

The effect of *Cannabis sativa* on testosterone level and seminal fluid analysis of male Wistar rats

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ABSTRACT

The effects of *Cannabis sativa* on the testosterone level and seminal fluid analysis were studied. A total of twenty-four adult male rats were randomly divided into four experimental groups. Group 1 (control) were fed on 5% ethanol. Group 2,3 and 4 were fed 20mg of *cannabis* in 5% ethanol, 20mg of *cannabis* in 5% ethanol +100mg Vitamin C, and 20mg *cannabis* in 5% ethanol +1ml honey respectively for 21 consecutive days. The result showed a statistically significant decrease in percentage motile sperm and an increase in the percentage dead sperm ($p < 0.05$). The decrease in the sperm count were not statistically significant. Also the changes in Testosterone hormonal levels were not statistically significant. However, those adverse effects caused by *cannabis* were reversed by the concomitant administration of vitamin C and honey. Also Vitamin C improved the quality of seminal fluid more than Honey.

Key words: *Cannabis sativa*, Honey, Seminal fluid analysis. Testosterone, *Cannabis sativa*.

INTRODUCTION

Cannabis is a psychoactive substance obtained from the plant *Cannabis sativa* (Turner et al, 1980). It is also known as Indian hemp, Marijuana (McPherson, 2004) or ganja (Turner et al, 1980).

The plant and the products have a long history of use both as a medicinal agent and intoxicant (Elsophya 2005). According to Wikipedia (2008), evidence of inhalation of cannabis smoke can be found as far back as the Neolithic age as indicated by charred Cannabis seeds found in a ritual brazier at an ancient burial site in the present Romania.

Like the Rastafari, some modern Gnostic sects have asserted that Cannabis is the 'tree of life'. Other religions founded on the past century that treat cannabis as a sacrament are: THC Ministry, the way of harmony, Cathaism, Cannabis Assembly and the church of Cognizance.

It is a drug of abuse and therefore its production and use is illegal in most parts of the world as legislated in Marijuana tax Act of 1937, 1961 convention on Narcotic drugs and 1988 United Nations Convention against Illicit Traffic in Narcotics, drugs and psychotropic substances. In United Kingdom it is re-classified as class c drugs at par with tranquillizers (Lewis 2008).

The phytochemical analysis of the leaves and flower of Cannabis is known to contain "9-tetrahydrocannabinol (THC) as the biological active substances (McPherson 2001) and the route of exposure is by smoking and cooking it in food.

Cannabis is known to have some effects on the body systems. In the brain and Central Nervous System, it produces alteration in motor behaviour, perception, cognition, memory, learning and regulation of body temperature (Huestis et al 1992). In respiratory system, the smoke is potentially carcinogenic, reduces

lung function and increases chances of bronchitis, pharyngitis and asthma-like conditions (Huestis *et al.*, 1992). In the reproductive system Grossman (2006) reported that *Cannabis* affect 'swimming behaviour' of sperm but the fertility effects associated with *Cannabis* is unclear (Berkow *et al.*, 1997).

Therefore this work is aimed at investigating the effect of *Cannabis* on the seminal fluid analysis, testosterone levels and the effect of antioxidant substances such as honey and vitamin C on the parameters analysed.

MATERIALS AND METHOD

Animals

Twenty-four adult male rats of the wistar strain weighing between 120-150g were purchased from the animal house unit of the College of Health Sciences of Delta State University, Abraka. They were kept in cages of same sizes in the animal unit throughout the duration of the experiment.

The animals were randomly divided into four experimental groups of six rats each. The animals were weighed before the commencement and at the end of the experiment using electronic weighing scale.

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| Group 1 | (Control) fed 5% ethanol + rat chow + water ad libitum |
| Group 2 | fed 20mg of cannabis in 5% ethanol +rat chow +water ad libitum |
| Group 3 | fed 20mg of cannabis in 5% ethanol +100mg vitamin C +rat chow + water ad libitum |
| Group 4 | fed 20mg of cannabis in 5 % ethanol + 1ml Honey + rat chow + water ad libitum |

Vitamin C, a product of Roche Nigeria Limited were purchased from the university health centre.

Honey was purchased from an accredited agent of Forever Living Products.

Method of *Cannabis* extraction and administration

The leaves of the plant *Cannabis sativa* were harvested from plants grown in Abbi, Delta State under the authority of National drug law

enforcement agency (NDLEA). The leaves were authenticated by a botanist. Leaves were air-dried and ground with the aid of electric blender a product of kenwood, Japan. The extraction was done using absolute alcohol (ethanol) for 8 hours using Soxhlet extractor by the method of Sailani and Moeini, 2007. A concentration of 20mg per ml was obtained. Extracts were administered orally between 8.00 and 11.00 daily for 21 consecutive days.

Sample collection and analysis

After an overnight fast, the animals were sacrificed by inhalational anaesthesia and blood collected by cardiac puncture. Approval was gotten from the local research ethics committee.

The right and left epididymis were dissected from the caput to the boundary between the cauda and the first part of the vas deferens.

For the sperm motility, one epididymis of the rat was minced, a few drops of physiological saline were dropped on the slide, the tissue were moved back and forth a few times until semen is transferred to the slide. Under a microscope using a neubaer hemocytometer at 40x objective lens the active progressive sperm movement, sluggish sperm movement and non-motile were compared by the methods of Linder *et al.*, 1988, Liobet *et al.*, 1991, and Nwangwa *et al.*, 2007.

Determination of hormonal assay

The collected blood was allowed to clot and the serum put in a test tube and centrifuged at 300 rev per minutes. The supernatants were collected with the aid of a pasteur pipette and testosterone levels determined by ELISA method using ELISA hormonal kits.

Statistical analysis

The results were expressed as mean \pm SEM. The students' t-test was used for the evaluation of statistical significance. SPSS (13.0 version) statistical software was also used for analysis. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The data obtained from the above study is as shown in the tables below.

Table 1: Shows the weight of Testes and final body weight of the rats after the administration of Cannabis, honey and Vitamin C

	Gp 1.n=6 (control) fed 5%ethanol	Gp 2.n=6 (fed 20mg Cannabis in 5% ethanol	Gp 3.n=6 (fed 20mg Cannabis in 5%ethanol +100mg Vit C	Gp 4. N=6 (fed 20mg Cannabis in 5%ethanol +1ml Honey
Wt. of Testes(g)	0.69 ± 0.03	0.92 ± 0.01*	0.82± 0.04*	0.81 ± 0.04*
Initial b.wt(g)	126.76 ± 3.64	125.01± 2.92	125.21± 3.42	125.46 ± 3.00
Final b.wt(g)	170.43 ± 3.46	160.63 ±2.23	161.35 ± 4.61	155.13± 3.06
Difference in b.wt (g)	43.68 ± 0.85	35.55± 1.51*	35.63± 1.92*	29.50± 0.96*

Data is given as mean ± SEM. b.wt = Body weight

*Statistically significant

Table 2: ; shows the effects of Cannabis, Honey and Vitamin C on the seminal fluid analysis and Testosterone level in the rats

	Gp 1.n=6 (control) fed 5%ethanol	Gp 2.n=6 (fed 20mg Cannabis in 5% ethanol	Gp 3.n=6 (fed 20mg Cannabis in 5%ethanol +100mg Vit C	Gp 4. N=6 (fed 20mg Cannabis in 5%ethanol +1ml Honey
Sperm count ×106/mL	56 ± 3.28	53± 3.55	79 ± 1.19*	72± 1.25*
% motile(%)	71± 3.71	60 ± 3.95*	87± 1.38*	78 ±2.43
% Dead (%)	29 ± 3.71	40 ± 3.95*	13± 1.38*	22± 2.43*
Testosterone Level (ng/ml)	0.16 ± 0.02	0.11± 0.27	0.12± 0.01	0.11± 0.01

*statistically significant.

Data is given as mean ± SEM

DISCUSSION

Honey is a natural sweet substance produced by honeybees from the nectar of flowers (Martos *et al.*, 2000). It has been effective in treating many ailment such as wounds, cough etc as a result of its antibacterial, antifungal and antiviral effects (Krishua, 2007). It is recommended for men and women with problems of infertility as it is known to be rich in glucose and fructose which these organs important in fertility utilizes. It is considered to be fertility boosting super food (Esminger et al 1983). Vitamin C has proven to be beneficial in treating male fertility because it protects sperm from oxidative damage and has been shown to improve

sperm quality in smokers (Brystrianyk, 2006).Vitamin C according to a study improved the three parameters for measuring sperm quality. In this research, the administration of cannabis has shown to have a statistically significant decrease in the percentage motile sperm, and a statistically significant increase ($p < 0.05$) in the percentage dead sperm but no statistical significant effect in the total sperm count. This finding is consistent with the report of Rossato et al, 2005 that cannabinoids and endocannabinoids negatively influence sperm function. Cannabis also caused a statistically significant decrease in the total body weight of the rats and statistically significant increase in the weight of the testes.

However, the concomitant administration of Vitamin C and Honey has shown to significantly reduce the adverse effects of cannabis administration on the quality of seminal fluid. Vitamin C also proved to have a better semen enhancing properties than Honey.

Cannabis, honey and Vitamin C did not have any statistically significant effects on the level of testosterone in the experimental animals, which means the infertility that may be associated with *Cannabis* administration may not be a hormonal defect but rather a seminal fluid functional disorder.

CONCLUSION

This present research has shown that *Cannabis* has an adverse effect on the quality of

seminal fluid and could lead to infertility in those who take it. The work also showed that the administration of vitamin C and Honey can improve remarkably the quality of the sperm and therefore individuals with infertility problem should be encouraged to take it as it protects the sperm from oxidative damage. *Cannabis* also does not affect the testosterone level of the rats. Individuals should therefore be discouraged from intake of *Cannabis* especially those in the reproductive age group.

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REFERENCES

- Berkow, R. *et al.*, The Merck manual of medical information (Home edition), 449. Retrieved, April 2008 (1997).
- Brystrianyk, R., Vitamin C supplement help reverse infertility Health sentinel (Home edition) Retrieved April 2008 (2006).
- Elsohly, M.A., Chemical constituents of Cannabis :The complex mixture of natural cannabinoid P1 Retrieved April 28, 2008 (2005).
- Esminger, A.H., Esminger, M.E., Kondale, J.E., Robson. TRK, Food and Nutrition Encyclopaedia. California. Papus press (1983).
- Grossman, J., Stoned sperm, cannabis can affect fertility. about.com Retrieved March 21: 2008 (2006).
- Huestis, M.A., Heringfield, J.E., and Cone, E.J., Blood cannabis: absorption of THC and formation of 11-OH-THC and THC-COOH during and after marijuana smoking *J. Ana. Tox* **16**(5): 276-282 (1992).
- Krishua, M. (2007) Honey, Cholesterol, high cholesterol, importance, infertility, sexual virility. Ayurveda. Retrieved September 2008
- Lewis, S., Informed choice <http://bbcnews/uk/northernireland/cannabisdamagesmalefertility> (2008).
- Linder, R.E., Rehnberg, G.L., Strader, L.F., Diggs, J.P., Evaluation of Reproductive parameters in adult male wister rats after subchronic exposure (gavage) to Benomyl. *J. Toxicol. Environ. Health* **25**: 285-289 (1988).
- Liobet, J.M., Irvent, J.J., Ortega, A., Domingo, J.L., Influence of chronic exposure to uranium on male reproduction in mice. *Fundam. Appl. Toxicol.* **6**: 821-825 (1991).
- Martos, J., Ferres, F., Thomas-Barberan, F., Identification of flavonoid markers for the botanical origin of Eucalyptus Honey *J. Agric. Food. Chem* **48**(3):1498-1502 (2000)
- McPherson, G., Black's Students medical Dictionary AC and Black publishers London 95-96 (2004).
- Nwangwa, E.K., Igweh, J.C., Uzuegbu, U.E., Adegor, E.C., The effect of quinine therapy on the seminal fluid analysis and histology of testes of male rats. *Biosc. Biotech. Res. Asia.*, **4**(1): 111-116 (2007).
- Rossato, M., Popa. I.F., Fengo, M., Clari, G and Forest, C., Human sperm express cannabinoid receptors cb1, the activation of which inhibits motility, acrosome reaction and mitochondrial function. *J. Clin. Endocrin and Metabol.* **90**(2): 984-991 (2005).
- Sailani, M.R., and Moeini H., Effects of Ruta graveolens and cannabis sativa alcohol extract on spermatogenesis in adult wistar rats, *Ind. J. Urol.* **23**: 257-260 (2007).
- Wikipedia. [http://cannabis\(drug\)healthissuesandtheeffectsofcannabis](http://cannabis(drug)healthissuesandtheeffectsofcannabis). Retrieved March 16 (2008).