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Derangement in Sperm Kinematics following Pre-pubertal Exposure to Cannabis sativa: Ameliorating effect of Vitamin C.

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Abstract **Original Research Article**

Background of study: Motility is an important function of the male gamete which enables it to actively reach and penetrate the female gamete for fertilization. Long term use of Cannabis sativa has been associated with decreased male fertility indices including sperm motility. The characterization of the spermatozoa movement has not been evaluated especially in rats pre-pubertally exposed to cannabis sativa. ***Methodology:*** Forty pre-pubertal male rats were randomly assigned into 4 groups of ten rats each. Group 1 served as the control, group 2 was the low dose cannabis group exposed to cannabis smoke for 5 minutes daily, group 3 was the high dose cannabis group exposed to cannabis smoke for 10 minutes daily while group 4 was exposed to cannabis smoke for 10 minutes daily and treated with 2.8mg/kg vitamin C daily. Duration of administration and treatment was 28 days after which animals were euthanized and cauda epididymis from each testis was dissected out for evaluation of sperm motility indices. ***Resuts:*** The results showed significant derangement in sperm kinetics including total motility ($P<0.05$), progressivity ($P<0.05$), velocity of active path ($P<0.05$), curvilinear velocity ($P<0.05$), amplitude of lateral head ($P<0.05$), beat cilia frequency ($P<0.05$) and wobbling rate ($P<0.05$) in the high dose group compared with control. These abnormal sperm kinematics were significantly ameliorated by vitamin C. ***Conclusion:*** In conclusion, cannabis smoke impairs several aspects of the kinematics of sperms in pre-pubertally exposed rats but which were ameliorated by vitamin C.

Keywords: Sperm motility indices cannabis prepubertal vitamin C, derangement.

INTRODUCTION

Estimates have it that one out of every four couples has some level of reproductive issues (Rutsein and Shah, 2004). It is also reported that 40%-50% of all cases of infertility is attributable to male factor (Duckitt, 2003). Infertility is of a great concern not only because of the need for continuity of species, but also due to its social and economic implications (Schmidt *et al.* 2005). At the testicular level, fertility can be impaired by interference with the spermatogenic process which may cause asthenozoospermia (reduced sperm motility) and teratozoospermia (Ostermeier *et al.* 2002).

Motility is an important function of spermatozoa which enables it to actively, reach and penetrate the female gamete for fertilization. Motility of sperm therefore is a strong predictive marker of male fertility and is also a key factor in artificial reproductive technology (ART) (The Practice Committee of the American Society for Reproductive Medicine, 2008). Indices of sperm motility include total mobility which is general movement of the sperm, progressivity, the general ability of sperms to travel a good distance; velocity of active path (VAP), the distance covered by sperms in the average direction of movement ; velocity of straight line (VSL), the straight line distance between the starting and ending points of sperm trajectory. Others include velocity of curved line travelled or curvilinear velocity which is the average velocity measured over the actual point-to-point track followed by sperm; linearity (LIN) of the curvillinear trajectory calculated as VSL/VCL x 100; amplitude of lateral head (ALH) deviation, the maximum lateral displacement of a sperm head; beat cilia frequency (BCF) and wobbling rate (Slotter *et al.*, 2006). These motility variables correlate well with fertility rates (Bollendorf *et al.* 1996) while some have been found decreased in oxidative stress states (Ariagno *et al.* 2017).

Sperm motility is contributed by its complex structural and molecular signaling mechanisms with the flagellum containing the machinery necessary to propel the spermatozoa forward. Asthenozoospermia has been

associated with sperm structural and genetic abnormalities (Leigh *et al.* 2009), mitochondrial DNA mutation (Kao *et al.* 2004), oxidative stress as well as alteration in the microenvironment of the sperms (Aribo *et al.* 2018, Makler *et al.*, 1981). The defective motility indices would invariably lead to infertility (Kao *et al.* 2004, Cantalk and Aidemir, 2013).

Cannabis sativa is a botanical plant product believed to have been in use for over 500 years (Bennett, 2010). In Nigeria Cannabis is widely grown across the country especially in Ondo, Edo, Delta, Osun, Oyo and Ogun states (Seshata, 2013) and is a major source of West African consumed cannabis (Ministry of Health, 2007). Though an illegal herb in Nigeria, it is consumed mainly by young men who smoke it or use it as vegetables to prepare meals.

Cannabis sativa contains several phytocannabinoids, terpenoids and phenolic compounds and has founds it health benefits in the management of cardiovascular, cancerous and neurodegenerative and inflammatory diseases (Andre *et al*, 2010). Aside several negative effects of the herb like psychosis, it has been found to impair male reproductive function (Du Plessis *et al*, 2015, Alagbonsi *et al.* 2016), the possible mechanism of action being oxidative stress (Mandal and Das, 2010).

Vitamin C is a naturally and synthetically occurring molecule/antioxidant (Yamamoto *et al.* 2002). In combination, it has been found to ameliorate cannabis-induced male reproductive damage (Udokang and Udom, 2019).

Evidence abounds that early life events affect health indices in later life (Drake *et al.* 2005, Drake *et al.* 2011). Researches have also shown that health disorders in adult life could be a manifestation of manipulations of fetal, neonatal or pre-pubertal exposures resulting in permanent alteration in the structure and function of tissues and organs which may cause altered physiology in adulthood (Myatt 2006, Perobelli, 2014). Most of the work done on effect of cannabis sativa on male reproductive function has focused on adult rats and total motility of sperms (Udokang and Udom, 2019, Alagbonsi *et al.*, 2016, Hsaiao and Clavijo 2018). There has been no literature on its effect on sperm motility indices following prepubertal exposure and hence this research.

MATERIALS AND METHODS

Approval and collection of Cannabis sativa for use

Approval for use and collection of 200g of dried cannabis sativa leaves were obtained from the State Office of the National Drug Law Enforcement Agency (NDLEA), Uyo.

Ethical approval

This was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo.

Experimental animals

Forty pre-pubertal (28-30 days old) male wistar rats were obtained from the Faculty of Basic Medical Sciences, University of Uyo, and raised in the Animal House of same. They were housed in wooden cages under a 12hour light and 12hour dark cycle and allowed free access to rat feeds (Vital Feed Mills Nigeria) and water. Acclimatization period was two weeks.

Experimental Protocol

The rats were randomly divided into four groups of ten rats each. Group 1 (control) received 2ml/kg distilled water orally. Group 2 (low dose cannabis sativa smoke) was exposed to the smoke for 5 minutes daily. Group 3 (high dose cannabis sativa smoke) was exposed to the smoke for 10 minutes daily. Group 4 (high dose cannabis smoke+ vitamin C) was exposed to high dose of cannabis sativa smoke for 10 minutes and treated with vitamin C at a dose of 2.8mg/kg by oro-gastric gavaging.

Preparation of Vitamin C solution

Each tablet of vitamin C (Emzor Pharmaceuticals, Nigeria) equivalent of 100mg vitamin C was dissolved in 100ml distilled water giving a concentration of 1mg/ml and administered at 2.8mg/kg of rat weight.

Preparation of Cannabis Sativa smoke

This was done by wrapping 1g of dry cannabis sativa leaves with a Rizla rolling paper and burnt with a red hot charcoal after which it was placed on a stainless plate in a smoke chamber. The smoke chamber was made with a polyethelene material (60cm x 50cm x 40cm). During each smoking session, all rats in necessary groups (2-4) were placed inside the smoke chamber and exposed to the smoke for their respective durations.

Initial Smoking Session

This was done to ascertain the tolerable inhalational dose for the rats. In the first instance 5 rats were exposed to smoke in the chamber prepared with 2g of cannabis sativa. Following this, mortality of one rat, aggressiveness of two rats and extreme weakness of another two were observed. In the second trial, three of the rats became extremely weak while one was aggressive but no mortality when 5 rats were exposed for 15minutes in a smoke prepared with 1g of cannabis sativa. During the 3rd attempt, none of the rats developed aggressiveness, weakness or mortality following exposure of 10 rats to smoke prepared with 1g cannabis sativa for 10minutes. This last trial therefore became our standard.

Collection and analysis of samples

At the end of the experimental period, the rats were euthanized and testes dissected out. The cauda epididymis of each testis was dissected out and several incisions of about 1mm made on it and the tissue suspended in 1ml semen buffer solution to allow spermatozoa swim up. Semen analysis was carried out according to the Tilley (2007) and WHO (2015) criteria. Motility indices or sperm kinematics was evaluated using the Computer Assisted Sperm Analysis (CASA) technique. Freshly collected semen samples from the epididymis as described above were liquefied at 37˚C. Video recordings were made from four different fields of the chamber using a magnification objective on the microscope. Analysis was carried out based on capturing sequences of 64 frames per field and counting a minimum of 100 spermatozoa. Motility indices analysed were total motility in (10^6/ml), progressivity, velocity of active path (VAP) in pm/s, curvilinear velocity (VCL) in pm/s, velocity of straight line, (VSL) in pm/s, amplitude of lateral head (ALH) in pm, beat cilia frequency (BCF) in Hz, linearity (LIN) of sperm cells in % and wobbling rate in %.

STATISTICAL ANALYSIS

Data were expressed as Mean ± SEM and differences between means evaluated using analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. Values of P<0.05 were considered statistically significant. Graph Pad Prism 7.0 Software (Graphpad Inc, USA) was used for statistical analysis.

RESULTS

The results showed a significantly reduced sperm motility in the high dose marijuana (HMJ) compared with control (P<0.05) and low dose marijuana (LMJ) smoke (P<0.05). Motility was however significantly increased in the high dose marijuana + Vitamin C (HMJ+ Vit C) compared with the HMJ group (P<0.05) as in fig 1. Progressivity was significantly lower in the HMJ compared with control (P<0.05) as in fig 2. Velocity of active part of sperms (VAP) was significantly decreased in the HMJ group compared with control (P<0.05) but which was significantly increased in the HMJ + Vit C compared with HMJ smoke group (P<0.05) as in fig 3. Curvillinear velocity (VCL) was significantly decreased (P<0.05 each) in the HMJ smoke and the HMJ + Vit C compared with control (fig 4). Amplitude of lateral head (ALH) was significantly decreased (P<0.05) in HMJ and HMJ + Vit C compared with control (fig 5). Significant decreases in beat cilia frequency (BCF) (P<0.05 each) were observed in LMJ, HMJ and HMJ + Vit C compared with control (fig 6). There were no significant differences in linearity (fig 7). Significant increase in wobbling rate (WR) in LMJ and HMJ compared with control (p<0.05) and HMJ compared with LMJ (0.05) were observed but the rate was significantly decreased in HMJ + Vit C (p<0.05 each) compared with control, LMJ and HMJ groups (fig 8).

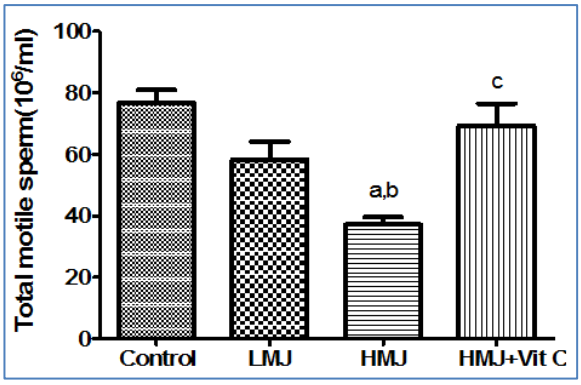

Fig-1: Comparison of total motile sperm in experimental groups. a= p<0.05 vs control. b= p<0.05 vs low *Cannabis sativa* smoke (LMJ*)* treated group. c= p<0.05 vs high *Cannabis sativa* smoke (HMJ*)* treated group.

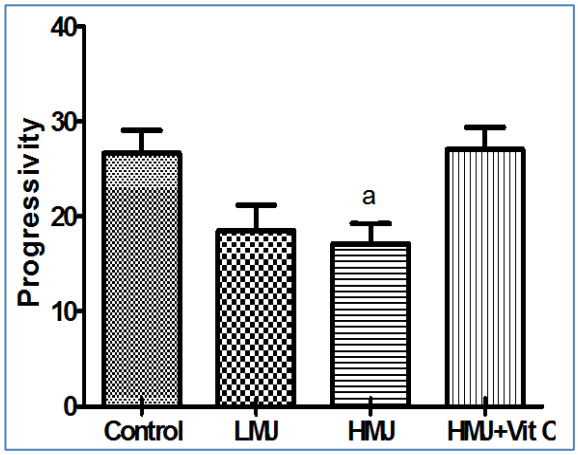

Fig-2: Comparison of progressivity of sperms in experimental groups. a= p<0.05 vs control group

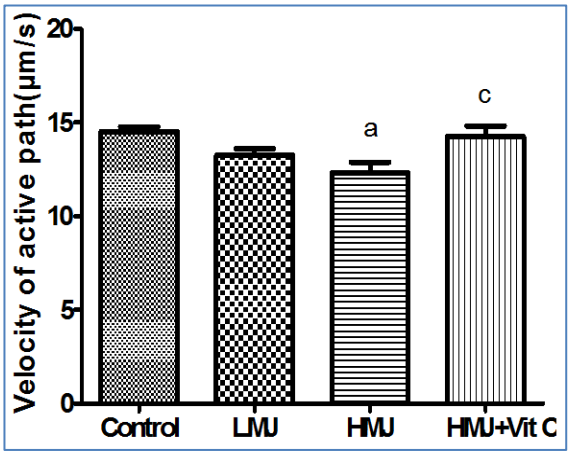

Fig-3: Comparison of velocity of active path of sperm cells in experimental groups. a= p<0.05 vs control group. c= p<0.05 vs high *Cannabis sativa* smoke (HMJ*)* treated group.

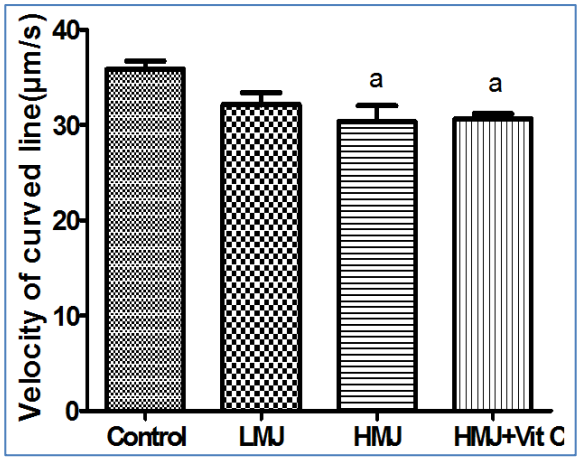

Fig-4: Comparison of curvillinear velocity in experimental group. a= p<0.05 vs control group.

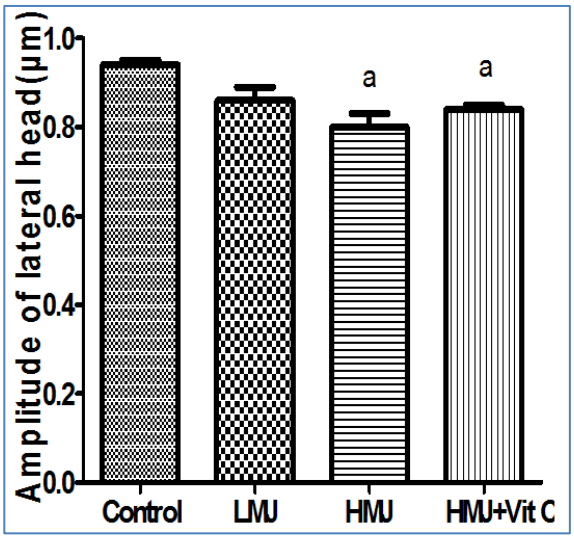

Fig-5: Comparison of amplitude of lateral head of sperm cells in experimental group. a= p<0.05 vs control group.

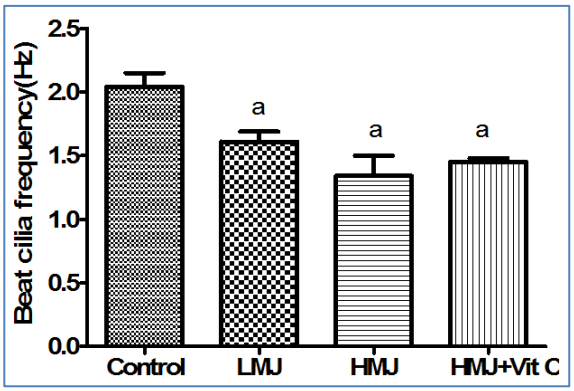

Fig-6: Comparison of beat cilia frequency of sperm cells in experimental groups. a= p<0.05 vs control group. b= p<0.05 vs low dose *Cannabis sativa* smoke (LMJ*)* treated group. c= p<0.05 vs high dose *Cannabis sativa* smoke (HMJ*)* treated group

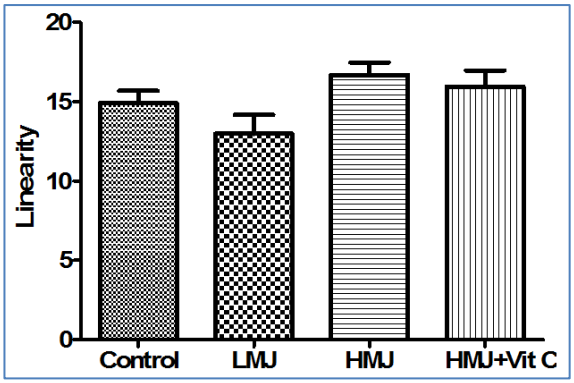

Fig-7: Comparison of linearity of sperm cells in experimental groups

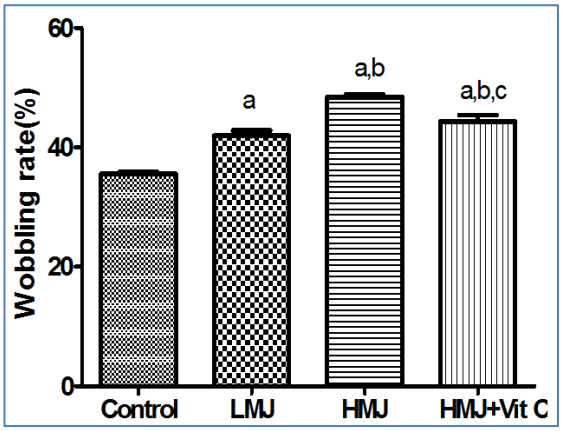

Fig-8: Comparison of wobbling rate in experimental groups. a= p<0.05 vs control group. b= p<0.05 vs low dose *Cannabis sativa* smoke (LMJ*)* treated group. c= p<0.05 vs high dose *Cannabis sativa* smoke (HMJ*)* treated group

DISCUSSION

A healthy reproductive function is essential not only for the continuity of species but also for social and economic reasons. Reproductive function can be affected negatively by several substances including Cannabis sativa (Udokang and Udom, 2018, Sansone *et al.* 2018). Pre-pubertal exposure to substances has been known to affect organ/tissue function in later life (Veeramachaneni *et al*. 2001, Aprioku and Ugwu, 2016). Most of the antifertility effect of Cannabis sativa studied including its effects on total sperm motility is on adult animals (Banerje *et al* 2011). The present study sought to evaluate the effect of pre-pubertal inhalational exposure to Cannabis Sativa smoke on indices of sperm motility.

Our results showed a significantly reduced general sperm mobility in the high dose of marijuana-exposed group compared with the control and LMJ groups. Also generally the progressivity was significantly reduced in the HMJ group compared with control in line with what previous researchers have found (Udokang and Udom, 2018, Alagbonsi *et al.*, 2016). Other Kinematics measurements or indices evaluated viz velocity of active path, velocity of curved line travelled, amplitude of lateral head of sperm and

beat cilia frequency and wobbling rate of sperm cells were significantly lower in the HMJ group compared with control most of which were significantly ameliorated by vitamin C.

Sperm motility is contributed by its complex structural and molecular signaling mechanisms, the flagellum containing the machinery needed to propel the spermatozoa forward. Several factors including intrinsic and extrinsic factors are known to affect sperm motility and its kinematic measurements. Extrinsic factors like rise in temperature, P^H and osmolarity or altered microenvironment affect its motility (Makler *et al.* 1981, Aribo *et al.*, 2018). Intrinsic anatomical or functional defects especially in the flagellum may also be responsible for the impaired motility observed (Kumar *et al*, 2019). Exposure to Cannabis sativa smoke is said to cause spermatotoxicity via oxidative stress (Ishaka and Luqman, 2019). Ariagno *et al.* (2017) observed reduced curvilinear velocity, straight line velocity and amplitude of lateral head in testes with oxidative stress. Oxidative stress has the ability to cause DNA mutation (Kao *et al.*, 2004) which may lead to structural and functional defect in the sperm and ultimately impaired motility.

The impairment in the sperm mobility indices occurred despite the fact that the rats were exposed to the smoke during pre-puberty days. This suggests a lingering effect of cannabis smoke on organ/tissue function. This is understandable since cannabis is highly lipophilic and in prolonged administration, fatty acid conjugates of tetrahydrocannabinoid and 11-hydroxytetrahydrocannabinoids are formed increasing their stability and extended storage in the tissues and is said to have a longer half-life in chronic than naïve users (Musshoff and Madea, 2006). Its large volume of distribution and slow elimination increases its half-life (Dackis *et al.*, 1982, Haestis, 2007).

Co-administration of vitamin C with cannabis demonstrated ameliorating effects on various indices of sperm motility listed above. This too is understandable. One of the possible mechanisms for tissue damage by Cannabis is via oxidative stress (Ishaka *et al.*, 2017). Vitamin C is a potent antioxidant (Traber and Stevens, 2011). This results agree with various researches that studied ameliorating effect of vitamin C on impaired sperm motility from different insults (Sadeghzadeh *et al.*, 2019) including tetrahydrocannabinoid (Alagbonsi and Olayaki, 2020).

CONCLUSION

We therefore conclude that pre-pubertal exposure to cannabis sativa smoke impairs indices of sperm motility which are ameliorated by co-administration of vitamin C.

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