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Neuroendocrinological, Metabolic and Toxicological Effects of Chronic Cannabis Use among Male in Lagos

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ABSTRACT

Controversies surround the use of cannabis and there have been discrepancies in the results of various studies on cannabis carried out on animals and on human beings. The aim of this study was to investigate the toxicological, metabolic, reproductive and neurochemical effects of chronic cannabis use among some male users in Lagos, Nigeria. Twenty male chronic cannabis smokers with ages between 20-35 years and average duration of cannabis use of 5 years were recruited along with ten male age matched controls that were not using cannabis. Blood samples were collected from all the subjects for the analyses of plasma dopamine, norepinephrine, Prolactin (PRL), Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone levels. Random blood sugar, plasma cholesterol, High Density Lipoprotein (HDL)-cholesterol, Low Density Lipoprotein (LDL)-cholesterol, triglycerides, total plasma protein, serum Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST) were also estimated. The results showed that smokers had significantly higher levels of dopamine, norepinephrine and testosterone. There was also significantly higher levels of serum ALT and AST in the smokers' group. However, the other reproductive hormones and the metabolic parameters checked were all not significantly different between the two groups. We concluded that chronic cannabis use is associated with increased catecholamine neurotransmitters and increased testosterone and liver toxicity while there are no effects on PRL and gonadotropins as well as metabolism.

Key words: Cannabis, toxicity, reproductive endocrinology, catecholamine neurotransmitters, metabolic effects

INTRODUCTION

Cannabis remains the most widely used illicit drug worldwide due to its affordability and availability (Bauman and Phongsavan, 1999). Besides, cannabis is a major controversial drug as there are numerous conflicting and controversial reports concerning its psychological and physiological effects (Richardson, 2010). Many reports have linked cannabis smoking to the development of psychosis (Hall and Degenhardt, 2008). Certain studies have also suggested that cannabis smoking is only a form of self-medication in people with psychotic symptoms rather than a causative factor in development of psychosis (Abdelrahman *et al.*, 2012). Recently, there seems to be an increase in the number of reports lending support to cannabis psychosis. Further, a review of the evidence surrounding the acute impact on memory concluded that cannabinoids

impair all aspects of short-term memory, especially short-term episodic and working memory (Ranganathan and D'souza, 2006). Apart from the psychological effects, various reports have shown that cannabis smoking has significant physical effects on the body (Fant *et al.*, 1998; Hashibe *et al.*, 2006). Cannabis has been found to increase heart rate by 20-50%. This is the most immediate effect and occurs within a few minutes after cannabis intake. After, cannabis use a sudden change of posture from lying down to standing up may produce orthostatic hypotension, a feeling of 'light-headedness' and faintness that is often the earliest indication of intoxication in naive users (Jones, 2002). Other physical effects of cannabis include reddening of the eyes due to congestion of the conjunctival blood vessels, lowering of the body temperature, dry mouth, reduced intraocular pressure and relaxation of the muscles (Grotenhermen, 2007). Cannabinoids have also been shown to affect the immune system (Friedman *et al.*, 2003) Clinical studies and survey data have found that cannabis increases appetite (Tehranipour *et al.*, 2009). The Δ^9 -tetrahydrocannabinol (THC) which is the main constituent of cannabis has been shown to have effect on both the action and the release of insulin (Frisher *et al.*, 2010). This may explain why cannabis has been employed to self-medicate in diabetes. Cannabis has also been reported to have medicinal use in treating depression (Henquet and Kuepper, 2010). Most of the experiments in the cannabis research make use of animal models. However, there are huge discrepancies in the results obtained from human studies. Even studies conducted using human subject have shown variations in the results. These variations may not be unconnected with the population used in a study. In our experiment, we chose to study the effects of chronic cannabis use among males in Lagos, Nigeria. We studied the toxic, metabolic and neurochemical effects of chronic cannabis use among male cannabis users in Lagos. Since there are claims among the user that cannabis among other uses, increases male virility we also studied some reproductive endocrinology parameters in this male population.

MATERIALS AND METHODS

Case: The subjects in the case group were twenty in number and were recruited from Idi Araba and Mushin areas of Lagos. They were all males within the ages of 20-35 and all had smoked cannabis for at least five years. They all were screened to ensure that none was using any drugs capable of interfering with the neurochemical, endocrinological and metabolic parameters that were assayed.

Control: The control group consisted of twenty volunteers who were age matched with the case group. None of them was using cannabis or any drugs capable of interfering with the parameter checked.

Sample collection: After interviewing the participants about use of cannabis and to determine if they qualify to participate in the study, blood samples were taken from them by venipuncture after they gave informed consent. These were transported on ice pack to the laboratory and were immediately separated to get the plasma samples used for the experiments.

Neurochemistry: Neurochemical assays for dopamine and norepinephrine were run by ELISA method using TriCat ELISA (IBL International, Hamburg, Germany). The *in vitro* diagnostic quantitative determination of noradrenalin (norepinephrine) and dopamine in human plasma has been performed according to the manufacture protocol.

Hormonal assays: Assays for prolactin, follicle stimulating hormone (FSH), Luteinizing Hormone (LH) and testosterone were carried out by Microwell Elisa (Diagnostic Automation Inc. USA).

Other biochemical assays: Random blood sugar, Plasma total protein, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides concentrations were all determined by enzymatic assay methods using relevant analytical kits (RANDOX, United Kingdom).

Statistical analysis: All determinations were in triplicates. The results are presented as Mean±standard deviation. The means were compared using student's t-test and value of $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Many controversies surround cannabis use as there have been several studies on cannabis with disparate results. Many of the results were confounded by factors such as the study populations. Most of the experimental studies were conducted using animal models (Brown and Dobs, 2002) and in some circumstances it may be difficult to extrapolate results from animal studies on human. This is because there are subtle physiological differences between the species such as in drug metabolizing enzymes. Even among humans, there have been reports that were not in agreement with one another (Hall and Solowij, 1998). We carried out our study using local cannabis smokers in some parts of Lagos, Nigeria to see if we would be able to replicate some of the previously reported results within our local population. The results of the catecholamine neurotransmitters showed a statistically significant difference between the case i.e., the cannabis smokers and the non-smokers. Both the smokers' values and the non-smokers' values still fell within the acceptable range for humans, however the smokers showed higher plasma dopamine levels compared to the non-smokers (Table 1). In line with some previous studies (Mirochnick *et al.*, 1997; Cheer *et al.*, 2004), we found that smokers showed higher levels of plasma dopamine and norepinephrine than non-smokers. Dopamine is a neurotransmitter with a well-established reward activation action. Activities that are rewarding are known to elicit dopamine release in parts of the brain (Bressan and Crippa, 2005). Many drugs of addiction have been found to increase the dopamine release in certain parts of the brain and this activates the reward centers leading to sustained drug use (Shaabani *et al.*, 2011). Cannabinoids have also been reported to indirectly induce dopamine release after binding the cannabinoid receptor CB1 in the nucleus accumbens (Cheer *et al.*, 2004). This may explain the observed higher dopamine in the smokers' group in our study. All the pituitary hormones-prolactin, follicle stimulating hormone and luteinizing hormone assayed were found not to be significantly different between the case and the control groups (Table 1). However, there was a significant increase in testosterone level in the smoking group in spite of the fact that

Table 1: Plasma levels of hormones and neurotransmitter in case and control subjects

Hormone/neurotransmitter	Control	Case
Dopamine (pg mL ⁻¹)	29.84±8.41	51.57±21.54*
Norepinephrine (pg mL ⁻¹)	247.20±73.85	385.98±102.31*
PRL (IU L ⁻¹)	12.33±2.78	11.29±3.44 ^{ns}
FSH (IU L ⁻¹)	9.30±2.36	7.67±2.93 ^{ns}
LH (IU L ⁻¹)	6.03±1.78	7.35±1.56 ^{ns}
Testosterone (IU L ⁻¹)	3.30±1.12	4.29±1.29*

*Significant when compared to control, ns: Not significant when compared to control

Table 2: Analysis of metabolic parameters in case and control subjects

Parameters	Control	Case
Total protein (g L ⁻¹)	71.50±7.10	73.55±6.43 ^{ns}
Glucose (mmol L ⁻¹)	5.15±1.10	4.27±1.22 ^{ns}
Triglycerides (mmol L ⁻¹)	0.91±0.09	1.08±0.13 ^{ns}
Total cholesterol (mmol L ⁻¹)	4.15±1.05	4.70±0.92 ^{ns}
HDL-cholesterol (mmol L ⁻¹)	1.40±0.10	1.41±0.08 ^{ns}
LDL-cholesterol (mmol L ⁻¹)	2.15±0.25	2.64±0.31 ^{ns}

ns: Not significant when compared to control

Table 3: Serum liver enzyme activities in case and control subjects

Activity (IU L ⁻¹)	Control	Case
ALT	7.20±2.54	8.90±2.97*
AST	5.90±1.79	9.50±2.54*

*Significant when compared to control

there was no significant difference in the levels of prolactin and the gonadotropin-FSH and LH. Table 2 shows the values for plasma total protein, glucose, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol. All these metabolic parameters revealed no significant difference between the case and the control group. In contrast, the liver toxicity marker enzymes, ALT and AST showed values that were different between groups (Table 3). Marijuana, Δ^9 -THC and other cannabinoids were reported to acutely alter Hypothalamic-pituitary-gonadal (HPG) function and affect reproductive function by acting at the hypothalamus either directly through GnRH or indirectly through other modulators (Murphy *et al.*, 1998). This effects include the inhibition of FSH, LH and testosterone secretion (Symons *et al.*, 1976) but we did not observe the same trend in our result. Prolactin is an inhibitor of the gonadotropins and itself is under the tonic inhibition of dopamine (Reschini *et al.*, 1980). Contrary to the expectation based on the higher dopamine levels in the smokers' group, there was no significant difference between the prolactin levels in the two groups, in agreement with (Block *et al.*, 1991). The reason for the higher levels of testosterone observed in the smoking group in spite of no difference in the gonadotropin is not clear, but it may rather be an associative relationship than a causative one. Many studies have shown that aggressive behaviors are associated with higher testosterone levels (Archer, 2004; Alqasoumi *et al.*, 2012). Cannabis smokers are more likely to engage in such behaviours that are linked with increased testosterone level. It also has to be mentioned at this point that the subjects we recruited for the case group are well known within the society for engaging in such behaviors. This group of people is also likely to take virility enhancing substances as they place high value on virility as a measure of manhood. This may explain to some extent the higher testosterone we observed in the smoking group. Similarly, some other components of marijuana may have testosterone elevating effects, this possibility cannot be ignored. Studies have shown that norepinephrine also plays key role in the brain reward system (Ordway *et al.*, 2007). Observing higher norepinephrine levels in the presence of higher dopamine is logical considering the fact that norepinephrine is synthesized from dopamine in just one step reaction catalyzed by dopamine β -hydroxylase (Cooper *et al.*, 1986). Many regions of the mesolimbic dopaminergic system, including the Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA), amygdala and the bed nucleus of stria terminalis have been reported to receive noradrenergic input (Alheid and Heimer, 1988). Lesion of noradrenergic neurons in the Locus Coeruleus (LC) decreases dopamine release in the NAc

(Grenhoff *et al.*, 1993) and conversely, activation of the LC's noradrenergic neurons increases the activity of dopaminergic neurons in the VTA (Lategan *et al.*, 1990; Sameri *et al.*, 2011). We observed a significant increase in the liver enzymes showing that cannabis smoking may have a somewhat toxic effect on the liver. This agrees with the reports of Borini *et al.* (2004). It should be noted that cannabis smoking does not only lead to ingestion of cannabinoids but several other substances also which are products of combustion of plants and some other organic materials. These include tar and carbon monoxide. Combustion of organic materials can also produce various polycyclic aromatic hydrocarbons which can be toxic to body tissues as well as the liver. It may therefore be too hasty to conclude that cannabinoids were responsible for liver toxicity observed. More studies are needed to be able to understand fully the various effects of cannabis on smokers. Understanding the effects of cannabis is very imperative given the many controversies surrounding the use of the drug. This will affect policy making in drug regulation and control. Further studies can also reveal any possible pharmacologically active substance that may have a good medicinal value, present in the drug. More human studies should be conducted using animal model, taking into account the possible effect of race, nutrition and lifestyle and also the species of cannabis used. Even in the animal studies administration of cannabis in way closely related to the human usual forms of consumption should be adopted rather than the administration of purified THC or other cannabinoids.

REFERENCES

- Abdelrahman, S.H., I.M. Mousa, S.M.E. Khojali and A.A. Ismail, 2012. Trypanocidal effect of *Cannabis sativa* on experimental camel trypanosomosis. *Res. J. Med. Plant*, 6: 281-285.
- Alheid, G.F. and L. Heimer, 1988. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid and corticopetal components of substantia innominata. *Neuroscience*, 27: 1-39.
- Alqasoumi, S., T.H. Khan, M. Al-Yahya, I. Al-Mofleh and S. Rafatullah, 2012. Effect of acute and chronic treatment of common spices in Swiss albino mice: A safety assessment study. *Int. J. Pharmacol.*, 8: 80-90.
- Archer, J., 2004. Sex differences in aggression in real-world settings: A meta-analytic review. *Rev. Gen. Psychol.*, 8: 291-322.
- Bauman, A. and P. Phongsavan, 1999. Epidemiology of substance use in adolescence: prevalence, trends and policy implications. *Drug Alcohol Depend.*, 55: 187-207.
- Block, R.I., R. Farinpour and J.A. Schlechte, 1991. Effects of chronic marijuana use on testosterone, luteinizing hormone, follicle-stimulation hormone, prolactin and cortisol in men and women. *Drug Alcohol. Depend.*, 28: 121-128.
- Borini, P., R.C. Guimaraes and S.B. Borini, 2004. Possible hepatotoxicity of chronic marijuana usage. *Sao. Paulo. Med. J.*, 122: 110-116.
- Bressan, R.A. and J.A. Crippa, 2005. The role of dopamine in reward and pleasure behaviour: Review of data from preclinical research. *Acta Psychiatr. Scand.*, 427: 14-21.
- Brown, T.T. and A.S. Dobs, 2002. Endocrine effects of marijuana. *J. Clin. Pharm.*, 42: 90S-96S.
- Cheer, J.F., K.M. Wassum, M.L. Heien, P.E. Phillips and R.M. Wightman, 2004. Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J. Neurosci.*, 24: 4393-4400.
- Cooper, J.R., F.E. Bloom and R.H. Roth, 1986. *The Biochemical Basis of Neuropharmacology*. 5th Edn., Oxford University Press, Oxford, pp: 289.

- Fant, R.V., S.J. Heishman, E.B. Bunker and W.B. Pickworth, 1998. Acute and residual effects of marijuana in humans. *Pharmacol. Biochem. Behav.*, 60: 777-784.
- Friedman, H., C. Newton and T.W. Klein, 2003. Microbial infections, immunomodulation and drugs of abuse. *Clin. Microbiol. Rev.*, 16: 209-219.
- Frisher, M., S. White, G. Varbiro, C. Voisey and D. Perumal *et al.*, 2010. The role of cannabis and cannabinoids in diabetes. *Br. J. Diabetes Vasc. Dis.*, 10: 267-273.
- Grenhoff, J., M. Nisell, S. Ferre, G. Aston-Jones and T.H. Svensson, 1993. Noradrenergic modulation of midbrain dopamine cell firing elicited by stimulation of the locus coeruleus in the rat. *J. Neural. Transm. Gen. Sect.*, 93: 11-25.
- Grotenhermen, F., 2007. The toxicology of cannabis and cannabis prohibition. *Chem. Biodivers.*, 4: 1744-1769.
- Hall, W. and L. Degenhardt, 2008. Cannabis use and the risk of developing a psychotic disorder. *World Psychiatry*, 7: 68-71.
- Hall, W. and N. Solowij, 1998. Adverse effects of cannabis. *Lancet*, 352: 1611-1616.
- Hashibe, M., H. Morgenstern, Y. Cui, D.P. Tashkin and Z.F. Zhang *et al.*, 2006. Marijuana use and the risk of lung and upper aerodigestive tract cancers: Results of a population-based case-control study. *Cancer Epidemiol. Biomarkers Prev.*, 15: 1829-1834.
- Henquet, C. and R. Kuepper, 2010. Does cannabidiol protect against the negative effects of the THC?. *Br. J. Psychiatry*, 197: 259-260.
- Jones, R.T., 2002. Cardiovascular system effects of marijuana. *J. Clin. Pharmacol.*, 42: 58S-63S.
- Lategan, A.J., M.R. Marien and F.C. Colpaert, 1990. Effects of locus coeruleus lesions on the release of endogenous dopamine in the rat nucleus accumbens and caudate nucleus as determined by intracerebral microdialysis. *Brain Res.*, 523: 134-138.
- Mirochnick, M., J. Meyer, D.A. Frank, H. Cabral, E.Z. Tronick and B. Zuckerman, 1997. Elevated plasma norepinephrine after in utero exposure to cocaine and marijuana. *Pediatrics*, 99: 555-559.
- Murphy, L.L., R.M. Munoz, B.A. Adrian and M.A. Villanua, 1998. Function of cannabinoid receptors in the neuroendocrine regulation of hormone secretion. *Neurobiol. Dis.*, 5: 432-446.
- Ordway, G.A., M.A. Schwartz and A. Frazer, 2007. *Brain Norepinephrine: Neurobiology and Therapeutics*. Cambridge University Press, Cambridge, UK.
- Ranganathan, M. and D.C. D'souza, 2006. The acute effects of cannabinoids on memory in humans: A review. *Psychopharmacology*, 188: 425-444.
- Reschini, E., C. Ferrari, M. Peracchi, R. Fadini, M. Meschia and P.G. Crosignani, 1980. Effect of dopamine infusion on serum prolactin concentration in normal and hyperprolactinaemic subjects. *Clin. Endocrinol.*, 13: 519-523.
- Richardson, T.H., 2010. Cannabis use and mental health: A review of recent epidemiological research. *Int. J. Pharmacol.*, 6: 796-807.
- Sameri, M.J., A. Sarkaki, Y. Farbood and S.M.T. Mansouri, 2011. Motor disorders and impaired electrical power of pallidal EEG improved by gallic acid in animal model of Parkinson's disease. *Pak. J. Biol. Sci.*, 14: 1109-1116.
- Shaabani, R., M. Jahanshahi, M. Nowrouzian, Y. Sadeghi and N.S. Azami, 2011. Effect of morphine based CPP on the hippocampal astrocytes of male Wistar rats. *Asian J. Cell Biol.*, 6: 89-96.
- Symons, A.M., J.D. Teale and V. Marks, 1976. Effects of delta-9-tetrahydrocannabinol on the hypothalamic-pituitary-gonadal system in the maturing male rat. *J. Endocrinol.*, 67: 43-44.
- Tehranipour, M. and S. Ebrahimpour, 2009. Evaluating the effect of aquatic extract of *Cannabis sativa* seed on spatial memory consolidation in rats. *J. Biol. Sciences*, 9: 884-888.