



IMMUNOTOXICITY OF HEROIN AND CANNABIS IN A SAMPLE OF EGYPTIAN POPULATION

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ABSTRACT

Addiction is one of the world's major health problems with large direct health costs (psychiatric and physical) as well as massive indirect costs to the society. The present study aimed to investigate the toxic effect of heroin and cannabis addiction on immune system and the role of reactive oxygen species (ROS) in immunotoxicity. 84 individuals; whose ages ranged from 18 to 38 years were classified into 6 groups: Group I, male subjects addicted to heroin; group II, male subjects addicted to Cannabis; group III female subjects addicted to heroin; group IV, female subjects addicted to Cannabis; group V, control male subjects; group VI, control female subjects. The control groups comprised 24 persons; while the study group included 60 addicts. 10 mL urine samples were screened for detection of substance abuse. Four mL blood was collected from each subject after taking informed consent and was divided as follows: Two mL for detection of cytokines; interleukin 2 & 6 (IL2 & IL6) by using flow cytometry and the other two mL for studying ROS (reduced glutathione; thiobarbituric acid reactive substances {TBARS} and protein oxidation) by using colorimetric assay. The results revealed a significant decrease in serum IL-2 in both heroin and cannabis males & females addicts compared to control group (64.9 ± 2.999 & 62.93 ± 2.198 respectively) and (64.45 ± 1.703 & 61.67 ± 2.182 respectively). While there is no significant difference in the IL-6 level. Regarding ROS; there is significant decrease in protein thiols level in both heroin and cannabis males & females addicts compared to control group (418.8 ± 13.10 & 406.8 ± 14.99 respectively) and (412.9 ± 14.35 & 408.8 ± 14.67 respectively). While, there are significant increases in both TBARS and protein oxidation in both cannabis and heroin males and females addicts in comparison to the control group. It could be concluded that heroin and cannabis addiction might play a role in the immunotoxicity through effects on the ROS.

KEYWORDS: Addiction, interleukin-2, interleukin-6, lipid peroxidation, protein oxidation, reduced glutathione, ROS.

INTRODUCTION

Addiction is a disorder of the brain characterized by an impaired ability to refrain from using a psychoactive substance despite serious negative consequences.^[1]

Cannabis is the third most commonly used drug after tobacco and alcohol.^[2] Cannabis has consistently been shown to be the most widely used illicit drug in various communities around the world. About 147 million people, 2.5% of the world population, consume cannabis.^[3] Ingestion of cannabis sativa preparations such as marijuana (leaves and flowering tops) or janja (resin) result in an intoxication characterized by sedation, cognitive dysfunction, failure to consolidate short-term memory, alteration in time assessment, perceptual changes, motor incoordination and poor executive function. Whether long term heavy cannabis use is

associated with gross anatomical abnormalities in cannabinoid receptor-rich regions of the brain was studied.^[4]

The main function of the endocannabinoid system is to regulate synaptic neurotransmission. The cannabinoid (CB1) endocannabinoid system regulates synaptic neurotransmission of excitatory and inhibitory circuits. In response to depolarisation and Ca^{2+} fluxes, endocannabinoids are released that inhibit further neurotransmitter via stimulation of presynaptic CB1 receptors. As a regulator of neurotransmission, the cannabinoid system seems to influence many different functions.^[5]

Moreover; heroin is a highly addictive drug. It is the most abused and the most rapidly acting of the opiates.

Heroin (diacetylmorphine) is processed from morphine; it influences different physiological functions in the body, including the reactions of the immune system.^[6]

The complexity of the heroin's effects results from the wide distribution of opioid receptors. These receptors have been demonstrated on various cell types, including cells of both the nervous and immune system. Heroin can thus regulate functions of the immune system either directly by acting on the opioid receptors on lymphocytes and macrophages, or can influence the reactions of the immune system through its effects on the nervous system.^[7, 8]

As regard cytokines; they are secreted by white blood cells as well as variety of other cells (fibroblasts, endothelial cells and epithelial cells) in the body in response to inducing stimuli, and are not constitutively expressed. They were in fact based on the realization that cells other than those of the immune system also secrete biologically active substances, that the concept of cytokines was proposed.^[9]

Cannabinoids, especially the major psychoactive component tetrahydrocannabinol, exert immunomodulatory effects that alter normal functions of T and B lymphocytes, natural killer cells, and macrophages in human and animals.

The first evidence that cannabinoids might modulate cytokine production was found in the mid-1980s¹⁰. Consequences of such cannabinoid immunomodulation are not fully understood.^[11] On one hand, pre-clinical studies link cannabinoid-1 receptor activation to inflammation and atherosclerotic effects.^[12] On the other hand, activation of cannabinoid-2 receptors primarily is found to mediate anti-inflammation and immunosuppression.^[13; 14; 15] Much of this work is pre-clinical, but there has been a steady increase in evidence from human studies.

Reactive oxygen species (ROS) are products of normal cellular metabolism. Most of the body's energy is produced by the enzymatically controlled reaction of oxygen with hydrogen ion in oxidative phosphorylation occurring within the mitochondria during oxidative metabolism. During this enzymatic reduction of oxygen to produce energy, free radicals are formed¹⁶. Several studies demonstrated that acute and chronic exposure to morphine may result in a significant decrease in GSH levels in rodent and human brain as well as in mouse liver.^[17; 18; 19]

Heroin was shown to induce a decrease in the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in the mouse brain²⁰. Moreover, morphine induced a decrease in GSH levels in the rat²¹ and rabbit brains, and also a decrease in unsaturated fatty acids in the rabbit nervous system.^[22]

The goal of the present study is to investigate the immunotoxicity of heroin and cannabis addiction and to explain if this effect is through changes in the reactive oxygen species (ROS).

SUBJECTS AND METHODS

Approval from the Ethical Committee of Ain Shams University-Faculty of Medicine was taken besides an informed consent from each subject to participate in the research.

1) Subjects: This study was conducted on 84 subjects who were recruited from the Addiction Unit, Faculty of Medicine Ain-Shams University, Cairo, Egypt. They were divided into two groups

A. Study group: 60 males & females addicts who were diagnosed by Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), 4th edition. They were further divided into 4 groups

Group I: Male addicts to heroin (n = 15).

Group II: Male addicts to cannabis (n = 15).

Group III: Female addicts to heroin (n = 15).

Group IV: Female addicts to cannabis (n = 15).

B. Control group: 24 healthy male & female subjects; divided into two control groups (12 each).

C. Exclusion criteria: Those who had diabetes, a history of liver disease, an unstable cardiovascular, peripheral vascular, respiratory, or gastrointestinal disease and a malignancy.

D. Each participant was subjected to

1. History taking to get sociodemographic criteria regarding age, sex and residence.
2. Complete medical examination.

3. Sampling

- On the first day of admission 10 mL urine were collected in the morning between 9 AM and 12 PM for screening of cannabis and heroin.
- On the second day, four mL blood sample was collected on ethylenediaminetetraacetic acid after being sure that all subjects do not receive any treatment from the first time of admission to the time of blood sampling in the second day.

All addicts and control subjects were selected as heavy smokers (smoking more than 40 cigarettes/day, of any type of cigarettes) to neglect the effect of nicotine smoking when comparing between addict and control groups. Moreover, all addict subjects selected from the chronic ones (addict for more than 6 years).

METHODS

Blood samples were allowed to clot for 15 minutes and centrifuged at 4000 rpm for 15 minutes. The yielded serum was aliquoted and stored at - 20°C until the time

of analysis. The separated serum was used for determination of IL2^[23] & IL6^[24] protein sulfhydryl groups.^[25] Protein oxidation and thiobarbituric acid reactive substances as modified by Sushmakumari *and coworkers* (1989).^[26]

Measurement of ROS: Determination of serum protein sulfhydryl groups (GSH): The protein SH-groups react with S-S linkage of 5, 5'-dithiobis (2-nitrobenzoic acid) [DTNB] (Ellman's reagent) at pH 7.4 to yield a coloured product, 5-thio-2-nitrobenzoic acid [NBA], which can be measured colorimetrically at 412 nm^[25] (Koster *et al.*, 1986).

- Determination of thiobarbituric acid reactive substances (TBARS): Lipid hydro-peroxides and conjugate dienes that are formed due to reaction with oxygen can decompose to form numerous other products including alkanals, alkenals and malondialdehyde (MDA)^[27] (Halliwell and Gutteridge, 1992). Lipid peroxidation products were measured in the serum using thiobarbituric acid assay modified according to the suggestions of Sushmakumari *and his coworkers* (1989).

- Determination of Protein oxidation: Protein oxidation was quantified using the interaction between 2,4-dinitrophenylhydrazine (DNPH) and the carbonyls generated from the oxidative modification of proteins to yield a chromophore that absorbs strongly at 380 nm^[28] (Levine *et al.*, 1990).

Immunological analysis: Flow cytometric analysis was used to measure lymphocyte phenotypic subsets was done by indirect immunofluorescence using monoclonal anti-human antibodies. For surface antigens staining, blood samples were lysed using lysine solution, washed with phosphate buffered saline (PBS) once or twice until complete red blood cells lysis and then resuspended in appropriate amount of PBS. The cells were stained with different fluorescently labeled monoclonal antibodies (mAb) according to manufacturer recommendations (Dakocytomation, Denmark and Beckman Coulter, France).

Screening of substance abuse: using drug testing kits (BabtMad LTD).

Statistical Analysis: Statistical analysis of obtained data was carried out through analysis of variance (ANOVA) and Student's t-test. The significance of results was ascertained at P<0.05. Data were expressed as mean $\pm$ SEM. The GraphPad InStat and GraphPad Prism software (SanDiego, USA) were used to conduct the statistical analysis.

RESULTS

Table (1) reveals the serum level of IL2 (pg / ml) in all the studied groups (n = 84). There is significant difference between the mean value of serum IL2 in heroin and cannabis male and female groups as compared to the control groups.

Table (2) shows the serum level of IL6 (pg / ml) in all studied groups (n = 84) There is no significant difference between the mean value of serum IL6 in heroin and cannabis male and female groups as compared to the control groups.

Table (3): illustrates the serum level of glutathione (μ mol/l) in all studied groups (n = 84). There is significant decrease in serum glutathione level in heroin and cannabis male and female groups as compared to the control groups.

Table (4) shows the serum level of TBARS (mmol/dl) in all studied groups (n = 84). There is significant increase in serum TBARS level in heroin & cannabis male and female groups as compared to the control groups.

Table (5) demonstrates the serum level of protein oxidation (μ mol/l) in all the studied groups (n = 84). There is significant increase in serum protein oxidation level in heroin & cannabis male & female groups as compared with control groups.

Table (1): The Serum Level of IL2 (pg/ml) in All Studied Groups (n = 84).

Gender	Groups	N	Range	Mean $\pm$ SE
Males	Control	12	115-217.3	152.4 $\pm$ 7.334
	Heroin	15	50.1-97.10	64.9 $\pm$ 2.999 ^a
	Cannabis	15	52.9-74.30	64.45 $\pm$ 1.703 ^a
Females	Control	12	130.5-218.1	163.5 $\pm$ 7.559
	Heroin	15	46.70-77.60	62.93 $\pm$ 2.198 ^b
	Cannabis	15	46.50-79.70	61.67 $\pm$ 2.182 ^b

^a Significantly lower than the male control group at $p < 0.001$; ^b Significantly lower than the female control group at $p < 0.001$; SE = standard error of mean.

Table (2): The Serum Level of IL6 (pg/ml) in All Studied Groups (n = 83).

Gender	Groups	N	Range	Mean $\pm$ SE
Males	Control	12	14.90-32.40	20.67 $\pm$ 1.285
	Heroin	15	15.30-24.80	20.67 $\pm$ 0.696
	Cannabis	15	14.90-25.00	19.79 $\pm$ 0.0.707

Females	Control	12	17.70-25.20	20.80 $\pm$ 0.662
	Heroin	15	16.40-23.20	19.69 $\pm$ 0.5896
	Cannabis	15	14.90-24.90	20.49 $\pm$ 0.725

SE = standard error of mean.

Table (3): The Serum Level of Glutathione ($\mu\text{mol/l}$) in All Studied Groups (n = 83).

Gender	Groups	N	Range	Mean $\pm$ SE
Males	Control	12	552.9-663.2	605.9 $\pm$ 9.762
	Heroin	15	301.5-485.3	418.8 $\pm$ 13.10 ^a
	Cannabis	15	327.9-491.2	412.9 $\pm$ 14.35 ^a
Females	Control	12	545.6-714.7	610.5 $\pm$ 15.21
	Heroin	15	322.1-486.8	406.8 $\pm$ 14.99 ^b
	Cannabis	15	310.3-480.9	408.8 $\pm$ 14.67 ^b

^a Significantly lower than the male control group at $p < 0.001$; ^b Significantly lower than the female control group at $p < 0.001$; SE = standard error of mean.

Table (4): The Serum Level of TBARS (mmol/dl) in All Studied Groups (n = 83).

Gender	Groups	N	Range	Mean $\pm$ SE
Males	Control	12	0.1370-0.1990	0.1745 $\pm$ 0.0059
	Heroin	15	0.2610-0.4470	0.3571 $\pm$ 0.01643 ^a
	Cannabis	15	0.2430-0.4170	0.3221 $\pm$ 0.01456 ^a
Females	Control	12	0.1400-0.1990	0.173 $\pm$ 0.0059
	Heroin	15	0.2580-0.4310	0.3279 $\pm$ 0.0159 ^b
	Cannabis	15	0.2270-0.3710	0.3169 $\pm$ 0.0141 ^b

^a Significantly higher than the male control group at $p < 0.001$; ^b Significantly higher than the female control group at $p < 0.001$; SE = standard error of mean.

Table (5): The Serum Protein Oxidation level ($\mu\text{mol/l}$) in All Studied Groups (n = 83).

Gender	Group	N	Range	Mean $\pm$ SE
Males	Control	12	30.68-71.36	54.81 $\pm$ 2.773
	Heroin	15	87.73-165.9	121.7 $\pm$ 6.270 ^a
	Cannabis	15	76.82-154.8	116.6 $\pm$ 5.021 ^a
Females	Control	12	55.91-79.54	67.29 $\pm$ 2.116
	Heroin	15	84.54-165.9	125.0 $\pm$ 8.047 ^b
	Cannabis	15	76.82-163.2	119.8 $\pm$ 7.521 ^b

^a Significantly higher than the male control group at $p < 0.001$; ^b Significantly higher than the female control group at $p < 0.001$; SE = standard error of mean.

DISCUSSION

Cytokines are regulating proteins, which adjust the activity of their target cells, especially in the hematogenic system. Cytokines play fundamental roles in the regulation of immune response and inflammation. The production of cytokines by immune system cells may be stimulated by specific or non-specific stimulants.^[29] Changes in cytokine levels have been proven in mice addicted to heroin^[30]

Regarding IL-2 the present study revealed that heroin and cannabis male and female addicts showed decreased level of IL-2 in relation to the control group. This could explain the increased incidence of infection which may occur in a lot of addicts to heroin and cannabis. This is in agreement with Pacifici and colleagues 2003, Shay and coworkers 2003.^[31, 32]

On the other hand, there was no significant change in IL6 level in heroin and cannabis addicts. This is in contrast to the study of Thomas *et al.* (2000)^[33] which found that

cannabis smoking alone resulted in a decrease in production of IL-6. Our results may be explained by previous research that showed a positive association between age and IL-6.^[34]

Furthermore; the present results demonstrated the association between heroin and cannabis addiction and oxidative stress. Oxidative stress was evaluated by measuring reduced glutathione, protein oxidation and lipid peroxidation.

The present results showed that heroin and cannabis were induced a decrease in serum reduced glutathione in male and female. Loss of cellular GSH can occur by free radical-mediated oxidative activity and the generation of ROS results in generation of oxidized glutathione.^[27] The generation of ROS had several undesirable consequences, including the impairment of cellular energetic and defense systems and the promotion of malignant transformation.^[35, 36] Cell death induced by cannabis smoke is largely necrotic. These deleterious

effects of cannabis smoke could have serious implications for tissues in direct contact including lung macrophages and surface epithelial cells in the upper aerodigestive tract and the tracheobronchial mucosa. This is in accordance with the study of Xu and coworkers (2006).^[37]

In addition, the current study stated that TBARS, an index of lipid peroxidation, was significantly increased in heroin and cannabis male and female addicts in relation to the control groups. Elevated gastric malondialdehyde reflects an intensification of lipid peroxidation process. These results are the same as Ozmen and colleagues, 2007.^[22] However, it has been extremely difficult to establish a direct cause and effect relationship between oxygen radicals formation and the actual lesion induced by heroin and cannabis. Lipid peroxidation mediated by oxygen radicals is believed to be an important cause of destruction and damage to cell membranes, because a single initiating event that hydroxyl radical or metal ion-free radical complexes abstract methylene hydrogen atom from polyunsaturated fatty acid side chains in to lipid peroxides, which alter the structural integrity and biochemical functions of membranes.^[38, 39]

Furthermore; the present results revealed that heroin and cannabis male and female addicts showed elevated protein oxidation levels suggest that the oxidation of these proteins may be an early component of the sequence of events leading to mucosal edema and inflammation which may be caused due to addiction. Cellular proteins are also believed to be a target of oxidative damage. One indication of oxidative damage to protein is the carbonyl content of the proteins.^[40] Protein damaged by oxidation are normally degraded quickly in the cell, but in cell where degradative protease activity is overwhelmed or lost by inactivation or decreased production, the oxidized proteins accumulates as in aging, diabetes, and inflammation.^[41] The accumulation of oxidized proteins can impair cell function and eventually leads to cell damage. Therefore, protein oxidation may be an early sign of cellular injury.

CONCLUSION

From the current work it could be concluded that heroin and cannabis addiction in both males & females lead to significant decrease in serum IL2 and glutathione with significant increase in serum TBARS and protein oxidation. These effects are due to production of ROS and thus affect the immune system and expose patients to infections.

REFERENCES

- 1 Nestler EJ and Malenka RC. The Addicted Brain. Scientific American, 2004; 290: 78-85.
- 2 Fattore L, Fadda P, Spano MS, Pistis M and Fratta W. Neurobiological mechanisms of cannabinoid

- addiction. Molecular and Cellular Endocrinology, 2008; 286: 97-107.
- 3 World health organization site (accessed in 12/2015):http://www.who.int/substance_abuse/facts/cannabis/en/.
- 4 Murat Y, Nadia S, Colleen R, Sarah W, Alex F, Christos P, Dan I and Lubman MB. Regional Brain Abnormalities Associated With Long-term Heavy Cannabis Use. Arch Gen Psychiatry., 2008; 65(6): 694-701.
- 5 Wilson RI and Nicoll RA. Endocannabinoid signalling in the brain. Science., 2002; 296: 678-682.
- 6 Inturrisi CE. Clinical pharmacology of opioids for pain. Clin. J. Pain., 2002; 18: S3-S13.
- 7 Collier JK, Hutchinson MR. Implications of central immune signaling caused by drugs of abuse: mechanisms, mediators and new therapeutic approaches for prediction and treatment of drug dependence. Pharmacol Ther., 2012; 134: 219-245.
- 8 Hutchinson MR, Shavit Y, Grace PM, Rice KC, Maier SF, Watkins LR. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. Pharmacol Rev., 2011; 63: 772-810.
- 9 Cohen S, Bigazzi PE and Yoshida T. Cytokine: more than a new word, a new concept proposed by Stanley Cohen thirty years ago. Cytokine, 2004; 28: 242-247.
- 10 Blanchard DK, Newton C, Klein TW, Stewart WE, Friedman H, In vitro and in vivo suppressive effects of delta-9-tetrahydrocannabinol on interferon production by murine spleen cells. Int. J. Immunopharmacol., 1986; 8: 819-824.
- 11 Klein TW. Cannabinoid-based drugs as anti-inflammatory therapeutics. Nat. Rev. Immunol., 2005; 5: 400-411.
- 12 Dol-Gleizes F, Paumelle R, Visentin V, Marés AM, Desitter P, Hennuyer N, Gilde A, Staels B, Schaeffer P, Bono F. Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. Arterioscler. Thromb. Vasc. Biol., 2009; 29: 12-18.
- 13 Klein TW, Cabral GA. Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. J. Neuroimmune Pharmacol., 2006; 1: 50-64.
- 14 Pacher P: Cannabinoid CB1 receptor antagonists for atherosclerosis and cardiometabolic disorders: new hopes, old concerns? Arterioscler. Thromb. Vasc. Biol., 2009; 29: 7-9.
- 15 Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Vitoretti LB, Mariano-Souza, DP, Quinteiro-Filho WM, Akamine AT, Almeida VI, Quevedo J, Dal-Pizzol F, Hallak JE, Zuardi AW, Crippa JA, Palermo-Neto J. Cannabidiol, a nonpsychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: role for the

- adenosine A(2A) receptor. *Eur. J. Pharmacol.*, 2012; 678: 78–85.
- 16 Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.*, 2007; 39: 44–84.
- 17 Zhang YT, Zheng QS, Pan J, Zheng RL Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. *Basic Clin. Pharmacol. Toxicol.*, 2004; 95(2): 53–58.
- 18 Guzman DC, Vazquez IE, Brizuela NO, Alvarez RG, Mejia GB, Garcia EH, Santamaria D, deApreza M, Olguin HJ. Assessment of oxidative damage induced by acute doses of morphine sulfate in postnatal and adult rat brain. *Neurochem. Res.*, 2006; 31(4): 549–554.
- 19 Payabvash S, Beheshtian A, Salmasi AH, Kiumehr S, Ghahremani MH, Tavangar SM, Sabzevari O, Dehpour AR: Chronic morphine treatment induces oxidant and apoptotic damage in the mice liver. *Life Sci.*, 2006; 79(10): 972–980.
- 20 Qiusheng Z, Yuntao Z, Rongliang Z, Dean G and Changling L. Effects of verbascoside and luteolin on oxidative damage in brain of heroin treated mice. *Pharmazie*, 2005; 60: 539–543.
- 21 Guzman DC, Vazquez IE, Brizuela NO, Alvarez RG, Mejia GB, Garcia EH, Santamaria D, Apreza MR and Olguin HJ. Assessment of oxidative damage induced by acute doses of morphine sulfate in postnatal and adult rat brain. *Neurochem. Res.*, 2006; 31: 549–554.
- 22 Ozmen I, Naziroglu M, Alici HA, Sahin F, Cengiz M and Eren I. Spinal morphine administration reduces the fatty acid contents in spinal cord and brain by increasing oxidative stress. *Neurochem. Res.*, 2007; 32: 19–25.
- 23 Hatakeyama M and Taniguchi T. "Interleukin-2" in *Peptide Growth Factors and Their Receptors*. Springer-Verlag, New York, 1990; 523.
- 24 Bauer J and Herrmann F. Interleukin-6 in clinical medicine. *Ann. Hematology* 1991; 62: 203-213.
- 25 Koster JF, Biemond P and Swaak AJ. Intracellular and extracellular sulphhydryl levels in rheumatoid arthritis. *Annals of the Rheumatic Diseases*, 1986; 45: 44-46.
- 26 Sushmakumari S, Jaydeep A, Kumar JS and Menon VP. Effect of carnitine on malonyldialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. *Indian J Exp Biol.*, 1989; 27: 134–137.
- 27 Halliwell B and Gutteridge JMC. Free Radicals in Biology and Medicine. *Arch Biochem. Biophys.*, 1989; 246: 501-514.
- 28 Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz A, Ahn B, Shaltiel S and Stadtman E. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.*, 1990; 186: 464-478.
- 29 Arababadi MK, Pourfathollah AA, Jafarzadeh A, Hassanshahi G, Daneshmandi S, Shamsizadeh A. Non-association of IL-12 and IFN γ polymorphisms with cytokines serum level in occult HBV infected patients. *Saudi J Gastroenterol*, 2011; 17(1): 30-5.
- 30 Pacifici R, di CS, Bacosi A, Pichini S, Zuccaro P. Pharmacokinetics and cytokine production in heroin and morphine-treated mice. *Int J Immunopharmacol*, 2000; 22(8): 603-14.
- 31 Pacifici R, Zuccaro P and Pichini S. Modulation of the immune system in cannabis users. *JAMA.*, 2003; 289(15): 1929-1931
- 32 Shay AH, Choi R, Whittaker K, Salehi K, Kitchen CM, Tashkin DP, Roth MD and Baldwin GC. Impairment of antimicrobial activity and nitric oxide production in alveolar macrophages from smokers of marijuana and cocaine. *J. Infect. Dis.* 2003; 187: 700–704.
- 33 Thomas WK, Brian L, Catherine AN and Herman F The cannabinoid system and cytokine network. *Proceedings of the Society for Experimental Biology and Medicine.*, 2000; 225: 1-8.
- 34 Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panagiota M, Invidia L, Celani L, Scurti M, Cevenini E, Castellan G, Salvioli S Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans *Mech. Ageing Dev.*, 2007; 128: 92–105.
- 35 Wallace KB, Eelss JT, Madeira VMC, Cortopassi G and Jones DP. Mitochondria-mediated cell injury. *Fundam. Appl. Toxicol.*, 1997; 38: 23–37.
- 36 Repine JE, Bast A and Lankhorst I. Oxidative stress in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.*, 1997; 156: 341–357.
- 37 Xu B, Wang Z, Li G, Li B, Lin H, Zheng R and Zheng Q. Heroin-administered mice involved in oxidative stress and exogenous antioxidant-alleviated withdrawal syndrome. *Basic Clin. Pharmacol. Toxicol.*, 2006; 99: 153–161.
- 38 Bankson DD, Kestin M and Rifai N. Role of free radicals in cancer and atherosclerosis. *Clin. Lab. Med.*, 1993; 13: 463–480.
- 39 Niki E. Antioxidants in relation to Lipid Peroxidation. *Chemistry and Physics of Lipids*, 1987; 44: 227-253.
- 40 Stafford RE, Mak IT, Kramer JH and Weglicki WB. Protein oxidation in magnesium deficient rat brain and kidney. *Biochem Biophys Res Comm.*, 1993; 196: 596-600.
- 41 Oliver Cn, Ahn BA, Moerman EJ, Goldstein S and Stadtman ER. Age related changes in oxidized proteins. *J Biol Chem.*, 1987; 262: 5488-5491.