



EVALUATION OF THE EFFECT OF *XYLOPIA AETHIOPICA* ON RENAL FUNCTION INDICES OF RATS.

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ABSTRACT

The effects of ethanolic, aqueous and Dimethyl Sulphur oxide extracts of *Xylopiya aethiopicica* on the renal function of Rabbits was studied. *Xylopiya aethiopicica* (African guinea pepper) is used mainly as spices, postpartum tonic to induce placental discharge at postpartum by traditional birth attendants (TBA). A total of thirty (30) Rabbits were recruited for the study. The Rabbits were divided into four (4) experimental groups and the control group. Group 1 comprised of six Rabbits weighing averagely 1.4kg, each was given 1ml of Dimethyl sulphur oxide extract of *X. aethiopicica* daily. Group 2 had a mean weight of 1.3kg and received 1.63ml of cold water extract each daily. Group 3 received same volume as group 2 but hot water extract of *X. aethiopicica*. Group 4 received 1.75ml each of ethanolic extract of the plant seed. The control group received 1m each of distilled water once daily for a period of 30 days. The results showed a significant increase ($p < 0.05$) in the levels of chloride ion in all the methods of extracts when compared with the control. Potassium ion levels were significantly increased in in both the Dimethyl Sulphur oxide and cold water extracts of *X. aethiopicica*. There was also a significant increase in creatinine values for the cold water extract when compared with the control group and sodium and bicarbonate levels were also increased significantly for the hot water and ethanolic extracts respectively. In this study, it can be deduced that *Xylopiya aethiopicica* caused a significant increase in the level of creatinine in Rabbits treated with cold water extract, indirectly affecting the renal function. It is recommended therefore, that further studies should be advocated to ascertain and to provide reasonable estimates of the human risk associated with the consumption of *Xylopiya aethiopicica* in hot water extract as is used to in this part of the globe.

KEYWORDS: *Xylopiya aethiopicica*, renal function, electrolyte, postpartum tonic.

INTRODUCTION

In Africa especially south of the Sahara, the use of plants and its extract for the treatment and management of diseases has been in existence since ancient times (Woode *et al.*, 2011). Traditional medicine accounts for about 80% of the health needs of the rural populace in most regions of Africa, *Xylopiya aethiopicica* (*X. aethiopicica*) is one of the medicinal plants, whose parts are of high medicinal value in many countries of Africa (Eze, 2012). *X. aethiopicica*, a West African “pepper tree”, with straight stem and smooth bark, remains ever green with a consistent aroma (Nnodim, *et al.*, 2011). It is a slim, tall tree of about 60–70 cm in diameter that can reach up to 15– 30 m tall, with a straight stem and a slightly stripped or smooth bark. The fruits are rather small and look like twisted bean-pods. When dry, the fruit turn dark brown, cylindrical, 2.5 to 5 cm long and 4 to 6 mm thick. The contours of the seeds are visible from outside. Each pod contains 5 to 8 kidney-shaped seeds of approximately 5 mm in length. The hull is aromatic, but

not the seeds (Dan *et al.*, 2004). It is commonly known as “African guinea pepper” or “Ethiopian pepper”. “Uda” as it is called in South Eastern Nigeria, is wide spread in tropical Africa, Zambia, Mozambique and Angola (Eze, 2012). The prolonged usage of these herbal products without proper monitoring of the usage had brought about a number of health related problems (Leke, 2008). In Nigeria, it is found all over the lowland rain forest and most fringe forest in the Savanna zones of Nigeria. Negro pepper as it is also known has been used as a pepper substitute in Europe and India (Eze, 2012). *X. aethiopicica* is widely distributed in the West African rainforest from Senegal to Sudan in Eastern Africa, and down to Angola in Southern Africa (Irvine, 1961; Burkhill, 1985).

The essential oil has been well characterized with linalool, β -trans-ocimene, α -farnesene, α -pinene, β -pinene, myrtenol, β -phellandrene, and 3-ethylphenol as the major volatile constituents (Tairu *et al.*, 1999).

Researchers describe that the intense 'peppery note' of the oil of the fruit largely comes from linalool and provides that characteristic aroma of the ground, dried, smoked fruits of *X. aethiopica*. The essential oil yield varies from 2.0% to 4.5%. The essential oils of the stem bark (0.85%) and the leaves (0.5%) of *X. aromatica* have also been investigated. The bark oil consists mainly of α -pinene, trans-pinocarveol, verbenone and myrtenol and differs significantly from that of the leaf oil (spathulenol, cryptone, beta-caryophyllene and limonene). (Dan *et al.*, 2004).



Figure 1. The fruits from *Xylopia aethiopica* still attached to the tree (left), the sun dried fruit (middle) and *X. aethiopica* leaves (right).

Plants produce many chemical compounds, which have potential value in the treatment of diseases, but a number of them could also be poisonous. Chemical compounds with beneficial effects have been isolated and biologically assayed to establish their medicinal activity. Modern drugs used in orthodox medicine have also been sourced from plants (Sofowora, 2001). *X. aethiopica* is a medicinal plant of great repute in West Africa and contains a variety of complex chemical compounds (Adegoke *et al.*, 2003). Almost every part of the plant is used in traditional medicine for managing various ailments including skin infections, candidiasis, dyspepsia, cough and fever (Irvine, 1961; Burkhill, 1985; Mishana *et al.*, 2000). A major advantage of using *X. aethiopica* as a food preservative is that foods preserved by this spice may qualify as a functional food since it has many health benefits such as anti-tumour, anti-asthmatic, anti-inflammatory, antimicrobial (Okigbo *et al.*, 2005; White, 2006; Okigbo and Igwe, 2007), hypotensive and coronary vasodilatory effects (Fleischer, 2003). The seeds contain bitter principles, alkaloids, glycosides, saponins, tannins, sterols, carbohydrate, protein and free fatty acid, mucilage's and acidic compounds some of which might be responsible for the reported uses (Woode *et al.*, 2011).

The microbiological activity of *X. aethiopica* essential oil against *Escherichia coli*, *Staphylococcus aureus* or *Aspergillus flavus*, among other microorganisms, has been well established (Tatsadjieu *et al.*, 2003; Asekun, 2004; Konnings *et al.*, 2004). It is also noticed that the features of the ether extract of *X. aethiopica* are favorable to its incorporation in the resins used for the manufacture of the paintings (Ajiwe *et al.*, 1998).

Fruit decoction of *X. aethiopica* is used to treat bronchitis, asthma, infertility, arthritis and rheumatism and as postpartum tonic. Traditional medicine practitioners and traditional birth attendants (TBA) also use decoction of the seeds to induce placental discharge postpartum and according to Burkhill (Burkhill, 1985), because of its traditional usefulness after child birth, it was employed in government hospitals in Ghana and was deemed to have abortifacient properties. The use of medicinal plants in traditional medicine have also generated a lot of interest and concern about their efficacy and safety margin, since 65-70% of the Nigerian population patronize traditional medicine practitioners in their various forms and methods (Bubayero, 1998; Sofowora, 2001). The plant contains high amount of copper, manganese and zinc. Key constituents are diterpenic and xylopic acids, and these within the fruits extracts show activity as an antimicrobial against Gram positive and negative bacteria. However, it has not been shown to be effective against *E. coli* (Batschauer de Borba *et al.*, 2005).

The plant is therefore considered to be a useful herb preventing and/ or ameliorating certain diseases such as asthma, bronchitis, neuralgia, rheumatism, amenorrhoea in females (Burkhill, 1985) and has also been reported to have antimicrobial effect (Karioti *et al.*, 2004).

The effects of *X. aethiopica* extract on some biochemical and haematological parameters have been studied. Chrissi *et al.* reported that the extract caused a significant increase in the level of haemoglobin, total white blood cell count (TWBCC) and Neutrophil count in the treated animals. Increase in these parameters could be due to a direct effect of the extract on haemopoietic activity in the experimental animals. It however did not affect red blood cell count (RBCC), and hematocrit (HCT). The extract also cause a significant increase in serum total protein, Albumin, Globulin, high density lipoprotein (HDL) and total Cholesterol levels as well as indirect and total bilirubin dose dependently while decreasing serum ALT. It did not however have a significant effect on Renal function test (urea and creatinine). The present findings may be responsible for the usefulness of *X. aethiopica* fruit in our local setting as an immune booster and postpartum tonic. (Chrissi *et al.*, 2011).

The extract also induced a significant increase in total protein, albumin and globulin in the treated animals compared to the control group. Albumin binds and transports metal ions, bilirubin, and drugs. Its levels is used to assess the synthetic function of the liver. Significant increase in the levels of these parameters is an indication that the extract had stimulated its synthesis in the liver. Serum protein levels are regulated via synthesis in the liver and its levels thus reflect the synthetic ability of the liver (Rothschil *et al.*, 1972). The extract also causes a significant reduction in the level of serum ALT but did not affect the levels of AST, GGT and alkaline Phosphatase. It is known that an increase in

the enzymatic activity of ALT, AST and ALP in the serum directly reflects hepatocellular damage (Benjamin *et al.*, 1978). Results of the enzyme analysis therefore suggest that extract of *X. aethiopica* has no hepatotoxicity with reference to the doses used in the study. This finding is similar to the result of Taiwo and colleagues (Taiwo *et al.*, 2009). There was no significant change in the serum direct bilirubin levels. However there was a significant increase in the serum levels of total and indirect bilirubin in the treated rats compared to the control animals.

MATERIALS AND METHODS

Materials

Rabbits, *X. aethiopica*, ethanol, hot and cold water, laboratory miler, weighing balance, beakers, measuring cylinders, automatic pipettes, glass pipettes, test tubes, test tube racks, sodium heparin bottles, plain bottles and spatula.

Animals

Thirty (30) Rabbits weighing averagely 1.3kg were used in the study. The animals were allowed to acclimatize to the laboratory condition (temperature 24-28°C and 12 hour light-dark cycle) at the Animal House, Madonna University Elele campus, Rivers State for two week before commencement of the experiment with free access to solid pellet diet (bought from Guinea feed Nigeria PLC) and water throughout the study. All animals were handled according to international guidelines for handling experimental animals (APS, 2002).

Plant material

Dried fruits of *X. aethiopica* were bought from Relief Market Owerri Imo State, Nigeria and identified by Chief Pharm F. N. Osuala, Head of Department, Pharmacognosy Department, Faculty of Pharmacy Dr. Abraham Mensah, Department of Pharmacognosy, Madonna University Elele campus, Rivers State Nigeria.

Preparation of extract

The dried *X. aethiopica* was grinded into fine powder using laboratory miler. After weighing the powder, the extracts were prepared using cold maceration method thus:

Ethanol Extraction

251g of the powder was dissolved in 1255ml of ethanol and left covered for 48hours. The mixture was sieved to get a homogenous extract free from debris and put in oven to evaporate the ethanol leaving the fruit extract which is in a semi solid form dissolved out with dimethyl sulphur oxide.

Aqueous Extract

Cold Water Extraction

251g of the powder was dissolved in 1255ml of cold water and left covered for 48hours. The mixture was sieved to get the filtrate extract. The aqueous solution is allowed to evaporate to get a gel-like filtrate weighing 14.9g dissolved in 37.25ml of water.

Hot Water Extraction

251g of the powder was dissolved in 1255ml of boiling hot water for 5minutes and then allowed to cool which the mixture was sieved to get filtrate extract. The aqueous solution is allowed to evaporate to get a gel-like filtrate weighing 30.3g dissolved in 75.75ml of water. The ED50 was found to be 500mg/kg of the extract.

Experimental Design

The laboratory animals were grouped into four groups of 6 each (with group 5 being the control) and administered thus daily for 30days.

Group 1 (weighing 1.3kg) received 1ml of Dimethyl sulphur oxide extract.

Group 2 (weighing 1.3kg) received 1.63ml of cold water extract

Group 3 (weighing 1.3kg) received 1.63ml of hot water extract

Group 4 (weighing 1.3kg) received 1.75ml of ethanolic extract

All groups received 500mg/kg of the different extracts aside the 6 control animals.

The physical appearance and activity of the Rabbits were observed throughout the exposure period.

Blood collection

On the 30th day, blood was collected from all the groups by cardiac puncture for renal function studies into lithium heparin bottles.

Laboratory methods and procedures for sample analysis

All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly observed.

Measurement of sodium, potassium, chloride and bicarbonate was done using Ion selective electrode analyzer model 6000.

Measurement of serum Urea and Creatinine were done using the Urease-Berthelot and Jaffe-Slot alkaline picrate methods respectively.

Statistical Analysis

The data generated from this study were analyzed using the SPSS version 22.0 statistical package. The first value indicates the mean value while the second value indicates the standard deviation of the parameters analyzed. The p-value was calculated to be less than 0.05 indicating a significant value and non-significant for values higher than 0.05.

RESULTS

The mean \pm standard deviation of serum creatinine level which is $13.4 \pm 2.1 \mu\text{mol/L}$, mean urea level which is $6.4 \pm 0.7 \text{mmol/L}$, mean sodium level which is $99 \pm 5.6 \text{mmol/L}$ and mean ($p > 0.05$) compared to the mean control creatinine level which is $12 \pm 1.4 \mu\text{mol/L}$, urea level which is $6.9 \pm 0.5 \text{mmol/L}$, sodium level which is $92.5 \pm 1.6 \text{mmol/L}$ and bicarbonate level which is 16 ± 0

mmol/L , while mean potassium level which is $5.5 \pm 0.9 \text{mmol/L}$ and mean chloride level which is $92.5 \pm 5.8 \text{mmol/L}$ of group 1 animals were significantly increased ($p < 0.05$) compared to their respective mean control levels which are $3.7 \pm 1.2 \text{mmol/L}$ and $84.5 \pm 4.3 \text{mmol/L}$ bicarbonate level which is $16.4 \pm 0.7 \text{mmol/L}$ of group 1 animals were not significantly different.

Table 1. Comparing the effect of various methods of extraction *Xylopi aethiopia* on renal function indices.

	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/)	Sodium (mmol/)	Potassium (mmol/)	HCO ³ (mmol/)	Chloride (mmol/)
Control	12.75 ± 1.4	6.98 ± 0.53	97.2 ± 1.6	3.67 ± 1.29	16 ± 0	84.5 ± 4.28
Dimethyl Sulphuric oxide	13.58 ± 2.11	6.367 ± 0.73	99 ± 5.59	5.5 ± 0.88	$16.42 \pm .74$	92.52 ± 5.8
p-values	0.443325883	0.126328564	0.466435956	0.016440559	0.195651156	0.02106226

Table 2. The effect of Dimethyl Sulphuric oxide methods of extraction of *Xylopi aethiopia* on renal function indices of rats compared with control.

	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/)	Sodium (mmol/)	Potassium (mmol/)	HCO ₃ (mmol/)	Chloride (mmol/)
Control	12.75 ± 1.4	6.98 ± 0.53	97.2 ± 1.6	3.67 ± 1.29	16 ± 0	84.5 ± 4.28
Cold water	16.95 ± 2.68	6.1 ± 0.87	118.7 ± 0.46	4.733 ± 0.38	24.42 ± 1.4	96.1 ± 2.52
p-values	0.006988904	0.060846788	2.94087E-11	0.04052134	3.98913E-08	0.00019318

Table 3. The effect of Cold water methods of extraction of *Xylopi aethiopia* on renal function indices of rats compared with control.

MEDIUM OF Extraction	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/)	Sodium (mmol/)	Potassium (mmol/)	HCO ³ (mmol/)	Chloride (mmol/)
Dimethyl Sulphuric oxide	13.58 ± 2.11	6.37 ± 0.73	99 ± 5.59	5.50 ± 0.88	$16.42 \pm .74$	92.52 ± 5.8
Cold water	16.95 ± 2.68	6.10 ± 0.87	118.7 ± 0.05	4.73 ± 0.38	24.42 ± 1.4	96.1 ± 2.52
Hot water	14.22 ± 2.89	17.23 ± 3.49	109.5 ± 10.8	7.90 ± 0.17	19.83 ± 0.68	93.37 ± 3.5
Ethanol	13.90 ± 2.87	10.15 ± 0.57	105.5 ± 0.51	4.92 ± 0.04	14.25 ± 0.93	88.1 ± 1.93
p-values	0.06824714	2.92355E-11	2.28972E-06	5.44173E-09	3.07613E-16	0.000170802

Table 4. The effect of Hot water methods of extraction of *Xylopi aethiopia* on renal function indices of rats compared with control

	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/)	Sodium (mmol/)	Potassium (mmol/)	HCO ₃ (mmol/)	Chloride (mmol/)
Control	12.75 ± 1.4	6.98 ± 0.53	97.2 ± 1.6	3.67 ± 1.29	16 ± 0	84.5 ± 4.28
Hot water	14.22 ± 2.89	17.23 ± 3.49	109.5 ± 10.8	7.9 ± 0.17	19.83 ± 0.68	93.37 ± 3.5
p-values	0.291697043	3.21496E-05	0.020506321	1.22283E-05	8.07079E-08	0.002873907

Table 5. The effect of Ethanol methods of extraction of *Xylopi aethiopia* on renal function indices of rats compared with control

	Creatinine ($\mu\text{mol/L}$)	Urea (mmol/)	Sodium (mmol/)	Potassium (mmol/)	HCO ₃ (mmol/)	Chloride (mmol/)
Control	12.75 ± 1.4	6.98 ± 0.53	97.2 ± 1.6	3.67 ± 1.29	16 ± 0	84.5 ± 4.28
Ethanol	13.9 ± 2.87	10.15 ± 0.57	105.5 ± 0.51	4.92 ± 0.04	14.25 ± 0.93	88.1 ± 1.93
p-values	0.400141099	1.67605E-06	3.37515E-07	0.060038615	0.001006643	0.045035874

DISCUSSIONS

There was significant increase in the mean value of potassium and chloride in the animals treated with (ED50, 500mg/kg) dimethylsulphuric oxide (group 1) when compared with the control animals ($p < 0.05$) whereas, creatinine, urea, sodium and bicarbonate showed no significant difference when compared with the control animals ($p > 0.05$). This shows that the dimethylsulphuric acid used to dissolve out the ethanolic extract increased the levels of potassium and chloride having no significant effect on creatinine, urea, sodium and bicarbonate. The increased potassium may suggest that dimethylsulphuric oxide has caused slight lysis of the red cells while the cause of increase in chloride level is unknown.

There was significant increase in creatinine, potassium and chloride in animals treated with (ED50, 500mg/kg) cold water extract of *Xylopiya aethiopic*a when compared with the control animals ($p < 0.05$) whereas, urea, bicarbonate and sodium showed no significant difference when compared with the control animals ($p > 0.05$). This result is in agreement with that of Ogbonna *et al.*, 2008 who stated that the elevation in the plasma creatinine concentration indirectly suggests kidney damage specifically renal filtration mechanism (Wasan *et al.*, 2001).

There was significant increase in the levels of sodium and chloride in animals treated with (ED50, 500mg/kg) hot water extract of *Xylopiya aethiopic*a when compared with the control animals ($p < 0.05$) whereas, creatinine, urea, potassium and bicarbonate showed no significant difference ($p > 0.05$). This result is in agreement with the work of Eric *et al.*, 2011, who found out that *Xylopiya aethiopic*a had no significant effect on the renal function test (creatinine). Also, this result is in agreement with the work of Amaechi *et al.*, 2011 where there was increase in sodium level when compared with the control. This finding, they said, is suggestive of a mild hypernatraemic effect. Hence, it probably may favour an improvement in renal function by increasing sodium reabsorption.

Furthermore, there was significant increase in bicarbonate and chloride levels in animals treated with (ED50, 500mg/kg) ethanolic extract of *Xylopiya aethiopic*a when compared with the control animals ($p < 0.05$) whereas creatinine, urea, sodium and potassium showed no significant difference ($p > 0.05$). This result is in agreement with the work of Eric *et al.*, 2011, who found out that *Xylopiya aethiopic*a had no significant effect on renal function test (creatinine).

CONCLUSION

In this study, it can be deduced that *Xylopiya aethiopic*a caused a significant increase in the level of creatinine in Rabbits treated with cold water extract, indirectly affecting the renal function, while the hot water and the ethanolic extract had no significant effect on the renal function. Consuming the different extract employed in

this study for a long period may cause an imbalance in the electrolytes which is important in regulating the body's acid/base balance. Boiled extract of *Xylopiya aethiopic*a taken as postpartum tonic by newly delivered women in different parts of the world has proven to have no negative effect on the renal functioning rather a replenishing effect evident by the augmentation of total protein levels with increase in urea levels, although it is not taken for a long time by postpartum mothers.

It is recommended therefore, that further studies should be advocated to ascertain and to provide reasonable estimates of the human risk associated with the consumption of *Xylopiya aethiopic*a in hot water extract as is used to in this part of the globe.

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