Histomorphological Examination of Reproductive and Accessory Organs Exposed To Alcohol and Cannabinol In Male Adult Rats

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Abstract
Alcohol and cannabinol belong to the class of illicit and recreational drugs because of the relative ease by which they can be obtained, however, experimental studies have revealed their deleterious effects on the male reproductive system. This study investigates the histomorphological examination of reproductive organs (testis and epididymis) of rats treated with alcohol and cannabinol. Twenty five (25) adult male rats were randomly grouped into five (5) groups of five rats each. Group 1 served as the control, Group 2 received methanol (2mg/kg bw), Group 3 received alcohol alone (3g/kg bw as 25%v/v), Group 4 received cannabinol alone (10mg/kg bw), Group 5 received both alcohol (3g/kg bw as 25%v/v) and cannabinol (10mg/kg bw). Animals were treated daily for a period of 8 weeks via oral administration and at the end of the experiment the rats were sacrificed via cervical dislocation and the reproductive organs of choice were harvested for histology. The microscopic studies of the male reproductive organs revealed degeneration of the cytoarchitecture of these organs (testes) treated with alcohol and/or cannabinol and did not show any detrimental effect on the histology of the epididymis. However, the results depict that treatment with alcohol and cannabinol caused morphological degradation of the reproductive organs (especially the testes), with maturation arrest, and lack of germ cells with absence of spermatozoa which gives an insight into the toxicity activities of these drugs on male reproductive organ and this suggests that both alcohol and cannabinol administration can damage the integrity of the reproductive organ (testis) and not the accessory organ (epididymis) as observed in this study.

Keywords: Alcohol; cannabinol; histology; reproductive organ; accessory organ; cytoarchitecture

1. Introduction
Alcohol and other illicit drugs particularly cannabinoids (cannabinol) are on the increase amongst the young (Miller and Plant, 1996). Alcohol is otherwise known as ethanol and is regarded as food staple in some culture (Libier, 1991), it is also known to have embryotoxic effects; it can as well disrupts the testicular-blood-barrier (Farghali et al., 1991); exposure to chronic ethanol can inhibits the development of the maturing germ cells and/or promotes degeneration of germ cells and enhances the occurrence of apoptosis (program cell death), (Zhu et al., 2002). However, cannabinol on the other hand belongs to the three major cannabinoids that are isolated from Cannabis Sativa L and has been reported to exert negative effects on the reproductive functions and male sex hormone (testosterone) in mammals (Dalterio and Bartke, 1979; Dalterio et al., 1981; Harclerode, 1984; Brown and Dobs, 2002). Studies have shown that cannabinoids or endocannabinoids have important actions in the reproductive system andit has been shown that marijuana is associated with the suppression of Luteinizing hormone (LH) secretion and shortened luteal phase in women (Mendelson et al., 1986). Over the past years there has been substantial report that cannabinoids have negative effects on reproductive functions of both male and female mammals(Mendelson et al., 1986; Tyrey, 1980; Kolodny, 1974; Paria and Dey, 2000, Maccarrone et al., 2000). Cannabinoids generally have been reported to have negative influence on sperm functions which have been demonstrated in mammalian tissues, including both male and female reproductive tracts. Furthermore, no study has investigated the histomorphological examination of reproductive organs (testis and epididymis) in animal model exposed to alcohol and/or cannabinol, and these are the key components of male reproduction. Therefore the purpose of this study was to examine the effects of alcohol and/or cannabinol on the histomorphology of rats’ testes and epididymis.
2. Materials and Methods

2.1 Animals

Wistar strain albino rats (200-220g) were used for the study. They were housed in wire mesh cages under controlled light (12L:12D cycles) and temperature (24±) conditions. Twenty five male rats were used for the study and were grouped into five groups and each group contained five rats each. The study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in care and use of animal.

2.2 Experimental Groups

Group 1 was the control, group two rats were treated with methanol (2mg/kg bw) group 3 was the Alcohol treated group (3g/kg bw as 25% v/v), group 4 was Cannabinol treated group (10mg/kg bw), and group 5 was treated with Alcohol (3g/kg bw as 25% v/v) plus Cannabinol (10mg/kg bw). Drugs and vehicle administration was done orally using an oral cannula for 8 weeks. Drugs given to each rat was based on its body weight (in grams). The animals were weighed before and after the treatment periods.

2.3 Organ collection and Histomorphological Study

At the end of the experimental periods, animals were sacrificed by cervical dislocation. The animals were dissected and organs of interest; testis, seminal vesicle, prostate, and epididymis, were removed and cleared of adherent tissues. The organs harvested were fixed in bouin’s fluid, passed through ascending series of ethanol baths, cleared in xylene and embedded in paraffin. Tissues were sectioned at 5 µm and stained with Haematoxylin and Eosin (H&E) to observe the photomicrography of the organs. The slides were then examined at magnifications of ×100 under optical microscope.

3. Results and Discussion

Photomicrograph of reproductive organs (testis and epididymis)

Figure 1: Group 1(Control) testis. Photomicrograph of a testicular section 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). H&E. X100.
Figure 2: Group 2 (methanol) testis. Photomicrograph of a testicular section showing mostly normal seminiferous tubules, they show normal germinal cell layers and normal lumen containing some strands of spermatozoa. Very few seminiferous tubules show incomplete maturation. The interstitial cells appear normal. H&E. X100.

Figure 3: Group 3 (alcohol) testis. Photomicrograph of a testicular section showing several abnormal seminiferous tubules (white arrow) with maturation arrest and the germinal cell layer (spanned) shows lack of germinal cells. The interstitial cells shows hyperplasia (slender arrow). H&E X100.
Figure 4: Group 4 (cannabinol) testis. Photomicrograph of a testicular section showing several abnormal seminiferous tubules (white arrow) with maturation arrest. The germinal cell layer (spanned) shows lack of germinal cells. The lumen (black arrow) show lack of spermatozoa. The interstitial cells shows hyperplasia. (slender arrow) H&E X100.

Figure 5: Group 5 (cannabinol+alcohol) testis. Photomicrograph of a testicular section showing several abnormal seminiferous tubules (white arrow) with maturation arrest and the germinal cell layer (spanned) shows lack of germinal cells. There is presence of fat degeneration. The interstitial cells shows hyperplasia. (slender arrow) H&E X100.
Figure 6: Group 1 (Control) epididymis. Photomicrograph of epididymal section showing epididymal ducts with normal epithelial cell lining. The tunica propria lining appear normal (blue arrow). The lumen of the ducts are filled with spermatozoa (slender arrow). The interstitial tissues appear normal (white arrow). H&E X100

Figure 7: Group 2 (Methanol) epididymis. Photomicrograph of epididymal section showing epididymal ducts with normal epithelia cell lining. The tunica propria lining appear normal (blue arrow), some of the lumen of the ducts shows strands of spermatozoa (slender arrow). The interstitial tissues appear normal (white arrow). H&E X100
**Figure 8:** Group 3 (Alcohol) epididymis. Photomicrograph of epididymal section showing epididymal ducts with normal epithelia cell lining. The tunica propria lining appear normal. (Blue arrow) The lumen of the ducts are filled with spermatozoa (slender arrow). The interstitial tissues appear normal. H&E X100

**Figure 9:** Group 4 (Cannabinol) epididymis. Photomicrograph of epididymal section showing epididymal ducts with normal epithelia cells lining. The tunica propria lining appear normal. (blue arrow) The lumen of the ducts are filled with spermatozoa(slender arrow). The interstitial tissues appear normal. H&E X100
The epididymis has a significant role in male reproduction and it has been established that spermatozoa are produced in the germinal epithelia of the seminiferous tubules and are transported to the epididymis for the onward transport, maturation and storage of spermatozoa (19, 20,21). However, the testis also contributes greatly to the functions of the male reproductive system; it produces testosterone, the principal male sex hormone; it also serves as the site for generating sperm. Within the testes are the coiled masses of tubules known as the seminiferous tubules, which is responsible for the production of sperm cells. Histomorphological examination of the reproductive organs; especially the testes of animals treated with alcohol and cannabinol revealed abnormal seminiferous tubules, maturation arrest and lack of germinal cells. However, interstitial cells show hyperplasia associated with incomplete spermatogenesis in the groups treated with alcohol and/or cannabinol in comparison with the control group. Both human and laboratory animal testes are susceptible to damage by substances which are toxic and can produce genetic disorder and disruption of the cytoarchitecture of the reproductive organs (testes) (13). Several reports established that epididymis and accessory sex organs need continuous androgenic stimulation for the preservation of their normal structural and functional integrity (14). Studies have shown that the secretion of various proteins by the principal cells of the epididymis into the epididymal lumen influences sperm maturation (17, 20).

4. Conclusion
In conclusion, the present study showed the effects of alcohol and/or cannabinol oral administration on the cytoarchitecture of the reproductive organ (testis) and the accessory organ (epididymis). The results indicate no detrimental effects of alcohol or cannabinol administration on the histological features of the epididymis of adult male rats, however, deleterious effects were observed on the histological features of the testes in the alcohol and/or cannabinol treated groups. Thus alcohol and/or cannabinol have direct effects on the testis in male rat.
References