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The Antihypertensive Properties of *Moringa oleifera* Crude Leaf Extract in Epinephrine-treated Rats

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Among the plants reported to have antihypertensive properties is *Moringa oleifera*. The present study tested the blood pressure-lowering effects of the crude ethanolic leaf extracts of the plant on Sprague-Dawley rats treated with epinephrine. The same effects were also compared with those of a hypotensive drug, nifedipine. The experiment involved using a tail-cuff plethysmograph to measure the blood pressure of a test animal subcutaneously administered with epinephrine at a dose of 0.2 mg per kg body weight (mg/kg BW), as well as extracting blood for measurement of selected blood parameters at different time intervals. The selected variables were measured before and after the administration of the leaf extract in one test group. The mean values were then compared with those of a group given normal saline solution only, and with another group given the nifedipine. The data revealed that the ethanolic leaf extracts of *M. oleifera* given at a concentration of 462.5 mg/kg BW in the test animal can decrease the following parameters to baseline values at about an hour following epinephrine administration, namely: blood pressure changes from 162 mm·Hg to 118 mm·Hg, hemoglobin content from 15.1 g/L to 14.08 g/L, hematocrit levels from 0.45 to 0.42, and erythrocyte count from 5.28 x 1012/L to 4.92 x 1012/L. The antihypertensive effect of the crude extract is attributed to the presence of glycosides reported in previous literature as having vasodilatory effects. The isolation of such bioactive agents from *M. oleifera* and testing for their possible dose-dependent hypotensive properties, singly or in combination, are among the recommendations for future research by this study.

I. INTRODUCTION

Hypertension is a serious condition that can lead to cardiovascular disease and stroke. The therapeutic agents for this disorder include pharmaceutical beta blockers, calcium channel blockers, and direct acting vasodilators. Some of these agents have been identified in plant sources, such as the horseradish tree, *Moringa oleifera*, which is among the species identified as having hypotensive properties (Dangi *et al.*, 2002). The leaves of *M. oleifera* are a preferred material for the medicinal tests because of their high nutritive content. The leaves can be harvested in large amounts since the plant grows fast and is a perennial backyard presence in many residential and agricultural areas. Research in alternative medicine is ongoing to demonstrate its potential as a source of bioactive agents that can mitigate cardiovascular disorders (Andersen and Andersen, 1997). Long-term effects of including *M. oleifera* leaves in the diet provide health benefits for hypertensive subjects. However, much has yet to be learned about short-term effects of the leaf compounds if administered by means other than through diet.

An elevated blood pressure in non-disease states can result from sympathetic nervous activation by the administration of the hormone epinephrine (Badyal *et al.*, 2003). This study aims to investigate if the administration of ethanolic leaf extracts from *M. oleifera* can lower epinephrine-induced hypertension in test animals. Such an effect, along with measures of some selected blood parameters, is also compared with a known hypotensive drug, nifedipine.

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II. MATERIALS AND METHOD

A. Preparation of Leaf Extract

Fresh matured leaves. of *M. oleifera* were manually collected from the Tierra Pura Subdivision in Quezon City, Philippines. About 2 kg of leaves were macerated, homogenized and soaked in 95% ethanol for 48 hours. The homogenate was filtered through a cheesecloth to extract the juice, which was further filtered using Whatman qualitative filter paper #1. The resulting extract was then concentrated in vacuum to 400 ml using a Heidolp W2000 condenser with a rotating machine attached to a Sibata Circulating Aspirator WJ20 cooling system and Heidolp WB2000 water bath. The concentrated extract was lyophilized using a ChemLab SB4 freeze-dry machine.

The ethanolic extract was dissolved in normal saline solution with 10% propylene glycol solution before intraperitoneal administration (after Suarez *et al.*, 1997) in the experiment proper.

B. Animals and Preliminary Testing

33 male Sprague-Dawley rats, eight to ten weeks old, with weights ranging from 75 g to 150 g were procured from the animal facility of the National Institutes of Health (NIH) of the University of the Philippines in Manila. The rats were housed in uniform plastic cages, and were maintained with commercial pellets and water before and during experiment

Eighteen rats were then divided into six groups, corresponding to the following doses of leaf extract: 0, 57.81, 115.63, 231.25, 462.5, and 925 mg per kg body weight (mg/kg BW). The highest dose was half of the LD50 of the *M. oleifera* alcoholic leaf extract for mice, as cited by Singh *et al*, 1978. Just prior to extract administration, each rat was anaesthetized with 0.09 mg/kg of ketamine hydrochloride and then attached to a tail-cuff plethysmograph, in which the systolic blood pressure was read from the manometer in the device. The blood pressure of each rat was measured before the administration of leaf extract, and then again at 30 minutes after leaf extract administrated This procedure and all subsequent steps undertaken were compliant with IACUC guidelines upheld by the NIH.

The data obtained were subjected to a linear regression analysis using the statistical software Design Expert version 4. The data were run with fitted quadratic and cubic models for comparison. From the resulting preliminary data, out of the six dosages, the one that is associated with the greatest decrease in blood pressure was used in the experiment proper.

C. Experiment Proper

The study design is similar to an intervention method for a causal-comparative investigation wherein the effect of a potential treatment for an induced condition is compared among different test groups of animals. Fifteen rats were anaesthetized and randomly assigned to three groups, namely: (1) the NSS group, wherein each rat received 1 ml of normal saline solution; (2) the NIF Group, wherein each rat received 1.25 mg/kg $_{BW}$ of nifedipine (Calcibloc), and (3) the MOR group, wherein each rat received 462.5 mg/kg $_{BW}$ of leaf extract. Hypertension in each rat was induced through subcutaneous administration of epinephrine at a dose of 0.2 mg/kg $_{BW}$. The different groups received their respective treatments through intraperitoneal administration. Blood pressure using the tail-cuff plethysmograph was measured immediately before and 15 minutes after the administration of epinephrine, and then one hour after the administration of the respective treatments.

Blood samples were also taken from the tail of each rat during the aforementioned time intervals. These samples were sent to the Our Lady of Mt. Carmel Medical Center diagnostic laboratory (in Pampanga, Philippines) for the measurement of haemoglobin content, haematocrit ratio and erythrocyte count. The parameters were measured using micro-capillary centrifuge method and using Cell Dyne l700 blood analysis machine.

D. Statistical Analysis

All rats were randomly assigned to their respective test groups in both the preliminary test and experiment proper. The data obtained were subjected to analysis of variance at 5% level of significance using the statistical program SPSS version 12. Mean values of blood pressure, haemoglobin

content, haematocrit level, and erythrocyte count for each treatment group were compared using Duncan's multiple range test.

III. RESULT

A. Preliminary Results

Statistical analysis revealed decreased systolic blood pressure levels among normotensive test rats given the varying dosages of the ethanolic leaf extract. The linear regression model showed that as the dosage of crude ethanolic leaf extract increased, the level of blood pressure measured decreased. The regression coefficient for the linear model was estimated at 0.132, indicating a weak relationship between mean blood pressure and leaf extract dosage. However, the fitted quadratic and cubic models showed that the greatest decreases in blood pressure were produced by dosages 231.25 and 462.5 mg/kg $_{BW}$ of the extract (Figure 1). The dosage of 462.5 mg/kg $_{BW}$ was then selected to be used in the experiment proper based on the premise suggested by the linear model. This amount is also about a quarter of the reported LD50 of the plant extract for rodents.

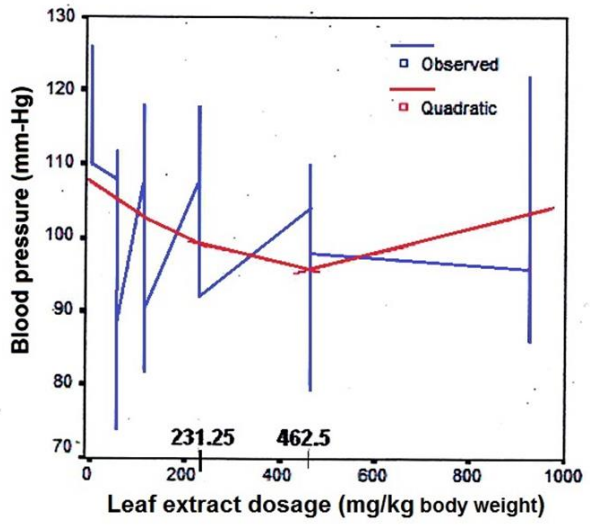

Figure 1. Generated quadratic model using Design Expert software, showing the relationship between measured blood pressure levels of test rats and leaf extract dosages based on preliminary test data

B. Systolic Blood Pressure

An increase in systolic blood pressure was observed 15 minutes after the administration of epinephrine in all the treatment groups of rats. From the respective baseline levels, the mean blood pressure rose to 156.8 mm·Hg in the NSS group, to 165.6 mm·Hg in the NIF group, and to 162 mm·Hg in the MOR group (Table 1). The mean blood pressures measured an hour after being given the respective treatments decreased to near baseline values in the NIF group, at 125.2 mm·Hg, and in the MOR group at 118 mm·Hg. However, the mean blood pressure of the NSS group did not return to the baseline value, even at 75 minutes time interval following epinephrine treatment.

Table 1. Mean blood pressure + S.D. (in mm·Hg) of the test groups of rats at different time intervals in the experiment. For each group, values with an asterisk in the last two columns indicates a significant difference ($P < 0.05$) from the values in the first time interval, while ns is not significant

Treatment groups	Time intervals		
	0 minutes	15 minutes	75 minutes
NSS group (given 1 mL normal saline solution)	106.6 ± 18.2	156.8 ± 12.8 *	158.4 ± 6.7 *
NIF group (treated with nifedipine at 1.25 mg/kg $_{BW}$)	124.2 ± 6.2	165 .6 ± 6.5 *	125.2 ± 3.63 ns
MOR group (treated with leaf extract at 462.5 mg/kg $_{BW}$)	109.6 ± 14.3	162 ± 2.4 *	118 ± 7.54 ns

C. Blood Parameters

The mean baseline values for all other measured blood parameters in the test animals did not vary significantly among all three test groups. Regarding haemoglobin content, haematocrit ratio, and erythrocyte count, the mean values were found to increase slightly 15 minutes after epinephrine administration in all three test groups. The mean values of these three blood parameters declined to about the level of the respective mean baseline levels only in the NIF and the

MOR groups of rats. The NSS group of rats had the highest mean values for the three aforementioned parameters among all test groups and these values did not decline to baseline levels even at the 75-minute time interval (Table 2).

Table 2. Mean values of haemoglobin content, haematocrit ratio, and erythrocyte count (+S.D.) in the test groups of rats at different time intervals in the experiment. For each group, the values with an asterisk indicate a significant difference ($P < 0.05$) from those values in the first-time interval, while ns is not significant

Treatment groups	Blood parameters	Time intervals		
		0 minutes	15 minutes	75 minutes
NSS group (given 1 mL normal saline solution)	Hemoglobin content (mg/100 mL)	13.8 ± 0.7	15.22 ± 0.5 *	16.8 ± 0.7 *
	Hematocrit ratio	0.41 ± 0.02	0.46 ± 0.01 *	0.5 ± 0.02 *
	Erythrocyte count (x 10^6/mL^3)	4.84 ± 0.52	5.3 ± 0.18 *	5.88 ± 0.25 *
NIF group (treated with nifedipine at 1.25 mg/kg $_{BW}$)	Hemoglobin content (mg/100 mL)	13.66 ± 0.52	15 ± 0.54 *	14 ± 0.03 ns
	Hematocrit ratio	0.41 ± 0.02	0.45 ± 0.02 *	0.42 ± 0.01 ns
	Erythrocyte count (x 10^6/mL^3)	4.8 ± 0.16	5.24 ± 0.21 *	4.9 ± 0.1 ns
MOR group (treated with leaf extract at 462.5 mg/kg $_{BW}$)	Hemoglobin content (mg/100 mL)	13.92 ± 0.43	15.12 ± 0.16 *	14.08 ± 0.73 ns
	Hematocrit ratio	0.42 ± 0.01	0.45 ± 0.01 *	0.42 ± 0.02 ns
	Erythrocyte count (x 10^6/mL^3)	4.88 ± 0.13	5.28 ± 0.10 *	4.92 ± 0.26 ns

IV. DISCUSSION

The elevation in rat blood pressure, 15 minutes after epinephrine administration, is expected since the sympathetic activation of heart rate results in higher cardiac output, which, in turn, increases blood pressure (Mayet and Hughes, 2003). Unlike the rats that were given normal saline solution, the mean blood pressures in rat groups given the leaf extract and nifedipine declined an hour after their respective treatments. These findings suggest that the possible presence of compounds in these treatments are antihypertensive. This effect is expected of nifedipine, which is a known calcium-channel blocker, that can selectively impede the inward activity of calcium channels in the vascular smooth muscle cells, resulting in arteriolar vasodilation. By decreasing the vigour of heart contractions which lessens the force with which blood is pumped into the arteries, calcium-channel blockers can lead to a decrease in blood pressure (Boura and Green, 1964).

The blood pressure-lowering effect of the administered leaf extract in the MOR group of rats appears to be to the same extent as that observed with nifedipine administration. This finding suggests that the potential effectiveness of a natural material which is similar to that of a pharmaceutical product within a short period of time (that is, within less than two hours after a hypertensive condition was chemically induced.)

The observed effect of the leaf extract may be attributed to the presence of mustard-oil glycosides, reported to have hypotensive activities (Caceres *et al*, 1992). Mustard-oil glycosides thiocarbamate glycosides niaziminin A and B, and 4-[4-O-acetyl-alpha-L-rhamnoxyloxy-benzyl] isothiocyanate, have been isolated in alcohol leaf extracts of *M. oleifera* (Faizi *et al.*, 1994). Jansakul *et al*, (1997) noted the hypotensive and negative chronotropic effects of thiocarbamate glycosides due to the muscarinic stimulation of the parasympathetic nervous system, or to beta-adrenergic-receptor antagonism, but also suggested that such effects could be due to calcium-channel blocking activity. All these mechanisms can result in a vasodilatory response leading to a lower blood pressure level. Thus, the potential benefit of the *M. oleifera* leaf extract to therapies for hypertension is apparent. However, the isolation of the key hypotensive agents from the extract and the assays for dose-dependent physiological responses to these agents are beyond the scope of the study. Given that only a single dose of the leaf extract was tested in the experiment proper, further investigations in the aforementioned statement are warranted.

The observed similar patterns in the changes of the mean haemoglobin content, haematocrit and erythrocyte count for the nifedipine-treated and leaf extract-treated rats suggest a relationship between these blood parameters and blood pressure changes. For example, Cinar *et al.* (1999) had reported that increased haematocrit is followed by increased blood viscosity and a rise in blood pressure. An increased haemoglobin content is typically associated with high erythrocyte count that could be linked to hypertension due to the association with high blood viscosity (Cinar *et al.* 1999, Ryan, 2015). The elucidation of mechanisms relating the hypertensive condition with blood parameters can render the latter as important clinical indicators of the disorder.

V. CONCLUSION

The administration of *M. oleifera* crude ethanolic leaf extract was found to lower the blood pressure in epinephrine-treated Sprague-Dawley rats. The results also indicate that the *M. oleifera* leaf extract can regulate alterations in selected blood properties, namely haemoglobin content, haematocrit ratio and erythrocyte count following epinephrine treatment. The crude ethanolic leaf extract, at the concentration tested in the present investigation, may have hypotensive properties similar to those of the calcium-blocking agent, nifedipine. The antihypertensive property of the leaf extract is attributed to the presence of mustard oil glycosides reported to have vasodilating effects that are relevant for hypertension therapy. A calcium channel blocking mechanism for such plant compounds is subjected to further research. Future investigations on the dose-dependent effects of the isolated active ingredients in the leaf extract, singly or in varying combinations (for optimal effectiveness), are also recommended.

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