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Oxidative Stress and Lipotoxicity of Bhang and Opium Addiction. Effects on Adrenal Gland Secretions

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ABSTRACT

This study was carried out to investigate the effect of bhang and opium addiction on hypothalamic pituitary adrenal axis hormones and the role of free radicals in lipotoxicity induced by bhang and opium addiction. 83 individuals, whose ages ranged from 23 to 35, 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; group F, control female subjects. Blood sampling from female groups (addicts and control) were taken during the follicular phase. Plasma and serum were separated to determine total cholesterol, triacylglycerol (TAG), HDL- and LDL-cholesterol, serotonin, dopamine, adrenaline, catalase, thiobarbituric acid-reactive substances (TBARS), protein oxidation and protein thiols. There was a significant increase in serum total cholesterol, TAG and LDL-cholesterol in male opium and bhang and female opium and bhang addicts, respectively in relation to the control group. However, the decrease in serum HDL-cholesterol in male and female addicts was not significant when compared to the control group. Moreover, there was a significant increase in serotonin (258.4 ± 0.692, 260.2 ± 0.734 and 259.5 ± 0.698, 259.8 ± 0.705), dopamine (86.95 ± 1.935, 82.66 ± 2.287 and 88.01 ± 2.108, 82.94 ± 2.360) and adrenaline (55.87 ± 2.212, 56.93 ± 1.830 and 57.57 ± 1.500, 58.40 ± 1.635) levels in male opium and bhang and female opium and bhang addicts, respectively compared to the control male (213.3 ± 1.483, 54.37 ± 1.432 and 36.67 ± 1.025) and control female (212.9 ± 1.445, 55.42 ± 1.320 and 35.83 ± 1.029) group, respectively. In addition, there was a significant increase in oxidative stress markers, TBARS, and protein oxidation and a decrease in antioxidants, catalase and protein thiols in male opium and bhang and female opium and bhang addicts, respectively in relation to the control group.

Keywords: adrenaline, dopamine, lipid peroxidation, protein oxidation, reactive nitrogen species, reactive oxygen species, serotonin

INTRODUCTION

Free oxygen radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems (Valko *et al.* 2006). Beneficial effects of ROS occur at low/moderate concentrations and involve physiological roles in cellular responses to anoxia, as for example in defense against infectious agents and in the function of a number of cellular signaling systems. One further beneficial example of ROS at low/moderate concentrations is the induction of a mitogenic response. The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress (Oborná *et al.* 2008). This occurs in biological systems when there is an overproduction of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other.

Nitric oxide (NO) is a key neuromodulator of corticostriatal synaptic transmission. Stimulation of dopamine receptor facilitates the neuronal NO synthase activity which increases the efflux of NO within the central nervous system (Park and West 2009). Moreover, dopamine may be metabolized intracellularly by monoamine oxidase (MAO). MAO catalyses the deamination of dopamine producing 3,4-dihydroxyphenylacetic acid (DOPAC) and hydrogen peroxide (H_2O_2). Dopamine autooxidation produces the superoxide anion $O^{\bullet}_2^-$ and H_2O_2, which may react with transition metal ions, via the Haber-Weiss/Fenton reactions, originating in the highly toxic hydroxyl $OH^{\bullet}$. Furthermore, $O^{\bullet}_2^-$ reacts with NO forming the highly toxic peroxinitrite (Cadet *et al.* 2007).

Overproduction of RNS is called nitrosative stress. This may occur when the generation of RNS in a system exceeds the system's ability to neutralize and eliminate them. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function (Valko *et al.* 2007).

Cannabis sativa, also known as marijuana, bhang or hashish depending on the particular preparation, is both a widespread illegal drug of abuse and a well-recognized medicinal plant (Iversen 2000). *C. sativa* contains about 60 phytocannabinoids, a handful of which are bioactive as defined by their ability to specifically interact with membrane-associated receptors, the cannabinoid receptors. The best-known phytocannabinoid is Δ^9-tetrahydrocannabinol (THC), which is thought to mediate most, if not all, of the psychotropic and addictive properties of *C. sativa* (Hall and Solowij 1998). Evidence suggest that some of the anti-inflammatory properties of *C. sativa* may be accounted for by cannabinol (CBN) and cannabidiol (CBD), two non-psychotropic phytocannabinoids that constitute promising lead compounds to develop cannabinoid-based anti-inflammatory medicines (Mackie and Stella 2006).

Both natural rewards (food, drink, and sex) and addictive drugs stimulate the release of dopamine from neurons of the presynaptic ventral tegmental area into the nucleus accumbens, causing euphoria and reinforcement of the behavior. In the case of natural rewards, there is a very rapid adaptive change, or habituation, after a few experiences,

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and the novelty or unexpectedness of the reward seems to play a major part in the initial response. The response to addictive drugs is not influenced by habituation, and each dose of the drug stimulates the release of dopamine (Di Chiara 1999).

Dopamine binds to a G-protein–coupled receptor with two main subtypes, D1-like receptors (D1 and D5) and D2-like receptors (D2, D3, and D4). D1-like receptors activate adenylyl cyclase, whereas D2-like receptors inhibit the enzyme. A membrane dopamine transporter moves the released neurotransmitter from the extracellular space back into the presynaptic neuron (uptake) (Durazzo *et al.* 2008).

Opioids release dopamine mainly by an indirect mechanism that decreases the activity of γ-aminobutyric acid (GABA)-inhibitory interneurons in the ventral tegmental area (De Vries and Shippenberg 2002; Huo *et al.* 2009). THC and other cannabinoids increase the efflux of dopamine in the nucleus accumbens and cell firing in the ventral tegmental area by their actions on CB_1 receptors in glutamatergic and non dopamine GABA-ergic neurons associated with the nucleus accumbens and ventral tegmental area (Robbe *et al.* 2001).

The aim of this study was to investigate the deleterious effect of drug addiction on redox equilibrium, lipid profile and adrenal gland secretions among Egyptian addicts.

SUBJECTS, MATERIALS AND METHODS

This study was carried out on 83 adult male and female subjects whose age ranged between 23 and 35. Both male and female subjects were recruited from the Addiction Unit, Faculty of Medicine Ain-Shams University, Cairo, Egypt. The control male and female subjects were recruited from the community through public announcement. All subjects were classified according to the substance abused into six groups: Group A, male subjects addict to opium (n = 15); Group B, female subjects addict to opium (n = 14); Group C, male subjects addict to bhang (n = 15); Group D, female subjects addict to bhang (n = 15); Group E, control male subjects (n = 12); Group F, control female subjects (n = 12).

Subjects were admitted to the hospital laboratory on the first day of hospital admission for urine sampling between 9:00 am and 12:00 pm for screening and selection of pure opium or bhang addicts. Participants were then subjected to medical (blood chemistry including random blood sugar, liver and kidney functions, hepatitis C virus and hepatitis B virus) and psychological evaluation, including past and recent drug use history and were excluded if they had diabetes, a history of liver disease, an unstable cardiovascular, peripheral vascular, respiratory or gastrointestinal disease, a malignancy or aminoria. On the second day, they were admitted to the laboratory (between 9:00 am and 12:00 pm) for blood sampling after overnight fasting. All subjects did not receive any treatment from the first admission to the time of blood sampling on the second day. All addicts and control subjects were selected from heavy smokers (smoking more than 40 cigarettes/day, of any type of cigarette) to neglect the effect of nicotine smoking when comparing addict and control groups. Moreover, all addict subjects were selected from chronic addict cases, i.e., addicts for > 6 years.

Fasting venous blood samples were withdrawn after 10 min rest in the sitting position and divided into two tubes, the first one allowed clotting for 15 min while the second tube contained 2.5% EDTA solution (1: 40 EDTA: blood ratio). The second tube was mixed gently then tubes were centrifuged at 4000 rpm for 10 min. The yielded serum and plasma was divided into aliquots and stored at -20°C until analysis.

The separated aliquots were used for the determination of serum total cholesterol (Richmond 1973), serum triacylglycerol (TAG) (Bucolo and Halord 1973), serum HDL-CH (Assmann *et al.* 1983), calculation of LDL-CH (Friedewald *et al.* 1972), serum serotonin (Lahir *et al.* 2004), plasma dopamine and adrenaline (Westermann *et al.* 2002), plasma lipid peroxidation product (Sushmakumari *et al.* 1989 as modified by Chaturvedi and Segale 2007), serum protein oxidation (Levine *et al.* 1990), plasma catalase (CAT) activity (Bisswanger 2004) and serum protein sulfhydryl groups (Koster *et al.* 1986).

Descriptive statistics were performed using Microsoft Excel 2007. All analyses and graphics were performed using GraphPad InStat v. 3 to perform one-way ANOVA with post-hoc test $P < 0.05$ followed by Tukey-Kramer's multiple comparison test (Windows v. 7, GraphPad Software 2007).

RESULTS

Table 1 shows the mean values and standard error of means for serum total cholesterol in the different studied groups. The serum level was significantly higher in addict groups (male and female, opium and bhang) than control male and female groups. Serum TAG was significantly higher in opium and bhang addict males than in the male control group (**Table 1**). It was also higher in the opium and bhang addict female groups than in the healthy female control group. However, there was no significant difference in the serum TAG level between opium and bhang addict male and female groups. There was no significant difference in serum HDL-CH between bhang and opium addict male or bhang and opium addict female groups when compared with control male and female groups (**Table 1**). The serum LDL-CH was significantly higher in opium and bhang addict male groups than in the male control group (**Table 1**). Although the level in opium and bhang addict female groups was significantly higher than in normal female control groups, the difference in serum LDL-CH between addict groups (opium and bhang, male and female) was statistically non-significant.

The serum serotonin level increased significantly in all addict groups (male and female, opium and bhang) compared to both male and female control groups (**Table 2**). There was no significant difference in serum serotonin level between male and female, opium and bhang addict groups. In addition, bhang addiction significantly increased the plasma dopamine level in both male and female addict groups compared to male and female control groups (**Table 2**). The plasma dopamine level in opium male and female addicts was significantly higher than their respective control groups. There was no significant difference between opium and bhang male and female addicts. The plasma adrenaline level in bhang and opium male and female addicts was significantly higher than their healthy control groups. The difference in plasma adrenaline level between the male and female addicts, whether opium or bhang, was statistically non-significant.

The plasma TBARS level was significantly higher in opium and bhang male and female addicts than in healthy

Table 1 Mean ± SEM of serum total cholesterol (mg/dl), TAG (mg/dl), HDL-cholesterol (mg/dl) and LDL-cholesterol (mg/dl) in different studied groups.

Groups		Total cholesterol	TAG	HDL-cholesterol	LDL-cholesterol
Male	Control	179.4 ± 4.130	99.40 ± 3.870	60.75 ± 4.090	98.95 ± 6.490
	Opium	265.7 ± 7.410 a	182.7 ± 7.410 a	49.33 ± 2.910	179.9 ± 8.630 a
	Bhang	270.1 ± 6.240 a	187.1 ± 6.240 a	47.93 ± 2.830	184.8 ± 7.730 a
Female	Control	171.3 ± 3.670	84.60 ± 3.580	58.83 ± 4.240	95.58 ± 4.910
	Opium	258.1 ± 6.460 b	175.1 ± 6.460 b	51.20 ± 3.250	171.8 ± 8.290 b
	Bhang	252.5 ± 7.290 b	168.4 ± 7.120 b	52.60 ± 2.630	166.3 ± 7.680 b

[a] Significantly higher than the male control group at $p < 0.001$ according to Tukey-Kramer's multiple comparison test
[b] Significantly higher than the female control group at $p < 0.001$ according to Tukey-Kramer's multiple comparison test
TAG: triacylglycerol
SEM: standard error of mean

Table 2 Mean ± SEM of serum serotonin level (ng/ml), plasma dopamine level (ρg/ml) and plasma adrenaline level (ρg/ml) in different studied groups.

Groups		Serotonin	Dopamine	Adrenaline
Male	Control	213.3 ± 1.483	54.37 ± 1.432	36.67 ±1.025
	Opium	258.4 ± 0.692 a	86.95 ± 1.935 a	55.87 ± 2.212 a
	Bhang	260.2 ± 0.734 a	82.66 ± 2.287 a	56.93 ± 1.830 a
Female	Control	212.9 ± 1.445	55.42 ± 1.320	35.83 ± 1.029
	Opium	259.5 ± 0.698 b	88.01 ± 2.108 b	57.57 ± 1.500 b
	Bhang	259.8 ± 0.705 b	82.94 ± 2.360 b	58.40 ± 1.635 b

[a] Significantly higher than the male control group at $p < 0.001$ according to Tukey-Kramer's multiple comparison test
[b] Significantly higher than the female control group at $p < 0.001$ according to Tukey-Kramer's multiple comparison test
SEM: standard error of mean

Table 3 Mean ± SEM of serum TBA-RS (mmol/dl), serum protein oxidation (μmol/l), plasma catalase activity (U/dl) and serum protein sulfhydryls (PrSHs) (μmol/l) in different studied groups.

Groups		TBA-RS	Protein oxidation	Catalase activity	Protein sulfhydryls
Male	Control	0.172 ± 0.018	64.58 ± 1.969	46.16 ± 0.548	653.7 ± 26.92
	Opium	0.327 ± 0.017 a	114.0 ± 4.882 a	25.88 ± 0.876 a	418.6 ± 13.56 a
	Bhang	0.330 ± 0.018 a	128.5 ± 5.292 a	25.92 ± 0.749 a	409.9 ± 14.29 a
Female	Control	0.163 ± 0.004	62.65 ± 2.280	46.13 ± 0.622	684.3 ± 26.41
	Opium	0.328 ± 0.191 b	128.8 ± 7.286 b	26.63 ± 0.960 b	417.8 ± 14.02 b
	Bhang	0.347 ± 0.180 b	123.5 ± 6.008 b	25.52 ± 0.889 b	404.3 ± 14.44 b

[a] Significantly higher than the male control group at $p < 0.001$ according to Tukey-Kramer's multiple comparison test
[b] Significantly higher than the female control group at $p < 0.001$ according to Tukey-Kramer's multiple comparison test
TBARS: thiobarbituric acid reactive substances
SEM: standard error of mean

male and female control groups (**Table 3**). Serum protein oxidation level was significantly higher in bhang and opium male and female addicts addict than in the respective male and female control groups (**Table 3**). There was no significant difference in plasma CAT activity between opium or bhang male and female addicts (**Table 3**). However, plasma CAT activity was significantly lower in all male and female addicts when compared to the healthy male and female control groups. Serum PrSHs decreased significantly in opium and bhang male and female addicts compared to the control male and female groups (**Table 3**).

DISCUSSION

The transition from sub-clinical cardiovascular disease to overt clinical disease is usually precipitated by acute coronary events such as unstable angina or myocardial infarction (Burke *et al.* 1997). Pathologic evidence indicates that rupture, erosion, and fissure of lipid-rich vulnerable atherosclerotic plaques are probably the most frequent underlying mechanisms that trigger the cascade of effects leading to acute coronary occlusion (Burke *et al.* 1997). Some hypotheses concerning the pathogenesis of this cascade focus on endothelial injury, the oxidation of LDL and their effects on the endothelium, the interaction of growth factors and cytokines leading to increased oxidative stress, increased free radical formation, destruction of NO, endothelial dysfunction, increased platelet aggregation, inflammation, proteolysis, impaired thrombolysis and thrombosis (Pahor *et al.* 1999).

Oxidative stress results from the metabolic reactions that use oxygen and represents a disturbance in the equilibrium status of prooxidant/antioxidant reactions in living organisms. Excess ROS can damage cellular lipids, proteins, or DNA inhibiting their normal function. Because of this, oxidative stress has been implicated in a number of human diseases as well as in the ageing process. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called "redox regulation". The process of "redox regulation" protects living organisms from various oxidative stresses and maintains "redox homeostasis" by controlling the redox status *in vivo* (Riso *et al.* 2009).

Siqueira *et al.* (2006) reported that the dyslipidemia of the metabolic syndrome (MS) confers an elevated cardiovascular risk and is characterized by increased concentrations of triglycerides, decreased HDL-cholesterol and qualitative alterations in LDL which renders it more atherogenic. Modified LDL is internalized by macrophages through scavenger receptors, originating foam cells and inducing an immune-inflammatory reaction (Shah *et al.* 2008).

Our results show increases in serum total cholesterol in opium addict male and female groups. These results are in agreement with those of Szmitko and Verma (2008), who reported that the accumulation of intra-abdominal fat and the subsequent development of visceral obesity rely on the body's mechanisms to store energy and stimulate the appetite. In addition, these authors reported that the endocannabinoid system has been implicated in the regulation of energy balance and has emerged as a critical target for the modulation of the visceral obesity and insulin resistance; its overactivity appears to be associated with the development of obesity. Moreover, Mohammadi *et al.* (2009) reported that opium consumption can have an aggravating effect in atherosclerosis formation related with hypercholesterolemia, mainly affecting the lipid profile.

In addition to catecholamines, adrenal chromaffin cells synthesize and secrete several neuropeptides, including opioids. Opioids are co-localized and co-released with catecholamines in bovine adrenal cells (Livett *et al.* 1981; Rico *et al.* 2005; Levitsky and Barneo 2009).

Dopamine is both a neurotransmitter and a neurotoxin, and changes in dopamine metabolism may induce oxidative stress and cell death in dopaminergic or surrounding cells (Jones *et al.* 2000). An increase in the level of monoamines may lead to neurotoxic effects often associated with oxidative stress, mitochondrial dysfunction, apoptosis and inhibition of neurogenesis (Cunha-Oliveira *et al.* 2008).

Bowers and Kantrowitz (2007) reported that plasma homovanillic acid (dopamine metabolite) is elevated in a number of newly admitted cannabinoid psychosis patients. There are several possibilities for this result. First, plasma homovanillic acid is elevated in a number of newly admitted psychosis patients (Bowers and Swigar 1987) which was the case in our first-episode psychosis groups (male and female addict groups). Second, acute cannabis administration can produce increased dopamine activity in animals and humans (Bowers and Morton 1994). Third, individuals with cannabis psychosis may also have a genetic vulnerability that can lead to increased dopaminergic activity (Henquet *et al.* 2006).

Current research has afforded significant preclinical evidence for the utility of cannabinoids in the treatment of depression. Overall, the evidence indicates that low doses of cannabinoid agonists exert anti-anxiety and antidepres-

sant effects in rodents; however, these dose-dependent effects can be modulated by other factors, such as previous experience with the drug or environmental manipulations that alter the level of stress experienced by the animal (Bortolato *et al.* 2007; Mato *et al.* 2010). Although the mechanism(s) by which cannabinoids modulate mood-related behavior is not entirely elucidated yet, administration of cannabinoids appears to produce effects similar to those observed following other antidepressant treatments, namely enhancement of serotonergic signaling and hippocampal neurogenesis (Hesketh *et al.* 2008).

Our results show significant increase in serum TBARS in opium addict male and female groups compared to their respective control groups. This result supports the previous findings of Ozmen *et al.* (2007), who found a significant increase in TBARS content of brain and liver of morphine addict rats compared to the control groups; moreover, the hearts of opiate addicted rats did not show a significant increase in TBARS, possibly because the heart is more resistant to opiate-induced lipid oxidative damage. On the other hand, our results show a significant increase in serum TBARS in bhang addict subjects (male and female) compared to their respective control groups. Mandal and Das (2009) found that THC significantly increased lipid peroxidation and decreased testicular lipid content in male albino mice at a lower dose. But the effects were at slightly lower high doses and at the time the drug was withdrawn (recovery dose).

Pan *et al.* (2005) reported a significant increase in protein oxidation products (carbonyl content) in brain and liver of opiates and bhang addict rats compared to the control group. In addition, Xu *et al.* (2006) found that heroin exposure induced an increase in oxidative DNA damage, protein oxidation and lipid peroxidation.

Cunha-Oliveira *et al.* (2008) reported that THC induces neurotoxicity, which has been attributed to the generation of free radicals. There are many examples in which neurotoxins are associated with oxidative stress. In investigations with THC, DNA strand breaks were also observed, which are commonly linked to ROS. Neuronal cell death was inhibited by aspirin, vitamin E, and other antioxidants, another indication of oxidative stress. Tapas and Niladri (2009) reported that CAT (a scavenger of H_2O_2) acts as a second line of defense, and decreases with graded doses of THC treatment and glutathione peroxide, a third line of defense, reduces at low dose of THC treatment, but not appreciably.

Regarding serum protein sulfhydryl groups our results are similar to those of Mandal and Das (2009) who reported a marked decrease of antioxidant enzyme systems (superoxide dismutase, catalase and glutathione peroxidase) and glutathione content in male albino mice at a lower dose. But the effects were slightly higher at a high dose and at the time the drug was withdrawn. Moreover, Guzman *et al.* (2006) and Cunha-Oliveira *et al.* (2008) reported that morphine induces a decrease in GSH levels in the rat. Thus, opiates caused an increase of ROS formation and a decrease of ROS defense in a vicious circle. When ROS level exceeds the antioxidant capacity, a deleterious condition known as oxidative stress occurs (Pan *et al.* 2005).

CONCLUSION

Opium and bhang addiction affect hypothalamic pituitary adrenal axis hormones, lead to undesirable changes in the lipid profile and enhance oxidative and nitrosative stress, which can lead to increased risk of cardiovascular diseases. Moreover, sudden abstinence induces changes in neurotransmitters and stress hormones.

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