A CONTRASTIVE EXAMINATION OF ADRENALECTOMY ON THE 
HEPATIC AND REPRODUCTIVE TISSUE OF THE MALE AND 
FEMALE ALBINO RATS

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Abstract

To evaluate the effect of quassin on female reproductive functions, 42 albino rats (35 females and 7 males) were used. The female albino rats were divided into seven groups of five rats each. Group I served as the control group and received distilled water while Groups II, III and IV rats were treated orally with 0.1 mg/kg, 1.0 mg/kg and 2.0 mg/kg body weight of quassin for 60 days respectively. Groups V, VI and VII rats were also treated orally with 0.1 mg/kg, 1.0 mg/kg and 2.0 mg/kg body weight of quassin for 60 days but were left untreated for another 30 days, to serve as the recovery groups. At the end of each experimental period, blood samples were collected from each rat. Fertility study was done by cohabiting one untreated male with the five female rats in each group for 10 days. Quassin did not adversely affect the weight of the kidney, heart, liver and the body of the rats. However, there was a significant decrease (P < 0.05) in the weight of the ovary and uterus in all the groups relative to the control. There was also a significant decrease (P < 0.05) in serum estrogen levels in quassin treated rats. The quassin treated rats had a significantly decreased (P < 0.05) mean litter number and weight. Histological studies show a disorganization and degeneration in the ovary while the uterus showed signs of vacuolation and disorganization. However, these effects were ameliorated after quassin was withdrawn from the rats. The results suggest that quassin has female anti-fertility properties, possibly acting via inhibition of estrogen secretion.

Keywords: Quassin, Female rat, Reproduction, Estrogen

Introduction

Plants all over the world have been used for the treatment of various human diseases since antiquity. They are still in use up till now in developing countries in form of folkloric or traditional medicines. Through experience, a lot of discoveries were made that tropical forest can provide many products apart from drugs, but also narcotics, hallucinogens and stimulants among many other products that make life more meaningful in remote areas (Soedigdo, 1980). Many plant species, half of them from tropical forest have been found to contain anti-fertility compounds. About 370 have been shown to provide assurance for safer and effective contraceptives suitable for both males and females (Maurga et al., 2004). Also, more than 600 medicinal plant species are believed to have potential abortifacient property; a good number of them from tropical forests (WHO, 1981).

Many of the herbs that are used medically have a traditional reputation for the purpose they are used for, but there is little or no scientific or medical documentation in respect of their active constituents, pharmacological actions or clinical efficacy. Relatively few herbal ingredients have
been subjected to rigorous scientific study with their pharmacological activities and active principles successfully investigated (Newell et al., 1996).

Many plant extracts have been reported to affect fertility in rodents. Gebri et al., [2005] reported that methanolic extract of Rumex steudelii decreased the number of implantation sites significantly. It was also showed that the extract of this plant did not affect the serum estrogen-progesterone ratio (Gebri et al., 2005). Nivsarkar et al. (2005) showed that Hibiscus rosa–sinensis flowers has anti fertility, abortifacient activity and exhibits anti-estrogenic activity as judged by increase in uterine weight. Kularni et al., (2005) reported that the alcoholic extract of lemon seeds exerted reversible anti-fertility effect in female mice by virtue of its anti-zygotic action. Maca (lepodiummeyeni) root had been acknowledged by Peruvians to improve sexuality and fertility (Gonzales et al., 2004). The methanol extract of Ricinus communis seed was also found to prevent implantation and when implantation occurred, it induced abortion in female guinea pigs (Makonnel et al., 1999).

Quassia amara belongs to the plant family of Simaroubaceae and is naturally distributed in several tropical countries. The ethnobotany database lists Amargo, Bitter wood, Quassia, Cuassia, Guabo, Hombre grande, Jamaica bark, palomuneco, pauamarelo, pauquassia, Quassia de caiena, Quassia amarga, Quassia wood, Surinam wood, and Wewegifi as other common names for this plant. Traditionally the bark and leaves are used in herbal remedies since they are rich in biologically active principles. Quassia amara also has antileukemic, antineoplastic, and anti-tumorous property (Kupchan and Streelman, 1976; Considine et al., 1983). The herb also has a prominent effect on the digestive system as an aperitif, astrangent, antpiulcerogenic, stomachic, antihelmintic, and laxative agent (Grieve, 1992). It has also been used as insecticide, larvicide, pediculicide, and vermifuge (Jenson, 1979; Park et al., 1987). Antimalarial activity of the plant extract has been carried out in mice in vivo (Ajaiyeoba et al., 1999). Several phytochemicals have been isolated from the bark of Quassia amara that can be broadly classified as quassinoids (Dou et al., 1996; Kitagawa et al., 1996).

Njar et al., (1995) studied the effect of Quassia amara L on the steriodogenesis in rat Leydig cells in an in vitro system and the result showed that the extract inhibited both basal and luteinizing hormone (LH) stimulated testosterone secretion from rat Leydig cells. Quassin and the alkaloid, 2-methoxycanthin- 6- one was isolated from the Quassia extract according to Njar et al., (1993). Raji and Bolarinwa, (1997) have also studied the anti-fertility activity of Quassia amara L in male rats. The crude methanol extract, quassin and the alkaloid were used and it was concluded that quassin appears to be the anti-fertility principle of Quassia amara L.

Although, there were many reports on the effects of quassin on the male reproductive functions; its effects on the female reproductive functions have not been reported. The present study was therefore designed to investigate the impact of quassin on the female reproductive functions such as fertility, serum estrogen levels and histology of the ovaries and uterus.

**Materials And Methods**

Extraction and purification of plant material: Stem bark of Quassia amara was collected at the botanical garden, University of Ibadan, Nigeria. A voucher specimen was deposited at the Forestry Research Institute of Nigeria (FRIN) herbarium, Ibadan. The stem bark was air-dried and pulverized with blender to obtain 1kg of the plant materials. This was carried out as described by Njar et al., 1993. The pulverized stem bark (1kg) was exhaustively extracted with methanol by means of Soxhlet apparatus and the extract
evaporated in vacuo. Water was added to the residue and the mixture extracted with hexane and then with CHCl3. The CHCl3 extract was dried using (anhydrous magnesium sulphate (MgSO4) and evaporated to give a residue (3.5g) called quassionoid. The residue (3.3g) was chromatographed on a silica gel column as previously described (Njar et al., 1993) to yield quassin.

Animals and treatments: Experiments were performed on 42 rats (35 females and 7 males) whose initial average weight ranged between 150g and 170g. They were obtained from the Animal House, College of Medicine, University of Ibadan, Oyo State, Nigeria. The animals were housed in wire mesh cages in the Central Animal House, College of Medicine, University of Ibadan and maintained in a well-ventilated room with a 12:12-hour light-dark at room temperature. Food and water were provided ad libitum. The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals. The rats were treated with varying doses of quassin dissolved in distilled water for 60 days (i.e. 0.1, 1.0 and 2.0mg/kg body weight of quassin (Raji and Bolarinwa, 1997). The female rats were divided into seven groups of five rats each. Group I served as the control group and were given 0.2mL of distilled water as solvent (vehicle) for dissolving the quassin. Groups II, III and IV rats were treated orally with 0.1mg/kg (low dose), 1.0 mg/kg (medium dose) and 2.0 mg/kg (high dose) body weight of quassin for 60 days respectively. Groups V, VI and VII rats were also treated orally with 0.1 mg/kg, 1.0mg/kg and 2.0 mg/kg body weight of quassin for 60 days but were left untreated for another 30 days.

Fertility studies: The experimental female rats were cohabited with untreated male in ratio 5:1 for a minimum of 10 days (Long and Evans, 1922). The presence of vagina plug was taken as indicator for positive mating. It was taken as the first day of pregnancy. Fertility test was calculated with the following formula as earlier reported (Raji et al., 2006).

\[
\text{% Fertility Success} = \left( \frac{\text{No. of Pregnant Female \times 100}}{\text{No. of Mated Female}} \right)
\]

The rats were allowed to give birth after pregnancy and the number and weight of the litter of each rat were recorded using a sensitive electronic balance made in England, the model is DT-1000 A with capacity of 0.1-1000g.

Blood sample collection: Blood (2ml) was collected from each animal via the retro-orbital sinus with 70μl heparinized capillary tube (Ezzai, 1995) and put into plain sample bottle for estrogen analysis. The sample was centrifuged at 3000 rpm for five minutes. The serum was used to analyze the level of estrogen.

Organ collection: After parturition, the animals were killed by cervical dislocation. The ovaries, uterus heart, kidney and liver were removed and cleared of adherent tissues before they were weighed immediately with an electronic weighing balance, model DT 1000 England with a capacity of 0.1 to 1000g.

Estrogen assay procedure: Blood samples were collected from the animals at their estrous phase and an enzyme –based immunoassay (EIA) system was used to measure estrogen level in serum samples collected. The EIA kit was obtained from Immunometrics (London, UK) and contained an estrogen EIA enzyme label, estrogen EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation.

Histological study: Ovary and uterus of the control and treated rats were fixed in Bouin's fluid for 6 hours before they were transferred
into 10% formalin for histological evaluation. The tissues were routinely processed and examined under the light microscope. Photomicrograph of the slide was then taken.

Statistical analysis: The results are presented as Means ± SEM for each group. Differences among groups were analyzed using one-way analysis of variance (ANOVA). P<0.05 was accepted as significant.

**Results**

Effect of quassin on body weight of female albino rats

There was no significant difference in the mean body weight of all the groups, before and throughout the treatment period when compared with the control group. However, there was a weight gain of 7.4%, 8.5%, 9.6%, 9.7%, 8.9%, 8.9%, 8.3% and 7.2% in the control, low dose, medium dose, high dose, and their respective recovery groups, when the weight after treatment (8th week) was compared with the weight before treatment.

Effect of quassin on organ weight of female albino rats

There was significant decrease (P< 0.05) in the mean weight of ovary of rats treated with 0.1, 1.0 and 2.0 mg/kg body weight of quassin when compared with the control group but there was insignificant decrease in the mean weight of ovary in their respective recovery groups when compared with the control as shown in table 2. There was significant decrease (P < 0.05) in the mean weight of uterus in 0.1, 1.0, 2.0 mg /kg treated and 2.0 mg /kg recovery group. There was insignificant increase in the mean weight of the uterus in the recovery group for 0.1 and 1.0 mg/kg body weight of quassin when compared with the control group as shown in table 2.

**Table 1:**

Mean body weight of female rats treated with quassin.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Before (g)</th>
<th>1st Week (g)</th>
<th>6th Week (g)</th>
<th>7th Week (g)</th>
<th>8th Week (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control (Vehicle)</td>
<td>168.2 ± 2.43</td>
<td>172.6 ± 2.52</td>
<td>179.8 ± 3.53</td>
<td>180.2 ± 3.57</td>
<td>180.7 ± 3.43</td>
</tr>
<tr>
<td>II. Low dose (0.1mg/kg)</td>
<td>153.2 ± 3.84</td>
<td>156.6 ± 4.29</td>
<td>163.0 ± 5.12</td>
<td>164.8 ± 5.38</td>
<td>166.2 ± 4.76</td>
</tr>
<tr>
<td>III. Medium dose (1mg/kg)</td>
<td>160.2 ± 3.09</td>
<td>162.6 ± 3.44</td>
<td>173.0 ± 4.42</td>
<td>174.4 ± 4.42</td>
<td>175.6 ± 4.32</td>
</tr>
<tr>
<td>IV. High dose (2mg/kg)</td>
<td>161.3 ± 3.04</td>
<td>163.4 ± 3.22</td>
<td>173.6 ± 2.94</td>
<td>175.2 ± 3.02</td>
<td>177.0 ± 3.15</td>
</tr>
<tr>
<td>V. Low dose recovery</td>
<td>151.5 ± 4.61</td>
<td>154.8 ± 4.92</td>
<td>162.4 ± 4.63</td>
<td>164.0 ± 4.85</td>
<td>165.0 ± 5.19</td>
</tr>
<tr>
<td>VI. Medium dose recovery</td>
<td>152.3 ± 5.65</td>
<td>154.6 ± 6.59</td>
<td>161.2 ± 7.56</td>
<td>162.6 ± 7.58</td>
<td>165.0 ± 7.40</td>
</tr>
<tr>
<td>VII. High dose. Recovery</td>
<td>155.2 ± 2.65</td>
<td>157.2 ± 2.78</td>
<td>164.4 ± 2.69</td>
<td>164.8 ± 2.35</td>
<td>166.4 ± 2.48</td>
</tr>
</tbody>
</table>
Table 2:
Mean organ weight of female albino rats treated with quassin

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Ovary (g)</th>
<th>Uterus (g)</th>
<th>Heart (g)</th>
<th>Kidney (g)</th>
<th>Liver (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control(Vehicle)</td>
<td>0.124 ± 0.008³</td>
<td>0.52 ± 0.04³</td>
<td>0.62 ± 0.02³</td>
<td>0.67 ± 0.04³</td>
<td>6.68 ± 0.15³</td>
</tr>
<tr>
<td>II. Low dose(0.1mg/kg)</td>
<td>0.082 ± 0.002³</td>
<td>0.42 ± 0.06³</td>
<td>0.56 ± 0.02³</td>
<td>0.59 ± 0.02³</td>
<td>5.52 ± 0.23³</td>
</tr>
<tr>
<td>III. Medium dose(1mg/kg)</td>
<td>0.060 ± 0.002³</td>
<td>0.44 ± 0.02³</td>
<td>0.50 ± 0.04³</td>
<td>0.70 ± 0.03³</td>
<td>5.92 ± 0.51³</td>
</tr>
<tr>
<td>IV. High dose(2mg/kg)</td>
<td>0.060 ± 0.002³</td>
<td>0.44 ± 0.02³</td>
<td>0.58 ± 0.04³</td>
<td>0.65 ± 0.05³</td>
<td>5.74 ± 0.19³</td>
</tr>
<tr>
<td>V. Low dose recovery</td>
<td>0.102 ± 0.004³</td>
<td>0.55 ± 0.04³</td>
<td>0.66 ± 0.02³</td>
<td>0.57 ± 0.04³</td>
<td>7.52 ± 0.63³</td>
</tr>
<tr>
<td>VI. Medium dose recovery</td>
<td>0.104 ± 0.004³</td>
<td>0.54 ± 0.03³</td>
<td>0.70 ± 0.04³</td>
<td>0.70 ± 0.02³</td>
<td>9.65 ± 0.62³</td>
</tr>
<tr>
<td>VII. High dose recovery</td>
<td>0.122 ± 0.001³</td>
<td>0.41 ± 0.03³</td>
<td>0.78 ± 0.02³</td>
<td>0.59 ± 0.03³</td>
<td>7.64 ± 0.08³</td>
</tr>
</tbody>
</table>

Table 3:
Mean value of fertility test of female rats treated with quassin.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Litter size</th>
<th>Litter weight</th>
<th>Positive mating %</th>
<th>Fertility%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control(Vehicle)</td>
<td>6.0 ± 1.05³</td>
<td>5.64 ± 0.26³</td>
<td>100³</td>
<td>100³</td>
</tr>
<tr>
<td>II. Low dose(0.1mg/kg)</td>
<td>4.6 ± 0.81³</td>
<td>4.52 ± 0.22³</td>
<td>100³</td>
<td>100³</td>
</tr>
<tr>
<td>III. Medium dose(1mg/kg)</td>
<td>3.5 ± 1.01³</td>
<td>4.61 ± 0.29³</td>
<td>100³</td>
<td>100³</td>
</tr>
<tr>
<td>IV. High dose(2mg/kg)</td>
<td>4.0 ± 0.24³</td>
<td>4.55 ± 0.26³</td>
<td>100³</td>
<td>100³</td>
</tr>
<tr>
<td>V. Low dose recovery</td>
<td>5.8 ± 0.38³</td>
<td>5.60 ± 0.40³</td>
<td>100³</td>
<td>100³</td>
</tr>
<tr>
<td>VI. Medium dose recovery</td>
<td>5.8 ± 0.52³</td>
<td>5.53 ± 0.23³</td>
<td>100³</td>
<td>100³</td>
</tr>
<tr>
<td>VII. High dose recovery</td>
<td>5.0 ± 0.49³</td>
<td>5.00 ± 0.14³</td>
<td>100³</td>
<td>100³</td>
</tr>
</tbody>
</table>

There was significant increase (P < 0.05) in the mean heart weight for 1.0 and 2.0 mg /kg body weight of quassin treated rats and there was also significant increase in their respective recovery groups when compared with the control. The 0.1 mg/kg body weight of quassin treated group showed a significant decrease (P < 0.05) and a significant increase (P < 0.05) in its recovery group when compared with the control as shown in table 2.

There was significant decrease (P< 0.05) in all the treated groups when compared with the control but their respective recovery group showed significant increase (P<0.05) when compared with the control as shown in table 2.

Effect of quassin on fertility of female albino rats

There was 100% positive mating in all the groups. This was confirmed by the presence of vaginal plug of the female rats, a day after cohabitation. There was significant decrease (P < 0.05) in the mean litter size in all the treated
groups when compared with the control while their respective recovery group showed an insignificant reduction ($P > 0.05$) when compared with the control as shown in table 3.

There was significant decrease ($P < 0.05$) in the mean litter weight in all the treated groups when compared with the control while their respective recovery group showed an insignificant reduction ($P > 0.05$) when compared with the control as shown in table 3. There was 100% fertility for the control and treated rats except for 2.0mg/kg body weight that had a significant decrease ($p<0.05$) as shown in table 3.

Effects of quassin on the histology of the ovary and the uterus of female albino rats.

The effects of quassin on the histology of the ovary (Fig.1) and uterus (Fig. 2) in female albino rats

Discussion

The results obtained from this study showed that quassin caused adverse effects on fertility in female rats, sufficient to cause reversible infertility. Female reproduction is functionally controlled by normal estrogen level which usually peaks during the estrous phase of the cycle. This gives an indication that quassin may act on the ovary (the source of estrogen) through altered endocrine functions associated with decreased estrogen level. The result of this study showed that the serum estrogen levels in all treated rats decreased significantly relative to the control.

The present study reports for the first time the effect of quassin on estrogen secretion. Previous studies have focused on the effect of quassin in the male. Evidence in support of the present findings could therefore be inferred only from the male studies. Njar et al., (1995) reported that quassin inhibited testosterone secretion in male rats, thereby causing infertility. The work of Raji and Bolarinwa, (1997) also confirmed this finding, suggesting that quassin inhibited testosterone in the Leydig cell. Thus, impairment in the production of testosterone which is a pre-hormone for estrogen is probably an indirect impairment on the production of estrogen.

The significant structural alterations in the histological sections of the ovary and uterus in quassin treated rats compared with the control further support the possible deleterious impact of quassin on female reproduction. Quassin induced degeneration of the follicular wall, may be responsible in part for the significant decrease in serum estrogen levels. This probably led to the anovulatory cycles and the consequent decrease in litter number and litter weight. The fetal morphology was not adversely affected which indicate that quassin might not affect fetal development if administered before conception (Garcia et al., 1997). Lucidi et al., (2003) suggested that steroidogenesis could be influenced by active development of the oocyte. It follows then that the atretic follicles in the histological sections could be due to a reduction in estrogen level. The hormonal and histological changes could lead to the significant reduction in the ovarian and uterine weights. Noteworthy was a slight increase in the serum level of estrogen in the recovery groups, following the withdrawal of quassin.
(b) 0.1mg/kg treated rat showing numerous developing follicles and corpora present, (Mag X 250 Hand E) (c) 1.0mg/kg treated rats showing prominent stroma and no developing follicle, (Mag X 250 Hand E) (d) 2.0mg/kg treated rats showing few follicle, (Mag X 250 Hand E) (e) 0.1mg/kg recovery group showing no lesion, (Mag X 250 Hand E) (f) 1.0mg/kg recovery group showing no lesion (Mag X 250 Hand E) (g) 2.0mg/kg recovery group showing large corpora albicans completely followed by parenchyma, (Mag X 250 Hand E)

Figure 2 Photomicrograph of uterus of (a) control rat with no lesion observed,(Mag X 250 Hand E) (b) 0.1mg/kg treated rat showing stratified squamous epithelium cells seen ,(Mag X 250 Hand E) (c) 1.0mg/kg treated rats showing swollen squamous epithelial cells seen,(Mag X 250 Hand E)(d)2.0mg/kg treated rats showing swollen squamous epithelial cells seen,(Mag X 250 Hand E) (e) 0.1mg/kg recovery group showing no lesion observed,(Mag X 250 Hand E)(f) 1.0mg/kg recovery group showing no lesion observed,(Mag X 250 Hand E) (g) 2.0mg/kg recovery group showing no lesion observed,(Mag X 250 Hand E)

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