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RESEARCH PAPER

EFFECTS OF DIETARY *XYLOPIA AETHIOPICA* ON SERUM ELECTROLYTES AND TRACE ELEMENTS IN MALE WISTAR RATS

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ABSTRACT

The effect of dietary intake of various doses of powdered whole fruits of *Xylopia aethiopica* on some selected serum electrolytes (Na^+, K^+, Cl^-, HCO_3^-, Mg^{2+}, Ca^{2+} and PO_4^{2-}) and trace elements (Cu^{2+}, Zn^{2+} and Mn^{2+}) were investigated in male Wistar rats (weighing 120-150g body weight). The control rats received normal rat feed, while test animals were fed with three graded diet doses that contained *Xylopia aethiopica* at 1.5% w/w, 2.5% w/w and 5% w/w. Both control and test animals received their food, plus drinking water, *ad libitum* for six weeks. At the end of the treatment, blood was then obtained from all the animals and used for the various analyses of the serum electrolytes and trace elements here in reported. The results showed significant ($p<0.05$) dose-dependent increases in mean serum concentrations of Ca^{2+}, HCO_3^-, PO_4^{2-}, Cu^{2+}, Zn^{2+},and Mn^{2+}. These alterations point to the positive effect of the spice on blood electrolyte homeostasis and its trace element ions content. It is speculated that these treatment-induced changes in serum electrolytes and trace elements play important roles in the documented antioxidant, anti-inflammatory, antihypertensive, spasmodic and antifertility activities of the fruits of *Xylopia aethiopica*.

Key words: Xylopia aethiopica, fruits, electrolytes, trace elements

INTRODUCTION

The dried fruits of *Xylopia aethiopica* is a common spice known for its strong aromatic quality, and used in the preparation of two special local soups in south-eastern Nigeria, called “isi ewu” and “obe nta”. *Xylopia aethiopica* is also used as a postpartum tonic in alleviating after–birth wounds and as lactation aid (Murray, 1995). Others uses include for the termination of unwanted pregnancy due to its abortifacient properties when administered in combination with the root of *Blighia sapida* (Sapindaceae) (Muanya, 2008), to increase menstrual flow when administered in combination with leaves of *Newbouldia laevis* (Bignoniaceae), or chieftaincy leaf, as well as, for the induction of labour to achieve delivery in southern Africa, when seven dried fruits of *Xylopia aethiopica* and 21 leaves of *Rouwulfia vomitaria* are orally administered in combination.

However, several researchers have reported that the intake of *Xylopia aethiopica* causes diminished reproductive performance in experimental animals, as regards sperm count reduction in males (Nwafor, 2013). Also, histological studies of the rat gonads following the intake of *Xylopia aethiopica*, have demonstrated changes in the testicular and ovarian architecture in male and female rats respectively, as well as litter size reduction in female rats (Onyebuagu, 2012).

This wide range of effects by *Xylopia aethiopica* is hypothesized to be mediated by biochemical events that involve signaling pathways which require electrolytes and trace elements. For example, the increase in gastrointestinal tract smooth muscle activity by *Xylopia aethiopica* in experimental animals (Nwafor and Kalio, 2006) is mediated via transmembrane extracellular fluid (ECF) calcium ion influx into the myofibrils to engender muscle contraction. Somova *et al*, (2001) also reported that *Xylopia aethiopica* demonstrated diuretic and natriuretic effects which were similar to that of chlorothiazide, thus, suggesting the inhibition of Na^+ and K^+ ion reabsorption in the renal tubules of the kidneys. The effect of electrolyte concentration on oocyte development was studied by Hisataka *et al*, (2004) and they observed that supplemental magnesium ions increase blastulation rate, follicular size and oocyte maturation in experimental animals. Linder (1991) noted that the main sources of body electrolytes and trace metals are from the diet via intestinal absorption.

The roles and importance of electrolytes and trace metals in man cannot be over-emphasized, as their imbalance or deficiency has been implicated in many disease etiologies, and impairment of body functions. For example, hypokalemia (low blood K^+ level) interferes with electrical events in the heart, since action potential and heart muscle contraction depend on membrane potential that is sensitive to plasma potassium ion concentration (Guyton and Hall, 2003; Fox, 1991). Trace elements are stored (accumulate) in the organs – kidneys, liver, pancreas and gonads (McCormick and Cunningham, 2003), where they mediate cellular functions. For example, depletion of zinc in the testes has been implicated in testicular atrophy, while age and decline in testicular activity are correlated with increased zinc in the prostate and decreased zinc in the testes (Oldereid et al, 2005).

On the other hand, Zinc is a component of the antioxidant called copper-zinc superoxide desmutase (CuZnSOD), and deficiency of zinc could lead to increase in tissue oxidative damage (Disilvestro and Blotein-Fuji, 1997). The antioxidant activity of *Xylopia aethiopica*, by virtue of its flavonoid content (Odukoya et al, 2005) and the high concentration of mono-unsaturated fatty acids which inhibit oxidation of LDL-cholesterol (Ezekwesili et al, 2010) have been reported. However, the underlying mechanisms behind most of the biologic actions of this spice are yet to be fully elucidated. The objective of this study therefore, is to determine the effect of dietary intake of the fruits of *Xylopia aethiopica* on some selected serum electrolytes and trace elements in Wistar rats in order to shed more light on its mode of action.

MATERIALS AND METHODS

Preparation of Animals: Young virgin male Wistar rats, weighing 120-150g body weight were used in this study. The rats were bought from the Animal House Unit of Department of Pharmacology, Niger Delta University, Bayelsa State, Nigeria, and housed in the research laboratory of the Department of Physiology, Niger Delta University.

The rats were randomly divided into four groups of five rats per group and housed in standard rat cages for two weeks to acclimatize, prior to the study. They were maintained under standard husbandry conditions of light (13 hours) and darkness (11 hours), room temperature of $28^oC \pm 2^oC$, and fed with standard rat chow (Pfizer Feeds, Aba, Nigeria) and tap water *ad libitum,* during the acclimatization period.

Preparation of Plant Material: The whole dried fruits of *Xylopia aethiopica* were purchased from the local market in Ekpoma, Edo State, Nigeria, washed in clean water and air-dried for 10 hours, prior to drying in the oven at 40^oC for 12 hours. The dried fruits were then ground into powdered form. This was achieved by first pounding the whole dried fruits into small pieces using wooden mortar and pestle, and then grinding the pieces into powdered form using mechanical grinding machine.

The desired amount of the powdered *Xylopia aethiopica* was measured using a sensitive digital weighing balance (Zohaus, Model CS 200, N.J. USA). The eventual prepared diet dose also contained cooked cassava starch as binder, and produced into dried crumbs, since Wistar rats prefer to hold their food with both hands to eat, while crouching on their hind limbs.

Treatment groups: Two categories of treatment diets were made available for use in this study. The control diet that contained normal rat chow and edible cassava starch only, and three treatment diets that contained the powdered *Xylopia aethiopica* at levels of 1.5% w/w, 2.5% w/w and 5% w/w, with cassava starch as binder (and part of the feed).

Duration of treatment: The rats were fed with the respective diet doses and tap water, *ad libitum*, for six weeks.

Sample collection and blood handling: At the end of the dietary treatment period, the rats were anesthetized in a chloroform chamber, and blood was obtained via cardiac puncture. Blood taken from each animal was put in pre-labeled non-heparinized sample tubes, and then placed in iced water for three hours. The blood samples were then centrifuged at 7000 rpm for 10 minutes. The serum was then taken out and put into freshly labeled sample tubes and stored at 15^oC until ready for use in determination of serum electrolytes and trace elements.

Sample analyses: The serum concentrations of Zn, Cu and Mn were determined as described by (Roeschlau *et al*., 1974) as follows: An aliquot of serum was predigested with a mixture of concentrated HNO_3, followed by further digestion with a mixture (10:3) of concentrated HNO_3 and perchloric acid ($HClO_4$). The acid samples were covered with a watch glass and heated in 50ml pyrex beaker on electric hot plate till all traces of HClO4 were eliminated – indicated by cessation of white fumes in the beaker. The remaining liquid in the beaker (about 2.5ml) was made up to 25ml with dionized water and then assayed for Zn, Cu, and Mn, using an atomic absorption spectrophotometer (Javrel – Ash Model 82-362). The serum concentrations of cations were estimated by the use of flame photometry, while the serum anion concentrations were estimated by titration methods (Tietz, 1995).

Statistical analyses: Data were presented as means ± SD. Data of test groups and control groups were analyzed statistically using Student's t-test. The level of significance of the difference between test groups and control data were determined and $p<0.05$ were considered to imply statistical significance.

RESULTS

The dietary intake of the varying doses of *Xylopia aethiopica* by the male Wistar rats produced various changes in the serum cation, anion and trace element concentrations in the animals. The results on the serum cation concentrations showed significant dose-dependent increased in the mean level of Ca^{2+} in the rats, compared to the corresponding control value. The mean serum concentrations of Na^+, K^+ and Mg^{2+} were not significantly different from the control values (see table 1).

The serum anion concentrations showed dose-dependent significant increases in the mean levels of HCO_3 and PO_4^{2-} in the treated rats, compared to the controls (see table 2).

The results of the trace element analyses demonstrated that the *Xylopia aethiopica* treatment diet caused dose-dependent increases in the mean serum concentrations of Zn, Cu, and Mn in the rats, and the increases were significant ($p<0.05$) at the treatment doses of 2.5% w/w and above (see table 3).

Table 1: Mean serum cation concentrations in male Wistar rats following dietary intake of doses of *Xylopia aethiopica*

Dietary Doses	Serum Cation Concentrations (mEq/L)			
	Na^+	**K^+**	**Ca^{2+}**	**Mg^{2+}**
Group I (Control)	138.01±2.04	2.66±0.30	4.80±0.36	2.60±0.82
Group II (1.5% w/w)	137.41±1.88	2.81±0.26	5.00±1.18	3.80±0.38
Group III (2.5% w/w)	137.66±2.41	2.88±0.22	5.80±0.41*	3.71±0.77
Group IV (5% w/w)	138.28±1.48	2.97±0.28	6.20±0.30*	3.24±1.08

Data presented as Means± SD; ***significantly different compared to control.

Table 2: Mean serum anion concentration in male Wistar rats following dietary intake of doses of *Xylopia aethiopica*

Dietary Doses	Serum Anion Concentrations (mEq/L)		
	Cl^-	**HCO_3^-**	**PO_4^-**
Group I (Control)	98.22±2.80	23.81±0.40	1.82±0.60
Group II (1.5% w/w)	97.60±1.70	24.70±0.60	2.40±0.24
Group III (2.5% w/w)	95.60±1.70	28.80±0.07*	3.11±0.77*
Group IV (5% w/w)	95.20±1.60	29.20±0.40*	3.62±0.82*

Data presented as Means± SD; ***significantly different compared to control.

Table 3: Mean serum concentration of trace elements in male Wistar rats following dietary intake of doses of *Xylopia aethiopica*

Dietary Doses	Serum Anion Trace elements (mEq/L)		
	Zn^{2-}	Cu^{2-}	Mn^{2-}
Group I (Control)	2.28±0.56	12.21±2.60	0.39±0.66
Group II (1.5% w/w)	3.61±0.47	15.83±3.10	0.65±0.11
Group III (2.5% w/w)	4.26±0.66*	26.58±4.20*	0.92±0.02*
Group IV (5% w/w)	7.94±1.28*	48.62±3.60*	1.38±0.68*

Data presented as Means± SD; ***significantly different, compared to control.

DISCUSSION

The dietary intake of whole fruits of *Xylopia aethiopica* by male Wistar rats for six weeks caused marked alterations in the serum levels of several electrolytes and trace elements. There were dose-dependent increases in the serum concentrations of Ca^{2+}, $HCO3^{-}$, $PO4^{2-}$, Cu, Zn, and Mn, while the serum levels of Na^{+}, K^{+}, Cl^{-} and Mg^{2+} were not significantly altered. The observed changes in serum concentrations of the electrolytes and trace elements have important implications with respect to the uses of *Xylopia aethiopica* for medicinal and dietary purposes. The dose-dependent increase in the serum level of Ca^{2+} in response to dietary intake of *Xylopia aethiopica* is noteworthy, in view of the role of calcium ions in smooth muscle activities. The spontaneous action potential and muscle contraction in uterine smooth muscle are dependent on ECF Ca^{2+} concentration, since ECF (serum) ionized Ca^{2+} is the main source of calcium for smooth muscle contraction. The treatment-induced increase in Ca^{2+} level observed in this study could explain the increase in uterine smooth muscle activity *in vitro* in albino rats exposed to *Xylopia aethiopica* (Nwafor, 2013), and the folkloric uses of *Xylopia aethiopica* as postpartum tonic, and to terminate unwanted pregnancy (Muanya, 2008). However, Mg^{2+} tend to block these responses by competing with calcium for the same membrane channels, thus, checking the possible pressor effect of the elevated Ca^{2+} level (Ebeigbe and Aloamaka, 1987; Bethlet and Wester, 1983). Moreover, dietary calcium intake have been reported to possess membrane stabilization property – by having direct effect on membrane Na^{+} and K^{+} permeability (Furspan et al, 1989), thus with a potential to checking the pressor effect the Ca^{2+}. At the doses used in this study, there were no significant changes in the serum levels of Mg^{2+}, Na^{+} and K^{+} ions, on which membrane potential depends, and thus, the risk of spontaneous smooth muscle activity due to changes in resting membrane potential may be remote, in spite of the elevated serum Ca^{2+} ion level.

The observed lack of significant changes in the serum levels of Na^{+}, K^{+}, and Mg^{2+} in this study could also represent the absence of renal toxicity in the treated rats, which could have caused increase in serum levels of these electrolytes due to failure of the affected renal tubules to excrete excess Na^{+}, K^{+} and Mg^{2+} in urine (Ezeilo, 2009). It is also possible that the lack of significant changes in the above cations in this study could be due to the documented diuretic activity of *Xylopia aethiopica* (Somova et al, 2001), by which these electrolytes which are regulated by renal excretion were not retained in the treatment-induced diuresis under normal kidney function. Furthermore, retention of Na^{+} may also have been prevented by the natriuretic effect of increase in serum Ca^{2+} caused by intake of *Xylopia aethiopica*. Dietary Ca-loading has been reported to cause decrease in blood pressure via natriuresis (Zemel et al, 1988; Saito et al, 1989). The increase in serum Ca^{2+} level also signifies that the reabsorptive capacity of the kidneys for calcium, mediated via the production of vitamin D_3 which enhances calcium reabsorption by the kidneys (Ezeilo, 2009) and thus, the integrity of the kidneys, was not compromised by the dietary *Xylopia aethiopica* treatment.

The significant increase in the mean serum HCO_3^{-} concentration at the 5% w/w dosage is noteworthy, and may represent some form of metabolic alkalosis. However, HCO_3^{-} regulation appears to be associated with Cl^{-} regulation in an inverse relationship. Hence, the slight decrease in serum Cl^{-} level as the serum HCO_3^{-} level increases in this study may represent a physiological response to ensure that the total anionic concentration equals the total cationic concentration in the ECF in order to maintain ECF ionic homeostasis (Ezeilo, 2009). The relatively slight change in serum Cl^{-} concentration observed in this study appears to be associated to the equally slight change in serum Na^{+} concentration, since their transport and regulation operate in consonance. Na^{+} transport is usually accompanied by Cl^{-} transport, and increase in one is balanced by increase in the other to ensure ECF ionic balance.

The significant increase in serum concentration of PO_4^{2-} at 2.5% w/w dosage and above matches the increase in serum Ca^{2+} concentration, and this may be related to the metabolism of both ions in the body, since body calcium and phosphate ions are regulated by the same calcium-regulating hormone, parathormone (PTH), and both ions play complementary roles in bone metabolism (Guyton and Hall, 2003; Fox, 2001).

The dose-dependent increase in the serum concentrations of Zn, Cu and Mn caused by dietary *Xylopia aethiopica* may be due to the high content of these elements in the fruits (Smith, 1996; Nwafor, 2013), and these elements may also have conferred on the spice its natural antioxidant activity (Odukoya et al, 2005; Nwafor, 2013). This assertion is supported by the report by Ezekwesili et al, (2010) who observed that methanolic extract of *Xylopia aethiopica* fruits stabilized erythrocyte membranes via actions against lipid peroxidation and free radical activity of red blood cell membranes – through promoting active ion (as opposed to osmosis) transport across the membrane, or possibly raising the oncotic pressure of the ECF. The documented high content of Cu, Zn and Mn (which are components of the natural antioxidants called Copper-zinc superoxide desmutase, and manganese superoxide desmutase) in *Xylopia aethiopica* are implicated in protection against oxidative damage to tissues, free radical activity and lipid peroxidation of cell membranes (Disilvestro and Blotein-Fuji, 1997).

Conclusion

Dietary intake of fruits of *Xylopia aethiopica* at 2.5% and above caused dose-dependent increases in serum concentrations of Ca^{2+}, HCO_3^-, PO_4^{2-}, Cu, Zn, and Mn, and these alterations did not appear to have adverse effect on electrolyte homeostasis in the treated Wistar rats. These treatment-induced changes in serum electrolytes may play important roles in the documented antioxidant, anti-inflammatory, antihypertensive and spasmodic activities of the fruits of *Xylopia aethiopica*.

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AUTHOR(S) CONTRIBUTION

All three authors (Onyebuagu, P.C., Pughikumo, D.T., and Erigbali, P.) participated actively in this study. Onyebuagu, P.C. was in charge of the experimental design, processes and other experimental aspect of the research work. Pughikumo, D.T. assisted in the in the interpretation/discussion of relevant clinically related observations of the study. Erigbali, P. assisted in the analysis and discussion aspects of the study.