THE PROTECTIVE EFFECTS OF KOLAVIRON ON THE RENAL FUNCTIONS OF FEMALE WISTAR RATS TREATED WITH CLOMIPHENE CITRATE.

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ABSTRACT

This research work accessed the effects of kolaviron (a methanolic extract of Garcinia kola seeds) on microanatomy of kidneys and biochemical parameters with a view to determining its relationship to renal functions treated with clomiphene citrate. A total of thirty adult female Wistar rats were used for this experiment. The animals were randomly divided into six (6) groups: A, B, C, D, E and F with five (5) animals in each group. Group A, were the control group that were given distilled water orally once daily for 14 days; Group B were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days; Group C were given kolaviron orally at concentration of 100 mg/kg twice daily for 14 days; Group D were given clomiphene citrate orally at concentration of 0.50 mg/kg body weight for 5 days; Group E were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days after which clomiphene citrate were administered at concentration of 0.50 mg/kg body weight for 5 days; Group F were given kolaviron orally at concentration of 100 mg/kg body weight twice daily for 14 days after which clomiphene citrate were administered at concentration of 0.50 mg/kg body weight for 5 days. Five milliliters (5 ml) of blood were collected by ocular puncture with the aid of capillary tubes from the animals for biochemical analysis. At the end of the experimental period, the animals were anesthetized by chloroform inhalation. The kidneys were removed and fixed in 10% formal saline for Haematoxylin and Eosin staining. The results showed that Relative kidney weight (F=1.595, df=5, P<0.05) was not increased significantly across the groups. Potassium (F=0.754, df=5), chloride (F=0.529, df=5), interstitial calcium (F=0.335, df=5), total calcium (F=0.840, df=5), silver ions (F=0.517, df=5), and pH (F=0.785, df=5) were not reduced significantly (p=0.05) when group A was compared with all the groups but sodium ions reduced significantly (F=19.426, df=5, P<0.05 ), creatinine increased significantly (F=2.698, df=5, P<0.05). The histoarchitecture of the kidneys in groups A, B and C had normal renal tubules and Bowman’s capsules; group D were distorted; while groups E and F had evidence of recovery from distortion. It was concluded that clomiphene citrate have adverse effects on the renal functions in female wistar rats while kolaviron ingestion have protective effects.

KEY WORDS: kolaviron, kidney, biochemical, renal functions and clomiphene citrate.

INTRODUCTION

In humans the kidneys are located in the abdominal cavity, more specifically in the paravertebral gutter and lie in a retroperitoneal position at a slightly oblique angle. There are two, one on each side of the spine. The asymmetry within the abdominal cavity caused by the liver typically results in the right kidney being slightly lower than the left, and left kidney being located slightly more medial than the right. The left kidney is approximately at the T12 to L3 vertebral level. The kidneys are organs that serve several essential regulatory roles in most animals. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme renin.

Clomid is a popular drug used as part of a Post Cycle Therapy and for that purpose, it’s become a staple of many body builder’s protocol. The medical purpose for Clomid is for women to aid in ovulation. In men, the application of Clomid is theorized to spur an elevation of follicle stimulating hormone and luteinizing hormone. As a result, natural testosterone production may be increased. When LH is elevated so is estrogen and it’s for this reason some people do not get the expected “anti estrogen” effect of Clomid, though it can act as an estrogen antagonist (occupying existing estrone sites) if estradiol levels are high. This effect can be beneficial to the athlete, especially at the conclusion of a cycle when endogenous testosterone levels are subnormal. Clomid will eventually raise testosterone levels over its period of intake, though in the interim it may present some estrogenic-like side effects, including headaches, mood swings and lessened sex drive. The testosterone elevating effects also dissipate once dosing stops so a supplement strategy to maintain natural production should be in place after cessation of the use of Clomid. Users often take the drug in a dosage of 50-100mg a day for 4-6 weeks following the end of a cycle. Clomid is kidney toxic and should not exceed this length. Users often add substances such as HCG and various supplements to support natural testosterone recovery to make their proximal convoluted tubule plan even more sound and effective.

This study was conducted to examine clomiphene citrate and kolaviron, and their possible effect on the renal function was examined whether clomiphene citrate has a detioriating effect on the kidney in female wistar rats. Despite this, the antioxidant property of kolaviron cannot but me mentioned. The production of antioxidants in the body declines as one ages, thus necessitating supplementation from nutritional sources. Flavonoids are rich sources of antioxidants. Antioxidants in the body protect cells and provide numerous other functions important to health. They have the ability to scavenge free radicals, making them harmless. Flavonoids have been proposed to play a useful role in protecting the central nervous system against oxidative and excitotoxic stress. Nutritional supplements of the antioxidants come from plants sources. Garcinia kola (GK) is one of such plants. Garcinia kola Heckel (family GUITIFERAE) is a herb grown in Nigeria and has a characteristic astringent bitter and resinous taste. The plant has been referred to as a “wonder plant” because every part of it has been found to be of some form medicinal value. However, the scientific knowledge of its use has not been extensively explored.
medicinal importance. *Garcinia kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhea, laryngitis, bronchitis and gonorrhoea. The seed is masticatory and also used to prevent and relieve colic, chest colds, and cough and can as well be used to treat headache.

Studies have also reported the use of this plant for the treatment of jaundice, high fever, purgative and as chewing stick. The plant usefulness in the treatment of stomach ache and gastritis were also previously recorded. The phytochemical compounds isolated from *G. kola* include oleoresin, tannins, saponins, alkaloids, cardiac glycosides. Other phytochemical compounds so far isolated from *G. kola* seeds are biflavonoids such as kolaflavone and 2-hydroxybiflavonols. The folklore remedies of *Garcinia kola* for the treatment of various infections caused by pathogens were also mentioned. Kolaviron (KV), the predominant constituent in *G. kola*, contains biflavones (GB1, GB2 and kolaflavanone) and has been reported to prevent hepatotoxicity mediated by several toxins. Likewise, kolaviron exhibited hypoglycemic effects in normal and alloxan- and streptozotocin-induced diabetic animals. Also, kolaviron has been reported to elicit strong antioxidant activity, both in vivo and in vitro experimental models. Due to the antioxidant properties of kolaviron, it has the potential to protect the cells from toxic damage.

**MATERIAL AND METHODS**

**Animals:** A total of thirty adult female Wistar rats were used for this experiment. The animals were fed with standard rat pellet and given water liberally in the animal holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife. A total of thirty adult female Wistar rats were used for this experiment. The animals were randomly divided into six (6) groups: A, B, C, D, E and F with five (5) animals in each group. Group A, were the control group that were given distilled water orally once daily for 14 days; Group B were given kolaviron orally at concentration of 100 mg/kg body weight twice daily for 14 days; Group C were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days; Group D were given kolaviron orally at concentration of 0.50 mg/kg body weight for 5 days; Group E were given kolaviron orally at concentration of 100 mg/kg twice daily for 14 days; Group D were given kolaviron citrate orally at concentration of 0.50 mg/kg body weight for 5 days; Group E were given kolaviron orally at concentration of 200 mg/kg body weight once daily for 14 days after which kolaviron citrate were administered at concentration of 0.50 mg/kg body weight for 5 days; Group F were given kolaviron orally at concentration of 100 mg/kg body weight twice daily for 14 days after which kolaviron citrate were administered at concentration of 0.50 mg/kg body weight for 5 days. Five milliliters (5 ml) of blood were collected by ocular puncture with the aid of capillary tubes from the animals for biochemical analysis. At the end of the experimental period, the animals were anaesthetized by chloroform inhalation. The kidneys were removed and fixed in 10% formal saline for Haematoxylin and Eosin staining. All animals were handled in accordance with guidelines for animal research as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals.

**Extraction of kolaviron:** Extraction of Kolaviron was achieved by the procedure previously described by Iwu and modified by Braide. Briefly, *Garcinia kola* seeds were peeled and air dried in the laboratory (25-28 °C) and ground into powdered form. The powdered seeds were extracted with n-hexane, in a Soxhlet extractor. The defatted, dried marc was repacked and then extracted with methanol in a Soxhlet extractor. The extract was concentrated and diluted to twice its volume in distilled water and partitioned with chloroform. The concentrated chloroform fraction gave a yellow-brown solid known as kolaviron. This was air dried and ground into fine particles.

**Histological analysis:** The animals were anaesthetized by chloroform inhalation. The peritoneal cavities were opened so that the kidneys can be excised and weighed using a Metler sensitive balance. They were fixed in 10% formal saline (pH 7.1) for histological procedures. Kidneys were routinely processed for paraffin wax embedding. Then, 5μ thick paraffin cross sections of the tissues were mounted on slides and stained using routine haematoxylin and eosin method. Microscopy was conducted on an Olympus microscope (Tokyo, Japan) and images were captured and processed by an attached eyepiece camera.

**RESULTS**

**Relative kidney weight:** There was no significant (p< 0.05) increase in the relative kidney weight of female wistar rats when control was compared with experimental groups (Table I).

**Concentrations of urea and creatinine:** There was no significant decrease in the concentrations of Urea when the control group was compared with the kolaviron administered groups irrespective of the concentration. Likewise with the concentration of clomiphene citrate administered group (group D). The concentrations of creatinine increased significantly (p<0.05) when kolaviron administered groups were considerably higher compared with the control and clomiphene administered groups (Table II).

**Concentrations of the electrolytes:** The average concentrations of the electrolytes in the serum were analysed and the results are stated in Table III below. Using one way ANOVA with Duncan multiple range test (DMRT), sodium ions and carbon dioxide differs significantly across the groups (p< 0.05). When sodium ion concentration in the control group (group A) (148.00±1.0) was compared with all the experimental groups (group B measured 142.10±0.00; group C measured 143.90±2.30; group D measured 143.50±0.3; group E measured 144.20±2.90; group F measured 136.30±6.45), they reduced significantly. When carbon dioxide concentration in the control group (group A)(14.70±0.30) was compared with all the experimental groups (Group B measured 17.3±0.30; group C measured 16.55±0.45; group D measured 16.95±0.75; group E measured 20.30±0.40; group F measured 15.80±0.00.), they increased significantly. While potassium, chloride interstitial calcium, total calcium, silver ions and pH were not significantly different (p< 0.05) in all the groups.

**Histological studies of the kidney:** H & E staining of the kidneys revealed that the nephrons of the kidneys in groups A, B and C were intact while there was evidence of distortion in renal tubules and Bowman’s capsules of the nephrons of group D while there were clear cut proof of recovery in groups E and F (Plates I-A-F).

**DISCUSSION**

This study showed that relative kidney weight was not increased significantly (p> 0.05) which opposed the work of Uko that reported a decrease in body mass gain in rats fed *G. kola* and this was associated with reduced feed...
consumption and a decrease in weight gain. The mode of extraction of kolaviron in this study made it to have no effect on the composition rate of the animals therefore, the weight was not affected.

Observations from the data collected indicated the influence of the extract on the serum did not significantly decreased the concentration of urea which is an indication that it had no adverse effect on the kidney while the concentration of creatinine increased significantly when control is compared with kolaviron and clomiphene citrate administered groups.

Sodium ions and carbon dioxide differ significantly while potassium, chlorine, interstitial calcium, total calcium, silver ions and PH values were not significantly different, which means that kolaviron and clomiphene citrate had no effect on them but did have effect on sodium ions and carbon dioxide. This supported an work which stated that the effect of vitamin C on electrolyte profile was not significant10. This was corroborated by the study of Eteng et al., (2006), who also obtained a non-significant increase in serum Na+ following administration of vitamin C to Wistar rats. The study is also in agreement with the findings which revealed an insignificant change in serum Na+ and K+ following vitamin C administration11. However, this study revealed a significant increase in serum Ca2+ level. Sodium and chloride are the most abundant electrolytes in the extra-cellular fluid and to a large extent determine plasma osmolarity.22,23 Kolaviron being an antioxidant make it possible for it to maintain the electrolytes in the serum. The significant effects it has on sodium ion showed that the ingestion of kolaviron significantly reduces its concentration in the serum of female wistar rats. Also, Carbon dioxide concentration was raised significantly in the groups that were given kolaviron which was proven of more energy production and more carbon dioxide to be excruded by the lungs. Potassium, chlorine, interstitial calcium, total calcium, pH and silver ions had a reduction in concentrations but not significantly. These showed that ingestion of kolaviron had a good antioxidative effect on the serum of female wistar rats.

In PLATE 1A-F, the renal tubules and bowman’s capsules were intact in groups A, B and C but group D showed appreciable distortion in renal tubules and bowman’s capsules while those of groups E and F were at their recovery stages. This work opposed the report that observed mild hydropic degeneration in cells of the renal proximal tubular epithelium and attributed the changes to the flavonoids contained in G. kola seeds24. This study proved that clomiphene citrate has a deteriorating effect on the histoarchitecture of the kidney while kolaviron has ameliorating effects on it which could be as a result of it antioxidant property.

REFERENCES

Table 1: Effect Of Kolaviron on relative kidney weight in female wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Relative kidney weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP A</td>
<td>3.2000±0.24290a</td>
</tr>
<tr>
<td>GROUP B</td>
<td>3.2500±0.15346b</td>
</tr>
<tr>
<td>GROUP C</td>
<td>3.9250±0.27500ab</td>
</tr>
<tr>
<td>GROUP D</td>
<td>3.6900±0.34641c</td>
</tr>
<tr>
<td>GROUP E</td>
<td>3.8500±0.29580ab</td>
</tr>
<tr>
<td>GROUP F</td>
<td>3.8000±0.12247a</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for parameters coded Relative kidney weight in each group. a, within column signifies that means with different letters differ significantly at p<0.05 while means with the same letters does not differ significantly at p<0.05 (using one way ANOVA with Duncan multiple range test).

Table 2: Effect of Kolaviron on urea and creatinine in female wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea (g/dL)</th>
<th>Creatinine (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP A</td>
<td>6.3360±1.62599a</td>
<td>10.1000±0.70738a</td>
</tr>
<tr>
<td>GROUP B</td>
<td>6.5760±0.33625b</td>
<td>17.0100±0.86518b</td>
</tr>
<tr>
<td>GROUP C</td>
<td>5.9100±0.53732a</td>
<td>17.9900±3.23961a</td>
</tr>
<tr>
<td>GROUP D</td>
<td>5.9500±0.66537b</td>
<td>15.7900±2.49470b</td>
</tr>
<tr>
<td>GROUP E</td>
<td>5.5420±0.19048a</td>
<td>18.5240±0.91024a</td>
</tr>
<tr>
<td>GROUP F</td>
<td>6.5540±0.52171a</td>
<td>15.4400±1.85160a</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for 2 biochemical parameters coded as urea and creatinine in each group. a,b,ab, within column signifies that means with different letters differ significantly at p<0.05 while means with the same letters does not differ significantly at p<0.05 (using one way ANOVA with Duncan multiple range test).

Table 3: Effect kolaviron on the concentrations of electrolytes in female wistar rats (mg/ml).

<table>
<thead>
<tr>
<th>Group</th>
<th>Potassium</th>
<th>Sodium</th>
<th>Chlorine</th>
<th>Int. Calcium</th>
<th>Total calcium</th>
<th>Acid based level</th>
<th>Carbon-dioxide</th>
<th>Silver</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP A</td>
<td>5.6250±0.7650a</td>
<td>148.0000±10000b</td>
<td>105.7000±63000b</td>
<td>1.2900±0.0000b</td>
<td>2.5100±0.02000b</td>
<td>8.4550±0.23500a</td>
<td>14.7000±0.30000a</td>
<td>27.6000±7.0000a</td>
</tr>
<tr>
<td>GROUP B</td>
<td>4.8700±0.41000a</td>
<td>142.1000±00000b</td>
<td>100.7000±0.10000b</td>
<td>1.2550±0.01500b</td>
<td>2.4450±0.02500b</td>
<td>8.3100±0.10000a</td>
<td>17.3000±0.30000a</td>
<td>24.1000±0.40000a</td>
</tr>
<tr>
<td>GROUP C</td>
<td>4.7950±0.29500a</td>
<td>143.9000±23000b</td>
<td>102.7000±1.00000b</td>
<td>1.2900±0.11000b</td>
<td>2.5200±0.21000b</td>
<td>8.2100±0.14000a</td>
<td>16.5500±0.45000a</td>
<td>24.6500±1.75000a</td>
</tr>
<tr>
<td>GROUP D</td>
<td>4.8700±0.18000a</td>
<td>143.5000±0.30000b</td>
<td>102.1350±0.75000b</td>
<td>1.0950±0.00500b</td>
<td>2.1350±0.00500b</td>
<td>8.1150±0.00500a</td>
<td>16.9500±0.75000a</td>
<td>24.4000±0.30000a</td>
</tr>
<tr>
<td>GROUP E</td>
<td>4.5420±0.33000a</td>
<td>144.2000±2.30000b</td>
<td>102.6500±1.50000b</td>
<td>1.1750±0.16500b</td>
<td>2.3000±0.32000b</td>
<td>8.3100±0.16000a</td>
<td>20.3000±0.40000a</td>
<td>21.2000±2.30000a</td>
</tr>
<tr>
<td>GROUP F</td>
<td>4.7600±0.32000a</td>
<td>136.2500±6.45000b</td>
<td>98.8000±4.20000b</td>
<td>1.2250±0.03500b</td>
<td>2.3850±0.06500b</td>
<td>8.3900±0.06000a</td>
<td>15.8000±0.00000a</td>
<td>21.6000±2.30000a</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM for electrolytes in each group. a,b,c,ab, within column signifies that means with different letters differ significantly at p<0.05 while means with the same letters does not differ significantly at p<0.05 (using one way ANOVA with Duncan multiple range test).
PLATE IA-F: Photomicrograph of tranverse section of kidney showing normal bowman’s capsules (arrows) and Renal tubule (R) in A, B and C; but distorted in D, recovery stages in E and F. H &E. X 400.