Bayero Journal of Pure and Applied Sciences, 3(1): 183 - 187
Received: February, 2010
Accepted: May, 2010

EFFECT OF ORAL ADMINISTRATION OF AQUEOUS EXTRACT OF *CASSIA OCCIDENTALIS* L. SEEDS ON SERUM ELECTROLYTES CONCENTRATION IN RATS

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ABSTRACT
The effect of aqueous extract of C. occidentalis seeds on serum electrolytes concentration, in rats, was investigated in order to assess the acclaimed hypotensive effect of the seeds and also the seeds extract's relation with acid – base balance of the body. Serum concentrations of sodium, potassium, chloride and bicarbonate ions were determined in 64 rats grouped into 4. Groups I, II and III rats were orally administrated with the seeds extract at a daily dose of 30 mg/kg, 60 mg/kg and 90 mg/kg respectively for 1,2,3 and 4 weeks each. Group IV served as control. Concentration of sodium, potassium, chloride and bicarbonate in the serum of control rats were found to range from 130 – 155 mEq/L, 3.4 - 8.7 mEq/L, 80 – 107 mmol/L and 20 -35 mmol/L respectively. The results of this study showed no significant difference (P<0.01) when serum sodium, chloride and bicarbonate levels in the rats orally administered with 30 mg/kg for 1 – 2 weeks were compared with control rats . However, there was significant increase (P<0.01) in serum potassium levels at 3rd and 4th weeks of experiment. Oral administration of aqueous extract of C. occidentalis seeds at a daily dose of 60 mg/kg was found to show significant increase (P<0.01) in serum potassium level and a significant decrease (P<0.05) in serum sodium levels irrespective of duration of administration (weeks). This indicates that the seeds extract have hyperkalaemic and hyponatremic effect at a daily dose of 60 mg/kg and as such can have hypotensive effect at this dose. Serum levels of chloride and bicarbonate showed significant decrease (P<0.01) when the daily dose is 90 mg/kg for ≥ 1 week. This indicates that the seeds extract dosage of 90 mg/kg could lead to disturbance in acid-base balance.

Key words: C. occidentalis, Electrolytes, Acid-base Balance, Hypotension.

INTRODUCTION

Nowadays, the use of plants as excellent sources of therapeutic agents is attracting the interest of researchers in the area of ethnomedicine (Sofowora, 1993). Studies have shown that many plant materials used as food have some medicinal activities (Gbile and Adesina, 1989; Odebiyi and Sofowora, 1993). Disease treatment and prevention, in Nigeria, have for a long time been handled by traditional healers (Ebomoyi *et al.,* 2004). Their practice involves the use of herbs, animals, mineral substances or the use of rituals and incantations. The use of herbs, however, survived best (Ebomoyi *et al.,* 2004). Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effects (Farnsworth, 1989). Many medicinal plants have been found and put into use by traditional healers in the management of many ailments for many years (Sofowora, 1993). One of the problems of using plant materials as medicines is that in many cases no definite doses are prescribed, often resulting in overdoses (Sule and Mohammed, 2006).

Cassia occidentalis Linn belongs to the family *Leguminosae,* sub-family *caesalpinoidae*. It is botanically classified as both *Cassia occidentalis* and *Senna occidentalis.* It is a small, erect, annual herb that can be up to 2m tall and is found abundantly in the rain forest and tropical areas of the world. Its seeds, found in long seed pods, are sometimes roasted and made into coffee-like beverage. *C. occidentalis* has a rich history in natural medicine and the parts of the plant used include roots, leaves and seeds (Saraf, 1994).

Clinical researches demonstrated *in vitro* anti-bacterial, anti-fungal, anti - parasitic and anti-malarial properties of *C. occidentalis* leaves (Sammy, 2000). The leaves and roots also demonstrated anti-inflammatory, anti-hepatotoxic, smooth muscle relaxant, inhibition of haemolysis and lipid peroxide formation activities. They are also used for treatment of asthma, bronchitis, gonorrhea and constipation (Caceres, 1991; Sammy, 2000; Gasquet, 1993; Hussain, 1991; Saraf, 1994). Although the seeds of *C. occidentalis* are used in herbal medicine for treatment of diseases like hypertension as well as consumption as coffe -like beverage, several researches (Sadique, 1987; Saraf, 1994) have demonstrated the toxicity of the fresh and dried roasted seeds. Ingestion of large amounts by grazing animals has been reported to cause toxicity problems and even death in cows, horses and goats (Sadique, 1987; Saraf, 1994). Studies have shown that oral administration of aqueous extract of *C. occidentalis* seeds at a daily dose of 60 mg/kg for ≥ 1 week causes significant increase in serum urea, creatinine as well as liver specific enzymes (Abubakar and Sule, 2008 a and b).

Indiscriminate usage of *C. occidentalis* seeds in treatment of diseases in Nigeria and many other countries justify carrying this research and many of its kind. This study intends to investigate the effect of aqueous extract of *C. occidentalis* seeds on serum electrolytes levels in rats. This will lead to assessing the relationship between *C. occidentalis* seeds extract and body fluid osmolarity as well as acid – base homeostasis.

MATERIALS AND METHODS

Collection and Extraction of Seeds

Dried seeds of *C. occidentalis,* collected from within Kano metropolis, Kano State -Nigeria, were roasted and ground to powder. The powder (15 g) was dissolved in 1dm^3 of distilled water for 24 hours. The resulting mixture was then filtered and the residue was weighed. The weight of the residue was found to be 5g. Hence, 10g of the powdered seeds dissolved in 1dm^3 of distilled water and the concentration of the resultant extract becomes 10g/dm^3. The seeds extract was stored in a refrigerator at 8^{o}C until use.

Animals

Male albino rats (64) used in this study were obtained from Pharmacology Department, Aminu Kano Teaching Hospital (AKTH), Kano-Nigeria. The rats (4 per plastic cage) were allowed to acclimatize for three (3) days in the animal house of the Department of Pharmacology, AKTH, before the commencement of the experiment. The rats were allowed free access to normal feed and drinking water.

Design of Experiment

The rats used in this study were divided into four (4) groups of sixteen (16) rats each. Groups I, II and III were study groups while group IV served as control. Each group was sub-divided into four sub-groups of four (4) rats each. Rats in groups I, II and III were orally administered with a daily dose of 30 mg/kg body weight (0.54cm^3), 60 mg/kg body weight (1.08 cm^3) and 90 mg/kg body weight (1.62 cm^3) of aqueous extract of *C. occidentalis* L. seeds respectively for 1, 2, 3 and 4 weeks each. Group IV rats were administered orally with distilled water instead of the aqueous seeds extract. The seeds extract were administered, in the morning, once in every twenty four hours throughout the study period.

Serum Sample Collection and Analyses

Using clean test tubes, blood samples were collected from the 64 rats studied at the end of week of oral administration of aqueous extract of *C. occidentalis* seeds. The blood samples were then centrifuged and sera samples obtained were used for the analyses of electrolytes (sodium, potassium, chloride and bicarbonate) levels.

Serum sodium and potassium concentrations were determined using flame emission photometry method of Magoshes and Vallee (1956), which was based on the desolvation of solution containing these elements by a flame, leaving solid (salts), which dissociates to neutral ground state atoms. These atoms become excited in the flame, thus moving to a higher energy state and the excited atoms fall back to the ground state, light of characteristic wavelength is emitted (590 nm for sodium and 770 nm for potassium). This light then passes through a suitable filter onto a photosensitive element and the amount of current produced is measured and this is proportioned to the amount of sodium or potassium present in the original sample.

Serum chloride concentration was determined using the titration method of Schales and Schales (1941). The method is based on the precipitation of chloride ions in serum using mercuric nitrate. When chloride ion is titrated with standard solution of mercuric ion, undissociated but soluble mercuric chloride, $HgCl_2$, is formed. The excess mercuric nitrate reacts with diphenylcarbazone to produce a violet colour.

Titration method of Van Slyke and Neil (1924) was employed in determining the concentration of bicarbonate ion in sera samples. The method is based on the release of carbon dioxide from bicarbonate ion in serum with dilute hydrochloric acid. The excess acid was then titrated with sodium hydroxide using phenol red as indicator.

Statistical Analysis

Student 't' test was used to analyse the data.

RESULTS

The serum concentration of sodium, potassium, chloride and bicarbonate ions in control rats and rats orally administered with aqueous extract of *C. occidentalis* at a daily dose of 30, 60 and 90 mg/kg body weight for 1, 2, 3 and 4 weeks each, are shown in Tables 1, 2, 3 and 4 respectively. Also the variation for each electrolyte at the different weeks is shown in figures 1, 2, 3 and 4.

The concentration of serum sodium, potassium, chloride and bicarbonate ion in control rats were found to range from 130 – 155 mEq/L, 3.4 - 8.7 mEq/L, 80 – 107 mmol/L and 20 – 35 mmol/L respectively. These values were found to have lower limits slightly lower and upper limits slightly higher than the reported normal ranges of 135 – 145 mEq/L for sodium, 3.3 – 5.5 mEq/L for potassium, 95 – 105 mmol/L for chloride and 24 – 31 mmol/L for bicarbonate ions in serum (Magoshes and Vallee, 1956; Schales and Schales, 1941; Van Slyke and Neil, 1924).

Table 1: Serum sodium, potassium, chloride and bicarbonate ions concentration in rats orally administered with aqueous extract of *Cassia occidentalis* seeds for one week.

Seeds extract Dosage (mg/kg)	Serum Sodium Concentration (mEq/L)	Serum Potassium Concentration (mEq/L)	Serum Chloride Concentration (mmol/L)	Serum Bicarbonate Concentration (mmol/L)
Control	145.00 ± 7.07	5.83 ± 2.16	95.00 ± 6.27	32.50 ± 6.45
30	141.50 ± 6.24	5.20 ± 1.12	96.75 ± 11.64	32.50 ± 6.45
60	128.75* ± 8.54	7.90 ± 1.04	87.50 ± 5.57	26.25 ± 6.29
90	113.75** ± 11.09	10.20* ± 1.17	81.50* ± 7.05	15.00** ± 4.08

n = 4 in each case, All values are presented as mean ± standard deviation. Values with superscripts * and ** means significant difference at P<0.05 and P<0.01 respectively when compared to control.

Table 2: Serum sodium, potassium, chloride and bicarbonate ions concentration in rats orally administered with aqueous extract of *Cassia occidentalis* seeds for two weeks.

Seeds extract Dosage (mg/kg)	Serum Sodium Concentration (mEq/L)	Serum Potassium Concentration (mEq/L)	Serum Chloride Concentration (mmol/L)	Serum Bicarbonate Concentration (mmol/L)
Control	137.50 ± 6.45	5.75 ± 1.02	95.00 ± 12.36	26.25 ± 4.79
30	145.50 ± 11.45	5.60 ± 1.36	95.00 ± 8.49	27.50 ± 6.45
60	126.25* ± 6.29	7.98** ± 0.45	90.00 ± 6.22	22.50 ± 6.45
90	105.00* ± 16.83	11.45** ± 0.59	90.00 ± 3.46	20.00* ± 4.08

n = 4 in each case, All values are presented as mean ± standard deviation. Values with superscripts * and ** means significant difference at P<0.05 and P<0.01 respectively when compared to control.

Table 3: Serum sodium, potassium, chloride and bicarbonate ions concentration in rats orally administered with aqueous extract of *Cassia occidentalis* seeds for three weeks.

Seeds extract Dosage (mg/kg)	Serum Sodium Concentration (mEq/L)	Serum Potassium Concentration (mEq/L)	Serum Chloride Concentration (mmol/L)	Serum Bicarbonate Concentration (mmol/L)
Control	147.50 ± 6.45	4.23 ± 0.84	92.75 ± 6.75	28.75 ± 4.79
30	143.75 ± 4.79	7.05** ± 0.89	96.75 ± 11.64	28.75 ± 8.54
60	127.50* ± 10.41	9.18** ± 0.67	77.50** ± 3.22	22.50 ± 6.29
90	111.25* ± 20.16	10.13** ± 1.04	85.00 ± 12.03	18.75* ± 8.54

n = 4 in each case, All values are presented as mean ± standard deviation. Values with superscripts * and ** means significant difference at P<0.05 and P<0.01 respectively when compared to control.

Table 4: Serum sodium, potassium, chloride and bicarbonate ions concentration in rats orally administered with aqueous extract of *Cassia occidentalis* seeds for four weeks.

Seeds extract Dosage (mg/kg)	Serum Sodium Concentration (mEq/L)	Serum Potassium Concentration (mEq/L)	Serum Chloride Concentration (mmol/L)	Serum Bicarbonate Concentration (mmol/L)
Control	140.00 ± 10.80	5.80 ± 0.85	96.50 ± 6.03	30.00 ± 4.08
30	137.50 ± 6.45	6.50* ± 2.04	105.00 ± 4.40	30.00 ± 4.08
60	115.00* ± 12.91	9.90** ± 0.91	86.50* ± 4.73	21.25 ± 4.79
90	117.50* ± 6.45	11.73** ± 1.32	71.75** ± 6.18	12.50* ± 8.66

n = 4 in each case, All values are presented as mean ± standard deviation. Values with superscripts * and ** means significant difference at P<0.05 and P<0.01 respectively when compared to control.

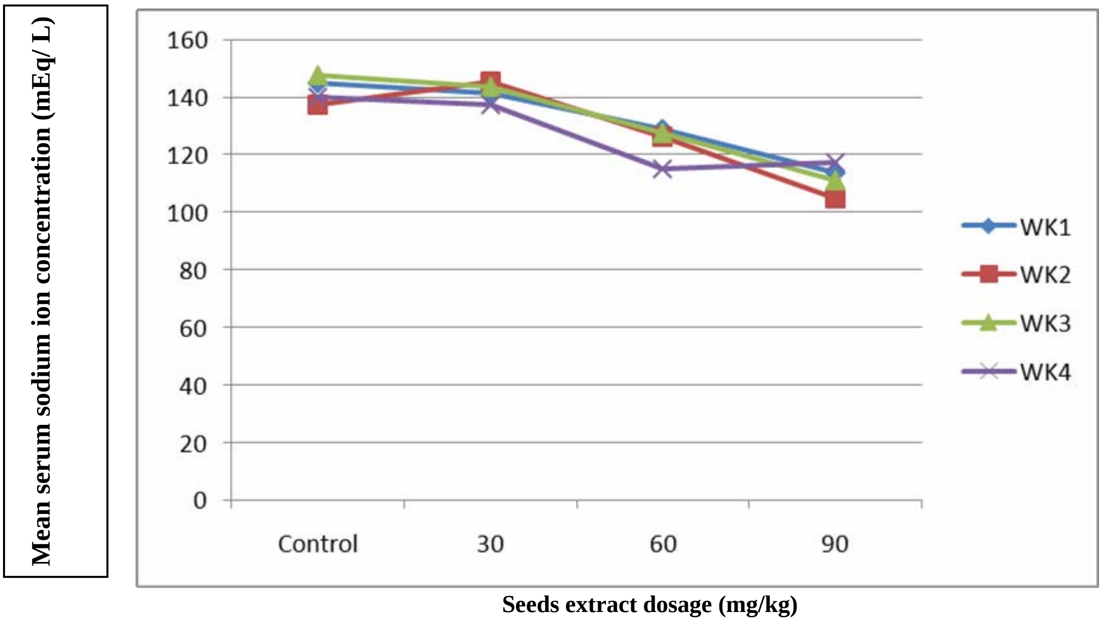

Figure 1: Mean serum sodium ion variation at different weeks of *C. occidentalis* L. aqueous seeds extract oral administration.

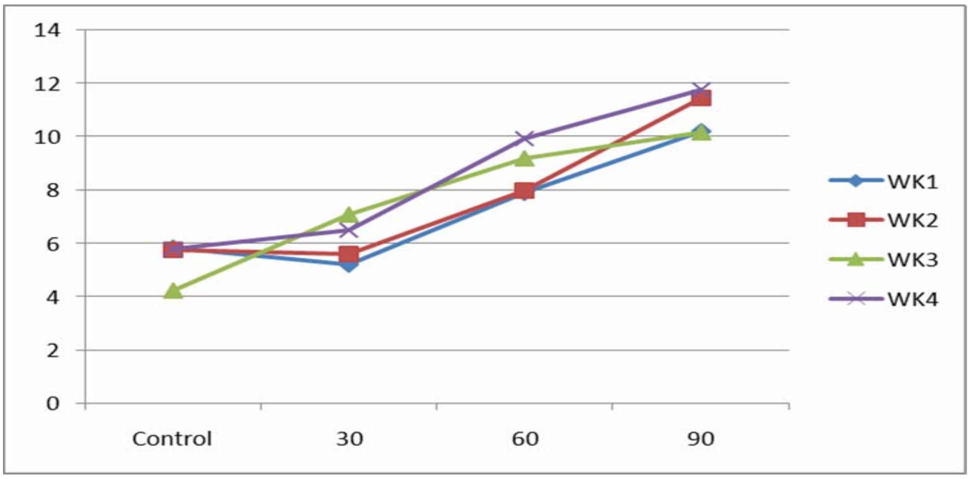

Figure 2: Mean serum potassium ion variation at different weeks of *C. occidentalis* L. aqueous seeds extract oral administration.

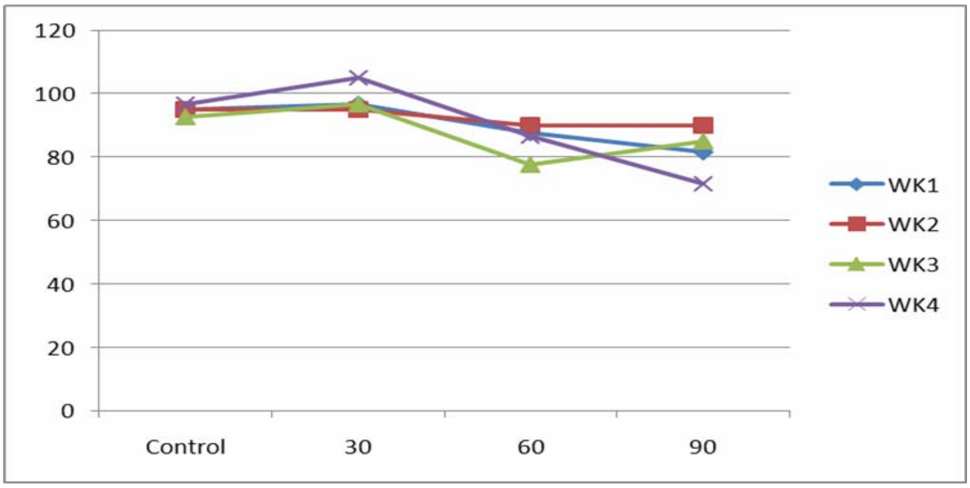

Figure 3: Mean serum chloride ion variation at different weeks of *C. occidentalis* L. aqueous seeds extract oral administration.

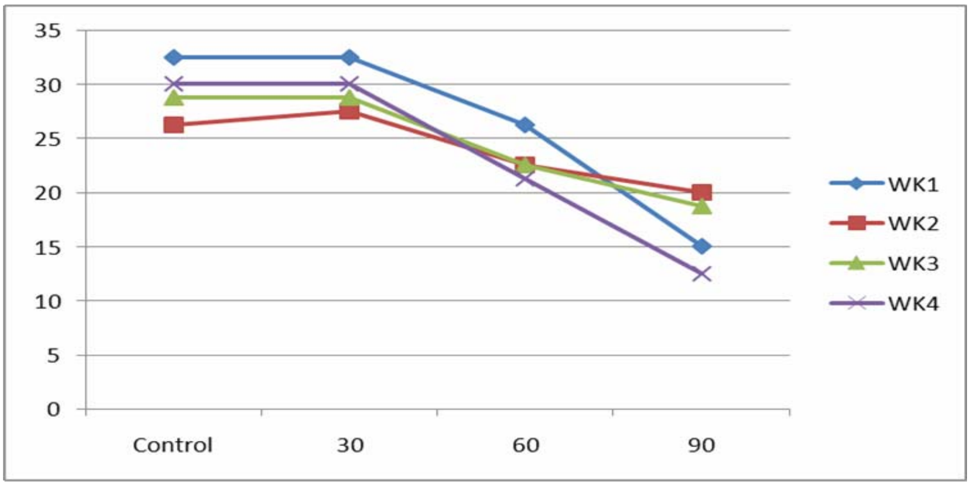

Figure 4: Mean serum bicarbonate ion variation at different weeks of *C. occidentalis* L. aqueous seeds extract oral administration.

DISCUSSION

The results obtained in rats orally administered with aqueous extract of *C. occidentalis* seeds at a daily dose of 30 mg/kg for up to two weeks showed no significant alterations in serum sodium, potassium, chloride and bicarbonate levels when compared with the control. However, oral administration of the extract at a daily dose of 30 mg/kg for $\geq$ three weeks caused significant increase ($P<0.01$) in serum potassium concentration compared with control. This result indicates that oral administration of *C. occidentalis* seeds at a daily dose of 30 mg/kg for $\geq$ three weeks could have hyperkalaemic effect. Hyperkalaemia, or excess potassium in the blood, occurs in cases of renal failure because the kidneys loose the ability to excrete the mineral. Severe dehydration will also produce hyperkalaemia. The consequences of this condition are muscle weakness and cardiac arhythmias that lead to heart failure (Kruetler, 1980).

Oral administration of aqueous extract of *C. occidentalis* seeds at a daily dose of 60 mg/kg for one week showed no significant changes in serum potassium, chloride and bicarbonate levels with control. However, there was significant decrease ($P<0.05$) in serum sodium levels. When the duration of administration was $\geq$ two weeks, significant increase ($P<0.01$) in serum potassium as well a significant decrease ($P<0.01$) in serum chloride concentration accompanied the significant decrease in serum sodium concentration. These results indicate that oral administration of aqueous extract of *C. occidentalis* at a daily dose of 60 mg/kg for $\geq$ two week could lead to hyperkalaemia as well as hyponatremia. This could lead to alteration of the osmotic pressure of body fluid. This is due to the fact that abnormal concentration of sodium and/or potassium in serum can affect the osmotic pressure of the body fluid which is related to blood pressure (Healy, 1995; Cheesbrough, 2002). The relationship between sodium and hypertension is based on the capacity of this mineral to "attract" water. When the serum sodium level increase, the body retains water, thereby decreasing the serum sodium concentration. Blood pressure, which depends in part on blood volume, increases as retained water increases. Reverse is the case when the serum sodium level decreases. In this way, serum levels of sodium can affect blood pressure. However, sodium is not the sole cause of hypertension as hypertension appears to be multifactorial in origin (Kruetler, 1980).

Oral administration of aqueous extract of *C. occidentalis* seeds at a daily dose of 90mg/kg for $\geq$ one week was found to cause significant decrease ($P<0.05$) in serum sodium, chloride and bicarbonate levels as well as significant increase ($P<0.05$) in serum potassium level compared with control. Therefore, daily dosage of both 90 mg/kg and 60 mg/kg body weight for 1 week causes significant decrease ($P<0.05$) in level of sodium. However, oral administration of the aqueous extract of *C. occidentalis* seeds at daily dose of 90 mg/kg for $\geq$ one week led to significant alteration/decrease ($P<0.05$) in serum chloride and bicarbonate levels. Therefore, oral administration of seeds aqueous extract of *C. occidentalis* seeds at a daily dose of 90mg/kg for $\geq$ one week could lead to disturbances in acid - base balance. Also figures 1, 2, 3 and 4 showed that the changes in serum electrolytes concentration in study rats is both seeds extract concentration and duration of administration dependent.

CONCLUSION

The results of this study (Table) indicate that aqueous extract of *C. occidentalis* seeds could have hyperkalaemic as well as hyponatremic effect when administered orally at a daily dose of 60 mg/kg for $\geq$ one week. This could be the possible way of affecting the tonicity of body fluid, hence affecting blood pressure. The seeds extract may also cause disturbance in acid – base balance when the dose is 90 mg/kg body weight. However, studies have shown that at a dose of 60 mg/kg, *C. occidentalis* seeds extract may cause liver and/or kidney dysfunction (Abubakar and Sule, 2008 a and b).

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