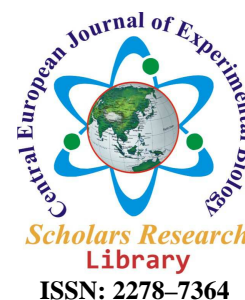




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An assessment of the diuretic effect of *Vernonia amygdalina* aqueous extract on wistar rats

Odewusi Odeyinka Olufunsho¹ and Tope-Ajayi Ayodele Abimbola²

¹Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria

²Department of Medical Laboratory Science, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria

ABSTRACT

The research was set to assess the diuretic effect of *V. amygdalina* extract on Wistar rats. Assessment of diuresis was carried out on five groups of rats. Three groups were injected intraperitoneally with varying concentrations of *V. amygdalina* extract in normal saline, each group consisting of eight rats. Groups iv and v were injected with furosemide (0.1mg/100g body weight) and saline respectively. The dosage of extract administered to groups i, ii, iii, iv and control are 25, 50, 75, 0.1 (furosemide) and 0 mg/100g body weight, while the mean urine output in ml/rat/hour for groups I to v are 1.050 ± 0.064 , 1.285 ± 0.079 , 1.465 ± 0.055 , 4.621 ± 0.319 and 0.95 ± 0.045 respectively. A statistically significant difference was observed when the urine output of groups i, ii and iii and iv were compared to that of the control group. These values reveal a fairly proportionate increase in urine output in response to increasing dosage of extract. *V. amygdalina* aqueous extract perhaps possess a dose dependent diuretic property due to the presence of phytochemical diuretic factor(s)/principle(s) which, if properly harnessed, can be used in the treatment of retentive disorders of water metabolism.

Keywords: Diuretic activity, Water, *V. amygdalina*,

INTRODUCTION

The essentiality of water to life is incontrovertible. It is in it that all the metabolic processes of our systems take place. In man, it is important for the transport and excretion of waste products via the kidneys in urine and also through the lungs, skin and faeces [1]. The maintenance of body water homeostasis depends on the balance between water intake and water excretion [2]. The 70kg individual's body has about 42 liters of water distributed between the intracellular and extracellular compartments [3, 4] the extracellular fluid further can be divided into the intravascular and interstitial fluid sub compartments [4]. The major means of water output from the body is through urine; other forms are through perspiration and respiration and are termed as insensible. Cheng et al. [2] defined the state of water homeostasis as the balance between input and excretion. Urine is defined as a filtrate of blood produced by the kidney, passed through the ureters, stored in the bladder and passed out through the urethra [5] and is therefore the major means of water excretion. In the online article authored by Welch titled "WATER METABOLISM: made easier", a summary of what happens when an individual takes a water load and then is deprived of water for several hours is explained. The article states "The individual starts with a normal state of hydration, electrolytes and plasma osmolality (POsm), then drinks 2 litres of water in 30 minutes. The water is

absorbed, reduces serum $[Na^+]$ and plasma osmolality and suppresses vasopressin release. The low vasopressin causes the renal collecting ducts to become relatively impermeable to water and maximally dilute urine ensues. If the individual drinks nothing for several hours, water is lost in the urine, breathe and sweat causing the plasma osmolality to increase; this causes vasopressin levels to increase when the threshold for its release is reached. The collecting ducts then becomes more permeable to water until maximum urinary concentration is reached. Water continues to be lost in urine, breath and sweat until the threshold for thirst is reached, when drinking again satisfies thirst, water is absorbed from the gut, reducing plasma osmolality, thirst drive, vasopressin levels and urine concentration". The above italicized can be described as the physiological response to a water load and water deprivation. In addition to this, aquaporins also play a part in renal water handling. Aquaporins are a family of water channels that mediate water transport [7]. It is however worth mentioning that newer concepts are being uncovered as regards the regulation of water homeostasis. For instance in vivo studies by Baron *et al.* [8] demonstrated that secretin has an antidiuretic effect on normal human and dogs. This finding was substantiated by Waldum *et al.*[8] when a significant increase in water excretion was observed after an intravenous infusion of natural secretin. Oxytocin has also been reported by Terashima *et al.*[10] to possess vasopressin-like effects on the collecting ducts of the nephrons of rats. Though both hormones possess vasopressin like effects, they appear to exert their effects in controlling water homeostasis through vasopressin independent mechanisms [2]. It is therefore the opinion of the investigator in this present research that substances that have effects on human water metabolism are likely to abound in nature

Retentive disturbances of water homeostasis such as hypervolaemia, SIADH and oedema are routinely treated with the use of diuretics. These drugs cause an increase in urine output per unit time. Their use are not without side effects, for example loop diuretics cause metabolic alkalosis, carbonic anhydrase inhibitors cause metabolic acidosis[3, 4]. Whitby *et al.* [3] went on to state that thiazide diuretics cause glucose intolerance. In light of these, new lines of drugs needs to be discovered and characterized, as there is an ever increasing need to limit toxic drugs[11]. If this is to be done, resort to phytochemicals could be the solution. Information on the active ingredients and curative actions of the medicinal plants was acquired by the introduction of European scientific methods [12]. *Vernonia amygdalina* is a Nigerian plant, the extract of which have been reported to possess: Natriuretic properties[13] hepatoprotective properties [14], chemotherapeutic effects against breast cancer cells [15],antibacterial effects [16]. Oyagbemi and Adejimi [17] and Adedapo *et al.*[18] reported its anticoccidial and antihelminthic activities respectively. Not more than nothing has been said about its diuretic property except that it is used in the treatment of kidney diseases [19]. It should be noted that the presence of phytochemicals is the rationale underlying the inclusion of plant parts in various native medicinal preparations [20], Bearing this in mind; this research work was designed to assess and ascertain the effects of metabolic extract of *Vernonia amygdalina* on renal water retention.

MATERIALS AND METHODS

2.1 Sources of materials

Freshly harvested *Amygdalina* leaves and young shoots were collected from forest lands adjoining Achievers University, Owo, Ondo State, Nigeria. The botanists in the Plant Science Unit of the Biological Sciences Department identified the plant.

2.2 Bitter leaf extraction:

The sample leaves were washed with water, dried at room temperature for two and a half weeks and grounded into powder using a blender. 400g of the ground leaf was dissolved in 1litre of water and placed in an oven at 40°C for 3 hours. The extract solution was then obtained by filtering. The extract obtained was condensed and later dried at 45°C for 1 week in an oven.

2.3 Preparation of standard *V.amygdalina* leaf extracts solution

About 200mg of the bitter leaf extract was dissolved in 5ml of normal saline at a temperature of 50°C.This was then made up to 10ml with distilled water to make a 200mg/10ml(20mg/ml) bitter leaf extract stock solution. 2.5mg/ml, 5mg/ml and 7.5mg/ml bitter leaf extract solution was prepared by appropriate dilution of stock with sterile normal saline.

2.4 Laboratory animals

Forty (40) Wistar rats (age 10 to 12 weeks, mean weight 105 grams) were used in this research. These animals were obtained from the animal house and were reared under standard animal management practices throughout the duration of research. They were quarantined in deep litter cages with absorbent materials to absorb moisture (8 rats per cage) 7 days before the commencement of supplementation and also throughout the period of the research. The animals were fed with chow pellets from CAPS feeds industry and water *ad libitum* in standard quantity all through.

2.5 Grouping of experimental animals and dosage of extract

Wistar rats were divided into five treatment groups (I - V) of eight (8) rats each. Each rat in groups I, II and III were supplemented through peritoneal injection with 2.5mg/100g body weight/day, 5mg/100g body weight/day and, 10mg/100g body weight/day bitter leaf extract solution respectively for 10 days, so that the effect observed, if due to treatment, should likely show a multiplying effect. Group IV was supplemented with furosemide (0.1mg/100g body weight) while the rats in group V were not supplemented but each rat was injected intraperitoneally with 1ml/100g body weight of normal saline.

2.6 Preparation of lab animals

The rats were placed in the metabolic cages at 8:00 pm of every day prior the days of injection. Chow pellets and fluid were withdrawn from the cages 2 hours before the intraperitoneal injection of extracts. The injection of extracts and the assessment of the volume of urine produced were done in an air conditioned environment set to a temperature of 22°C.

2.7 Assessment of Diuresis

The method used was an adaptation of the method as previously described by [21]. At the beginning (t=0 minutes) of the experiment, the urinary bladder was emptied by gentle compression of the abdomen. Once the bladder was emptied, the extracts and standard furosemide solution were then administered, as appropriate, by intraperitoneal injection. At the end (t=120 minutes) of this period, the rest of the urine in the bladder was collected by abdominal compression to ensure complete emptiness of the bladder. The urine so collected was added to the sample of urine collected into calibrated pipettes at the base of the cage. The total urine sample collected was measured and noted. The volume of urine voided/ rat/hour was computed and recorded.

2.8 Statistical analysis

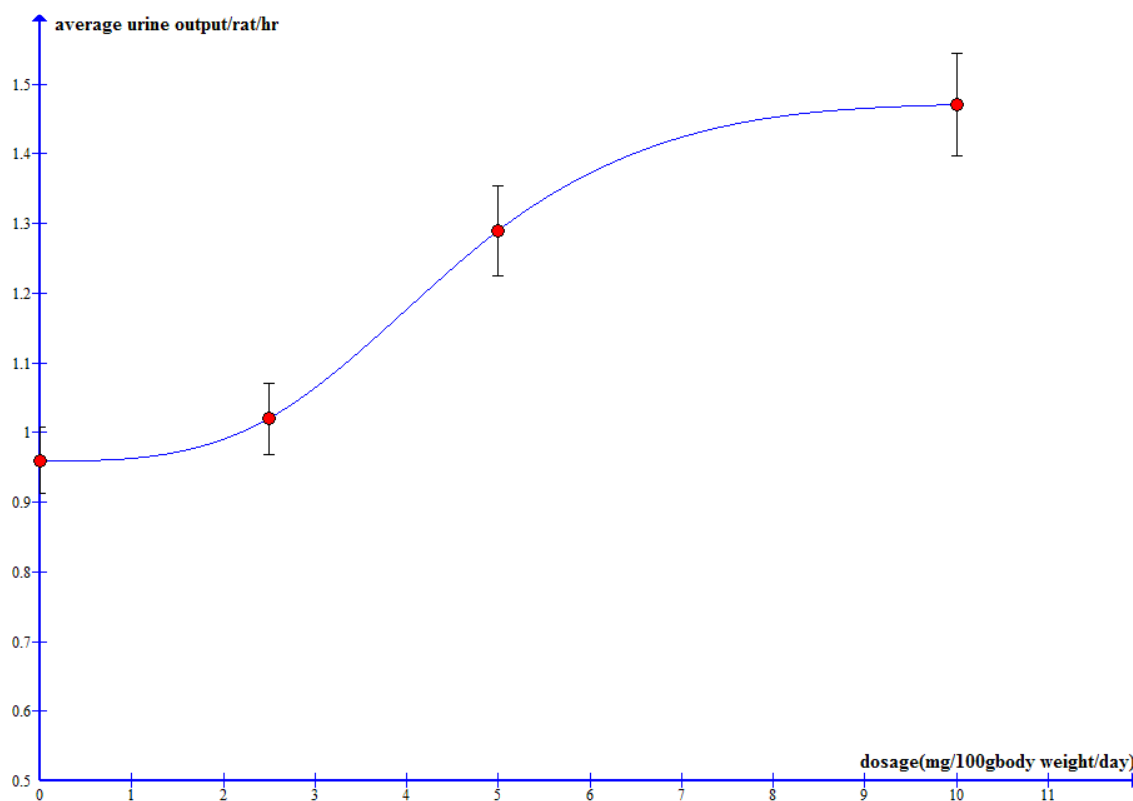
All values are expressed as mean \pm standard deviation. Statistical analysis was performed using the student's t test. Differences were considered to be significant or otherwise at $p < 0.05$.

RESULTS

The mean water excretion in ml/rat/hour of groups i, ii, iii iv and control are 1.05 ± 0.064 , 1.285 ± 0.079 , 1.465 ± 0.055 , 4.621 ± 0.319 and 0.95 ± 0.045 respectively (Table 1). Further illustrating the diuretic response of rats to aqueous V *.amygdalina* extract is the dose response curve shown on figure 1, which clearly shows a spinal surge in plasma urine output as the administered extract increases.

Table 1. The mean urine voided, the standard deviation (SD), the P value and students' t when the volume of urine voided of the supplemented groups were compared with that of an unsupplemented group

Group	Mean \pm SD (ml/rat/hour)	Student's t	Significance	Remarks
Group I	1.050 ± 0.064	7.081	0.006	Significant
Group II	1.285 ± 0.079	6.295	0.008	Significant
Group III	1.465 ± 0.055	17.514	0.000	Significant
Group IV	4.621 ± 0.319	32.212	0.000	Significant
Control	0.952 ± 0.045	Not Applicable	Not Applicable	Not Applicable



Graph i. showing the mean urine output in response to various doses of *A. amygdalina* administered

DISCUSSION

Diuresis could be defined as an increase in the frequency or volume of urine voided per unit time. It is a most desirable phenomenon in clinical conditions where water and or sodium tend to be retained such as hypertension, heart failure, nephritic syndrome and cirrhosis [3, 22]. It is of opinion that diuretic substances abound in nature and are yet to be tapped. Various research works have made the phytochemical constituents of *V. amygdalina* known. It has been known to contain carbohydrates, saponins, alkaloids, tannins, proteins and steroids, flavonoids and glycosides [23]. The presence of water soluble alkaloids and flavonoids in plant parts have been known to confer diuretic properties on them as stated by Ranju *et al.* [24] in fact to be more specific, some of the plants with potent diuretic activity have been found to contain benzyl isoquinoline type of the alkaloids [25]. In this research, a significant increase in urine output was seen in all supplemented groups when diuresis due to *Vernonia amygdalina* supplementation was compared to control. The values seen reveal a proportionate increase in urine output in response to increasing dosage of extract as explained by Graph i. This confirms the presence of a diuretic substance, factor or principle in the extract; it would also imply that the phytochemical constituent of *V. amygdalina* responsible for diuresis is dose dependent. There was also a significant variation between the urine volume seen in furosemide treated and control rats, but the diuresis observed as a result of furosemide supplementation is higher than that observed in control. This observation could be due to the fact that crude ethanolic extract was used in this study.

This research work ventured into and found out that crude aqueous extract of *V. amygdalina* possess diuretic properties and that, the diuresis seen is dose dependent. It is the opinion of the investigators in this research that the diuretic effect of *V. amygdalina* is most probably linked to its effect on renal sodium handling, in fact this seems to be the rationale behind the use of diuretics, as diuresis most definitely favours sodium excretion [3, 26]. It is possible that flavonoids and alkaloids present in the extracts of *V. amygdalina* exerted diuretic effect by inhibiting tubular reabsorption of water and electrolytes as such action has been suggested for some other plants [24, 27]. Certain flavonoids were found to exert their diuretic activity by binding with adenosine A1 receptor associated with the diuretic action [28]. The diuretic activity of *V. amygdalina* may be through any of these mechanisms since it is

rich in alkaloids and flavonoids [23]. An advantage of using herbal mixes as diuretics is that they produce very little acute toxicity and in general they can be considered as mild and good drugs, in comparison to other diuretics used nowadays in the therapeutics [21, 24]. Further research could be carried out to characterize the diuretic chemicals in *V. amygdalina* extract. It will also increase knowledge and understanding if the systemic, cellular and molecular mechanisms of the action of this extract on both animal and human is investigated.

CONCLUSION

As it has been clearly stated above, this research work ascertained that the peritoneal administration of *V. amygdalina* extract favours water excretion in rats. The phytochemical constituents responsible for this characteristic could be isolated, purified and characterized. The resultant drug when administered would probably have little or lesser side effects than most synthetic drugs presently in use, the rationale being that, if the inclusion of *V. amygdalina* leaves in stews and soups have not been associated with any side effects on the long term, the use of the purified phytochemicals responsible for diuresis would most probably bring about no side effect if used in the prescribed dose.

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