Anti-inflammatory and Diuretic Activities of *Moringa oleifera*Lam. (Moringaceae) and *Andrographis paniculata* Burm.f. (Acanthaceae) Co-administered in Rats

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ABSTRACT

Moringa oleifera Lam. (Moringaceae) and *Andrographis paniculata* Burm.f. (Acanthaceae) are commonly used medicine plants in several parts of Nigeria. The leaf extract of *M. oleifera* (MO) has been extensively studied and used traditionally for its anti-inflammatory and diuretic activities. Because of the wide medicinal benefits of these plants they are often used together in polyherbal formulation(s).

This study was aimed at evaluating some biological activities of MO and *A. paniculata* (AP) administered singly and in combination. This aim was pursued using *in vivo* acute toxicity study, formalin induced rat paw edema and diuretic assay.

The leaf extracts MO and AP were observed to be safe up to 5000 mg/kg body weight. The extract of MO and AP at 100 mg/kg body weight displayed 69.44 and 10.10 % inhibition of rat paw edema respectively, while the combination of extract elicited weaker inhibition (11.29 %) of paw volume. The extract of MO and AP when administered singly and in various combination at different doses elicited similar diuretic activity compared to the hydrochlorothiazide except at a combined dose of MO 50 mg/kg + AP 50 mg/kg, where the urine volume (1.27±0.60 mL) was lower compared to the negative control (1.62±0.52 mL).

The extracts of MO and AP were observed to be relatively safe. The extract of MO had significant anti-inflammatory and diuretic activities compared to AP. However the combination of both plants extracts led to inhibition of the anti-inflammatory and diuretic activities of MO.

Keywords: *Moringa oleifera*, *Andrographis paniculata*, Combination, Anti-inflammatory, Diuretic activity, Polyherbal formulation

INTRODUCTION

Man has depended on plants for his food and medicines from ancient times[1] and over 80% of Africans still use phytomedicines for their health care needs[2]. Several plants have been implicated in the treatment of different disease conditions[3]. Medicinal plants are often used in combination to manage different disease conditions[4]. Polyherbal formulations include two or more herbs co-formulated. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity[5].

Moringa oleifera (MO) is a commonly used as food and medicine in several parts of Nigeria. It is locally called *Ewe igbale* (Yoruba), *Okweoyibo* (Igbo) and *Zogali* (Hausa) among the three major Nigerian ethnic groups and commonly referred to as drumstick tree[6]. The leaves and seeds of the plant have been reported to have numerous health benefits. It is also claimed to have nutritional, medicinal, chemopreventive potentials and is a rich source of antioxidants[7, 8].Studies conducted on various parts of the plant have been reported. The hydro-alcoholic extract of the seeds was studied and reported to have anti-inflammatory activity against acetic acid-induced colitis in rats[9]. MO had been co-administered with other medicinal principles in several studies. In a report, Curcumin was observed to enhance the antioxidant potential of MO root extract in a beryllium induced toxicity model[10]. The anti-inflammatory activity of MO root extract was enhanced when combined with *Citrus sinensis* fruit rind extract in acetic acid-induced ulcerative colitis in mice[6].

Andrographis paniculata (AP) is another commonly used medicinal plant in several parts of the world that belongs to the Acanthaceae family. It is an erect annual herb, extremely bitter and commonly known as "king of bitters"[11]. Similar to MO, AP is also used traditionally in treatment of different diseases and has been reported to have a broad range of pharmacological effects including anticancer, antidiarrheal, anti-hepatitis, anti-HIV, anti-hyperglycemic, anti-inflammatory, antimicrobial, antimalarial, antioxidant, cardiovascular, cytotoxic, hepatoprotective and immune stimulatory[12]. Because of its wide medicinal benefits AP is a major ingredient of polyherbal formulations[13].

The leaf extract of MO has been extensively studied and used traditionally for its anti-inflammatory and diuretic activities[14, 15]. This study was aimed at evaluating the anti-inflammatory and diuretic activity of MO and AP administered singly and in combination.

METHOD

Plant Collection

The leaves of MO and AP were collected from the Demonstration Farm of the Department of Pharmacognosy and Drug Development, University of Ilorin. The plants collected were identified and confirmed as MO and AP. The leaves were air-dried and milled into fine powder.

Preparation of the Plant Extracts

The powdered leaves of MO and AP (250g each) were separately extracted with methanol by maceration for 72 hours at room temperature (25^{o}C). The crude extract was filtered, concentrated *in vacuo*, weighed and stored until needed.

Animals

Albino rats of both sexes were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Ilorin Nigeria. The animals were housed in metal

activity cages and kept in a well-ventilated room. Animals were fed with standard laboratory feed and provided access to water *ad libitum*. A cycle of light and dark (12 h light and 12 h dark) and a temperature of 24 ± 2°C were maintained in the room. The animals were acclimatized for one week before use. All experiments were conducted in accordance with the guidelines set by University of Ilorin Animal Ethics Committee.

Acute Toxicity Testing

The acute toxicity study for MO extract was conducted by determining the dose of the extracts that will kill 50 % of the animal population (LD_{50}) using the method described by Lorke[16]. The study was conducted in two phases using a total of thirteen rats. In the first phase, nine rats were randomly divided into 3 groups (n =3). Group 1, 2 and 3 animals were given 10, 100 and 1000mg/kg body weight (b.w.) of the extract via oral cannula respectively, to establish the range of doses producing any toxic effect. The animals were observed periodically for 24 h for signs of toxicity. Thereafter animals were observed for mortality, weight loss, tremor or paralysis. In the second phase, higher doses (1000, 1600, 2900, 5000mg/kg b.w.) of the extract were administered orally to a new set of four rats (one rat per dose) to further determine the correct lethal dose. The same procedure was repeated with AP extract to estimate the dose of the extract lethal to 50 % of the animal population (LD_{50}).All the animals were observed frequently on the day of treatment and the animals were monitored daily for 2 weeks for signs of acute toxicity or lethality. Recovery and weight gain were seen as indications of surviving acute toxicity.

Anti-Inflammatory Activity

Formalin-Induced Rat Paw Edema

The anti-inflammatory activity of MO and AP extracts when administered singly and in combination was determined using a modified method described by Sudipta and co-worker[17]. The animals were randomly divided into five groups (n = 6).Acute inflammation of the rat paw was induced by subplantar administration of 0.1 mL of 1% $^{v}/_{v}$ formalin in normal saline in the right paw. Wistar rats were divided into five groups (n=3). Group1 received normal saline (*i.p*). Groups 2, 3 and 4 were pre-treated with the MO100 mg/kg body weight, MO50 mg/kg +AP 50 mg/kg body weight and AP100 mg/kg body weight respectively. Animals in groups 5 were treated with indomethacin (10mg/kg). After 1 hour, 0.1mLof 1% $^{v}/_{v}$ formalin was injected into the plantar surface of the left hind paw of each rat. The paw volumes were measured with a plethysmometer at 0 hour and 3 hours after the administration of formalin.

The ratio of the anti-inflammatory effect of extracts was calculated by the formula below:

$$\text{Anti-inflammatory activity (\%)} = \frac{1 - D}{C} \times 100$$

Where D = Difference in paw volume after extracts were administered to the rats

C = Control group paw volume.

Evaluation of Diuretic Activity

The diuretic activity of MO and AP extracts administered singly and in combination was determined using the methods used by Lahlou *et al*.,[18].The male albino rats were randomly assigned into seven groups (*n* =5). Diuretic activity of the extracts was carried out via two routes of administration (oral and intraperitoneal routes).The negative controls were treated with 0.2 mL normal saline, while the positive controls group was treated with standard drug (hydrochlorothiaz -ide 10 mg/kg body weight). The other five groups were treated with different doses of

the extracts as follows: MO100 mg/kg, MO75 mg/kg+AP25 mg/kg, MO 50 mg/kg+AP 50 mg/kg, AP25 mg/kg+MO75 mg/kg and AP 100 mg/kg respectively. Each rat was placed in a metabolic cage 24 hours prior to commencement of the experiment for adaptation and then fasted overnight with free access to water. The rats were pretreated with 1mLnormal saline (0.9% NaCl) solution subcutaneously at 1.5 h and 0.5h prior to the start of the experiment to impose a uniform water and salt load [19]. Immediately after administration, the rats were individually placed in a metabolic cage. Urine was then collected and measured 1, 2, 3, and 4 hours after dosing. The urine volume and pH was determined for each group. The same procedure was repeated for intra-peritoneal route of administration using fresh set of rats.

Statistical Analysis

The data obtained from the study were expressed as Mean ± standard error of the mean (SEM) and analyzed using one–way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test. The difference between the means of treated groups and the non-treated control group was evaluated using paired Student's t-test. A probability value of $p \leq 0.05$ was considered to denote a statistically significant difference. The statistical software used was GraphPad Prism version 6.0 software.

RESULTS

Table 1: *In vivo* acute toxicity study of *M. oleifera* and *A. paniculata* extracts.

Extract	Percentage Yield (%$^{w}/_{w}$)	Group	Phase I(*n* =3)/group		Phase II(*n* =1)/group	
			Dosage (mg/kg)	Mortality	Dosage (mg/kg)	Mortality
M. oleifera	5.66	**1**	10	0/3	1000	0/1
		2	100	0/3	1600	0/1
		3	1000	0/3	2900	0/1
		4	-	-	5000	0/1
A. paniculata	8.86	**1**	10	0/3	1000	0/1
		2	100	0/3	1600	0/1
		3	1000	0/3	2900	0/1
		4	-	-	5000	0/1

Table 2: Anti-inflammatory activity of *M. oleifera* and *A. paniculata* extracts in formalin-induced rat paw edema

Treatment (n = 6)	Dose(mg/kg)	Percentage(%)Inhibition
Normal saline		
MO	100	69.44±2.15**
MO+AP	50:50	11.29±1.36*
AP	100	10.10±1.01*
Indomethacin	10	48.30±2.14

Key
MO = *M. oleifera*extract
AP = *A.paniculata*extract
* = No significant difference (P> 0.05) when compared with control group..There is no need to indicate with a * if there is no significant difference
.
** = Significant difference (P< 0.05) when compared with control group

Table 3: Effect of *M.oleifera* and *A. paniculata* on urine volume and pH

Treatment (n = 5)	Dose (mg/kg body weight)	Oral Administration		Intraperitoneal Administration	
		Urine Output		Urine Output	
		(mL)	pH	(mL)	pH
Control		1.62 ± 0.52	5.83 ± 1.53	2.70 ± 0.00	5.80 ± 0.48
Hydrochlorothiazide	10	2.76 ± 0.45	6.95 ± 0.58	5.22 ± 0.52	7.21 ± 0.31
MO	100	2.18 ± 0.63*	7.15 ± 0.52*	1.24 ± 0.32*	5.60 ± 1.41*
MO + AP	75+25	1.86 ± 0.42*	6.84 ± 0.58*	0.32 ± 0.23*	4.0 ± 1.63*
MO + AP	50+50	1.27 ± 0.60	4.55 ± 1.90	0.40 ± 0.40	1.31 ± 1.31
MO + AP	25+75	1.60 ± 0.86*	4.32 ± 1.85*	0.90 ± 0.32*	4.93 ± 1.23*
AP	100	1.54 ± 0.49*	4.92 ± 1.23*	2.10 ± 0.42 *	7.28 ± 0.22*

Key
MO = *M. oleifera*extract
AP = *A.paniculata*extract
* = No significant difference (P> 0.05)when compared with control group
** = Significant difference (P< 0.05)when compared with control group

RESULTS AND DISCUSSION

The leaves of MO and AP had the yield of 5.66 % $^{w}/_{w}$ and 8.86 % $^{w}/_{w}$ respectively, as shown in Table 1. The leaf extract of AP however had a higher yield compared to the leaf extract of MO. Air dried plant material have been observed to provide the advantage of preserving the phytochemical constituents of the plant material compared to fresh plant

material extraction, interestingly in a previous study by Vongsak *et al*.,[20], it was observed that there was no significant difference in total phenolic content between the fresh and dried *MO* leaves but a higher flavonoids content was in dried sample. Cold maceration method has been adopted and widely used in medicinal plants research, it is often preferred because it is easy and guarantees the stability of the phytochemical constituents, unlike soxhlet extraction or hot continuous extraction that provide higher yield and requires a smaller volume of solvent compared to maceration but has the short fall that phytochemical principles in the extract maybe denatured due to exposure to high temperature[21].

From the acute toxicity study of the leaf extract MO and AP it was observed that both extracts produce no mortality at even at 5000 mg/kg body weight as displayed in Table 1. Also, no mortality was observed after the period of two weeks. The LD_{50} value for both MO and AP extract was estimated to be greater than 5000 mg/kg. In previous human studies MO leaf extract was found to be safe even at high doses[22]. The result of the study is also in agreement with another report where AP was observed to relatively safe[23].

The anti-inflammatory activity of MO and AP is shown in Table 2. At dose of 100 mg/kg body weight the extract of MO and AP exhibited 69.44 and 10.10 % inhibition of the paw edema respectively. However, the combination of MO and AP exhibited weaker inhibition (11.29 %) of paw volume compared to MO (69.44 %). Under the same experimental conditions the standard drug (indomethacin) exhibited 48.30 % inhibition of the paw edema at 10 mg/kg body weight. The extract of MO displayed a significant ($P<0.05$) suppression of the rat paw edema about six times better than AP extract. The leaf extract of MO have been reported to have anti-inflammatory activity. The anti-inflammatory effect of leaf extract was attributed to the ability of the extract to inhibit cyclooxygenase enzyme and subsequent inhibit prostaglandin synthesis[24]. In another report, the hydroethanolic extract of MO leaves was observed to significantly inhibit the secretion of NO production and other inflammatory markers such as prostaglandin E2, tumor necrosis factor alpha, interleukin (IL)-6, and IL-1β[25]. MO had also been reported to display antioxidant activity[9]. While andrographolide, isolated from AP had been reported to reduce the inflammation caused by histamine, dimethyl benzene, and adrenaline[26]. However, the combination of both extracts displayed a weak suppression of the rat paw edema similar to AP (100 mg/kg body weight). This suggests antagonism of the anti-inflammatory activity of the MO leaf extract by the AP leaf extract. The ability of the MO and AP extracts to influence urine volume and pH as shown in Table 3. Hydrochlorothiazide used as the positive diuretic in both the oral and intraperitoneally routes increased the urine volume by 2.76 ± 0.45 and 5.22±0.52 respectively. The various dose combinations of extracts had similar diuretic activity compared to the hydrochlorothiazide except the MO 50 mg/kg+AP 50 mg/kg combination where the urine volume (1.27±0.60 mL) was lower compared to the negative control (1.62±0.52 mL). MO 50 mg/kg+AP 50 mg/kg combination appears to have antidiuretic. It was observed that MO 100 mg/kg and MO 75 mg/kg+AP 25 mg/kg displayed higher diuretic activity than AP 100 mg/kg and MO 25 mg/kg+AP 75 mg/kg. This is perhaps due to the complete absence or small amounts of AP in the dose. This may suggest that MO possesses some diuretic activity while AP appears to possess antidiuretic activity. This may explain why there was a decrease in the urine volume with increase in concentration of AP in the doses. When the extracts were

administered orally and intraperitoneally there was no significant change in the pH of the urine except in the MO 50 mg/kg+AP 50 mg/kg combination. However, only MO100 mg/kg (po), MO 75 mg/kg+AP 25 mg/kg (po) and AP 100 mg/kg (*i.p.*) maintained a urine pH similar to the normal (negative control). Unlike AP, MO has previously been evaluated for diuretic activity. The study is in consonance with previous diuretic studies of MO[27, 28].

Conclusion

The methanol extracts of MO and AP are relatively safe with LD_{50} greater than 5000 mg/kg body weight. MO had significant anti-inflammatory and diuretic activities. In contrast to MO, AP extract displayed antidiuretic activity. The co-administration of AP with MO does not potentiate the anti-inflammatory nor diuretic activity of MO but the combination resulted in the antagonism of the anti-inflammatory and diuretic activity of MO. Thus, this study has not found sufficient scientific evidence, hence rationale to support the co-administration of MO and AP as either an anti-inflammatory or diuretic agent.

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