The Effect Of Xylopia Aethiopica Fruits Extract On The Sperm Production And Testicular Oxidative Status In Male Wistar Rats.

1Nnodim Johnkennedy,1Nwanjo Harrison Ugo,1Nwosu Dennis, and 2Uche Uchegbu

1Department of medical laboratory science, faculty of health science, Imo State University overri, Imo State, Nigeria and Department of Microbiology Federal Medical Centre Owerri

ABSTRACT

BACKGROUND: The plant, xylopia aethiopicafruits locally called “uda” by the southern eastern part of Nigeria. This is highly valued plant in Igbo land. Among the Igbo’s; the fruits are used as spices; and aqueous decoction is used especially after child birth probably for its antiseptic properties and to arrest bleeding. This plant has a wide spectrum of biological activities and have played a crucial role in traditional medicines because of their valuable physiological and pharmacological properties OBJECTIVE: This study was done to determine the effect of Xylopiaaethiopica fruits extract on the sperm production and testicular oxidative status in male wistar rats. MATERIALS AND METHODS: Twenty male rats weighing 160-220g were used for the research work. Water and rat feeds were provided ad libitum. The male wistar rats were randomly divided into two groups of 10 rats each. Group 1 which is the control group received normal saline only while group 2 was administered with 200mg of the X. aethiopica extracts by oral compulsion. These rats were sacrificed after 28days RESULTS: The sperm count (19.6 ± 4.9x10^6 /ml) and sperm motility (14.2 ± 2.7%) of male wister rats administered with 200mg of extract were significantly decreased when compared with the control (121.5± 9.2x10^6/ml and 95.17±2.6%) (P<0.05). Similarly, the level of superoxide dismutase[SOD](15.61± 5.3 µmol/l), catalase[CAT](4.92±1.81µmol/l),glucose peroxidase[GPX](0.19±0.12nmol/l) and reduced glucothione[GSH](0.64± 0.09nmol/l) in male rats administered with 200mg X. aethiopica were significantly decreased when compared with the control(SOD:42.61± 2.5µmol/l, CAT:18.14± 2.8µmol/l,GPX:1.2±0.14nmol/l and GSH:3.17± 0.12nmol/l respectively)(P<0.05). On the other hand, the level of lipid peroxidation product[Malondialdehyde MDA](4.16± 0.18nmol/l) in male wistar rats administered with 200mg extracts was significantly increased when compared with the control(0.92± 0.14nmol/l). (P<0.05) CONCLUSIONS: These observations probably suggest that X. aethiopica extracts could be associated with damage to the spermatogenic process as well as causing oxidative stress to the tests.

Key words:

Introduction

Recently, there has been increasing demand for the use of plant products in treating some disorders. This is due to low cost, easy availability and lesser side effects. Hence, plant material are continuously scrutinized and explored for their beneficial effects[1]. One such plant is xylopia aethiopica which has been used in the treatment of digestive system hypermotility (diarrhea), bronchitis, stomach aches, febrile pains and rheumatism. This fruit of xyllopia aethiopica has been reported to act as antioxidant, hypolipidemic and hypoglycaemic agent hence, confirming to its use as an antidiabetic agent used in traditional medicine for treating diabetes. The plant, xylopia aethiopicafruits locally called “uda” by the southern eastern part of Nigeria. (That is the Igbo’s). It is an evergreen, aromatic tree, growing up to 20metres high. This is highly valued plant in Igbo land. Among the Igbo’s; the fruits are used as spices; and aqueous decoction is used especially after child birth probably for its antiseptic properties and to arrest bleeding[2]. This plant has a wide spectrum of biological activities and have played a crucial role in traditional medicines because of their valuable physiological and pharmacological properties[3]. The fruit have been found to contain volatile aromatic oil, fixed oil and rutin[4].

Researches on herbal medicine have attained an incredible global level in the recent past. The application of some plants constituents in pharmaceutical industries has gone long way in the elevation of the status of the traditional herbal medicine in Africa and in Nigeria in particular[5]. Hence herbal medicines have received greater attention as an alternative to clinical therapy leading to increasing demand [6]. The use of herbal drugs by elites and non-elites for the treatment of diseases in Imo State Nigeria is very common particularly in the rural area. Ogbonnaet al[3] state that experimental screening method is important in order to ascertain the safety and efficacy of herbal product as well as establishing the active component of these herbal remedies. In Imo State Nigeria, the use of Xylopiaaethiopicaas spices, antiinflamatory, worm expeller and anti-malarial is not uncommon. However, the folk medicine abhors the use of X. aethiopica among male counterpart upon the beneficial effect of the plant. It was speculated to cause oxidative stress in the system which has not been
scientifically proved. Hence, it is the purpose of this research work to provide information on the testicular oxidative stress and sperm production in male rats administered with X. aethiopica.

Materials and Method

**Plant materials:** The *Xylopia aethiopica* fruits were obtained from Ekeonunwa market in Owerri Nigeria. The botanical identification and authentication was confirmed by Dr. C. Okere (Head of Department of Plant Science and Biotechnology, Imo State University, Owerri). The plant material was sun dried for seven days. The dried fruit of *Xylopia aethiopica* were milled to get a coarse powder used for the extraction of the powder were macerated in a 400g percolator with 250ml of distilled water. The mixture was allowed to stand for 48 hours after which it was filtered. The filtrate was then placed in an oven to evaporate and the solid residue referred to as extract. The appropriate concentrations of the extract were made in distilled water for the experiment.

**Experimental Animals:** The male wistar albino rats weighing between 150 and 220g (ages 8 to 10 weeks) were used in this study. These animals were obtained from the Animals House of College of Medicine and Health Science, Imo State University Owerri Nigeria. They were kept under standard laboratory conditions, fed with commercial growers mash, product of Top feeds LTD, Sapele Nigeria. Water and feeds were provided *ad libitum.* The animals were left for two weeks to acclimatize and then divided into groups for experimentation.

**Experimental Design:** The animals were randomly assigned to two experimental groups (n=10 x 2 group). The first group of animals which served as control was given distilled water. Group 2 was administered with 200mg/kg body weight for 28 days. In all groups, the extract was administered through oral route. This treatment was by oral compulsion. All animals were allowed free access to food and water through out the experiment.

**Blood Collection:** Twenty four hours after the last doses were administered the animals were anaesthetized with chloroform vapour, quickly brought out of the jar and sacrificed. Whole blood was collected by cardiac puncture from each animal into clean dry test tubes and EDTA containers. The blood in the clean dry test tubes were allowed to stand for about 15 minutes to clot and further spun in a westerfuge centrifuge (Model 1384) at 10,000g for 5 minutes, serum was separated from the clot with Pasteur pipette into sterile sample tubes for the measurement of antioxidant profile.

**Sperm collection:** The epididymis was minced with anatomical scissors in 5ml of saline. It was placed in a rocker for 5 minutes and allowed to incubate at room temperature for 3 min. After the incubation, the supernatant fluid was diluted 1:100 with solution containing 5g sodium bicarbonate and 1ml formalin.

**Preparation of Erythrocyte Samples:**

Five milliliters of blood was drawn from the cubital median vein of the subjects into into plain tube and heparinized tube. The sample in plain tube was spun in a Wisperfuge (model 684) centrifuge at 1000g for 10 minutes and the serum collected into a clean dry bijou bottle.

The blood samples in heparinized tubes were centrifuged at 1000 x g for 10 min at 4°C and the upper phase was taken with a pasteur pipette into an eppendorf tube and stored at -4°C. The buffy coat on top of the erythrocyte layer was carefully removed and 10 mL isotonic NaCl solution was added.

Resuspended erythrocyte was centrifuged at 1000 x g for 10 min and the upper part removed again. Then 10 mL phosphate buffer solution (PBS) was added and the erythrocytes were centrifuged, and the upper buffer part removed by pasteur pipette. The erythrocytes were diluted 10 times with ice cold water, vortexed and stored at -40°C until used.

**Measurement of Catalase Activity:**

Catalase, (CAT, E. C.I.I. I. 6) enzyme converts H₂O₂, H₂O and 1/2 O₂. Catalase activity was measured by the Aebi[6] method. The principle of this method was based on the hydrolyzation of H₂O₂ and decreasing absorbance at 240 nm. The conversion of H₂O₂ into H₂O and 1/2 O₂ in 1 min under standard condition was considered to be the enzyme reaction velocity.

**Superoxide Dismutase (SOD) Enzyme Activity Determination** The superoxide dismutase [SOD (E.C.1.15.1.1)] enzyme, which catalyzes the dismutation of the superoxide anion (O₂⁻) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. SOD activity determination was based on SOD’s inhibition of the reaction of superoxide anion (O₂⁻), from xanthine by xanthine oxidase and the reduction of nitrobluetetrazolium (NBT)[6].

**Determination of MDA:**

MDA, as a marker for lipid peroxidation, was determined by the double heating method of Draper and Hadley (1990). The principle of the method was based on a spectrophotometric measurement of the colour
produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). In brief, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test-tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer at 532 nm. The concentration of TBARS was calculated by the absorbance coefficient of MDA-TBA complex 1.56 x 10^5 cm^-1 M^-1 and expressed in nmol/ml.

Statistical analysis: The results were expressed as mean ± standard deviation. The statistical evaluation of data was performed by using one-way anova (Analysis of variance) followed by Duncan’s multiple range test.

Results:
Table 1 showed the level of sperm count and sperm motility in control and male rats administered with 200mg/bw of Xylopia aethiopica

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm count (x10^6/ml)</th>
<th>Sperm motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>121.5 ± 9.2x10^6</td>
<td>95.17 ± 2.6</td>
</tr>
<tr>
<td>200mg of extract</td>
<td>19.6 ± 4.9x10^6</td>
<td>14.2 ± 2.7</td>
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*Significantly different from control at P<0.05

Table 2 showed the level of SOD (µmol/l) CAT (µmol/l) GPX (nmol/l) GSH (nmol/l) and MDA(nmol/l) in control and male rats administered with 200mg/bw of Xylopia aethiopica

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD (µmol/l)</th>
<th>CAT (µmol/l)</th>
<th>GPX (nmol/l)</th>
<th>GSH (nmol/l)</th>
<th>MDA(nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.61 ± 2.5</td>
<td>18.14 ± 2.8</td>
<td>1.2 ± 0.14</td>
<td>3.17 ± 0.12</td>
<td>0.92 ± 0.14</td>
</tr>
<tr>
<td>200mg of extract</td>
<td>15.61 ± 5.3</td>
<td>4.92 ± 1.8</td>
<td>0.19 ± 0.12</td>
<td>0.64 ± 0.09</td>
<td>4.16 ± 0.18</td>
</tr>
</tbody>
</table>

The sperm count (19.6 ± 4.9x10^6 /ml) and sperm motility (14.2 ± 2.7%) of male wister rats administered with 200mg of extract were significantly decreased when compared with the control (121.5± 9.2x10^6/ml and 95.17±2.6%) (P<0.05)[table 1]. Similarly, the level of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and reduced glutathione (GSH) in X. aethiopica were significantly decreased when compared with the control (SOD:42.61± 2.5µmol/l, CAT:18.14± 2.8µmol/l, GPX:1.2± 0.14nmol/l and GSH:3.17± 0.12nmol/l respectively)(P<0.05). On the other hand, the level of lipid peroxidation product (Malondialdehyde MDA) in male wister rats administered with 200mg extracts was significantly increased when compared with the control(0.92± 0.14nmol/l). (P<0.05)

Discussion
The application of some plants constituents in pharmaceutical industries has gone long way in the elevation of the status of the traditional herbal medicine in Africa and in Nigeria in particular.

In this study, the antioxidants were significantly depleted when compared with the control. The biochemical mechanism by which X. aethiopica causes reduction in enzymatic antioxidants is currently unclear. It is postulated that the consumption of this plant in male could cause oxidative stress and hence generate free radicals. These free radicals could then cause membrane damage through lipid peroxidation and protein oxidation. Therefore, the decrease in the activities of SOD, CAT, and GPX enhanced lipid peroxidation. This is evidenced by the increased level of MDA in this study.

Furthermore, it was observed that there was decreased sperm motility among the rats that was administered with the extract. This could be associated with oxidative stress induced by the consumption of the extract. The sperm is particularly vulnerable to lipid peroxidation because of the molecular anatomy of its plasma membrane. The increased oxidative stress damages the sperm membrane hence, reduced motility. This is in line with the work of Kalender and Yel[9]; Sachder and Davies [10].

In conclusion, these observations probably suggest that X. aethiopica extracts could be associated with damage to the spermatogenic process as well as causing oxidative stress to the testis.

References


4. Burkhill HM. The useful plants of west tropical Africa 2nd edition Royal Botanic


