Alteration in Hormonal level and Testicular Histomorphology in Rats Treated with Alcohol (ALC) and Cannabinol (CBN)

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ABSTRACT
This study explores the effects of chronic alcohol and cannabinol exposure on the testosterone levels and the histomorphological integrity/cytoarchitecture of the rats’ testes. Adults male Wistar rats were randomly divided into control (distilled water), methanol (2mg/kg bw), alcohol (3g/kg bw), cannabino (10mg/kg bw) and alcohol (3g/kg bw) plus cannabinol (10mg/kg bw) groups respectively. Alcohol and cannabinol were administered orally twice daily for 52 days (spermatogenic cycle in rats) and at the end of treatment, male reproductive organ (testis) was removed and cleared of adherent tissue and then fixed for histological examination. Blood samples were collected via retro-orbital sinus for hormone (testosterone) assay. Serum testosterone levels were measured using the enzyme-immunoassay (E.I.A.) technique. Hormonal assay showed significant reductions in the levels of testosterone (T) \((p < 0.05)\) in the alcohol alone, cannabinol alone and in the alcohol plus cannabinol treated groups. The Histological analysis of the treated groups showed severe reduction of the spermatic cells. The present study showed that following chronic alcohol and/or cannabinol administration the results showed significant reduction \((p<0.05)\) in testosterone levels and a detrimental effect on the histomorphology of the testes. Alcohol and/or cannabinol therefore exhibit inhibitory effects causing inhibition of testosterone as observed in this study.

Keywords: Alcohol, cannabinol, spermatogenesis, hormone, histomorphology.

1. Introduction
The use of drugs is on the increase among people of reproductive age. Alcohol and cannabinoids belong to the commonly abuse drugs among the youths and belongs to the most widely used drugs which can suppress reproductive functions and sexual behavior both in human and in experimental animals (Abel, 1980; Talabi et al., 2011). Alcohol consumption is often associated with poor semen production and sperm quality (Symons et al., 1976; Mendelson et al., 1977). Chronic consumption of alcohol can result to fertility disturbances (low sperm count, sperm motility and reduced serum testosterone level, testicular atrophy) both in men and laboratory animals (Abel, 1980; Mendelson et al., 1977). However, cannabinol also belongs to the class of illicit drug which can evoke deleterious effects on reproductive activities both in males and in females. Researchers have investigated that tetrahydro-cannabinol induced blockage of the release of gonadotropins and have resulted in lowered LH and FSH, both of which are responsible for the reduction of testosterone production by the Leydig cells of the testis (Dal terio and Bartke, 1981). Testosterone is essential in males during spermatogenesis, as it is being secreted by the testicular Leydig cells under the influence of luteinizing hormone (LH) and acts on the seminiferous epithelium; thus enhances spermatogenesis. The Leydig cells produce testosterone which is essential for the development of the male reproductive system. Interference with Leydig cell testosterone production in male adults causes diminution in male sexual behavior, atrophy of the testis, epididymis and accessory sex organs and impairment of spermatogenesis. However, the role of testosterone on spermatogenesis could be suppressed as a result of exposure to illicit drugs, recreational drugs and other synthetic drugs. Various substances have been implicated as Leydig cell toxicants and some of these substances act directly on Leydig cells, while others could act directly on the hypothalamic-pituitary-testicular axis. Acute and chronic administration of cannabinoids including tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD) has been reported to decrease sexual functions both in male and female rodents as well as in primates. The effects of cannabinoids on both male and female reproductive system have been studied (Marks, 1973; Symons et al., 1976; Kolodny et al., 1976). However, study has not
revealed the combined administration of alcohol and cannabinol on reproductive hormone levels and
the testicular cytoarchitecture in animal model. This study seeks to evaluate testosterone level and
testicular histomorphology in rats exposed to alcohol and cannabinol.

2. MATERIALS AND METHODS

2.1 Experimental Design

2.1.1 Animals

Thirty (30) healthy male Sprague-Dawley rats weighing 180-200 g (12 weeks old) were randomly
selected from the Laboratory Animal Center of NIPRD, Abuja. The rats were fed on standard diet
(Ladokun Feed and Flour Mills Ltd), water was given ad libitum and maintained under standard
conditions. The animal room was well ventilated with a temperature range of 25 - 27°C under
light/dark 12-12 h photoperiodicity. The rats were grouped into 5 groups of 5 rats each. The use of
the animals was approved by the Experimental Ethic Committee on Animal Use of National
Institute of Pharmaceutical and Research Development (NIPRD), Abuja, Nigeria and was
conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2 Experimental Groups

Group 1 was the control (distilled water), group 2 rats received (2mg/kg bw), group 3 was the alcohol
treated group (3g/kg bw), group 4 was cannabinol treated group (10mg/kg bw), and group 5 was
treated with Alcohol (3g/kg bw) plus cannabinol (10mg/kg bw). Drugs and vehicle administration was
done orally using an oral cannula for 48 days. Drugs given to each rat was based on its body weight
(in grams). The animals were weighed before and after the treatment period. The drug treated groups
were sacrificed after the 52 days of administration.

2.2.1 Anesthetic protocol and autopsy

The rats were examined on a daily basis throughout the experimental period for signs of toxicity.
At the end of the experiment the rats were sacrificed via cervical dislocation under 25% urethane
anesthesia (0.6 ml/ 100 g bw).

2.2.2 Organ Collection

The animals were sacrificed by cervical dislocation and were dissected and reproductive organ of
interest (testis) was removed, cleared of adherent tissue and weighed immediately using electronic
weighing balance.

2.2.3 Hormone assay

Blood was collected from each rat via cardiac puncture from which the serum was separated. Serum
testosterone hormone levels were measured using the enzyme-immunoassay (E.I.A.) technique. The
E.I.A. kits were produced by inteco (London, UK) and the optical density was read using a
spectrophotometer (Jenway, 6300 Spectrophotometer, UK) that was sensitive at wavelengths between
492–550 nm.

2.2.4 Histomorphological Evaluation

The testis from each group was harvested and fixed in bouin’s fluid for six hours and then
transferred into 10% buffered formalin for 24 hours after which it was transferred to 70% alcohol
for dehydration. The tissues were passed through 90% and absolute alcohol and xylene for
different durations before they were transferred into two changes of molten paraffin wax for 1 hour each in an oven at 65 °C for infiltration. They were subsequently embedded and serial sections cut using rotary microtome at 5 microns. The tissues were picked up with albumenised slides and allowed to dry on hot plate for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70% alcohol, 50% alcohol and then to water for 5 min. The slides were then stained with haematoxylin and eosin. The slides were mounted and the photomicrographs of the slides were then taken at magnifications of ×100.

2.2.5. Statistical Analysis
Data analysis was carried out using one way analysis of variance (ANOVA) supported by Newman-Keuls test when followed by Turkey’s multiple comparison pair with comparison was done between groups. Results were presented as mean ± SEM, and the differences were considered significant at p < 0.05.

3. Results

![Testosterone concentrations of rats exposure to alcohol and/or cannabinol](image)

**Figure 1. Testosterone concentrations of rats exposure to alcohol and/or cannabinol**
3.1.1 Histomorphology of experimental animals

A is designated as the control group showing normal seminiferous tubules (white arrows) with full maturation of spermatocytes. The germinal cell layer (spanned) shows developmental stages. The interstitial cells appear normal (slender arrow). B, is the group that served as the vehicle (methanol) for cannabinol, the testicular histology showed normal cytoarchitecture with normal seminiferous tubules and both primary and secondary spermatocytes. C, D and E were treated with alcohol, cannabinol and alcohol+cannabinol respectively; the cytoarchitecture showed very few seminiferous tubules showing incomplete maturation with widening of the interstitial space with interstitial cells showing hyperoplasia (black arrow). (X100, H&E).

Figure 1: Photomicrography of a testicular section treated with alcohol and/or cannabinol in Wistar rats
4. Discussion

Substances of abuse exhibit toxic effects in the reproductive functions in both humans and laboratory animals. Cannabinol and alcohol exerted dramatic reproductive toxicity by lowering the testosterone levels and causing a deleterious effect on the testes. Testosterone is the primary androgen that controls the functional activity of virtually all male reproductive tract structures. Spermatogenesis in seminiferous tubules, sperm maturation in the epididymis and the secretary activity of the accessory sex glands all require adequate levels of testosterone, although spermatogenesis in the rat can be maintained with intra-testicular testosterone concentrations as low as 20% of normal values. Oral administration of Δ⁹-tetrahydrocannabinol, the major active ingredient of cannabis (Marijuana), increases both plasma testosterone and LH concentrations in male mice as reported by (Daltero et al., 1982, 1984). Testicular histomorphological examination of groups treated with alcohol and/or cannabinol showed degeneration of spermatogenic cells in the seminiferous tubules and in complete spermatogenesis. However, there was an improvement in the testicular architecture in rats co-treated with quercetin alongside alcohol and/or cannabinol. The alteration in reproductive functions may also be linked to the decrease in the mass of the testes and inhibition of testicular androgenesis (testosterone), likewise reducing the serum concentrations of gonadotrophins (FSH and LH) as revealed in the present study. Testosterone is essential for the sequential stages of spermatogenesis and also responsible in the maintenance of spermatogenesis; it aids in the growth of external genitalia (penis, scrotum and other accessory sex organs including the genital ducts, seminal vesicles and prostate gland respectively. Moreover, the reduction in serum testosterone levels in the alcohol and/or nicotine treated rats could be associated with the inhibitory effect on the pituitary gland thereby altering reproductive functions. Although, the regulation of testosterone production or secretion is regulated by Luteinizing hormone (LH) or interstitial cell stimulating hormone (ICSH). LH is important in stimulating the Leydig cells and the quantity of testosterone is proportional to the concentration of LH. However, the reduction in the serum testosterone concentration observed in the rats treated with alcohol and/or cannabinol could be linked to the reduction in the serum levels of LH. The effect appears to be a direct response of the glandular tissues involved, as the release of testosterone does not require the presence of the pituitary, and the administration of testosterone does not inhibit the release of LH. The effect on testosterone is biphasic, causing rapid sustained increases in plasma levels at low doses and subsequent decreases at higher doses. Daltero et al., 1984, suggested that this may be responsible for the reported aphrodisiac effects of cannabis, which are arousal at low doses or in occasional users, followed by suppression of libido at high concentrations. The present result is in agreement with the study by Purohit et al., 1979, 1980) showed that both tetrahydrocannabinol and cannabinol decreased ventral prostate, seminal vesicular weight and plasma concentrations of testosterone and dihydrotestosterone in adult male rats after 10 days of subcutaneous injection at a dose of 10ng/kg of body weight. The serum testosterone levels of rats’ treatment with cannabinol at a dose of 10mg/kg of body weight showed a marked significant reduction in comparison to the control counterpart. Studies by Marks, 1973; Kolodny et al., 1976, also revealed that exposure to cannabinoids involving a mice model could be confusing because cannabinoids have been shown to both increase and decrease serum testosterone concentrations in peripheral blood depending on the dosage, time of blood sampling, route of drug administration and the interactions with the gonadotropins (FSH and LH) (Dalterio et al., 1976, 1979; Dalterio and Bartke, 1981). Reports by Dalterio et al., 1979; Frias et al., 2000, revealed alterations in the function of the hypothalamo-hypophyseal-gonadal axis in adult male mice following prenatal exposure to cannabinoids. This study is in alignment with earlier reports revealing the detrimental effects of
alcohol and cannabinol administration on the reproductive activities evident by the alteration derangement in the cytoarchitecture of the epididymis and testis respectively (Akintayo et al., 2015).

4.1 Conclusion
In conclusion the present study demonstrates that following chronic administration of alcohol and/or cannabinol caused reduction in serum testosterone levels and deleterious effects on the cytoarchitecture of the rats’ testes. Thus, alcohol and cannabinol are testicular toxins and are believed to produce site-specific lesion at such sites as the Leydig cell, germ cells and as well as Sertoli cell.

References


