Leaf Extracts Of Irvingia Gabonensis Increase Urine Output And Electrolytes In Rats

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Citation

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Abstract

The diuretic effect of the ethanol extract of the leaves of Irvingia gabonensis was assessed in adult wistar rats. Five groups of five adult wistar rats each were used for the study. Ethanol extract of Irvingia gabonensis was administered orally to the first and second groups of rats at doses of 50 and 100mg/kg respectively. The third and fourth groups were given Frusemide (5mg/kg) and Acetazolamide (5mg/kg) while the last group was used as positive control in the study. The parameters used to evaluate the diuretic effect of the different doses of the extract were cumulative urine volume, electrolyte changes and pH. The cumulative urine output was monitored at 3hrly intervals for 24hrs.The diuretic effect was compared with the control, acetazolamide (5mg/kg) and frusemide (5mg/kg). The extract (50 and 100mg/kg) produced a time related increase in urine output. The electrolyte excretion was also affected by the different doses of the extract especially the HCO_3^- ions. The 50 mg/kg extract produced an increase ($P< 0.03$) in Cl^- excretion compared with the control likewise the 100 mg/kg with a significant ($P<0.001$) increase in Cl^- excretion. Comparing the urinary excretion of electrolytes produced by the extract with acetazolamide, similar diuretic profile was observed like the pH and the increased excretion of HCO_3^-. Frusemide, a high ceiling diuretic had no effect on HCO_3^-, had a different pH but enhanced the urinary excretion of Na^+, K^+ and Cl^-. These results suggest that the active ingredient(s) in the ethanol extract of Irvingia gabonensis induces diuretic response comparable to that produced by acetazolamide.

INTRODUCTION

Irvingia gabonensis (O'Rorke) Baill var Excelsa (Keay 1989) is a species from the family of Irvingiaceae. It is found in the tropical forests and often seen in villages and towns in the forest region like Njala in Sierra leone, Yapo in Ivory Coast, Port Novo in Dahomey, Owerri, Calabar, Lagos and Abeokuta in Nigeria. The fruit pulp is acrid and bitter though it can be eaten. The seed act as a source of human food in Nigeria and known as ogbono. Chemical composition of oil extracted from Irvingia gabonensis seed kernels include: protein (8.33-8.71%), oil (34.28-73.82%), ash (2.06-3.8%) and carbohydrate (15.71-55%) and the major fatty acid was, C12:0 (36.6-39.37%), C14:0 (50.92-53.71%) and C16:0 (4.97-5.23%) (Matos et al, 2009).

Irvingia gabonensis is largely used in traditional and modern medicine for the treatment of several illnesses, as well as in industry (Lowe et al, 2000, Anegbeh et al, 2003). Different parts of the plant have been employed for this purpose and prepared locally as crude extracts of the stem, bark, roots, leaves and kernels. The seeds have been found to reduce fasting blood glucose levels in obese subjects (Ngondi et al, 2005). The stem bark has also been reported to have analgesic effects (Okolo et al, 1995). The powdered chocolate prepared from the kernels is applied to burns and used to make astringent remedies (Irvine, 1961). A decoction of the stem back of Kigelia Africana and leaves of Irvingia gabonensis is used to cure spleen infection (Sofowora 1986). The aqueous leaf extract of Irvingia gabonensis has been found to cause a significant dose-dependent decrease of gastrointestinal motility in mice (Abdulrahman 2004). Preliminary phytochemical screening of the aqueous leaf extract of Irvingia gabonensis revealed the presence of saponins, tannins, phenols and phlobatanins. It has been reported that saponins are of great pharmaceutical importance because of their relationship to compounds such as the sex hormones, diuretics, steroids, vitamin D and cardiac glycosides (Adedapo et al, 2009). Some studies have also demonstrated that several compounds like saponins, and flavonoids could be responsible for plants diuretic effects (Maghrani et al, 2005) and according to Lowe et al 2000, this plant is used for the treatment of several illnesses. No previous pharmacological or clinical study has been carried out to test the diuretic activity of this plant. Therefore this

study was designed to evaluate the diuretic effect of the ethanol leaf extract of Irvingia gabonensis in experimental animals in order to establish a pharmacological rationale for the traditional use of this plant.

MATERIALS AND METHODS

ANIMALS

Adult albino rats of both sexes weighing between 170 – 230g were used in the study. They were obtained form the animal house of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. Before the commencement of the studies all animals were allowed to acclimatize to the laboratory environment for at least five days and within this period they were watched closely and observed for any clinical signs, body weight changes, water and feed consumption. The experiments were carried out in accordance with the Guidelines for Laboratory Procedures laid down by the Ahmadu Bello University Zaria Ethics Committee on Research as well as the internationally accepted principles regarding the care and use of animals for experimental techniques.

PLANT MATERIAL

The leaves of Irvingia gabonensis were collected from the Herbarium Keeper of the Forestry Research Institute, Ibadan, Oyo State, Nigeria. A voucher specimen of the plant has been deposited with the Institute's Herbarium under voucher number FHI 103947 for future reference.

PREPARATION OF PLANT EXTRACT

The plant leaves were obtained in large quantities and left to dry at room temperature for two days after which they were dried in an oven at 35 – 40 C for 36 hrs. After that some of the leaves were ground into a coarse powder. The powdered leaves were kept in an air-tight glass container and stored in a dry place. 100g of the powder was subjected to Soxhlet extraction for 20 hrs at 50 – 55 C. The extract was concentrated and dried under vacuum. The percentage yield of the 100 g powdered leaves of Irvingia gabonensis was 7.67% from dry weight, a black crude extract with a sweet smelling (turpentine) flavor.

Pharmacological evaluation for diuretic Activity

In this experiment, the animals were randomly divided into five groups of five rats. Acetazolamide and Frusemide were the standard drugs used to screen the effects of the extract for the diuretic studies. Both male and female albino rats (wistar strain) were fasted overnight prior to test and screened for diuretic activities using the ethanol extract dissolved in normal saline. The first and second groups were given 50 and 100mg/kg of the ethanol extract, third group Frusemide 5mg/kg, fourth group Acetazolamide 5mg/kg, and the fifth group 2ml of normal saline. Immediately after administration, all were paired and kept in a quiet room to avoid unnecessary disturbance inside different metabolic cages capable of collecting their urine in a graduated cylinder free from faecal contamination. The output of the urine was measured for 24hours at 3hrs interval and route of administration of saline, frusemide, acetazolamide and extract was oral. At the end of the 24 hours the urine excreted was pooled in each group and an aliquot taken for estimation of Na^+, K^+, Cl^-, HCO_3^- contents and pH expressed as mmol/litre.

STATISTICAL ANALYSIS

Results are expressed as mean ± S.E.M. Statistical differences were analyzed using student's t-test where $P< 0.05$ was considered significant. SPSS version 15 software program was used.

RESULTS

DIURETIC EFFECT OF THE LEAF ETHANOL EXTRACT IN RATS

The results of screening the ethanol extract for diuresis are shown in Table 1, Fig.1 and Fig.2. The extract increased urine output after an hour but the response to frusemide and Acetazolamide was within the first 30 minutes. The control showed only slight increase in urine output in the day (Fig.1) Frusemide (5mg/kg) induced brisk diuresis which reached maximum after 30 minutes and stayed constant until after about 6 hours and then increased gradually for the next 24 hours. The extract (50 and 100mg/kg) on the other hand produced a continuous increase in urine volume throughout the period of the experiment (Fig.2). This is similar to the diuresis obtained with acetazolamide (Fig.1) which produced a significant ($P<0.05$) increase when compared with the control.

Figure 1

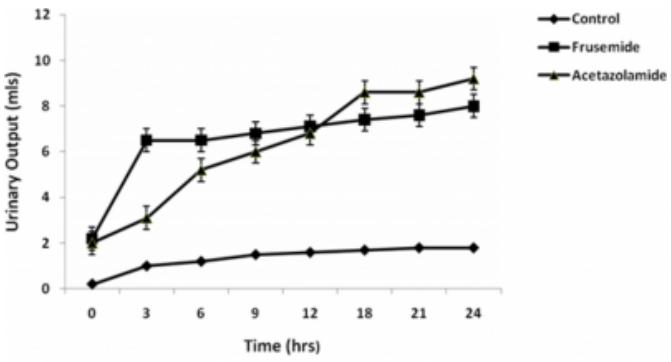

Fig. 1 Effect of standard diuretic drugs on urine output. Acetazolamide, 5mg/kg and Frusemide, 5mg/kg were given to rats and the cummulative urine output measured for 24 h. (◊) represents saline-treated (control). Results are expressed as mean±SEM, n=5

Figure 2

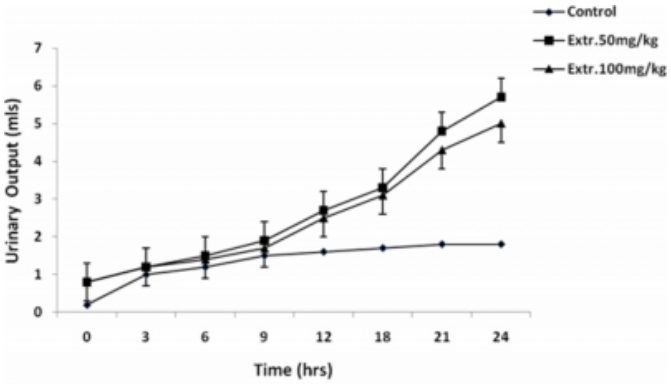

Fig.2 Effect of Ethanol leaf extract of *I. gabonensis* on urine output. 50mg/kg and 100mg/kg of the extracts were injected into rats and cummulative urine output measured for 24 h. (◊) represents saline-treated responses. Results are expressed as mean±S

Figure 3

Table 1. Effect of oral administration of ethanol extract of on electrolyte changes (mmol/l) and pH in the urine after 24hrs

Treatment dose mg/kg	Na^+	K^+ (mmol/litre)	Cl^-	$HCO3^-$	pH
Control	124±.70	130±.71	6.0±.70	-	7
Frusemide					
5mg/kg	190±.63	210±1.6	14±.32	10±1.4	12
Acetazolamide					
5mg/kg	110±2.1*	170±3.5	8±.92	320±8	9.3
Extract					
50mg/kg	96±.70	60±1.3	9±.89**	90±1.6	10
Extract					
100mg/kg	74±1.3	90±1.4	11±.51*	80±2.3	10

*P and **P represent P<0.001 and 0.03 respectively compared with the control group (students t-test)

Table 1 shows the electrolyte changes observed in the cumulative urine output. Frusemide was seen to enhance the increased excretion of Na^+, K^+ and Cl^-. The different doses of the extract (50 and 100mg/kg) enhanced the excretion of $HCO3^-$ with pH of 10 compared with control. This is similar to the effect of acetazolamide (carbonic acid anhydrase inhibitor) on $HCO3^-$ level in urine. 50mg/kg of the extract produced an increase ($P<0.03$) in Cl^- excretion compared with the control likewise the 100mg/kg of the ethanol extract with a significant ($P<0.001$) increase in Cl^- excretion. However, acetazolamide produced a significant increase ($P<0.001$) in Na^+ compared with the control.

DISCUSSION

The term diuresis has two separate connotations: One refers to the increase in urine volume per se while the other to the net loss of solute (electrolyte) and water (Irwin, 1990) and these are involved in the suppression of renal tubular reabsorption of electrolytes. The evidence of a diuretic response was observed in the rats treated with frusemide (Laxis^R), acetazolamide (Diamox ^{R}) and the extract. Frusemide inhibits electrolyte reabsorption in the thick ascending limb of loop of Henle. Micropuncture experiment has demonstrated a greatly enhanced excretion of Na^+ and Cl^- (Greger and Wangemann, 1987). Studies has shown that the high ceiling diuretics enhance the excretion of both Ca^{2+} and Mg^{2+} to an extent approximately equal to the increase in Na^+ excretion and also causes a greater depletion of K^+ (Sutton, 1985). Some of these were also observed in this study.

Acetazolamide is a potent reversible inhibitor of carbonic anhydrase. More than 99% of enzyme activity in the kidney is inhibited before physiological effects become apparent (Preisig, 1987).

As the reabsorption of water is reduced, volume of urine increases and pH of urine becomes alkaline. Also it increases urinary concentration of HCO_3^- accompanied by increases in Na^+ and K^+ ions with a fall in concentration of Cl^- ((Preisig, 1987). All these were observed with Acetazolamide and the different doses of the extract.

There was a greater excretion of Na^+ with the 50mg/kg of the extract than with the 100mg/kg which gave a greater depletion of K^+. This suggests that an increase in the dose of the extract can lead to hypokalemic alkalosis (Milton,1970) and this is in line with the pH of the urine but with the lower dose can lead to hyponatremia. The urine bicarbonate (HCO_3^-) and chloride (Cl^-) contents were almost the same (HCO_3^-

(90 and 80mmol/litre), Cl^- (9 and 11 mmol/litre)) for the 50 and 100mg/kg of the ethanol extract. The HCO_3^- content was much higher than frusemide treated animals.

The 24 hour cumulative urine output induced by the extract was higher than that of the loop diuretic agent but lower than that of acetazolamide. Considering the definition given to diuretics in the first paragraph, frusemide, acetazolamide and ethanol extract fall into this category. The leaf extract of Irvingia gabonensis may have similar mechanism of action to acetazolamide but different from that of frusemide, based on their effects on the concentrations of various ions in the urine and pH. However the presence of saponins in Irvingia gabonensis from the phytochemical screening may also have contributed to the diuretic effect as saponins have been reported to be involved in plant diuretic responses.

CONCLUSION

From this investigation, the different doses of the extract showed that Irvingia gabonensis has diuretic effect and this effect is similar to that produced by the carbonic anhydrase inhibitor, acetazolamide.

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