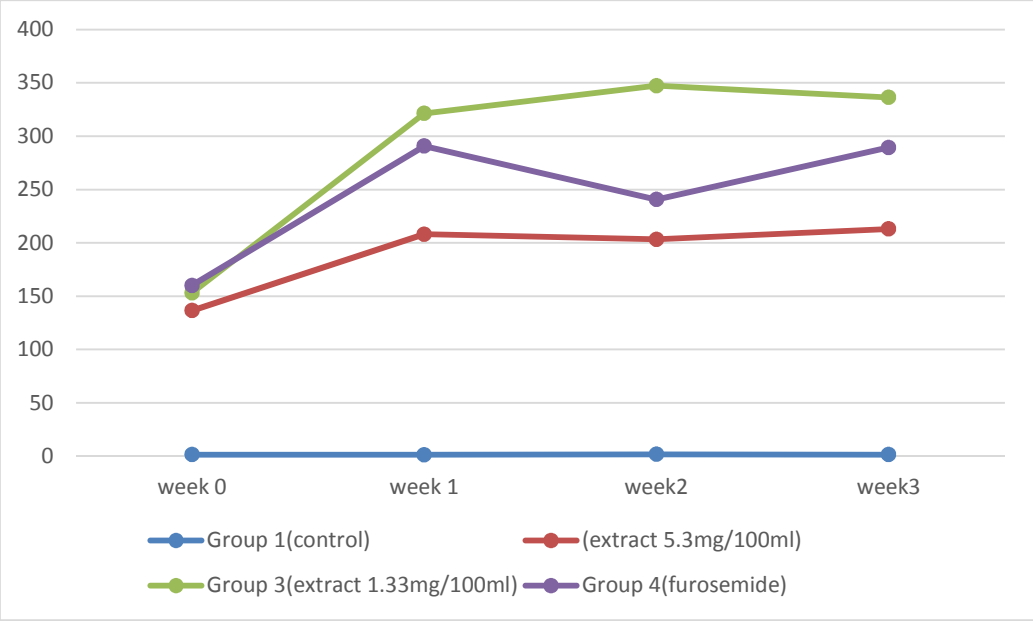

Figure 3.0 showing the The daily urinary volume of control rats and rats given extract (5.3mg/100ml), extract (1.33mg/100ml) and furosemide.

Table 3.3: The daily urinary Na+ excretion of control rats and rats given extract (5.3MG/100ML) and furosemide.

	WEEK 0	WEEK 1	WEEK 2	WEEK 3
Group 1 (control)	148 ± 2.45	129.67 ± 1.45	143.33 ± 5.78	173.0 ± 13.0
Group 2 (extract ,5.3mg/100ml)	136.5 ± 8.56	208.0 ± 17.24**	203.33 ± 10.48*	213 ± 16.56**
Group 3 (extract 1.33mg/100ml)	153.0 ± 4.32	321.33± 15.76***	347.33± 45.11***	336.33± 24.27***
Group 4 (furosemide)	160 ±6.9	290.67 ± 14.7***	240.67± 29.45***	289.33 ± 25***

n = 5, values are represented as Mean ± SEM
** represents values are significant at P< 0.01
*** represents values are significant at P< 0.001
NS. Represents values are not significant at P> 0.05 compared with the control.

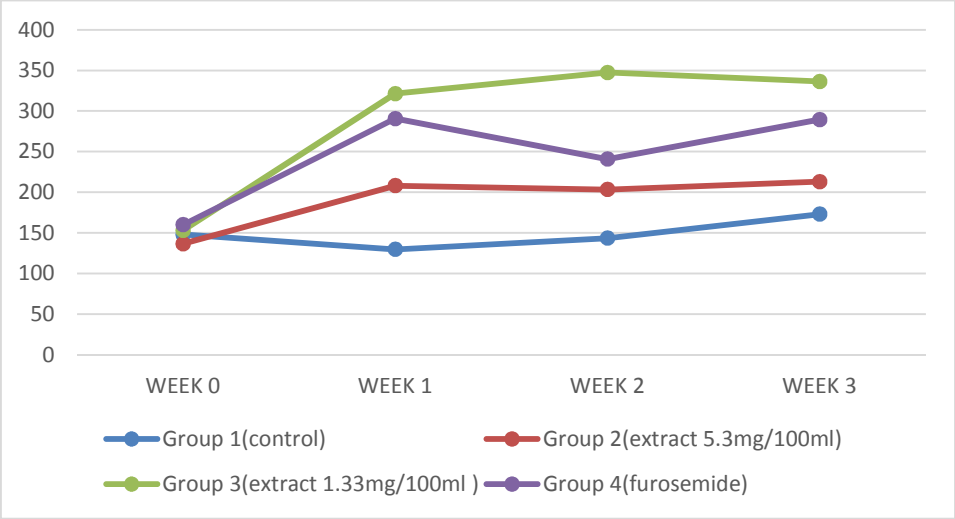

Figure 3.1: showing The daily urinary Na+ excretion of control rats and rats given extract (5.3MG/100ML) and furosemide.

Table 3.4: The daily urinary potassium excretion of control rats and rats given extract (5.3MG/100ML) extract and furosemide.

	WEEK 0	WEEK 1	WEEK 2	WEEK 3
Group 1 (control)	263.75 ± 12.34	246.67 ± 9.62	328.67 ± 42.49	309 ± 30.99
Group 2 (extract 5.3mg/100ml)	286.0 ± 25.46	305.67 ± 28.43 NS	402.0 ± 72.51 NS	331.67 ± 22.30 NS
Group 3 (extract 1.33mg/100ml)	291.27 ± 22.80	385.67 ± 11.92 NS	404.0 ± 55.85 NS	475 ± 63.06 NS
Group 4 Furosemide	280.12 ± 19.81	407.0 ± 34.78 NS	403.67 ± 43.05 NS	404.33 ± 22.33 NS

n = 5, values represents as Mean ± SEM *represents values are significant at P <0.05
*** represents values are significant at P< 0.001 'NS' represents values are not – significant at P >0.05 compared with control.

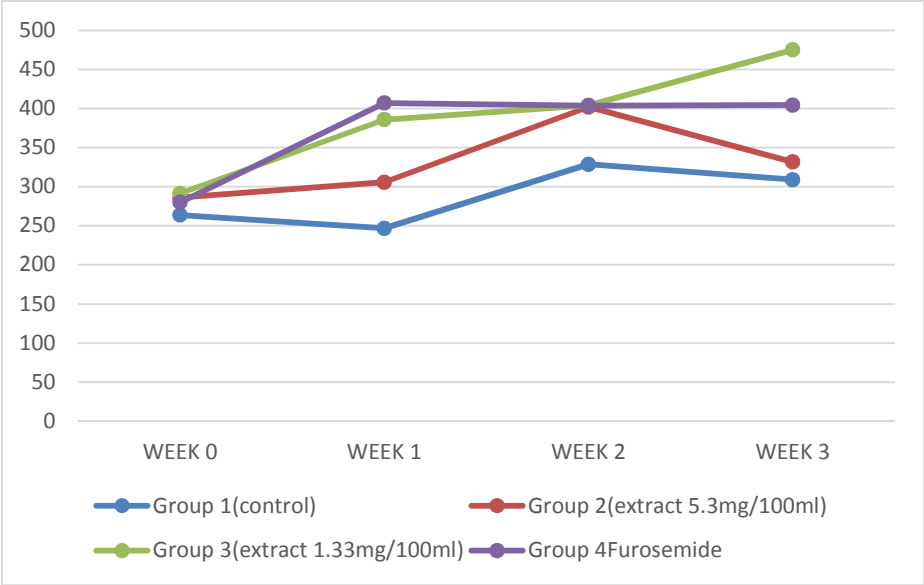

Figure 3.2 showing the daily urinary potassium excretion of control rats and rats given extract (5.3MG/100ML) extract and furosemide.

Table 3.5: The daily fluid intake of control rats and rats given extract (5.3MG/100ML), extract (1.33MG/100ML) and furosemide.

	WEEK 0	WEEK 1	WEEK 2	WEEK 3
Group 1 (control)	79.0 ± 1.7	59.67 ±1.15	89.00 ± 2.6	89.00 ±2.08
Group 2 (extract 5.3mg/100ml)	83.0 ± 1.37	42.0 ± 1.16***	44.67 ± 0.88***	46.33 ± 1.2***
Group 3 (extract 1.33mg/100ml)	80.91 ± 2.32	70.0 ± 1.16***	67.33 ±3.53***	70.0 ± 0.58***
Group 4 (furosemide)	85.43 ± 1.23	64.0 ±1.16	71.67 ±1.20***	69.67 ± 0.88

n = 5, values are represented as Mean ± SEM
*** represents values are significant at P, 0.001 compared with control.

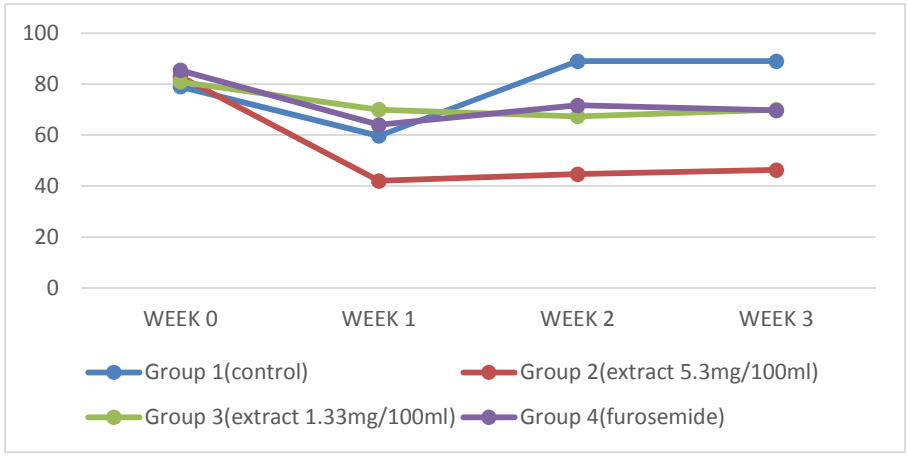

Figure 3.3 showing the daily fluid intake of control rats and rats given extract (5.3MG/100ML), extract (1.33MG/100ML) and furosemide

4.0 DISCUSSION

Result from this study demonstrate that oral water extract of *Musanga cecropiodes* produced an increase in daily urine output in normotensive rats.

In this study, two concentration 5.3mg/100ml and 1.33mg/100ml of *Musanga cecropiodes* were given to rats compared to control rats that drank water only and the rats that took furosemide, a standard diuretic drug. This study shows that at concentration of 1.33mg/100ml, *Musanga cecropiodes* significantly increased urine volume of rats compared to control. The diuretic effect observed here is similar to that of furosemide, a standard diuretic drug known to increase urine output, thus suggesting a diuretic effect by the *Musanga cecropiodes* at this concentration.

The dieresis was not likely to be due to increased fluid intake because fluid intake of rats on 1.33mg/100ml *Musanga cecropiodes* did not differ significantly from the water intake by the control rats, neither was it significantly different from the fluid intake by the rats on furosemide solution.

However, surprisingly, increasing the concentration of *Musanga cecropiodes* to 5.3mg/100ml did not result in grater diuretic effect, rather it led led to a significantly lower production of urine. This urine observation may be due to the fact that this group of rats, that drank 5.3mg/100ml *Musanga cecropiodes* drank significantly far less of the solution than the control rats, and rats that drank 1.33mg/100ml *Musanga cecropiodes* and the rats on furosemide. It is possible that at this concentration (5.3mg/100ml) the solution of *Musanga cecropiodes* has become bitter causing the rats to drink it sparingly resulting in a lack of a diuretic effect. This observation is similar to thyat reported by some earlier workers on *Hibiscus sabdariffa* and *Rhaptopetalum corcaeum* extract, plants that were also earlier showed to posses diuretic property (Mojiminiyi, et al., 2000, Ajagbonna, et al., and Ajagbonna and Onwuchekwa, 2000)

Substance that increase urine output are known to reduce blood pressure (Ajagbonna *et al.,* 2003,). The above finding suggest that at a concentration of 1.33mg/100ml, *Musanga cecropiodes* solution may be working as a diuretic and this may be an additional mechanism of its blood pressure lowering effect. Already, it had been reported that *Musanga cecropiodes* extract produced a fall in systolic and diastolic blood pressure in normotensive rats (Adeneye *et al.,* 2005). A therapeutically useful diuretic does not only increase sodium excretion (Adegunloye, 1996, Ajagbonna, *et al.,* 2002).

*Musanga cecropiodes (*1.33mg/100ml) in this study increase sodium excretion (table 3),this effect is similar to that observed with furosemide a standard drug that increases sodium excretion by blocking Na-K^+ ATPase (Adegunloye, 1996). This suggest that *Musanga cecropiodes* may have also reduse blood pressure by increasing sodium excretion (natriuresis)

Potassium excretion was higher in *Musanga cecropiodes* rats and furosemide treated ratsrats compared to control in this study. Diuretic like the thiazides are known to enhance excretion in tubules and collecting ducts which are subsequently reabsorbed in exchange for excreted potassium, (Okenwa, 1997). This may suggested a diversity in the diuretic action on *Musanga cecropiodes* which may be due to presence of one or more active component in water extract.

Phytochemical analysis of *Musanga cecropiodes* shows it contain alkaloids, tannins, flavonoids, cardiac glycoside, anthraquinones and reducing sugar. These substance alone or in combination may be responsible for the diuretic property observed in the study.

It can therefore be concluded that observation from this study support to a large extent the claims by the traditional medicine practitioner that water of *Musanga cecropiodes* may contain active substances that may be responsible for its diuretic property and therefore subsequently explain additional mechanism for the antihypertensive effects of this plant.

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