Effects of Kolaviron, the Major Constituent of *Garcinia kola*, on the Histology of the Hypothalamus, Pituitary, and Testes Using Adult Male *Wistar* Rats as a Model Organism

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Abstract

This study determined the effects of kolaviron on the histology of organs of the hypothalamic-pituitary-gonadal axis, mainly the hypothalamus, pituitary and testis. The aim was to ascertain if its consumption has deleterious effects on these organs. Thirty six adult *Wistar* rats divided into six groups of six animals each were used and kolaviron administered at 100, 200, 400 and 800 mg/kg body weight. The results showed that gross cellular depletion and desquamation of cells of testis significantly reduced number of cells in the hypothalamus and pituitary (P < 0.05). It significantly reduced the relative brain weight (P < 0.05). These findings suggest that kolaviron can alter the histology of the axis which may impair its reproductive function.

Keywords

Histology, Hypothalamus, Pituitary, Testis

1. Introduction

Kolaviron is the major constituent of *Garcinia kola*. The extract is one of the numerous plant products, and nutritional supplements that have been found to have a wide range of medicinal value. It is a drug of plant origin, which has numerous biochemical importances in the human body system. It is a defatted ethanol extract as well as a bioflavonoid of plant origin [1]. Documented the ability of kolaviron to inhibit hydroxy and superoxide
anion radicals, which are known to play important role in the process of lipid peroxidation. Its anti-oxidant action on lipoprotein has been reported [2]. Kolaviron has been found to significantly prevent hepatotoxicity mediated by galactosamine, amanita toxin and paracetamol [3]. It has a profound effect on the hormone, erythropoietin in the kidney [4] [5]. It is reported that kolaviron causes impotency in male subjects, which has made Garcinia kola an unwelcomed substance in some cases, especially where the man is battling with child bearing. Yet [1] have shown that kolaviron could be used in the treatment of male infertility problems associated with oxidative stress damage.

These suggest that it could have effect on the hormones of the hypothalamic-pituitary-gonadal axis. Considering the usefulness of kolaviron, it becomes necessary to determine its effects on the morphology of the organs, particularly the organs of hypothalamic-pituitary-gonadal axis-hypothalamus, pituitary and testis.

2. Materials and Methods

Thirty six adult male Wistar rats weighing 180 g - 200 g, aged 20 - 24 weeks, were used for this study. The animals were obtained from the Animal Holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife. The animals were fed with standard rat pellet and given water liberally. They were randomly assigned to six groups A, B, C, D, E, and F, of six rats each. Animals were housed in clean plastic cages under natural light and dark cycle, and at room temperature. Animals in group A served as primary control, those in group B served as secondary control. The animals in groups C, D, E, F, formed the experimental groups. All animals were handled with care in accordance with guidelines for animal research as detailed in the NIH guidelines for the care and use of laboratory Animal (NIH publication, 1985).

Kolaviron was extracted by the procedure of Farombi [6]. Briefly, Garcinia kola nuts were purchased from local market in Ile-Ife, Nigeria. The seeds were peeled and air dried in the laboratory. Air dried seeds were ground into powdered form. The powdered seeds were extracted with n-hexane, in a soxhlet extractor. The defatted, dried mass 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, which was allowed to dry in oven (40˚C), and ground to powdered form.

Kolaviron was administered at 100, 200, 400 and 800 mg/kg body weight to each of the animals in groups C, D, E, and F respectively. Animals in group B were administered corn oil, while those in group A (control) received normal saline [7]. All administration was by gastric intubation which lasted for eight weeks. The coin oil was obtained from Sigma Chemical, USA.

At the end of administration of Kolaviron (experiment), the animals were sacrificed by cervical dislocation; the hypothalamus, pituitary and testes were dissected out and fixed in 10% formal saline for histological studies. Haematoxylin and Eosin method Drury and Wallington, [8] was used to show the histology and morphology of the organs of the treated and control groups. Relative weight of brain, and testis were calculated.

Slides were viewed under a Leica DM750 microscope and digital photomicrographs taken by an attached Leica ICC50 camera. Photomicrographs of haematoxylin and eosin stained sections were imported on to OpenOffice.org™ (OOo-dev 3.4.0) software for histomorphometric analysis. One-way ANOVA was used to analyze data followed by Student Newman-Keuls (SNK) test for multiple comparisons. Primer for windows (McGraw-Hill, version 4.0.0.0) was the statistical package used to analyze data. Results were expressed as mean ± standard error of mean. P < 0.05 was set as accepted level of significant difference.

3. Results

3.1. Physical Observation

Animals in group F that were given 800 mg/kg body weight of kolaviron had increase in bolls of urine and watery stool, and these made the cage to be always wet. They also exhibited loss of body hair, and grooming was high. Macroscopic observation of the testes showed transparent covering from the outer layer in which the blood vessels were exposed with purplish colouration to the inner layer. For group E, administered 400 mg/kg body weight of kolaviron there was also increase in bolls of urine, and watery stool, like those of group F. For groups C, D, and primary and secondary controls, A and B, there was no apparent loss of body hair, and grooming was less apparent. The difference in mean body weight shown in Table 1 indicates that groups B, D, E and F had
significantly reduced body weight gain when compared to group A (control group); and also that group E was significantly lower when compared to group C (P < 0.05). Generally, there was weight gain in all groups of animals except for group E that showed slight lost in body weight. Table 2 shows relative brain, and testis weights. It indicates that there was no significant difference in relative brain weight (RBW) and testis weight in all the groups (P > 0.05). In group F that relative brain weight was significantly lower compared to controls A, and B, and experimental groups C, D, and E (P < 0.05).

The Table 3 shows mean values of cells counted for the hypothalamus, and pituitary. Neurons of hypothalamus in the secondary control group, 100, 200 and 400 mg/kg body weight of kolaviron groups were significantly lower than in the primary control group (P < 0.05). The neurons of hypothalamus in 100, 400 and 800 mg/kg body weight kolaviron were significantly higher than the secondary control group (P > 0.05), while the 200 mg/kg body weight of kolaviron showed significantly high value than in the 200 mg/kg body weight group. The neurons of hypothalamus in 100, 200 and 400 mg/kg groups were significantly lower in neurons than the 100 mg/kg body weight group (P < 0.05), and 200 mg/kg body weight was significantly lower than the secondary control group (P < 0.05). The 200 and 400 mg/kg body weight groups were significantly lower in neurons than the 100 mg/kg body weight group (P < 0.05), and 800 mg/kg showed significantly high value than the 100 mg/kg body weight of kolaviron (P > 0.05). 400 and 800 mg/kg body weight of kolaviron showed significantly high value than in the 200 mg/kg body weight group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
<th>Body Weight Gain/Loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>156.7 ± 6.41</td>
<td>171.7 ± 7.71</td>
<td>15 ± 2.58</td>
</tr>
<tr>
<td>Group B</td>
<td>152.5 ± 4.79</td>
<td>153.3 ± 3.80</td>
<td>0.83 ± 3.01*</td>
</tr>
<tr>
<td>Group C</td>
<td>155.8 ± 1.537</td>
<td>164.2 ± 4.55</td>
<td>8.33 ± 3.58</td>
</tr>
<tr>
<td>Group D</td>
<td>175.8 ± 3.01</td>
<td>178.3 ± 2.47</td>
<td>2.5 ± 2.14*</td>
</tr>
<tr>
<td>Group E</td>
<td>177.5 ± 3.10</td>
<td>174.2 ± 10.68</td>
<td>−3.33 ± 2.47*</td>
</tr>
<tr>
<td>Group F</td>
<td>200 ± 1.83</td>
<td>201.7 ± 5.11</td>
<td>1.68 ± 3.33*</td>
</tr>
</tbody>
</table>

Values are mean ± Standard Error of Mean (SEM) of data from the animals. *Implies significant difference when compared to Group A; §Implies significant difference compared with Group B; δ Implies significant difference compared with Group C; Φ Implies significant difference compared with Group D; §Implies significant difference between Groups E and F. Significance was verified with ANOVA. Student Newman-Keuls (SNK) test was used for multiple comparisons. P < 0.05; N = 6.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Brain Weight (g)</th>
<th>Right Testis Weight (g)</th>
<th>Left Testis Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>1.25 ± 0.08</td>
<td>0.40 ± 0.08</td>
<td>0.41 ± 0.07</td>
</tr>
<tr>
<td>Group B</td>
<td>1.38 ± 0.04</td>
<td>0.53 ± 0.13</td>
<td>0.50 ± 0.13</td>
</tr>
<tr>
<td>Group C</td>
<td>1.30 ± 0.03</td>
<td>0.56 ± 0.08</td>
<td>0.55 ± 0.08</td>
</tr>
<tr>
<td>Group D</td>
<td>1.17 ± 0.02§</td>
<td>0.68 ± 0.04</td>
<td>0.67 ± 0.03</td>
</tr>
<tr>
<td>Group E</td>
<td>1.26 ± 0.03</td>
<td>0.57 ± 0.08</td>
<td>0.59 ± 0.10</td>
</tr>
<tr>
<td>Group F</td>
<td>1.04 ± 0.04*§δ</td>
<td>0.69 ± 0.01</td>
<td>0.68 ± 0.01</td>
</tr>
</tbody>
</table>

Values are mean ± Standard Error of Mean (SEM) of data from the animals. *Implies significant difference when compared to Group A; §Implies significant difference compared with Group B; δ Implies significant difference compared with Group C; Φ Implies significant difference compared with Group D; §Implies significant difference between Groups E and F. Significance was verified with ANOVA. SNK test was used for multiple comparisons. P < 0.05; N = 6.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hypothalamus (/10³ µm²)</th>
<th>Pituitary (/10³ µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neurons</td>
<td>Granule Cells</td>
</tr>
<tr>
<td>Group A</td>
<td>364 ± 0.58</td>
<td>103.7 ± 0.88</td>
</tr>
<tr>
<td>Group B</td>
<td>238.3 ± 0.88*</td>
<td>156.3 ± 0.89</td>
</tr>
<tr>
<td>Group C</td>
<td>332.3 ± 0.66*</td>
<td>179 ± 0.57*</td>
</tr>
<tr>
<td>Group D</td>
<td>214 ± 0.58*</td>
<td>141.7 ± 1.20*</td>
</tr>
<tr>
<td>Group E</td>
<td>282 ± 0.57*</td>
<td>126 ± 0.57*</td>
</tr>
<tr>
<td>Group F</td>
<td>363 ± 0.57*</td>
<td>132 ± 0.57*</td>
</tr>
</tbody>
</table>

Values are mean ± Standard Error of Mean (SEM) of data from the animals. *Implies significant difference when compared to Control (Group A); §Implies significant difference compared with Group B; δ Implies significant difference compared with Group C; Φ Implies significant difference compared with Group D; §Implies significant difference between Groups E and F. Significance was verified with ANOVA. SNK test was used for multiple comparisons. P < 0.05; N = 6.
(P > 0.05). 800 mg/kg showed significantly high value when compared to groups (P > 0.05). The quantity of acidophils in the pituitary gland in the secondary control group, as in 100, 200, 400 and 800 mg/kg body weight groups were significantly lower compared to primary control group (P < 0.05). The quantity of basophils in the pituitary gland in 400 and 800 mg/kg body weight groups were significantly lower than the primary control (P < 0.05), while the secondary control, 100 and 200 mg/kg body weight groups showed high significant compared to the primary control group (P > 0.05).

The quantity of granule cells of hypothalamus in the secondary control group and in all the treated groups were significantly higher compared to primary the control group (P > 0.05). The value in the 100 mg/kg body weight group was significantly higher compared to secondary control group (P > 0.05), while 200, 400, and 800 mg/kg body weight groups were significantly lower compared to corn oil group (P < 0.05). The 400 and 800 mg/kg group were significantly lower compared to other groups (P < 0.05).

3.2. Results of the Heamatoxylin and Eosin Staining (Figures 1-4)

Figure 1. Photomicrograph of hypothalamus. (A) Primary control group; (B) Secondary control group; (C) 100 mg/kg; (D) 200 mg/kg; (E) 400 mg/kg; and (F) 800 mg/kg body weight. Observe normal neurons (N) that looks like the granule cells with distinct blue staining in primary control (A) and other groups (B, C, D, E, and F). Also seen are vacuolations around the neurons which are more prominent in the group F and less in the secondary control group B. Heamatoxylin and Eosin stain. ×400.

Figure 2. Photomicrograph of Pituitary. (A) Primary control group; (B) Secondary control group; (C) 100 mg/kg; (D) 200 mg/kg; (E) 400 mg/kg; and (F) 800 mg/kg body weight. Primary control showed the presence of the basic pituitary cells. The acidophils (AC) and basophils (BA) are well stained, and conspicuous. Few chromophobes (CH) were seen. The connective tissue (CT) was intact. Notice a gradual decrease in the cells and connective tissues of pituitary across the treated groups (C, D, E, and F). Heamatoxylin and Eosin stain. ×400.
4. Discussion

Many beneficial effects of kolaviron, the bioflavonoid complex of G. kola have been reported [3][9]-[12]. These effects have been attributed to its anti-inflammatory, antioxidant as well as its antigenotoxic abilities [13][14]. Kolaviron has been demonstrated to be hepatoprotective against dimethyl nitrosamine-induced liver damage [13], tetra chloride-induced liver damage [15], and ethanol-induced oxidative stress in the liver [16]. Also kolaviron has been shown to be analgesic [17], antispasmodic and spasmylytic [18]. This complex of G. kola has also been implicated in neuroprotective role against gamma-radiation-induced brain injury [19], 3-nitro-propionic and methamphetamine-induced neurotoxicity [20][21]. It has also been shown to possess hypoglycaemic and hypolipidaemic effects in streptozotocin-induced diabetes [12]. Kolaviron has also been demonstrated to
effects. The prolonged duration and the abuse of the substance *G. kola* resulted in reproductive damage, but may not exhibit these beneficial effects when taken alone, especially for a gestation that involves testicular degeneration characterized by generalized erosion and necrosis of the germinal epithelium as well as reduction of Sertoli cells population in EGEE-induced reproductive toxicity. These effects have also been observed following consumption of diets containing high levels of condensed tannins or flavonoid oligomers [25], and *G. kola* is known to possess considerable amounts of tannins [26], and flavonoids [1] and [27], with kolaviron, the flavonoid complex being the predominant constituent of *G. kola*. Also, it has been observed that chronic feeding on *G. kola* diet for as long as 6 weeks produced histological changes in duodenal villous epithelium likely to adversely affect the absorption of nutrients from the gut [25]. Decreased body weight gain following *G. kola* extract administration has been associated with reduced feed consumption, though this decrease has also been observed with accompanied decrease in food intake [23]. The present study showed kolaviron did not significantly affect testicular weight in treated animals. [23] Has also observed a similar result that *G. kola* extract did not alter testicular weight, though [28], observed a significant increase in testicular weight following administration of *G. kola* extract but with no accompanied effect on sperm count.

*G. kola* nut have been used as aphrodisiacs by the natives [29], and has been reported to possess sexual enhancing characteristics on male rats as evidenced by increased mounting, and intromission frequencies behaviours with increased number of subsequent ejaculations [28]. These behaviours have been indicated to be a measure of both libido and potency [28].

Studies on the effect of administration of *G. kola* or kolaviron only on microscopic anatomy of the brain and pituitary glands are scarce. Though Uko [23] no microscopic alterations in brains of rats were treated with extract of *G. kola*. In the present study, kolaviron was found to alter the histology of the hypothalamus, pituitary and testis of the male adult *Wistar* rats. The sections from the hypothalamus of rats administered with 400 mg/kg and 800 mg/kg per body of kolaviron showed hypertrophy of the neuronal fibres and atrophy of the granular cells. There was also an increase in the neurite plaque. Similar histological alterations were also observed with the sections of the pituitary of the male adult *Wistar* rats. The 400 mg/kg and 800 mg/kg per body weight of kolaviron also showed degeneration of the connective tissues and pituitary cells (acidophils and basophils) and other giant cells which were the secretory cell of the organ. Overall, there was little or not much effect of kolaviron in the brain (hypothalamus and pituitary), and this may probably be because of its neuroprotective effects as documented by Nwoha [20] [21]. However, the histology of the testes showed much more observable alternations than the pituitary and the hypothalamus. High dose of the kolaviron resulted in the degeneration of the cells in the tubules and the interstitial cells. [30] had earlier reported that prolonged administration of aqueous extract of *G. kola* resulted in degeneration of testicular cells in rabbit testes. Also [24] observed hypoplastic and degenerating seminiferous tubules with disorganized epithelial cells in testes of dogs fed with *G. kola* extract. In contrast, [31] reported that treatment with *G. kola* extract showed improvement in structural integrity of the seminiferous tubules, germinal epithelium and interstitial spaces in testes of rats following Lead II oxide induced injury. [32] has reported that kolaviron improved marked degeneration of the testes observed following reproductive toxicity in sub-lethally whole body gamma-irradiated rats. [33] also reported that kolaviron improved severe testicular degeneration characterized by generation generalized erosion and necrosis of the germinal epithelium as well as reduction of Sertoli cells population in EGEE-induced reproductive toxicity. These effects again suggest that *G. kola* and/or kolaviron may improve reproductive health in cases of complications or diseases resulting in reproductive damage, but may not exhibit these beneficial effects when taken alone, especially for a prolonged duration and the abuse of the substance *G. kola* should be avoided irrespective of many beneficial effects.

5. Conclusion

In conclusion, this study has been able to show that kolaviron at 800 mg/kg body weight alters the microanatomy of the hypothalamus, pituitary and testes thereby affecting the hypothalamic-pituitary-gonadal axis. This histological alteration suggests that kolaviron at high dose may impair reproductive functions in the male adult.
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References


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