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Sex Hormones Levels as Influenced by *Cannabis sativa* in Rats and Men

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Abstract: This study targeted the possible effects of chronic marijuana use on reproductive hormones. To pursue this effect, the levels of the testosterone, the Luteinizing Hormone (LH) and the Follicle Stimulating Hormone (FSH) were assayed in men users in Sudan and in *Cannabis sativa* extract treated rats. Results were compared to non using groups as controls. Luteinizing Hormone (LH), Stimulating Hormone (FSH) and testosterone levels showed significant changes after 10 days in rat groups. In all addicted men groups, only slight non significant changes were observed compared to non users. These findings supported previous studies and concluded that *C. sativa* can significantly influence the levels of sex hormones in rats.

Key words: Chronic marijuana, reproductive hormones, rats

INTRODUCTION

Alterations in endocrine function in conjunction with marijuana use have caused considerable concern. Researchers' efforts in many investigations in the recent past have made it abundantly clear that exposure to marijuana had significant effects upon the reproductive system on both male and female altered testicular function and depressed hormonal secretion (Harclerode, 1984); LH and FSH, secreted by pituitary gland are of major importance in reproduction in the male. The tetrahydro-cannabinol induced blockage of gonadotropins release and results in lowered LH and FSH, which are responsible for a reduced testosterone production by the leyding cells of the testis (Harclerode, 1984). Testosterone, LH and FSH levels were fell below normal laboratory ranges in 10.8% marihuana men users, range from 0.2-15.3 mg/ml, 3.2-30.8 U/ml and 1.3-12.2 U/ml respectively, but demonstrated no significant differences among user group frequency of marihuana use and age (Block *et al*., 1991). Acute and chronic marihuana smoking resulted in decreased plasma testosterone and LH concentrations and large doses produced oilgospeimia with decreased FSH (Kolodny *et al*., 1974). Similar result were observed in male mice studies as decreased plasma testosterone due to inhibition of pituitary LH release with decreased plasma FSH (Dalterio *et al*., 1978). Also, plasma LH concentration was significantly depressed when four healthy males smoked one or two marihuana cigarettes containing 2.8%-terahydrocannabiol (delta 9-THC), whereas, FSH and testosterone concentration showed no significant decrease (Cone *et al*., 1986). However, Fredrich *et al*. (1990) observed no influences of long term cannabis usage on plasma testosterone levels in 66 males Pakistani smoked cannabis daily for four years or drank cannabis regularly and measurement results were compared with those of 41 normal controls. Moreover, plasma LH concentration increased significantly in healthy menopausal female smokers, a 1gm marihuana cigarettes containing 1.83 delta 9-THC (Mendelson *et al*., 1985). Recent findings showed that, obvious changes detected in liver function and hematological indices were observed to be abolished after Long term *C. sativa* usage in addict men (Mukhtar and Elbagir, 2011) and Mukhtar *et al*. (2011). Then the length of the period of exposure to *C. sativa* could be significant factor that major changes resulted after the beginning exposure can be balanced and abolished after longer time of *C. sativa* use. In this work, sex hormones levels were followed in rats injected with *C. sativa* extract and in addict men, smoked *C. sativa* for different periods of time.

MATERIALS AND METHODS

The experimental subjects included twenty-four healthy rats (males and females), 6-9 months old, weighing between 70-200 gm, divided randomly into four groups a control and 3 *C. sativa* extract treatments, six animals were allotted to each group. The treated groups were injected with four doses of *C. sativa* petroleum extract intramuscularly for 10 days, in two days intervals as (0.2 mg/g body weight as low dose), (0.4 mg/g body weight as medium dose) and (0.6 mg/g body weight as high dose), respectively. The dose was prepared by weighing cannabis extract and calculated according to the animal body weight as described by (Mahfouz *et al*., 1975; Rsenkrantz and Esber, 1980). Blood samples were collected twice from the ocular vein of rats, after two days from the second dose and from the last dose respectively. The human blood samples were collected from twenty Sudanese male users addicted to *C. sativa* smoking and six healthy non users (control) of 18-60

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years old. Addicts were divided into three groups according to the duration of usage (3-8 years, mild users), (11-17 years, medium users) and (18-40 years, heavy users). All blood samples were used to determine changes in endocrine hormone LH, FSH and testosterone.

Luteinizing hormone LH quantification was measured by using radioimmunoassay Kit LHK-455 according to the methods described by Krieger *et al.* (1972), Nillius and Wide (1973) and Beitins *et al.* (1976). Follicle stimulating hormone concentration was determined using radioimmunoassay Kit IMK-456 according to the method described by Frenchimont (1973) and Shome and Barlow (1974). Testosterone hormone concentration was determined using enzyme immunoassay (ELA) Kits by Testo-EIA. (2001).

Statistical analysis was preformed by using completely randomized design. The data was tabulated and subjected to analysis of variance (on way ANOVA) using the Microsoft Computer Program as described by Steal and Torrie (1960).

RESULTS

The effects of different *Cannabis sativa* extract doses on LH hormone in rats are presented in Table 1. After the second dose no significant effect was seen in animals treated with low and high dose (0.2 and 0.6 mg/g) though they showed lower levels compared to the control group whereas, the group treated with medium dose (0.4 mg/g) showed high level. By the end of the experiment, animals treated with the high dose (0.6 mg/g) showed significant rise ($p<0.05$) compared to the control group. Table 2 presents the result of the plasma FSH hormone concentration in treated rats groups which showed reduced levels in all groups following the second dose compared to control group but after the last *C. sativa* extract dose, the FSH concentration represented higher values compared to the samples collected after the second dose and the value reported in the group treated with 0.4 mg/g was significantly ($p<0.05$) higher value than in the control group. Results of plasma testosterone level, in the treated groups of rats are presented in Table 3. The level of testosterone represented decreased values in all treated groups with significantly ($p<0.05$) lower level in the high dose group compared to the control group. The concentration of the testosterone hormone showed slight rise in samples collected after the last dose but still the group treated with high dose showed significantly ($p<0.05$) lower level compared to the control group.

Table 4 presents changes in the levels of plasma LH, FSH and testosterone hormone in addicts groups compared to the non users. No significant differences were observed in all groups of different levels of addiction compared to the non users group. The LH hormone concentrations in addicts reported slightly lower levels than in the control group. On the other hand FSH hormone and testosterone hormone in addict men group showed clear but not significant elevation compared to the normal level, in the non users group.

Table 1: The effect of different doses of *C. sativa* extract on plasma Luteinizing Hormone (LH) concentration U/L in rats (Mean ± SE)

Treatment	LH level after the second dose	LH level after the last dose
Control	1.12±0.48[a]	0.59±0.11[b]
Low dose 0.2 mg/g	0.43±0.13[a]	0.37±0.11[b]
Medium dose 0.4 mg/g	1.17±0.46[a]	0.24±0.08[b]
High dose 0.6 mg/g	0.47±0.14[a]	2.92±0.90[a]

Means with different letters in the same column are significantly different ($p<0.50$)

Table 2: The effect of different levels of *C. sativa* on Follicle Stimulating Hormone (FSH) concentration U/L in rats (Mean ± SE)

Treatment	FSH level after second dose	FSH level after last dose
Control	3.04±1.26[b]	2.09±0.75[b]
Low dose 0.2 mg/g	0.42±0.06[b]	1.09±0.36[b]
Medium dose 0.4 mg/g	1.38±0.62[b]	5.82±0.69[a]
High dose 0.6 mg/g	0.75±0.36[b]	2.62±0.50[b]

Means with different letters in the same column are significantly different ($p<0.50$)

Table 3: The effect of different levels of *C. sativa* on testosterone hormone concentration U/L in rats (Mean ± SE)

Treatment	Testosterone level after second dose	Testosterone level after last dose
Control	7.95±1.23[a]	8.90±1.05[a]
Low dose 0.2 mg/g	6.78±1.62[a]	10.65±1.39[a]
Medium dose 0.4 mg/g	5.85±1.89[a]	8.05±1.52[a]
High dose 0.6 mg/g	1.33±0.53[b]	3.05±0.88[b]

Means with different letters in the same column are significantly different ($p<0.50$)

Table 4: The effect of *C. sativa* on Luteinizing Hormone (LH) Follicle Stimulating Hormone (FSH) and testosterone hormone U/L in men (Mean ± SE)

Addiction periods	LH concentration	FSH concentration	Testosterone concentration
Control-non users	5.28±0.83[a]	2.06±0.45[a]	21.93±2.68[a]
Mild users	4.68±1.72[a]	3.55±0.79[a]	30.17±2.69[a]
Medium users	4.32±1.19[a]	3.36±1.85[a]	27.43±0.89[a]
Heavy users	4.27±1.23[a]	2.72±0.36[a]	32.59±5.60[a]

Means with different letter in the same column are significantly different ($p<0.05$)

DISCUSSION

In the present work, the effects of the length of exposure to *C. sativa* on the levels of the sex hormones were tested by using different doses of *C. sativa* extract in rats. The levels of the FSH, LH and the testosterone hormones were assessed during the course of the study. Also the same hormones were estimated in *C. sativa*, addicts, smoked for different periods of time and the values were compared to the levels in non users as

control. Results of LH level in rats serum (Table 1) showed no significant effect, in samples collected after the second dose, only by the end of the experiment rats treated with the high dose (0.6 mg/g) reported significant rise ($p<0.05$) compared to the control group. FSH hormone levels (Table 2) showed non-significantly decreased levels in all groups following the second dose compared to the control group. FSH levels after the last dose increased and the increase was significantly ($p<0.05$) higher in the group treated with the medium dose of (0.4 mg/g) compared to the control group. The testosterone hormone levels (Table 3) in rats performed similar to the FSH and showed decreased values after the second dose with significant ($p<0.05$) difference in the group received the highest dose. Also the levels increased after the last dose compared to their values after the second dose but still lower than the control group. These results were in line with findings of Ayalon *et al.* (1977); Besch *et al.* (1977); Collu *et al.* (1975) and Marks (1973). However, Smith *et al.* (1979) and Tyrey (1978) mentioned that the hormonal fluctuation might be in part, pressure to testicular development, resulting in phases of interruption in the function of sertoil and leydig cells, general acute cases or sub-acute cases. The transient decrease of the LH levels in the high dose treated rats and of the FSH levels in all groups can be explained as the suggested by Harris and Esber (1980) and Harclerode (1984) who stated that in response to drug metabolic stress cannabinoids transiently depress pituitary function as reflected by decrement in LH and FSH hormone. It is not known whether this effect occur directly through the pituitary gland or is mediated through the hormone-releasing factors and higher brain centers regulating sexual development and behavior. It might be speculated that acute administration of cannabinoids can affect nerve and hormone signals to the pituitary gland.

On the other hand in all addict men groups, LH, FSH and testosterone hormone values showed no significant change and were within normal levels. This is in agreement with the result reported by Block *et al.* (1991), who found that, chronic marihuana smoking did not alter endocrine hormone levels of men or women. Also Cushman (1975) reported that Plasma testosterone, FSH and LH levels obtained from healthy consecutive heterosexual male marijuana smoking University students did not differ significantly from those of normal controls.

Other authors reported adverse physiological and psychological effects of marihuana use which may include possible effects on reproductive function and obtained some affection of marihuana on reproductive organs. Mandal and Das (2009) found that Intra peritoneal injection of cannabis extract at low doses induced adverse effect on testes, histology findings revealed significant shrinkage of tubular diameter and detrimental changes in seminiferous epithelium of testis with resulting lowered serum testosterone and pituitary gonadotropins (Follicular Stimulating [FSH] and Luteinizing Hormones [LH]) levels. Abdel Mageed *et al.* (2005) showed clear deleterious effects effect of *C. sativa* smoke on rats' testicular tissue manifested by the degenerative changes encountered in the spermatogenic, supporting and interstitial leydig's cells accompanied by variable degree of maturation arrest and marked proliferation in the myoid cells surrounding the basement membrane was also evident. In other work, Chan *et al.* (2001) studied about the delta 9-Tetrahydrocannabinol (delta 9-THC) for its' potential carcinogenicity in rodents because it is the principal psychoactive ingredient in marihuana and it has potential medicinal uses. When delta 9-THC was administered to groups of male and female Fischer rats and B6C3F1 mice, serum FSH and LH levels in all dosed male rats and corticosterone levels in female rats were significantly higher than controls. delta 9-THC administration also induced testicular atrophy and uterine and ovarian hypoplasia.

In a recent study Saberivand *et al.* (2010) studied the inclusion of *Cannabis sativa L.* seed (hempseed) in the diet of rats, as model of menopause, for 3 weeks and suggested that hempseed may improve post-ovariectomy complications in rats and stated that *Cannabis sativa L.* has been used for the treatment of various gynecological diseases in traditional medicine. The potential of this plant to protect against complications of menopause has been raised but rarely studied.

Findings of the present work supported previous studies and concluded that dose and period length of exposure to *C. sativa* can influence the levels of sex hormones in users. Provision of careful clinical information is recommended, considering the length of exposure, may be helpful in evaluating accurate picture of the health consequences of marihuana use.

REFERENCES

Abdel Mageed, N., A.M. Abbas, E. Hassan and E. EL Abagy, 2005. Effect of Bhang on Testicular Tissue of Adult Male Albino Rats: A Histological and Ultrastructureal Study. National hepatology and tropical medicine research institute. http://www.nhtmri.org/Researches.htm.

Ayalon, D., I. Nir, T. Cordova, S. Bauminger, M. Puder, Z. Noar, R. Kashi, U. Zoi, A. Harell and H.R. Linder, 1977. Acute efforet of THC on hypothmopituitsary ovarian axis in the rat. Neuroendocrinology, 23: 31-42.

Beitins, J.Z., K.O. Loughlin, T. Ostrea and J.W.MC. Arthur, 1976. Gonadotrophin determination in time 3 hours urine collection during the menstrual cycle and LHRIA testing. J. Clin. Endo. Metab., 43: 46-55.

Besch, N.F., C.G. Smith, P.K. Bech and F.H. Kaufman, 1977. The effect of marihuana THC on the secretion of luteinizing hormone in the ovariectomized rhesus monkey. Am. J. Obstet. Gynecol., 128: 635-642.

Block, R.I., R. Farinpour and J.A. Schlechte, 1991. Effects of chronic marijuana use on testosterone, luteinizing hormone, follicle stimulating hormone, prolactin and cortisol in men and women. Drug Alcohol Dependence, 28: 121-128.

Chan, P.C., R.C. Sills, A.G. Braun, J.K. Haseman and J.R. Bucher, 2001. Toxicity and carcinogenicity of delta 9-tetrahydrocannabinol in Fischer rats and B6C3F1 mice. J. Mol. Med., 78: 613-25.

Collu, K., G. Leboeuf and J.R. Ducharme, 1975. Endocrine effect of chronic administration of psychoactive drugs prepateral male rat. 1-THC. Life Sci., 16: 5333-542.

Cone, E.J., R.E. Jhnson, J.D. Moore and J.D. Roache, 1986. Acute effect of smoking marijuana on hormones, subjective effects and performance in male human subject. Pharmcol. Biochem. Behav., 24: 1749-1754.

Cushman, Jr. P., 1975. Plasma testosterone levels in healthy male marijuana smokers. Am. J. Drug Alcohol Abuse., 2: 269-275.

Dalterio, S., A. Barlke, C. Roberson, D. Watson and S. Burstein, 1978. Direct and pituitary mediated effects of THC and cannabinol on the testis. Pharmacol. Biochem. Behav., 8: 673-678.

Frenchimont, 1973. Human gonandoprotein secrection in male subject. In James. V.H.T, Serio M. and Martini, L. (Eds). The endocrine function of the human testic. Academic Press Vol. 1, New York, pp: 439-458.

Fredrich, G., W. Nepita and T. Andre, 1990. Serum testosterone concentration in cannabis and a piate users. Beifr. Gericht. Med., 48: 6-66.

Harris-Rosenkrantz and H.J. Esber, 1980. Coannabinoid induced hormone change in monkey and rats. J. Toxicol. Enuiron. Healh, 6: 297-313.

Harclerode, J., 1984. Endocrine effect on marijuana in the male preclinical studies. NIDA. Res. Monogr., 44: 46-64.

Kolodny, R.C., W.H. Masters, R.M. Kolodner and G. Toro, 1974. Depression of plasma testosterone levels after chronic intensive marihuana use. N. Engl. J. Med., 290: 872-874.

Krieger, D.T., R. Ossowski, M. Fogel and W. Allen, 1972. Lake of circadina periodicity of human serum FSH and LH levels. J. Clin. Endo. Metab., 35: 619-623.

Mandal, T.K. and N.S. Das, 2009. Testicular toxicity in cannabis extract treated mice: Association with oxidative stress and role of antioxidant enzyme systems. Toxicol. Ind. Health, 26: 11-23.

Mahfouz, M., A.B. Marker, M.T. Ghoneim and H.M. Mikhail, 1975. Effect of hashish on brain gamma aminobutyric acid system, blood fibrinolytic activity and glucose and some serum enzymes in rats. Pharmazie., 30: 772-774.

Marks, B.H., 1973. Delta 9-THC and luteinizing hormones secretion. Prog. Brain. Res., 39: 331-338.

Mendelson, J.H., P. Cristofaro, D. Ellingboe, K. Benedikt and N.K. Mello, 1985. Acute effects of marijuana on leutinizing hormone in menopausal women. Pharmacol. Biochem. Behav., 23: 765.

Mukhtar, A.H. and N.M. Elbagir, 2011. Effect of *Cannabis sativa* on hematological indices in rats and men. Pak. J. Nutr., 10: 313-316.

Mukhtar, A.H., N.M. Elbagir and A.A. Gubara, 2011. The effect of *Cannabis sativa* on certain enzymes of clinical significance in rats and men. J. Pharmacognosy, 2: 10-13.

Nillius, S.J. and L. Wide, 1973. Radioimmunosorbent assay of FSH and LH hormone in serum and urine from men and women. Acta Endo-Copehge, 73: 3-58.

Rsenkrantz, H. and H.J. Esber, 1980. Cannabinoid-induced hormone hormone changes in monkeys and rats. J. Toxicol. Environ. Health, 6: 297-313.

Saberivand, A., I. Karimi, L.A. Becker, A. Moghaddam, S. Azizi-Mahmoodjigh, M. Yousefi and S. Zavareh, 2010. The effects of *Cannabis sativa* L. seed (hempseed) in the ovariectomized rat model of menopause. Methods Find Exp. Clin. Pharmacol., 32: 467-473.

Shome, B. and A.F. Barlow, 1974. Human follicle stimulating hormone. J. Clin. Endo. Metabo., 39: 199-202.

Smith, C.G., N.F. Besch, R.G. Smith and P.K. Besch, 1979. Effect of tetrahydrocannabinol on the hypothalamic pituitary axis in the ovariectomized rehesus monkey. Ferti. Steril., 13: 335-339.

Steal, K.G.D. and J.H. Torrie, 1960. Principles and procedures of statistics. Mcgraw Hill Book Co Inc., New York.

Testo-EIA, 2001. Testosterone hormone concentration determind using enzyme immunoassay (ELA) kits.

Tyrey, L., 1978. Suppression of serum LH in the ovariectomized by delta 9-THC. Endocrinol., 102: 1808-1814.