SERUM TESTOSTERONE LEVELS IN NIGERIAN MALE MARIJUANA AND CIGARETTE SMOKERS.

R.E. OKOSUN*; H.B.OSADOLOR2; O. USO3 and E.M. ADU4

ABSTRACT
The effects of marijuana and cigarette use on serum levels of testosterone, the principal androgen in man has been a matter of serious controversy; and there is a paucity of reports on the subject in Nigeria in West Africa south of Sahara. We therefore investigated the effects of the use of these substances on serum levels of testosterone in young adult Nigerian males. Serum testosterone was estimated using enzyme linked immunosorbent assay (ELISA) on micro plate format in 169 Nigerian males (ages 18 – 35); 55 of whom were marijuana smokers, 62 cigarette smokers and 52 age – matched never – smokers who served as controls. Results show a significantly decreased (p<0.05) serum testosterone in marijuana smokers (5.33± 2.8ng/ml) compared with controls (8.5±2.4ng/ml). The testosterone level of cigarette smokers (5.4±1.5ng/ml) was similar (p>0.05) to that of marijuana users but significantly lower than that of the control group. The Body Mass Index (BMI) of marijuana smokers (29.5±8.07kg/m2) was significantly higher (p<0.05) than that of cigarette smokers (22.8±2.08kg/m2) and non-smokers (23.2±2.39kg/m2) but no significant difference was observed between cigarette smokers and non-smokers. There was a strong positive relationship between serum testosterone levels and duration (r=0.63, p<0.05) as well as frequency (wraps/day), (r=0.36, p<0.05) of marijuana use. We conclude that smoking of marijuana or cigarette has depressive effects on serum testosterone concentration in males and that this could be a factor in male infertility and therefore, should not be ignored in the investigation of infertility in males, especially those presenting with borderline fertility potentials. The campaign against the use of these substances should be intensified by government and other relevant agencies.

INTRODUCTION
Marijuana is a mixture of crushed leaves, flowers and sometimes stems from the plant Cannabis sativa, which contains the psychoactive chemical Delta-9-tetrahydrocannabinol (THC) at various concentrations. There are countless street names for marijuana worldwide. In Nigeria, these include pot, herb, we-we, moroko, stone, igbo among others. Cigarette smoke on the other hand is a complex mixture of thousands of compounds containing relatively high concentrations of oxidants or pro-oxidants such as nicotine, carbon monoxide and cadmium. Marijuana is adjudged the most widely abused illicit drug worldwide with the attendant deleterious health and social implications.

Decreased serum levels of testosterone, the principal androgen in males, are often listed as the endocrine effects of marijuana. In animal models (rodents and primates) acute administration of cannabinoids has been shown to decrease testosterone and gonadotrophins levels and disrupt normal spermatogenesis. Chronic exposure is less dramatic than acute administration.
In human studies, however, reports on the effect of cannabinoid use on reproductive hormones including testosterone have been conflicting. In 1974, Kolodny et al, reported decreased testosterone levels in chronic marijuana smokers. Two years later, same researchers reported that marijuana smoking had acute but no chronic effect on serum testosterone. In other laboratory studies, researchers have been generally unable to replicate these findings.

Similar inconsistencies tray the reports on the effect of cigarette smoke (nicotine) on testosterone levels, demonstrating lower, similar or higher levels in healthy male smokers than in matched never-smokers.

These observed inconsistencies in the reports on the effects of marijuana and cigarette use on serum testosterone levels in males may not be due only to differences in experimental design and physiological factors, but may also be due to genetic, ethnic, cultural and environmental variations, thus underscoring the need for such studies in a given population. There is a dearth of information on the androgen status of the Nigerian male marijuana and cigarette smokers. This present effort, therefore, aims at assessing the testosterone levels of the Nigerian male marijuana and cigarette smokers and to compare the results with each other and with other reports elsewhere. We are yet unaware of any such study in Nigeria or elsewhere in West African sub-region.

**MATERIALS AND METHODS**

**Study Population:**

This study was carried out in Benin City metropolis in Edo State, South - South geopolitical zone of Nigeria. One hundred and sixty nine (169) apparently healthy Nigerian males (ages 18 - 35) were enlisted for the study. These consisted of 55 marijuana smokers, 62 cigarette smokers (test groups) and 52 never-smokers as controls. Individuals in the test groups have used their respective drugs for six months period and above. A well structured questionnaire was administered to all participants and informed consent obtained from all subjects and their smoking habits established after the purpose and implications of the study were thoroughly explained to them and well understood.

Venous blood samples were collected with minimum stasis from all participants into non-anticoagulated sample containers between 7.00am and 9.00am. These were allowed to clot, centrifuged at 3000rpm for 5 minutes and the resultant serum harvested and stored at -20°C until required for analysis which was done within 72 hours. The weight and height of participants were obtained and their Body Mass Index (BMI) calculated

**Biochemical Assay:**

Enzyme Linked Immunosorbent Assay (ELISA) method on micro plate format was used for the estimation of serum total testosterone. The kit used was the product of Dialab, Austral, Germany. The manufacturer's analytical procedures were followed and adhered to strictly. Replicates determinations of each sample were made and the mean used for the group statistical analysis. The intra and inter assay variations were 2% and 4% respectively.

**Statistical Analysis:**

The groups mean ± SD was calculated and significant difference between means evaluated using the unpaired student t-test. Statistical Package for Social Science (SPSS), version 16.0 software (SPSS Inc. Chicago, IL USA) for windows was used, with P ≤ 0.05 considered as statistically significant.
RESULTS:
The means of testosterone levels in marijuana and cigarette smokers were significantly decreased when compared with non-smokers. There was however no significant difference between the mean of testosterone levels in marijuana users and that of cigarette smokers (Table 1).

The BMI of marijuana users was significantly higher than those of cigarette smokers and non-smokers controls, while the latter had similar BMI (Table 2). There were positive correlations between serum testosterone levels and both duration ($r = 0.63$, $p<0.05$) and frequency (wraps/day) ($r = 0.48$, $p<0.5$) of cannabis use.

### Table 1: Serum testosterone of marijuana smokers, cigarette smokers and non-smokers.

<table>
<thead>
<tr>
<th></th>
<th>Serum Testosterone (ng/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marijuana smokers (n=55)</td>
<td>5.33±3.5</td>
<td></td>
</tr>
<tr>
<td>Cigarette smokers (n=62)</td>
<td>5.40±1.5</td>
<td></td>
</tr>
<tr>
<td>Non-smokers (n=52)</td>
<td>8.5 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>Marijuana vs Cigarette</td>
<td>0.10 = P&gt;0.05</td>
<td></td>
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<tr>
<td>Marijuana vs Non-smokers</td>
<td>3.96 = P&lt;0.05</td>
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<tr>
<td>Cigarette vs Non-smokers</td>
<td>5.96 = P&lt;0.05</td>
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</tbody>
</table>

### Table 2: BMI of marijuana smokers, Cigarette smokers and Non-smokers.

<table>
<thead>
<tr>
<th></th>
<th>BMI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marijuana smokers(n=55)</td>
<td>29.5±8.07</td>
<td></td>
</tr>
<tr>
<td>Cigarette smokers (n=62)</td>
<td>22.8±2.05</td>
<td></td>
</tr>
<tr>
<td>Non-smokers (n=52)</td>
<td>23.2±2.39</td>
<td></td>
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<tr>
<td>Marijuana vs Cigarette</td>
<td>4.96 = P&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Marijuana vs Non-smokers</td>
<td>4.44 = P&lt;0.05</td>
<td></td>
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<tr>
<td>Cigarette vs Non-smokers</td>
<td>0.68 = P&gt;0.05</td>
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DISCUSSION
In this study, we report a significantly decreased \((p \leq 0.05)\) serum testosterone levels in marijuana smoking Nigerian males compared with non-smokers. This is in consonance with earlier reports elsewhere\(^5\) but at variance with others who observed that chronic use of marijuana have no significant effect on serum testosterone in the population studied\(^6,7,8\). The decreased effect of marijuana on serum testosterone is attributed to inhibition of the gonadotrophin releasing hormone (GnRH) pulse generator in the hypothalamus by THC; an action mediated by central cannabinoid CB1 receptors located in the hypothalamus\(^4\). CB1 Receptors have also been found in the testis and ovaries of experimental animals\(^16\) suggesting a possible direct effect of cannabinoids on the gonads. Furthermore, THC inhibits binding of dihydrotestosterone (DHT) to the androgen receptors and non-cannabinoid components of marijuana extract have been shown to bind estrogen receptors. The extent to which these non-CB1 mediated pathways contribute to marijuana’s effect on the hypothalamic pituitary gonadal (HPG) axis has not been clarified\(^3\).

The observed significantly higher BMI of marijuana users than those of cigarette smokers and non-smokers in our study is in agreement with the work of\(^17\) who attributed this to the weight gain associated with marijuana smoking. Others\(^18,19\) have reported similar observation in patients on dronabinol; a drug that has THC as its basic component. This may be due to accumulation of breast tissue in men which results from increases in circulating estrogen/androgen ratio; a common feature with marijuana smokers. Marijuana has been associated with the development of gynecomastia in early case series\(^20\), but a case control study shows no association\(^21\). Nevertheless, given the effects of marijuana on HPG axis in males, and the possibility that non-THC components of marijuana smoke have affinity for estrogen receptors\(^3\), an association with gynecomastia appears plausible.

Our study also shows that cigarette smokers have significantly reduced testosterone levels compared with non-smokers. This corroborates the reports of earlier investigators\(^9,10,11\). The lowering effect of cigarette smoke is attributed to its nicotine content which produces free radicals and causes oxidative stress thereby altering the biological system. Oral administration of nicotine has been shown to lower serum testosterone in rats\(^22,23\). Contrary to our findings, many investigators\(^14,15\) have reported increased testosterone levels among male cigarette smokers versus non-smokers. Yet others\(^12,13\) have reported no significant differences in the levels of testosterone in male smokers compared to non-smokers.

These conflicting reports on the effects of marijuana and cigarette smoking on serum testosterone may be explained, in part, by difficulties in the accurate assessment of testosterone levels. Androgens display marked circadian fluctuations in plasma levels and considerable intra and inter-individual variability\(^15\). About 65 - 80% of circulating total testosterone is inactive and tightly bound to sex hormone binding globulin (SHBG), whereas the biologically active fraction circulates either free (1-3%) in circulation or loosely bound (20 – 35%) to albumin. Levels of total testosterone, therefore, can be directly affected by changes in SHBG levels. It has been suggested that the inconsistent observations may be due to differences in study design as well as a reflection of the development of tolerance; resulting in the down regulation and desensitization of CB1 receptors in the hypothalamus\(^15\). These may underline the
weakening of effects observed with chronic use. The observed association between testosterone levels and duration \((r=0.63, p \leq 0.05)\) as well as the frequency \((r=0.47, p \leq 0.05)\) of marijuana use in this study lends support to the suggested development of tolerance. The rate and the degree of tolerance may be influenced by variations in genetic, ethnic, cultural and environmental factors.

In this study, we found no significant difference \((p \geq 0.05)\) between serum testosterone in marijuana and in cigarette smokers. This appears to be the first time a comparative study of the effects of the use of these substances on testosterone levels in males is being reported in Nigeria. These authors are also unaware of any such studies elsewhere. The implication of this our finding is that cigarette smoking, though legally and socially acceptable, portends the same level of risk as does the use of the illicit marijuana in male fertility potential.

CONCLUSION

We have reported reduced levels of testosterone in cannabinoids users and in cigarette smokers and that the extent of reduction appears to be the same with both substances. This reduction, though statistically significant, the mean levels still fall within acceptable reference values of testosterone for males, explaining the observation that cannabis\(^5\) and cigarette\(^6\) use may not be a major factor in male fertility. However, our report suggests that men with borderline fertility potentials may tend towards infertility when involved in marijuana and or cigarette use. Thus this study underscores the urgent need for governments at all levels and relevant bodies to intensify the campaign against the use of these substances of abuse, especially by young male adults who are usually unaware of their fertility potentials (apart from erectile function) until when the need to father children arises. Furthermore, it appears unsafe to ignore marijuana and or cigarette use in the investigation of infertility in males attending fertility clinics, especially in Nigeria.

REFERENCES


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