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Anti-inflammatory and Diuretic Activity of Aqueous Extract of *Leptadenia hastata* Leaves in Wistar Rats

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Abstract

This study investigated the anti-inflammatory and diuretic effects of aqueous leaf extract of *Leptadenia hastata* (AELH) in Wistar rats. Acute toxicity study was carried out on the extract according to Lorke's method. In the anti-inflammatory test, egg albumin was used to induce inflammation. AELH was administered intraperitoneally to the rats at doses of 200, 400 and 800 mg/kg b.w respectively to the test groups while aspirin and normal saline were used as the positive and negative controls respectively. In the diuretic test, same doses of AELH as in the anti-inflammatory test were administered to the test groups while four standards (Furosemide, Hydrochlorothiazide, Spironolactone and Acetazolamide) and normal saline served as controls. Acute toxicity study revealed that AELH produced no mortality or signs of toxicity up to a dose of 5000 mg/kg. Results obtained also showed that the extract significantly ($P<0.05$) and dose- dependently inhibited the inflammation of the hind paws and also increased the volume of urine/ electrolytes excreted by the rats. It was concluded that the aqueous leaf extract of *Leptadenia hastata* possesses promising anti-inflammatory and diuretic activities in rats. Thus the plant could be useful in the management of inflammation and oedema associated with some health conditions.

Keywords: *Leptadenia hastata,* Inflammation, Oedema, Diuresis, Wistar rats

Introduction

Drugs which are used to increase urinary output and electrolytes excretion are known as diuretics (Aziz *et al.*, 2014). These drugs mostly act on different parts of nephrons and increase urine volume (Bharna & Rani, 2006). These drugs are known to relieve pulmonary congestion and peripheral oedema thereby decreasing cardiac work load, oxygen demand and plasma volume leading to decrease in blood pressure. The subclasses of diuretics include; thiazides, loop diuretics (e.g furosemide, bumetanide, torasemide), potassium sparing diuretics which are the weak diuretics, carbonic anhydrase inhibitors and osmotic diuretics. The use of plant based materials as diuretics has been reported. Plant such as *Allium sativa*, *Salvadora perica*, *Mentia viridis*, have been observed to have diuretic effects (Ekpeyong *et al.,* 2014; Muhammad *et al.,* 2014).

Inflammation is part of the complex biological response of vascular tissue to harmful stimuli such as pathogens, damaged cells, or irritant (Ferrero-Millian *et al.,* 2007). Acute and chronic inflammatory diseases are still one of the most important health problems in the world. Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system. However, their prolonged use often leads to serious adverse reactions such as gastric intolerance, bone marrow depression, water and salt retention. Consequently, development of new anti-inflammatory drugs with low side-effects is still necessary.

Only a small portion of medicinal plants as well as formulations used in traditional medicine are pharmacologically evaluated for both diuretic and anti-inflammatory activities and one of such plants is *Leptadenia hastata*. *Leptadenia hastata* belongs to the family asclepiadaceae widely used in Tropical Africa as vegetable (Burkil, 1985). The plant is medicinally important in the treatment of many ailments (Burkil, 1985; Oliver-Boyer, 1986; Aliero *et al*., 2001). Ethnobotanical information obtained from traditional medical practitioners in northern Nigeria revealed that *L. hastata* is used for the treatment of diabetes mellitus. The antibacterial and antimicrobial effects of *L. hastata* have been reported (Aliero and Wara, 2009) and the result of its toxicity studies showed that the plant is relatively safe (Tambuora *et al*., 2005).

Materials and Methods

Chemicals and drugs

All the chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor while drugs were obtained from a pharmacy shop.

Plant Collection and Identification

The leaves of *Leptadenia hastata* were collected from a natural habitat in Gaskiya area of Zaria, Kaduna State, Nigeria. The plants were identified at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University; Kaduna State, Nigeria.

Preparation of Extracts

The leaves of *Leptadenia hastata* were shade-dried for seven (7) days and pulverized using an electric blender. Two thousand (2000) gram of the pulverized leaves was soaked in distilled water for 72- hours. The resulting mixtures were filtered using Whatmann filter paper (Size No1) and the extract was concentrated using rotary evaporator. The extract of *Leptadenia hastata* shall henceforth be reffered to as AELH.

Acute Toxicity Study

The oral median lethal dose (LD_{50}) of the extracts was determined in rats according to the method of Lorke *et al.* (1983)

Anti-inflammatory activity

The anti-inflammatory activity of the extract of *Leptadenia hastata* was determined using the

method of Okoli and Akah, (2000). Wistar rats were grouped into seven groups. The animals were allowed to feed prior to the experiment but were fasted during the experiment. Treatments were administered orally. Group 1 served as positive control and administered 50 mg/kg aspirin. Group 2 was administered only normal saline thus served as negative control. Groups 3 to 5 were administered the extract at 200, 400 and 800 mg/kg respectively. The animals were left for 10 minutes after which 0.1 ml fresh egg albumin was injected into the sub planter of the right hind paw of each rat. The diameter of the hind paw was measured at 30 minutes interval for 2 ½ hours, using a vernier caliper.

Diuretic activity

The assessment of diuretic activity of AELH was carried out using the method of lipschiz *et al*, (1993). The albino rats were fasted for 10 hours prior to treatment. The experimental animals were divided into 8 groups. The animals in group 1 received normal saline which served as control. Group 2 animals were administered with furosemide, group 3 animals received Thiazide, group 4 animals were injected with spironolactone and group 5 animals were given Acetazolamide. Group 6 to 8 animals were the test group and were administered AELH at the dose of 200, 400 and 800 mg/kg. The treatment was carried out intraperitoneally. After the treatment, the animals were placed separately in aerated cages. Syringes were used to collect the urine produced after 24 hours. The animals were fasted during the test and were closely monitored. The total volume of urine and concentrations of sodium ion, potassium ion, chloride ion and bicarbonate in the urine were determined.

Statistical Analysis

Data were expressed as mean standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA and the values were considered statistically significant when p-value is less than 0.05.

Results

Acute Toxicity

The results of acute toxicity studies showed no mortality or physical signs of toxicity up to a dose of 5000 mg/kg of aqueous extract of *Leptadenia hastata*. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg (Lorke's method).

Table 1: Observations from the Acute Toxicity Study of the Aqueous Leaf Extract of *Anogeissus leiocarpus* in Rats

Treatment (mg/kg)	D/T	Observed Sign of Toxicity
AELH (10)	0/3	-
AELH (100)	0/3	-
AELH(1000)	0/3	-
AELH(1600)	0/1	-
AELH(2900)	0/1	-
AELH(5000)	0/1	-

D=death, T= No of animals treated

Effect on hind Paw diameter of Wistar Rats

Table 2 shows the effect of the extract on the paws of the rats. The extract significantly ($P < 0.05$) and dose- dependently reduced the inflammation of the paws of the Wistar rats. The standard drug used- aspirin, produced an average inhibition (15.42 ± 4.38) of the edema at 0 minute and 15.28 ± 4.32 after 150 minutes. The extract at the highest dose (800 mg/kg) used produced an average inflammatory inhibition of 14.36 ± 3.32 at 0 minutes and 8.29 ± 1.98 after 150 minutes. This was a better effect than that of Aspirin.

Table 2: Effect of Aqueous Leaf Extract of *Leptadenia hastata* (AELH) on hind Paw diameter (mm) of Wistar Rats

Treatment	0 mins	30 mins	60 mins	90 mins	120 mins	150 mins
Positive control	15.42±4.38^{b}	15.23±3.44^{b}	14.82±5.21^{b}	15.36±5.20^{b}	16.21±4.89^{b}	15.28±4.32^{b}
Negative control	12.21±3.43^{a}	13.78±2.56^{a}	12.78±3.11^{a}	13.47±5.31^{a}	13.45±3.11^{a}	13.79±3.45^{b}
AEL H(200 mg/kg)	15.24±3.45^{b}	15.61±3.22^{b}	14.89±4.69^{b}	14.37±4.21^{b}	14.51±3.77^{b}	13.74±3.45$_{b}$
AELH (400 mg/kg)	14.20±3.31^{b}	14.23±3.43^{b}	14.21±3.31^{b}	11.51±3.45^{a}	11.14±2.31^{a}	9.29±2.48^{a}
AELH (800 mg/kg)	14.36±3.32^{b}	13.58±3.18^{b}	13.20±4.67^{b}	11.23±4.78^{a}	10.35±2.38^{a}	8.29±1.98^{a}

Values shown are mean ± S.D. Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Effect on Volume of Urine produced by Wistar Rats

The extract significantly (P < 0.05) increased the volume of urine produced by the rats (Table 3). The negative control (saline) produced 0.83 ± 0.11 ml of urine and the positive controls (Furosemide, Hydrochlorothiazide, Spironolactone and Acetazolamide) produced 0.32 ± 0.16, 0.30 ± 0.20, 0.31 ± 0.19, 0.35 ± 0.14 ml respectively, while the highest dose of AELH(800 mg/kg) produced 0.94 ± 0.23 ml of urine and the lowest dose (200 mg/kg) produced 0.76 ± 0.25 ml of urine.

Table 3: Effect of Aqueous Leaf Extract of *Leptadenia hastata* (AELH) on Volume of Urine (ml) produced by Wistar Rats

Treatment	Volume of urine (ml)
Normal saline	0.83±0.11
Furosemide	0.32±0.16
Hydrochlorothiazide	0.30±0.20
Spironolacetone	0.31±0.19
Acetazolamide	0.35±0.14
AEL H(200 mg/kg)	0.76±0.25
AELH (400 mg/kg)	0.90±0.20
AELH (800 mg/kg)	0.94±0.23

Values shown are mean of three replicates

Effect on Serum Electrolytes excreted by the Wistar Rats

AELH produced significant ($P < 0.05$) changes in the excretion of electrolytes by the rats (Table 4). The excretion of sodium, chloride, potassium and bicarbonate ions due to treatment with the negative control (normal saline) was 80.12 ± 7.26, 78.35 ± 8.11, 16.35 ± 8.18 and 25.15 ± 8.45 respectively, while those of the highest dose of the extract were 145.21 ± 13.14, 143.30 ± 13.45, 25.38 ± 3.36 and 18.38 ± 3.25 respectively. The result obtained shows that electrolytes excretion increased in a dose- dependent manner (Table 4).

Table 4: Effect of Aqueous Leaf Extract of *Leptadenia hastata* (AELH) on Serum Electrolytes excreted by the Wistar Rats

Treatment	Na^+	Cl^-	K^+	HCO_3^+
Normal saline	80.12 ± 7.26^{a}	78.35 ± 8.11^{a}	16.35 ± 8.18^{b}	25.15 ± 8.45^{b}
Furosemide	147.23 ± 12.89^{cd}	143.12 ± 12.13^{c}	27.36 ± 2.32^{c}	23.27 ± 2.43^{b}
Hydrochlorothiazide	160.21 ± 17.12^{d}	158.18 ± 11.14^{d}	25.70 ± 7.54^{bc}	27.31 ± 7.27^{bc}
Spironolacetone	152.26 ± 13.18^{d}	145.33 ± 12.95^{a}	15.43 ± 3.68^{a}	23.44 ± 3.67^{b}
Acetazolamide	87.52 ± 6.21^{b}	86.15 ± 8.98^{b}	20.13 ± 8.35^{b}	46.85 ± 8.93^{c}
AEL H(200 mg/kg)	131.20 ± 12.11^{c}	130.13 ± 9.16^{bc}	21.56 ± 2.28^{b}	24.13 ± 2.73^{b}
AELH (400 mg/kg)	140.34 ± 12.92^{cd}	135.82 ± 12.21^{bc}	22.31 ± 7.44^{b}	23.39 ± 7.15^{b}
AELH (800 mg/kg)	145.21 ± 13.14^{cd}	143.30 ± 13.45^{c}	25.38 ± 3.36^{bc}	18.38 ± 3.25^{a}

Values shown are mean ± S.D. Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Discussion

Results of this study revealed the safety (acute) profile of the aqueous extract of *Leptadenia hastata* leaves as the toxicity study showed no mortality or signs of toxicity up to a dose of 5000 mg/kg. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg. Thus, the extract is not likely to produce toxic effects when consumed acutely.

The most widely used primary test to screen ant-inflammatory agent measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent. In this study, the extract significantly ($P < 0.05$) reduced the rate of inflammation of the paws (edema) of the rats, when compared with the control. This indicates that the leaf extracts had anti-inflammatory activity. The anti-inflammatory activity of medicinal plants is usually attributed to the presence of phytochemicals such as alkaloids, glycosides, flavonoids, phenolic compounds, steroids, saponins etc they contain (Shah *et al.*, 2011; Sengupta *et al.*, 2012). *Leptadenia hastata* leaves are known to contain alkaloids, flavonoids, saponins and tannins. The ability of the extract to inhibit inflammation of the rat paws increased with increase in the dose. Thus the anti-inflammatory effect of the extract is dose-dependent.

The extract also significantly ($P <0.05$) increased the volume of urine produced by the rats with a corresponding significant ($P <0.05$) increase in electrolyte excretion. This showed that the extract possesses diuretic activity. The diuretic activity of medicinal plants is usually attributed to their phytochemical content. Asif *et al.*, (2013) and Hallu and Engida-work, (2014) reported that the presence of phenolic compound, tannins, saponins, flavonoids, terpenoids, steroids, alkaloids and other secondary metabolites were responsible for the diuretic activity of medicinal

plants. The volume of urine produced by the rats and the excretion of electrolytes increased as the dose of the extract increased.

Conclusively, the aqueous extract of the leaves of *Leptadenia hastata* showed anti-inflammatory and diuretic properties. Thus the extract could be utilized in the management and treatment of inflammation and oedema associated with some health conditions.

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