SEX HORMONES AND BIOCHEMICAL PROFILES OF MALE GOSSYPOL USERS IN SOUTH-WESTERN NIGERIA

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Summary: The present study was designed to evaluate the effects of gossypol administration on sex hormones and biochemical parameters of male subjects. Twelve male subjects receiving 20mg daily gossypol at the family planning clinic of University College Hospital, Ibadan were studied. Blood samples collected from the subjects before, at 16 weeks and 28 weeks of treatment with gossypol were used to determine the blood levels of sex hormones and biochemical parameters. There were significant reduction in spermatozoa count (P<0.01), motility (P<0.01) and testosterone concentration (P<0.01) but elevated concentrations of luteinizing hormone (LH) (P<0.05) and follicle stimulating hormone (FSH) (P<0.01) following treatment with gossypol. However, the seminal fluid volume was unchanged (P>0.05). Serum concentrations of sodium, potassium, transaminases, and alkaline phosphatases during the period of treatment showed a significant downward trend (P<0.05 in each case). Whereas there was no consistent pattern in the serum concentrations of bilirubin, total protein and albumin. The findings of the present study suggest that gossypol is a potent male antifertility agent with capability of causing organ impairment.

Key Words: Gossypol, Male, Anti-fertility, Biochemical, Hormones, Contraception.

Introduction:
The need to reduce the global population at the rate at which global economic growth can sustain has led to the use of different contraceptive methods (King 1990). Much emphasis has been laid on female methods (Population report 1986, Sathuray 1988, Hug and Cleland 1989), rather than on male methods of contraception. Successes at developing male contraceptive methods have been restricted by the attendant harmful side effects of the contraceptive agents (WHO 1990, Vickery et al 1986). However, the use of gossypol (1,1', 6,6', 7,7'-hexahydroxy 5, 5' di-isopropyl-3, 3'-dimethyl[2,2'-binaphthalene]-8,8'-dicarboxaldehyde) as a potent male antifertility agent was first reported in China (NCGMAA 1978, Liu et al 1987, Waites et al 1998, Yu and Chan 1998). Since then several studies have been reported on the toxicity and anti-spermatogenic ability of gossypol on animal species (Xue et al 1980, Xue 1981), with limited studies in humans (Elsimar et al 1984, Elsimar and Jose 1988). Therefore, the present study was designed to evaluate the effects of gossypol on the gonads and some biochemical parameters.

Materials and Methods:
Subjects:
Twelve apparently healthy male subjects of proven fertility (married with children) between the ages of 38 and 55 years undergoing gossypol treatment in family planning clinic of University College Hospital Ibadan, were recruited as ‘test subjects’ and 10 age-matched apparently healthy male subjects of proven fertility without gossypol treatment served as ‘control subjects’ for the study. Selection for the study was based on sperm count greater than 20 million/ml on two different occasions of two weeks intervals. The blood chemistry parameters (transaminases, alkaline phosphatases, bilirubin, total protein, albumin, electrolytes) were carried out in the ‘test subjects’ before, at 16 and 28 weeks of treatment with gossypol. Whereas the blood level of sex hormones (testosterone, LH, FSH) and seminal fluid analysis (spermatozoa count, motility and seminal fluid volume) was
determined at the on-set of azoospermia in the gossypol treated subjects. However only a point blood sample was collected from control subjects for the analysis of sex hormones and seminal fluid. The subjects were counseled before the commencement of the study and were properly briefed about the procedures and aim of the study. Ethical approval was given for the study and the subjects gave informed consent.

**Drug treatment:**

Oral dosage of 20mg gossypol was administered to the 'test subjects' daily until the on-set of azoospermia/aegrooospermia. The drug was usually given to the test subjects at the clinic to ensure compliance.

**Methods:**

**Determination of plasma testosterone concentration:**

This was performed by double antibody technique. The principle depends on the ability of an antibody to bind its antigen. The technique was carried out using Immunochem 125 I double antibody testosterone RIA test kit. The procedure of the test is briefly described as given by the manufacturer of the test kit. In the assay, a limited amount of testosterone specific antibody was reacted with the corresponding radiolabelled testosterone. Upon the addition of an increasing amount of the testosterone in the serum sample, a correspondingly decreasing fraction of the labeled testosterone added is bound to the antibody. The bound labeled testosterone is then separated from the free labeled testosterone and the amount of radioactivity of the bound labeled testosterone in the samples and the standards are measured and the results of the standards are used to plot a standard graph of testosterone concentrations on a logit-log paper. The values of the testosterone in the plasma samples are then extrapolated from the testosterone standard curve.

**Determination of the Gonadotrophins-Luteinizing hormone (LH) and Follicle Stimulating hormone (FSH):**

This was determined in a simultaneous manner in a single tube using Becton Dickinson Simultrac LH/FSH radioimmunoassay test kit. The principle is based on the competition between the labeled gonadotrophins (125 I) and unlabelled gonadotrophins (LH & FSH) for the limited number of available respective antibody binding sites. In this reaction the level of radioactivity bound is inversely related to the concentration of the LH or FSH respectively in the samples. The procedure of assay is as described by the manufacturer. In brief, into appropriately labeled test tubes containing specified volumes of either the samples or the LH & FSH standards, was added limited amount of LH/FSH antisera and after incubation a limited amount of the tracers 125 I LH and 125 I FSH were added and after further incubation for one hour at room temperature the bound and unbound components were separated and the radioactivity of the bound fractions were counted using the gamma counter. A standard curve of LH and FSH was drawn on a separate logit-log paper from which the values of the respective sample tests were obtained respectively.

**Determination of other biochemical parameters.**

Determination of sodium and potassium was by flame photometer using Corning Clinical Photometer 410C with automatic aspirator. The concentrations of sodium and potassium were measured at wavelengths of 589nm and 768nm respectively. Alkaline phosphatase activity was determined by optimized spectrophotometric method as described by REC.GSCC (1972) and the transaminase enzymes activities were determined by method of Reitman and Frankel (1957). Serum total protein and albumin concentrations were measured by the Biuret method of Kingley (1942) and Bromocresol green method of Doumas and Watson (1971). Serum bilirubin concentration was determined by the Jendrassik and Grof method (1938). Seminal fluid analysis was performed as described in WHO manual (1992).

**Statistical analysis**

The parametric variables were compared using the Student's t-test while Chi-square (X²) was used to compare the non-parametric variables. The significant level was considered at P<0.05.

**Results**

A significant downward trend in serum sodium concentration (mmol/l) was observed by week 16 (P<0.05) and week 28 (P<0.05) of gossypol administration compared with the value before treatment (Table 1). Similarly, a downward trend in serum potassium concentration (mmol/l) was observed at week 16 (P<0.05) and week 28.
Fig 1: Serum enzymes, protein, bilirubin, sodium and potassium concentration before and during gossypol administration in male subjects (* P<0.05 compared with week 0).

(P<0.05) of gossypol administration compared with the value before treatment. Figure 1. In addition, serum aspartate and alanine transaminases (AST and ALT) activities (ui/l) were significantly reduced at week 16 (P<0.05) and week 28 (P<0.05) of gossypol treatment compared with their respective values before gossypol treatment. Similarly total protein and
albumin concentrations (g/l) were significantly reduced while serum bilirubin concentration (µmol/l) was significantly raised at week 16 (P<0.05 in each case) and week 28 (P<0.05 in each case) of gossypol treatment compared with their respective values before administration of gossypol. However, serum alkaline phosphatase (ui/l) activity was only significantly reduced by 28 week of gossypol administration compared with value before initiation of treatment (figure 1). Furthermore, serum activities (ui/l) of the transaminase enzymes were reduced significantly by week 28 of gossypol administration compared with their respective values by week 16 of treatment (P<0.05 in each case). On the other hand serum concentrations of bilirubin (µmol/l), protein (g/l) and albumin (g/l) were significantly raised by week 28 of gossypol administration compared with their respective values at week 16 of treatment (P<0.05 in each case).

The mean serum concentration of FSH in gossypol treatment subjects was significantly raised at onset of azospermia compared with the level in control subjects (18.3 ± 7.7 vs 11.2 ± 3.0 mIU/ml; P<0.05) (Table 1). In addition, the mean plasma LH in gossypol treatment subjects was significantly higher at the onset of azospermia (17.0 ± 5.3) compared with the value in control subjects (11.8 ± 1.9) mIU/ml; P< 0.05). However, the mean plasma testosterone concentration in gossypol treatment subjects was significantly reduced at the onset of azospermia (2.5 ± 0.5) compared with the value in the control subjects (5.8 ± 2.1) ng/ml; P<0.05 (Table 1).

The seminal fluid volumes in gossypol treatment subjects (2.6±1.3) was not significantly different from the value in the control subjects (3.2±1.0) milliliters, (P>0.05), while the spermatozoa count in the gossypol treated subjects (3.4±4.7) was significantly reduced compared with the value observed in the control subjects (53.4±13) x10⁶ per milliliter, p<0.01. In addition, the motility of the spermatozoa was significantly retarded in gossypol treatment subjects (28±16) compared with the value observed in the control subjects (86±6)% P<0.01. (Table 2).

Table 1: Mean (±1sd) plasma concentrations of gonadotrophins (iu/ml) and testosterone (ng/ml) in gossypol treatment and control subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>FSH (iu/ml)</th>
<th>LH (iu/ml)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gossypol treatment subjects (n=10)</em></td>
<td>18.3±7.7</td>
<td>17.0±5.3</td>
<td>2.5±0.5</td>
</tr>
<tr>
<td>Control subjects (n=10)</td>
<td>11.2±3.0</td>
<td>11.8±1.9</td>
<td>5.8±2.1</td>
</tr>
<tr>
<td>p-value</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

- 20-24 week of 20mg gossypol administration.

(±1sd) = plus/ minus 1 standard deviation

Table 2: Mean (±1sd) seminal fluid volume (ml), spermatozoa count/ml, and percentage motility (%) in gossypol treatment and control subjects.

Discussion
The antifertility action and side effects of gossypol has continued to be a research focus since the report of the discovery of this agent. In the present study, there is a strong indication that gossypol administration possesses antifertility properties as well as noticeable side effects. The downward trend in the serum concentrations of sodium and potassium in subjects administered gossypol may suggest effect of gossypol on electrolytes balance. In one of the subjects, the serum level of potassium was 2.6 mmol/l, though hypokalaemic paralysis was not observed. It was first reported in studies in China that gossypol do have some possible side effects (NCGMAA 1978, Elsimar et al 1984, Elsimar and Josc 1988) and from the present observation, it is suggested that gossypol effect may be on the electrolyte–regulating organ of the human system, possibly the kidney.
Table 2: Mean (±1sd) seminal fluid volume (ml), spermatozoa count/ml, and percentage motility (%) in gossypol treatment and control subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Spermatozoa (x10^6)</th>
<th>Seminal fluid volume (ml)</th>
<th>Percentage motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gossypol treatment subjects</td>
<td>3.4±4.7</td>
<td>2.6±1.3</td>
<td>28±16</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>53.4±13</td>
<td>3.2±1.0</td>
<td>86±6</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>P&lt;0.01</td>
<td>p&gt;0.05)</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

- 24 week of 20mg gossypol administration and 4 subjects presented with complete necrospermia.

(±1sd) = plus/ minus 1 standard deviation

The drop in serum transaminases and alkaline phosphatase activities was persistent in the subjects administered gossypol. This drop in activity is likely due to inactivating or inhibiting actions of gossypol on the enzymes. In fact, studies have reported accumulation of gossypol in the liver (NCGMAA 1978) and the extent of accumulation in the liver has been shown to correlate with toxicity (Frick et al 1988, Nair and Bhigwade 1990, Coutinho et al 2000). Hence gossypol may have accumulated in the liver of the subjects used in this study while exerting its effect by reducing liver enzymes activities. The above reason may also be responsible for the raised serum total bilirubin levels possibly due to it effect on the conjugating enzymes in the liver.

In the long term there seem to be recovery of the body's ability to regulate serum concentrations of total serum protein and albumin in subjects administered gossypol. These finding seem to suggest that the action of gossypol may have a defined or selective biochemical pathway, thereby affecting some organs that share similar pathways but not those that have different pathways.

Similarly, subjects administered gossypol showed abnormal sex hormones, azoospermia and necrospermia but the seminal fluid volume was unaffected. The ability to induce azoospermia/necrospermia might have been achieved by possible direct action of gossypol on the male reproductive organs. There seem to be intact hypothalamus-pituitary axis functioning in subjects administered gossypol but possible disruption of the negative feedback mechanism between the hypothalamus-pituitary axis and the gonad may exist. This assertion is drawn from the observed values of the gonadotrophins (LH& FSH) and testosterone in the subjects administered gossypol in the present study. The reduced serum concentration of testosterone in subjects administered gossypol may be a reflection of the effect of gossypol on testosterone synthesizing cells of the gonads. While the elevated serum gonadotrophins (LH& FSH) levels might have been due to suppression or failure of the gonads function.

Gossypol has been shown to inhibit follicle-stimulating hormone (FSH) and luteinizing hormone (LH) Gu et al 2000, Udoh et al 1992, Thomas et al 1991, Kolena et al 2001, Zhong et al 1990). Similarly, testicular architecture was reported distorted in gossypol users, while exfoliation and damage of the Leydig and Sertoli cells have been shown in males administered gossypol (Udoh et al 1992, Thomas et al 1991, Lan et al 1992, Nair and Bhigwade 1990, Gu et al 1990). These reports and the present observation in subjects administered gossypol strongly suggests the potentials of gossypol to distort the regulation of the gonads functions. Therefore, the finding of the present study in Nigerian male subjects administered gossypol reveal that gossypol is a potent male antifertility agent that have possible direct action on some organs and thus impairing their functions.

References


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