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Effect of Narcotic Addiction on Hypothalamic Pituitary Gonadal Axis Hormones

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ABSTRACT

Drug addiction is considered a serious societal problem, often leading to social maladaptation, decreased work productivity and job loss. Beside loss of control, drug addiction gradually leads to the development of tolerance and a demand to take higher doses in order to attain the desired stimulating effect. This study intended to investigate the effect of bhang (which consists of the leaves and flowering tops of male and female *Cannabis sativa* plant) and opium addiction on hypothalamic pituitary gonadal axis hormones. It was conducted on 83 individuals whose ages ranged from 23 to 35 years and who were classified into 6 groups. Group A: male subjects addicted to opium; group B female subjects addicted to opium; group C male subjects addicted to bhang; group D female subjects addicted to bhang; group E control male subjects and group F control female subjects. Blood sampling from female groups (addicts and control) were taken during the follicular phase. The results of our research revealed a significant decrease in serum total testosterone, follicle stimulating hormone (FSH), leutinizing hormone (LH) and prolactin in male addicts to opium in relation to the control group. Although the serum level of these hormones was significantly lower in males addicted to bhang when compared to the control group, the effect of opium was relatively more severe than the effect of bhang. However, the decrease in serum estradiol in male addicts was not significant when compared to the control group. Moreover, the results showed a significant decrease in serum testosterone, estradiol, FSH, LH and prolactin levels in female addicts to opium more than the female addicts to bhang when compared to the control group except for estradiol, for which the effect of bhang was more severe.

Keywords: bhang, drug abuse, estrogen, opium, progesterone, prolactin, testosterone
Abbreviations: CBD, cannabidiol; **CB1**, cannabinoid receptor 1; **CBN**, cannabiniol; **DA**, dopamine; **FAAH**, fatty acid amide hydrolases; **FSH**, follicle stimulating hormone; **GABA**, gamma-aminobutyric acid; **GnRH**, gonadotropin releasing hormone; **LH**, lutenising hormone; **PRL**, prolactin; **THC**, Δ-9-tetrahydrocannabinol

INTRODUCTION

Drug addiction is a chronic relapsing disorder in which compulsive drug seeking and drug-taking behavior persist despite serious negative consequences. Substances of abuse induce pleasant feelings (euphoria in the initiation phase) or relieve distress. Regular use of drugs induces adaptive changes in the central nervous system that lead to a state of tolerance to the drug, lack of physical independence, sensitization, craving, and relapse (Farre and Cami 2003; Cunha-Oliveira *et al*. 2008).

Opioids activate specific receptors (mu (μ), delta (δ), and kappa (κ)) that couple the G protein. The μ receptor has been implicated in mediating or modulating the rewarding effect of other drugs of addiction (e.g., cannabinoids) (Boothby and Doering 2007). The use of marijuana, hashish, and bhang produces feelings of relaxation and well-being and at the same time it impairs cognitive faculties and performance of psychomotor tasks. Overdose can induce panic attack and psychosis (Hall and Solowij 1998).

The long-term effect of taking high doses of cannabinoids is a complex and controversial subject. Although there is evidence that long-term use of cannabis impairs memory, the cause of marijuana, a motivational syndrome, loss of energy and drive to work, remains unclear (Bolla *et al*. 2002). Drugs of addiction are usually receptor agonists, such as endogenous neurotransmitters, that act on two different types of membrane receptors: ionotropic and metabotropic receptors. Ionotropic receptors (ligand-gated ion channels) mediate fast synaptic transmission (Farre and Cami 2003).

There is abundant evidence that the endocannabinoid system plays a role in the reproductive functions in both males and females, in both animals and humans (Wang *et al*. 2006; Francavilla *et al.* 2009). High levels of functional cannabinoid receptor 1 (CB_1), anandamide, and fatty acid amide hydrolase (FAAH) are present in the pre-implantation embryo and/or in the uterus (Guo *et al*. 2005).

Endocannabinoids may also affect the levels of various hormones required for normal fertility and reproduction. Although such findings may suggest the potential usefulness of CB_1 antagonists in the treatment of infertility problems, a note of caution is necessary because CB_1 knockout mice were reported to have impaired oviductal transport of embryos, leading to embryo retention. This suggests that treatment with CB_1 antagonists may facilitate ectopic pregnancy (Horne *et al*. 2008; Abel *et al.* 2009).

In males, anandamide adversely affects the fertilizing capacity of sperm, which express functional CB_1 receptors (Whan *et al*. 2006). On the other hand, there is preclinical evidence to suggest that blockage of CB_1 may be useful in the treatment of erectile dysfunction (Melis *et al*. 2006).

Opioids acts preferentially at different receptor sites leading to stimulatory or inhibitory effects on hormone release. Increasing opioid abuse primarily leads to hypogonadism but may also affect the secretion of other pituitary hormones. The potential consequences of hypogonadism include decreased libido and erectile dysfunction in men,

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oligomenorrhea or amenorrhea in women, and bone loss or infertility in both sexes (Vuong *et al*. 2010).

Follicle-stimulating hormone (FSH) and luteinising hormone (LH) are glycoproteins secreted by the anterior pituitary that acts directly on the testis in order to stimulate somatic cell function in support of spermatogenesis. In males, FSH receptor expression is limited to the testicular Sertoli cells (Rannikki *et al*. 1995; Adhikari and Liu 2009), while LH receptors are found primarily in the Leydig cells. In particular, the primary role of FSH in spermatogenesis is stimulation of Sertoli cell proliferation during pre-pubertal development (Heckert and Griswold 2002), whereas LH acts on regulation of testosterone synthesis within the adult testis.

The hypothalamic-pituitary-gonadal process of controlling the secretion of gonadal hormones, testosterone and estrogen, begins with the hypothalamus secretion of gonadotropin-releasing hormone (GnRH). GnRH stimulates the pituitary gland to secrete LH and FSH. These two hormones are released into the systemic circulation and interact with the gonads, the testes and ovaries, in order to secrete testosterone or estrogen, respectively (Rajagopal *et al*. 2003).

These sex hormones then feed back to the hypothalamus and pituitary to form a complete feedback loop. Testosterone and estrogen are essential for normal sexual and reproductive growth and behavior (Rajagopal *et al*. 2003).

This mechanism is also modulated by a complex series of outside influences; opioids are one of a number of such influences. Evidence shows that endogenous opioids can bind to opioid receptors primarily in the hypothalamus, but potentially also in the pituitary and the testes, to modulate gonadal function (Veldhuis *et al*. 1984; Vuong *et al.* 2010). Decreased release, or interference with the normal pulsatility of release of GNRH at the level of the hypothalamus, has been documented, with a consequent decreased release of LH and FSH from the pituitary. Since bhang and opium are one of the most illicit drugs of abuse in Egypt, this study intended to investigate the effects of opium and bhang addiction on hypothalamic pituitary gonadal axis hormones and their role in hypogonadism and infertility in both sexes.

SUBJECTS AND METHODS

This study was performed on 83 adult male and female subjects whose ages ranged between 23 and 35 years. The subjects were recruited from the Addiction Unit, Faculty of Medicine, Ain-Shams University, Cairo, Egypt while the control male and female subjects were recruited from the community through public announcement. Blood sampling from female groups (addicts and control) was done during the follicular phase. They were classified according to the substance of addiction into six groups: Group A: male subjects addicted to opium (n = 15). Group B: female subjects addicted to opium (n = 14). Group C: male subjects addicted to bhang (n = 15). Group D: female subjects addicted to bhang (n = 15). Group E: control male subjects (n = 12). Group F: control female subjects (n = 12).

Fasting blood samples were withdrawn after 10 min rest in the sitting position, allowing 20 min for coagulation, centrifuged at 4000 rpm for 10 min. Serum was separated and stored at -20°C for determination of serum total testosterone (Chen *et al.* 1991), serum estradiol (Ratcliffe *et al*. 1988), serum FSH, serum LH and serum prolactin (Uotila *et al*. 1981).

Descriptive statistics were done using Microsoft Excel 2007. All analysis and graphics were performed using GraphPad Prism v. 5 (Windows v. 7, GraphPad Software 2007).

RESULTS

Table 1 shows the descriptive statistic of serum total testosterone level (ng/ml) in different investigated groups. Serum testosterone was significantly lower in opium and bhang addict males compared with the healthy male group at $P < 0.001$. In female addicts to opium and bhang the serum testosterone level was significantly lower than the control female group at $P < 0.01$ and < 0.05, respectively.

Table 1 Serum total testosterone level (ng/ml) in different studied groups.

Groups		Number	Range	Mean ± SEM
Male	Control	12	4.600 – 6.200	5.208 ± 0.123
	Opium	15	1.600 – 3.800	2.533 ± 0.148 a
	Bhang	15	1.500 – 4.900	3.367 ± 0.258 a
Female	Control	12	0.4000 – 2.700	1.333 ± 0.251
	Opium	14	0.2000 – 0.600	0.3850 ± 0.033 b
	Bhang	15	0.2000 – 0.900	0.5600 ± 0.069 c

[a] Significantly lower than male control at $P < 0.001$
[b] Significantly lower than female control at $P < 0.01$
[c] Significantly lower than female control at $P < 0.05$
SEM: standard error of mean

Table 2 Serum estradiol level (pg/ml) in different studied groups.

Groups		Number	Range	Mean ± SEM
Male	Control	12	18.00 – 55.00	35.92 ± 4.126
	Opium	15	17.00 – 25.00	21.73 ± 0.726
	Bhang	15	18.00 – 29.00	23.27 ± 0.864
Female	Control	12	36.00 – 98.00	65.92 ± 6.948
	Opium	14	17.00 – 81.00	30.64 ± 4.767 b
	Bhang	15	4.000 – 50.00	22.80 ± 2.900 b

[b] Significantly lower than female control at $P < 0.01$
SEM: standard error of mean

Table 3 Serum FSH level (mIU/ml) in different studied groups.

Groups		Number	Range	Mean ± SEM
Male	Control	12	6.000 – 12.00	9.333 ± 0.497
	Opium	15	3.000 – 5.500	4.233 ± 0.206 a
	Bhang	15	2.500 – 6.000	4.333 ± 0.291 a
Female	Control	12	6.000 – 14.00	10.42 ± 0.802
	Opium	14	3.500 – 7.500	5.693 ± 0.338 b
	Bhang	15	3.000 – 7.500	5.840 ± 0.304 b

[a] Significantly lower than male control at $P < 0.001$
[b] Significantly lower than female control at $P < 0.01$
SEM: standard error of mean
FSH: follicle stimulating hormone

Table 4 Serum LH level (mIU/ml) in different studied groups.

Groups		Number	Range	Mean ± SEM
Male	Control	12	2.700 – 7.200	4.592 ± 0.374
	Opium	15	0.500 – 4.000	2.127 ± 0.242 a
	Bhang	15	0.500 – 3.500	1.860 ± 0.262 a
Female	Control	12	7.000 – 11.00	8.792 ± 0.415
	Opium	14	1.200 – 5.000	3.479 ± 0.272 b
	Bhang	15	2.000 – 5.400	3.707 ± 0.278 b

[a] Significantly lower than male control at $P < 0.001$
[b] Significantly lower than female control at $P < 0.01$
SEM: standard error of mean
LH: luteinizing hormone

Table 2 demonstrates the mean ± SEM of serum estradiol level (pg/ml) in different studied groups. Serum estradiol was significantly lower in opium and bhang female addict groups compared to its respective healthy female group at $P < 0.001$, while there was no significant difference between the two addict female groups. In the male control group, the mean ± SEM of serum estradiol level did not show a significant difference from opiates and bhang addict male groups.

The serum FSH level (mIU/ml) decreased significantly in opiates and bhang male addict groups compared with the male control group at $P < 0.001$. Opiates and bhang female addicts showed a significant decrease in serum FSH level compared to the female control group at $P < 0.001$. Our results show a non-significant difference between opiates and bhang addiction for FSH. The descriptive statistics (mean ± SEM) of FSH level (mIU/ml) in different addicts and control groups are illustrated in **Table 3**. Moreover, the data in **Table 4** demonstrates the mean ± SEM of serum LH level (mIU/ml) in different studied groups. It is clear that serum LH level was significantly lower in opiate and bhang male and female addicts in relation to control male and female groups.

Finally, **Table 5** shows the descriptive statistics of serum prolactin level (ng/ml) in the different investigated

Table 5 Serum prolactin level (ng/ml) in different studied groups.

Groups		Number	Range	Mean ± SEM
Male	Control	12	5.500 – 8.500	7.183 ± 0.299
	Opium	15	1.800 – 3.100	2.567 ± 0.122 a
	Bhang	15	1.500 – 3.400	2.567 ± 0.161 a
Female	Control	12	22.00 – 44.50	32.68 ± 2.586
	Opium	14	1.800 – 20.00	11.36 ± 1.585 b
	Bhang	15	5.500 – 21.00	11.96 ± 1.321 b

[a] Significantly lower than male control at $P < 0.001$
[b] Significantly lower than female control at $P < 0.01$
SEM: standard error of mean

groups. The data show a significant decrease in serum prolactin level in opium and bhang female addict groups compared to their respective control at $P < 0.001$, while there was no significant difference between the effect of opium and bhang addiction on the level of serum prolactin level in the two female addict groups. Moreover, in the male groups, there was a significant decrease in the serum prolactin level in opium and bhang addict groups compared to their control at $P < 0.05$.

DISCUSSION

Changes in sexual activity are commonly observed in addict subjects. The effects of drug addiction on sexual functions and sex hormones are one of the major scopes of investigations throughout the world (Seyed *et al*. 2007). Swartout and White (2010) demonstrated that increased marijuana use increased the severity of sexual aggression over time. Cannabinoids have long been known to have potent effects on reproduction and sexual behavior. Although some of these influences may be mediated at the levels of the pituitary and gonads (Pagotto *et al*. 2001), the primary effects have been ascribed to hypothalamic action because synthetic GnRH can rescue ovulation in cannabinoid-treated animals. Multiple factors regulate the activity of the GnRH neurons responsible for controlling fertility. Foremost among neuronal inputs to GnRH neurons are those using the amino acids glutamate and gamma-aminobutyric acid (GABA) (Constantin *et al*. 2010). Despite this, the mechanism of action of cannabinoids on GnRH function is obscure. The reported *in vivo* effects of cannabinoids agonists on LH secretion and/or ovulation in female rodents and humans suggests that endocannabinoids may play an important role in GnRH physiology and reproduction (Gammon *et al*. 2005; Finch *et al*. 2010).

The endogenous opioid system is activated in response to a number of stressors, both physical and psychological, and plasma β-endorphins increase with these stressors (Feldman *et al*. 1997). These stressors cause an increase in pro-opiomelanocortin (POMC) synthesis rate, as well as β-endorphin release from the arcuate nucleus of the hypothalamus. In men, social or psychological chronic stress causes erectile dysfunction, increases plasma levels of β-endorphins, and decreases sexual motivation (libido). Treatment with the opioid antagonist naltrexone improves these symptoms and increases penile blood flow. In healthy sexually-active men, naltrexone increases the number and intensity of orgasms, which suggests that endogenous opioids modulate orgasmic response and the perceived intensity of sexual arousal and orgasm in men (Retana *et al*. 2009).

Our results showed that the serum testosterone level was significantly lower in opium and bhang male addicts compared with the healthy male group. In female addicts to opium and bhang the serum testosterone level was significantly lower than the female control group. These results are in harmony with previous studies of Ceccarelli *et al.* (2006), Seyed *et al.* (2007) and Wisniewski *et al.* (2007), who reported that opioids exert important effects on plasma and central nervous system sex hormone levels; in particular, opioids induced hypogonadism. The different magnitude and time-course of the effects of the different opiates on testosterone and estradiol levels are likely due to their different mechanisms of action.

On the other hand, Daletrio *et al*. (1984) found maternal exposure to delta 9-tetrahydrocannabinol (THC), the major psychoactive constituent in marijuana and bhang, or to the non-psychoactive cannabinol (CBN) or cannabidiol (CBD) alters endocrine functions and concentrations of brain biogenic amines in their male offspring. Prenatal CBN exposure on day 18 of gestation resulted in decreased plasma FSH levels, testicular testosterone concentrations, and seminal vesicles weights, but increased plasma levels of LH and FSH post-castration in adulthood. Moreover, these authors found that the addition of THC to incubations of whole decapsulated mouse testes altered testosterone production differentially, depending on the specific gonadotropin used, the dose of THC and/or the amount of divalent cation present in the media.

Harclerode (1984) and AbolMaged *et al.* (2002) reported that marijuana affects a variety of hormones that are regulated by hypothalamic function and it appears that the psychoactive ingredient, THC, is the major compound responsible for this action. It is probable that THC affects these hormones through its ability to alter various neural transmitters in the hypothalamus or in the CNS, which impinge on the hypothalamus. The dopaminergic and serotonergic fibers seem to be particularly important. The two gonadotropins, LH and FSH, secreted by the pituitary gland are of major importance to reproduction in the male. Both gonadotropins appear to respond to a single releasing factor from the hypothalamus, GnRH, which is sensitive to catecholamine neurotransmitters. The THC-induced block of GnRH release results in lowered LH and FSH which is responsible for reduced testosterone production by the Leydig cells of the testis. Other hormones that might have a synergistic or antagonistic effect upon reproduction in the male are the adrenal cortical hormones, prolactin, thyroid hormones, and growth hormones. THC appears to depress prolactin, thyroid gland function, and growth hormone while elevating adrenal cortical steroids (Uberto *et al*. 2006).

Our result correspond to the previously reported results of AbolMaged *et al.* (2002), Ceccarelli *et al.* (2006) and Seyed *et al.* (2007) who reported that opioids exert important effects on plasma and central nervous system sex hormone levels, in particular opioid-induced hypogonadism. Moreover, Katz *et al*. (2009) reviewed that long-term opioid therapy for either addiction or chronic pain often induces hypogonadism owing to central suppression of hypothalamic secretion of gonadotropin-releasing hormone. Symptoms of opioid-induced hypogonadism include loss of libido, infertility, fatigue, depression, anxiety, loss of muscle strength and mass, osteoporosis, and compression fractures in both men and women, impotence in men, and menstrual irregularities and galactorrhea in women.

The serum FSH level was significantly decreased in opiates and bhang male addict groups compared with the control male group. Opiates and bhang female addicts show a significant decrease in serum FSH level when related to the female control group. This result corresponds to that of Seyed *et al*. (2007) who reported that a significant decrease in plasma FSH level in opium male and female addicts compared to the non-addict male and female control groups and disagrees with that of Wenger *et al*. (1999) who reported that CB_1 cannabinoid receptors are located in hypothalamic nuclei whose activation alters several hypothalamic neurotransmitters resulting in, among other things, decreased prolactin (PRL) and LH secretion from the anterior pituitary gland. Thus, the acute administration of THC produced a noticeable decrease in plasma PRL and LH levels, with no changes in FSH levels. This was paralleled by an increase in the contents of GABA, but not of DA in the medial basal hypothalamus and, to a lesser extent, in the anterior pituitary gland.

Regarding the serum LH level, the obtained results confirm the previous results of Seyed *et al*. (2007) who reported that opium addiction in Iranian male and female subjects significantly decrease the plasma LH level when these

addict subjects were compared with healthy non-addict male and female groups. In addition, Wenger *et al*. (1999) found a decrease in plasma LH level after THC administration.

Murphy *et al*. (1999) reported that acute treatment with THC, the major psychoactive constituent of marijuana, produces a dose-related suppression of LH secretion in ovariectomized rats. Wenger *et al*. (1999), Francavilla *et al*. (2009) and Wenger and Moldrich (2002) reported that the main psychoactive component of marijuana, THC, has mainly inhibitory effects on the regulation of reproduction. They found that exogenous and endogenous cannabinoids have only slightly different effects on reproductive parameters. These effects may occur via the central cannabinoid receptor. It is possible that the sites of action are at both hypothalamic and pituitary levels. The results further support the view that anandamide may be a central neurotransmitter or neuromodulator (Ranganathan *et al*. 2008). On the other hand, AbolMaged *et al*. (2002) and Moshtaghi *et al*. (2005) reported that there was enhanced plasma prolactin level in Iranian opium smokers men compared to the healthy control group. Moreover, Bart *et al*. (2005) found that nalmefene has partial kappa opioid agonist activity to induce elevation in serum prolactin in normal human volunteers.

CONCLUSION

Opium and bhang addiction affects hypothalamic pituitary gonadal axis hormones, leads to undesirable changes, and central suppression of hypothalamic secretion of gonadotropin-releasing hormone leading to loss of libido, infertility, impotence in men and menstrual irregularities in women.

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