Impact of marijuana smoking on liver and sex hormones: Correlation with oxidative stress

El-Shahat A. Toson

Chemistry Department (Biochemistry Division), Faculty of Science (Damietta), Mansoura University, Egypt.
eatoson@yahoo.com

Abstract: In recent years, there has been a dramatic increase in the number of marijuana users. Also, in the past two decades it was found that cannabinoids present in marijuana exert their biological effects via cannabinoids receptors CB1 and CB2. Such receptors act as crucial mediators in a variety of pathophysiological conditions including liver. This is because, a three-fold increase in CB1 receptors on isolated vascular endothelial cells was detected in cirrhotic human livers. Moreover, the liver plays a major role in the catabolism of the steroid hormones. Therefore, the aim of this study was to determine whether marijuana smoking can participates in liver damage and, therefore, in sex hormones abnormalities and/or oxidative stress, or not. In this study a group of marijuana smokers (n=90) with no history of liver diseases in addition to 25 of the healthy individuals with matched age and sex to that of the smoker group. In sera of marijuana smokers, the mean GGT activity was 86.6% higher than that of the control group and that of alkaline phosphatase (ALP) was 121.7% higher than that of the nonsmokers' group. In addition, the total bile acids, which were synthesized from cholesterol in the liver, as well as the acetyl cholinesterase (AChE) activity were 39.2% higher and 11.3% lower than those of the corresponding control values, respectively. However the mean nitric oxide level was dramatically increased in sera of marijuana smokers (210.8%), the C-reactive protein level was only 40 % higher in sera of marijuana smokers compared with those of the control group. Also, the mean SGPT activity was 19.4 % and that of bilirubin level was 39.1 % higher in sera of the smoker group than those of the healthy control group. With respect to the effect of the marijuana smoke on testosterone and its trophic pituitary hormone; luteinizing hormone, their levels were lowered by not less than 48.7% and 14.5% compared to those of the healthy control group, respectively. Moreover, the markers of the oxidative stress; namely glutathione (GSH), the total antioxidants capacity (TAC) and malondialdehyde (MDA), the first two were significantly reduced and the latter was elevated compared with those of the control group. Finally, negative correlations were found between testosterone and ALP, GGT and NO with a stronger negative correlation between the latter and testosterone. On the other hand, positive correlations were found between NO and both GGT and ALP with the strongest one between the latter and NO. Also, a positive correlation was found between GGT and ALP. These negative and positive correlations may lead one to conclude that marijuana smoke may participate in both liver and testicular damage via NO and its related radicals-dependent mechanisms.

[El-Shahat A. Toson. **Impact of marijuana smoking on liver and sex hormones: Correlation with oxidative stress**. Nature and Science 2011;9(12):76-87]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: marijuana; smoking; liver; sex hormones; oxidative stress

Introduction

In the past two decades, cannabinoids in Cannabis sativa (marijuana) have emerged as crucial mediators in a variety of pathophysiological conditions. Also, in recent years there has been a dramatic increase in the number of marijuana users and in the long-term health consequences of marijuana use. **In 1996, Adams and Martin** found that, a short term effects of marijuana use include memory loss, distorted perception, trouble with thinking and problem solving, loss of motor skills, decrease in muscle strength, increased heart rate, anxiety, and heart attack. Smoking marijuana also weakens the immune system **(Klein et al., 2003),** raises the risk of lung infections **(Dobson, 1999)** and had higher mutagenicity compared to tobacco smoke. The potentiality of marijuana to promote cancer of the lungs and other parts of the respiratory tract may be due to the fact that it contains irritants and carcinogens up to 70 percent more than tobacco smoke **(Wu et al., 1988).** Also, it was found that a control group smoking a single marijuana cigarette every other day for a year had a white-blood-cell count that was 39 percent lower than normal, thus damaging the immune system and making the user more susceptible to infection and sickness **(National Institute of Drug Abuse, 1997 and Klein et al., 2003).**

Awareness of the functions of marijuana use in liver pathophysiology is only recent, probably given the low level of expression of cannabinoid CB1 and CB2 receptor in normal liver. **In 2005, Hézode et al.** added that CB receptors regulate progression of experimental liver fibrosis. Moreover, **Klein et al. (2003)** illustrated that, cannabinoids via their binding with their receptors have been shown to modulate a

variety of immune cell functions in humans and animals including T helper cell development, chemotaxis, and tumor development. The latter binding also modulate cytokines and other gene products through signaling mechanisms **(Klein et al., 2003)**.

Hepatic fibrosis, the common response associated with chronic liver diseases, ultimately leads to cirrhosis, a major public health problem worldwide. Advanced cirrhosis is associated with generalized vasodilatation of unknown origin, which contributes to mortality. Cirrhotic patients are endotoxemic, and activation of vascular cannabinoid CB1 receptors has been implicated in endotoxin-induced hypotension. Compared with the non-cirrhotic controls, in cirrhotic human livers there was a three-fold increase in CB1 receptors on isolated vascular endothelial cells **(Bátkai et al., 2001).**

However, it has been shown that non-alcoholic fatty liver disease and cirrhosis are associated with a marked increase of the hepatic endocannabinoid system, which include increases in endocannabinoid and the hepatic CB receptors, both in humans and in rodents. Consequently, a growing number of cannabinoid-related hepatic effects are being unravelled **(Mallat and Lotersztajn, 2006).** Hence, hepatic CB1 receptors enhance liver steatogenesis in a mouse model of high fat-induced obesity, and contribute to peripheral arterial vasodilatation in cirrhosis, thereby promoting portal hypertension. In addition, CB1 and CB2 receptors elicit dual opposite effects on fibrogenesis associated to chronic liver injury, by promoting pro- and antifibrogenic effects, respectively **(Mallat et al., 2007).**

Research on the free radical gas, nitric oxide (NO), during the past twenty years is one of the most rapid growing areas in biology. NO seems to play a part in almost every organ and tissue. However, there is considerable controversy and confusion in understanding its role. The liver is one organ that is clearly influenced by NO. Acute versus chronic exposure to NO has been associated with distinct patterns of liver disease. NO also demonstrates antimicrobial and anti-apoptotic properties during acute hepatitis infection and other inflammatory processes. However, in the setting of chronic liver inflammation, when a large sustained amount of NO is present, NO might become genotoxic and lead to the development of liver cancer. Additionally, during prolonged ischemia, high levels of NO may have cytotoxic effects leading to severe liver injury **(Hon et al., 2002).**

Bile acids are synthesized from cholesterol in the liver and are essential for digestion. In the intestine, bile acids function in the solubilization and absorption of fats, certain vitamins, and cholesterol. Individually, bile acids are known to have hepatocellular toxicity both in vivo and in vitro. Furthermore, bile acids can not only promote cell proliferation but can also induce programmed cell death. **(Keitel et al., 2008).** A role for bile acids in liver regeneration has also recently been identified. These results were confirmed by the increase in bile acid levels after partial hepatectomy **(Huang et al., 2006).**

Acetylcholine (Ach), which is synthesized by the liver, has a great number of physiologic effects including enhancement of attention to sensory stimuli, improving sensory processing, encoding of memory for specific stimuli, modulation of cortical function and cognition. The action of acetylcholine is terminated via acetyl cholinesterase (AChE) which hydrolyzes it **(Benarroch and Eduardo, 2010). Hasselmo and Sarter (2011)** demonstrated that, not only the latter enzyme is competitively inhibited by the active Δ^9-THC but also its-induction of amyloid β-peptide (Aβ) aggregation is prevented by such cannabinoid derivative. The latter is the key pathological marker of Alzheimer's disease. This is because the computational modeling of the THC-AChE interaction revealed that THC binds in the peripheral anionic site of AChE, the critical region involved in amyloidgenesis.

Moreover, steroid hormones, including the sex hormones, are generally catabolized via their conversion into inactive metabolic excretion products in the liver **(Champ et al, 2005).** Based on such data, the levels of the previous hormones are related to the status of the liver and its function.

Therefore, the present study was designed to test if marijuana smoking can participates in liver damage, sex hormones abnormalities and/or oxidative stress, or not. For these reasons, the effects of marijuana smoke on serum levels of bile acids, choline esterase, gamma glutamyl transpeptidase (**γ**- GT) and alkaline phosphatase (ALP) activities, routine liver function tests, NO, testosterone, FSH, LH, malondialdehyde (MDA), glutathione reduced (GSH) and the total antioxidant capacity(TAC) were suggested to be evaluated in sera of marijuana smokers and in sera of the healthy control group, in this study.

2- Subjects and Methods

a-Subjects:

1-**Marijuana smokers:** Male marijuana smokers (n= 90) were only included in this study. Their ages were ranged from 20 - 30 years and those having any history of liver diseases or gave positivity for hepatitis C virus (HCV antibodies) or hepatitis B virus positivity (HBsAg) were excluded from this study. In addition, the smoking period was ranged from 5 - 10 years and each smoker was informed by

the nature of the research and gave samples with consent. In addition, after 12 hours fasting, blood samples were collected by venous blood puncture from each smoker and control subjects. Ethylene diamine tetra acetic acid (EDTA) disodium salt solution was added to one part of the blood sample to prevent its clotting and was used for hematological assays. The other part of each sample was left to clot, centrifuged and the serum samples were separated and kept frozen until its use.

2-Healthy individuals: A group of healthy males (n=25) with similar age and sex to those of marijuana smokers were included in this study. The same protocol of blood withdrawal used for marijuana smokers was also used during deals with the blood samples withdrawn from the healthy individuals.

b- Methods

b1- Glutathione (GSH) in RBCs and malondialdehyde (MDA) in liver tissues: GSH was determined in liver tissues and RBCs by the method of **Beutler et al. (1963)** but MDA was determined by the method of **Stock and Donnandy (1971)**.

b1 - Routine liver functions: Serum albumin was determined according to the method of **Doumas et al. (1971).** Also, the activities of serum glutamic pyruvic transaminase and that of γ-glutamyl transpeptidase (GGT) were determined by the method of **Reitman and Frankel (1957) and by Szasz et al. (1969),** respectively.

b2 - C-Reactive protein: CRP concentration was carried out according to **(Otsuji et al., 1982)**.

b3 - Nitric oxide : NO_2 was assayed by the methods of **Berkels et al. (2004).**

b4- Sex hormones profile: Quantitative estimation of Follicular stimulating hormone **(FSH)** in serum was carried out (using ELISA kit, obtained from DRG Instruments GmbH, Germany), according to the method described by **Marshall (1975)**. Quantitative estimation of Leutinizing hormone (**LH**) and Testosterone in serum was carried out (using ELISA kit, obtained from DRG Instruments GmbH, Germany), according to the method described by **Uotila et al. (1981)**.

b5 - **Total Bile acid:** Total serum bile acids (SBA) were measured by an enzymatic method using the Sigma Diagnostic† kit, which is based on the method of **Mashige et al. (1981).** SBA calibrators in concentrations of 5, 25, 50, 100 and 200 mmol/l from Sigma Diagnostics (Sigma-Aldrich, Dorset, UK) were used for construction of the standard curve.

b6 – **Total antioxidant capacity (TAC):** TAC was measured according to the method of ***Koracevie et al., (2001).***

b7 – **Complete Blood Count (CBC):** CBC was done Auto Hematology Analyzer (**D-Cell 60 Diagon Ltd Haungary**).

b8 – **Acetyl cholinesterase (AChE) activity**: AChE activity was assayed by the method of **Kndel et al. (1967)**.

RESULT

1-Serum γ- glutamyl transpeptidase, alkaline phosphatase and cholinesterase activities and total bile acids levels:

1-1-γ-Glutamyl transpeptidase (GGT) activity: The mean GGT activity in sera of the healthy control group was 13.4 ± 4.8 IU/l with a range of 8.2 - 21.3 IU/l. On the other hand, the mean GGT activity in sera of marijuana smokers was 25 ± 11.8 IU/l with a range of 3.0 - 49 IU/l. Moreover, the mean GGT activity was 86.6% higher than that of the control group (**Table 1**).

1-2-Alkaline phosphatase (ALP) activity: With respect to ALP mean activity, its control value was 17.5 ±5.7 IU/L with a range of 11- 32 KAUs but that of marijuana smokers was 38.8 ± 15.4 KAUs with a range of 10 - 90 KAUs. In addition, the latter mean activity was 121.7% higher than that of the nonsmokers' group (**Table 1**).

1-3- Acetyl cholinesterase (AChE) activity: The mean cholinesterase activity of the control group was 6.2 ± 1.2 and its individual values were ranged from 3.4 - 7.9. In addition, the mean cholinesterase activity of marijuana smokers was 5.5 ± 1.8 and its individual values were ranged from 1.0 - 9.0. Moreover, the mean activity of this enzyme in sera of the smoker's group was 11.3% lower than its corresponding activity of the non-smoking individuals (**Table 1**).

1-4-Total bile acids: The mean level of total bile acids in sera of marijuana smokers was 7.8 ± 3.5 *µmole/l* with a range of 2.0 -13 *µmole/l*. In addition, its mean value in sera of the healthy control group was 5.6 ± 2.0 *µmole/l* with a range of 2.5 - 8.3 *µmole/l*. Moreover, the total bile acids mean level of the smokers was 39.2% more higher than that of the healthy control group (**Table 1**).

2-Serum levels of C-reactive protein and nitric oxide:

2-1-C-reactive protein: The mean C-reactive protein level in sera of the healthy control group was 0.5 ± 0.2 with a range of 0.22 - 0.88 and that of marijuana smokers was 0.7 ± 0.3 with a range of 0.3 - 1.5. In addition, the mean C-reactive protein level was 40 % higher in sera of marijuana smokers than that of the control group (**Table 2**).

2-2-Nitric oxide (NO) level: The mean NO level in sera of the healthy individuals was 4.6 ± 0.7 µmole/l and its individual values were ranged from 3.6 - 6.0 µmole/l. On the other hand, the mean nitric oxide level in sera of marijuana smokers was 14.3 ± 6.4 µmole/l with a range of 3.0 - 30 µmole/l. Dramatically, the mean nitric oxide level in sera of

marijuana smokers was 210.8 % higher than that of the healthy non-smoking group (**Table 2).**

3- Parameters of routine liver function tests:

3.1- Serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxalloacetic transaminase (SGOT) activities: The mean SGPT activity in sera of the healthy control group was 25 ± 5.2 IU/ml with a range of 20 - 36 IU/ml and that of marijuana smokers was 31 ± 6.9 IU/ml with a range of 23 - 43 IU/ml. In addition, the mean SGPT activity was 19.4 % higher and was significantly elevated in sera of marijuana smokers compared with that of the control group. With respect to **SGOT** activity in sera of the healthy control group, its mean value was 26 ± 5.9 IU/ml with a range of 22 – 37 IU/ml and that of marijuana smokers was 29 ± 9.4 IU/ml with a range of 20 - 60 IU/ml. In addition, the mean SGOT activity was 10.3% higher but not significantly elevated than that of the healthy control group (**Table 2**).

3.2- Bilirubin: The mean bilirubin level in sera of the healthy control group was 0.67± 0.15 mg% with a range of 0.45 -0.87 mg% and that of marijuana smokers was 1.1 ± 0.5 mg% with a range of 0.5 - 1.8 mg%. In addition, the mean bilirubin level in sera of marijuana smokers was 39.1 % higher and was highly significantly elevated than that of the healthy control group ($P< 0.0001$ and **Table 3**).

3.3- Albumin: The mean albumin level in sera of the healthy control group was 4.5 ± 0.52 gm% with a range of 3.9 - 5.3 gm% and that of marijuana smokers was 4.35 ± 0.5gm% with a range of 3.9 - 5.3 gm%. In addition, The mean albumin level in sera of marijuana smokers was not significantly differ than that of the healthy control group (**Table 3**).

4-Serum levels of testosterone, leutinizing hormone (LH) and follicle stimulating hormones (FSH):

4-1-Serum levels of testosterone: The mean testosterone level in sera of the healthy control group was 11.9 ± 3.1 *pg/ml* with a range of 8.3-18.3 *pg/ml*. On the other hand, the mean testosterone level in sera of marijuana smokers was 6.1 ± 2.1 *pg/ml* with a range of 3.0 -10 *pg/ml* indicating that serum testosterone level was lowered by not less than 48.7% of that of the healthy control group (**Table 4).**

4-2-Serum levels of leutinizing hormone (LH): The mean level of the LH in sera of the healthy control was 6.2 ±1.9 *mIU/ml* with a range of 3.1 - 8.3 *mIU/ml* and that of LH of marijuana smokers was 5.3 ± 1.6 *mIU/ml* with a range of 2.0 - 8.8 *mIU/ml*. In addition, the bad effect of the marijuana smoke caused LH lowering by 14.5% compared with that of the healthy control group (**Table 4**).

4-3- Follicle stimulating hormone (FSH): The mean FSH level in sera of the control group was 5.1 ± 1.9 *mIU/ml* and its individual values were ranged from 2.6 - 8.3 *mIU/ml*. In addition, the individual values of FSH in sera of marijuana smokers was ranged from 1.0 - 8.0 *mIU/ml* with a mean value of 5.1 ± 1.9 *mIU/ml* (**Table 4**).

5- Oxidative stress:

5.1- Glutathione reduced (GSH): The mean GSH level of the blood of marijuana smokers was 2.5 ± 0.9 mM/ml cells with a range of 1.0 - 4.4 mM/ml cells. Also, the mean GSH of the blood of the control group was 4.1 ± 0.53 mM/ml cells and its individual values were ranged from 3.2 - 5.1 mM/ml cells **(Table 5).**

5.2- Malondialdehyde (MDA): The mean **MDA** of the backed red blood of marijuana smokers was 12.1 ± 0.4 $M\times10^{-6}$/ml packed cells with a range of 6.5 - 22 $M\times10^{-6}$/ml packed cells. However the mean value of such parameter in the backed cells of the control group was 2.3 ± 0.68 $M\times10^{-6}$/ml packed cells and its individual count was ranged from 31.0 - 3.6 $M\times10^{-6}$/ml packed cells **(Table 5).**

5.3- Total oxidants capacity (TAC): Mean **TAC** in sera of marijuana smokers was 0.65±0.21 with a range of 0.2-1.03 and mean value of such parameter in sera of the control group was 0.74±0.17 with individual values ranged from 0.42-0.97 **(Table 5).**

6-Parameters of blood picture:

6.1- Haemoglobin and red blood cells (RBCs): The mean haemoglobin concentration of the blood of marijuana smokers was 13.4 ± 1.7 gm% with a range of 9.0 - 16 gm%. Also, the mean RBCs count in the whole blood of marijuana smokers was 4.7 ± 0.7 *$\times10^6$/µl blood* and the individual count of such RBCs was ranged from 3.0 - 6.0 106/µl blood.

6.2- White blood cells (WBCs) and their differential counts: The mean total leucocytic count in the blood of marijuana smokers was 6.8 ± 2.0 $\times10^3$/µl blood with a range of 3.5 -12.4 ×103/µl blood. Also, the mean lymphocytic count of their blood samples was 59 ± 22% with a range of 24 - 91%. In addition, the mean count of the mixed leucocytes (eosinophils, basophils and the monocytes) was 13 ± 4.0 %. Moreover, the mean granulocytic count (neutrophils) in the smokers, blood was 28 ± 11% and its individual values were ranged from 15 - 40%.

Correlation:

Negative correlations were found between testosterone and ALP, GGT and NO with a stronger negative correlation between the latter and testosterone ($r= - 0.5$). On the other hand, positive correlations were found between NO and both GGT and ALP with the strongest one between the latter and NO ($r= 0.43$). Also, a positive correlation was found between GGT and ALP (Table 7 and 8).

Table (1): Mean γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP) and cholinesterase activities and total bile acids levels in sera of marijuana smokers compared with those of the healthy control group.

Parameters Group	GGT (IU/l)	ALP *(IU/L)*	Cholinesterase	Bile acids *(μmole/l)*
Control	13.4 ± 4.8 (8.2 -21.3)	17.5 ± 5.7 (11 - 32)	6.2 ± 1.2 (3.4- 7.9)	5.6 ± 2.0 (2.5 -8.3)
Marijuana smokers	25 ± 11.8* (3.0 - 49) [86.6 %]	38.8 ± 15.4** (10 - 90) [121.7 %]	5.5 ± 1.8 (1.0 - 9.0) [11.3 %]	7.8 ± 3.5* (2.0 -13) [39.2 %]

*= Significant and **= highly significant compared with those of the control group. Values between [] represent the percent of changes in the test results compared to those of the control group (= Test – control/Control x100)

Table (2): Mean serum glutamic pyruvic transaminase (SGPT), serum glutamic oxalloacetic transaminase (SGOT) activities and bilirubin and albumin mean levels in sera of marijuana smokers compared with those of the healthy control group.

Parameters Group	SGPT *(IU/ml)*	SGOT *(IU/ml)*	Bilirubin *(mg%)*	Albumin *(gm%)*
control	25 ± 5.2 (20 – 36)	26 ± 5.9 (22 – 37)	0.67± 0.15 (0.45 -0.87)	4.5 ± 0.52 3.9 - 5.3
Test	31 ± 6.9** (23 -43)	29 ± 9.4 (20 - 60)	1.1 ± 0.5** (0.5 - 1.8)	4.35 ± 0.5 (3.6 - 4.9)

*= Significant and **= highly significant compared with those of the control group. Values between [] represent the percent of changes in the test results compared to those of the control group (= Test – control/Control x100)

Table (3): Mean serum levels of C-reactive protein and nitric oxide in sera of marijuana smokers compared with those of the healthy control group.

Parameters Group	C-reactive protein (mg%)	Nitric oxide (μmole/l)
Control	0.5 ± 0.2 (0.22 - 0.88)	4.6 ± 0.7 (3.6 – 6.0)
Marijuana smokers	0.7 ± 0.3* (0.3 - 1.5) [40 %]	14.3 ± 6.4** (3.0 - 30) [210.8 %]

*= Significant and **= highly significant compared with those of the control group. Values between [] represent the percent of changes in the test results compared to those of the control group (= Test – control/Control x100)

Table (4): Mean serum levels of testosterone, luteinizing hormones (LH) and follicle stimulating hormones (FSH) in sera of marijuana smokers compared with those of the healthy control group.

Parameters / Group	Testosterone *(pg/ml)*	LH *(mIU/ml)*	FSH *(mIU/ml)*
Control	11.9 ± 3.1 (8.3 -18.3)	6.2 ±1.9 (3.1 - 8.3)	5.1 ± 1.9 (2.6 - 8.3)
Marijuana smokers	6.1 ± 2.1** (3.0 - 10) [48.7 %]	5.3 ± 1.6** (2.0 - 8.8) [14.5 %]	5.1 ± 2.2 (1.0 - 8.0) [0 %]

*= Significant and **= highly significant compared with those of the control group. Values between represent the percent of changes in the test results compared to those of the control group (= Test – control/Control x100)

Table (5): Mean levels of blood glutathione reduced (GSH), malondialdehyde (MDA) in red blood cells and total oxidants capacity (TAC) in sera of of marijuana smokers compared with those of the healthy control group.

Parameters / *Group*	GSH *(mM/ml cells)*	MDA *($M\times10^{-6}$/ml packed cells)*	TAC
Controls	4.1 ± 0.53 (3.2 - 5.1)	2.3 ± 0.68 (1.0 - 3.6)	0.74 ± 0.17 (0.42 – 0.97)
Marijuana smokers	2.5 ± 0.9** (1.0 – 4.4)	12.1 ± 0.4** (6.5 - 22)	0.65 ± 0.21* (0.2 - 1.03)

*= Significant and **= highly significant compared with those of the control group. Values between [] represent the percent of changes in the test results compared to those of the control group (= Test – control/Control x100)

Table (6):The mean haemoglobin levels, red blood cells, total leucocytes and platelets counts and the percentages of lymphocytes, neutrophils (granulocytes) and the mixed leucocytes (eosinophils, basophils and monocytes) in the blood of marijuana smokers compared with those of the healthy control group.

Group Parameters	Control	Marijuana smokers✪
Haemoglobin *(gm%)*	13.6 ± 1.7 11.3 – 17	13.4 ± 1.7 (9.0 - 16)
Red blood cells count	4.4 ± 0.6 3.9 - 5.6	4.7 ± 0.7 (3.0 - 6.0)
Total leucocytic count	8.4 ± 2.6 5.0 - 13.1	6.8 ± 2.0 (3.5 -12.4)
Platelets count	282 ± 34 219 – 357	232 ± 60 (87-393)
Lymphocytes *(%)*	38 ± 12 (25 - 44)	59 ± 22 (24 - 91)
Granulocytes (%)	59 ± 17 (49 - 66)	28 ± 11 (15 - 40)
Mixed *(%)*	8 ± 3.6 (6 – 10)	13 ± 4.0 (4.0 - 21)

✪= The RBCs count × 10^6/µl blood and that of total leucocytic count and platelets × 10^3/µl Blood.

Table (7): The positive and significant correlations between NO with GGT and ALP.

Markers		Correlation coefficients	P values
ALP	GGT	0.26	P< 0.03
NO	GGT	0.32	P< 0.03
NO	ALP	0.43	P< 0.03

Table (8):The negative and significant correlations between testosterone with NO and GGT.

Markers		Correlation coefficients	P values
Testosterone	ALP	- 0.4	P< 0.007
Testosterone	GGT	- 0.3	P< 0.03
Testosterone	NO	- 0.5	P< 0.0014

DISCISSION

Hepatitis C virus infection, alcohol consumption and non-alcoholic steatohepatitis are causative factors of hepatic cirrhosis. In addition, hepatic fibrosis is the response of the liver to chronic injury and is associated with portal hypertension, progression to hepatic cirrhosis, liver failure, and high incidence of hepatocellular carcinoma (HCC). On the molecular level, a large number of signaling pathways have been shown to contribute to the activation of fibrogenic cell types in the liver and the subsequent accumulation of extracellular matrix (ECM). Recent evidence suggests that the endocannabinoid system is an important part of this complex signaling network. In the injured liver, the endocannabinoid system is up regulated both at the level of endocannabinoids themselves and their CB1 and CB2 receptors as well (**Siegmund and Schwabe, 2008).** Moreover, awareness of the functions of marijuana use in liver pathophysiology is only recent. Nevertheless, suppression of the cause of hepatic injury is not always feasible and numerous efforts are directed at the development of liver-specific antifibrotic therapies **(Mallat and Lotersztajn, 2006).** Also, **Teixeira-Clerc et al. (2006)** added that CB1 receptors were highly induced in human cirrhotic samples and on liver fibrogenic cells. Moreover, The role of CB1 in hepatic fibrogenesis were confirmed by the decrease in the severity of such pathological state after CB1 inactivation. This is probably mediated by lowering of the hepatic transforming growth factor (TGF)-β1 and the reduction in the accumulation of fibrogenic cells in the liver after apoptosis and growth inhibition of hepatic myofibroblasts (**Teixeira-Clerc et al., 2006).** In the present study one can suggest that, upon binding of THC of marijuana smoke to CB1 receptors located on the fibrogenic cells of the liver, such receptors will be activated. The activated receptors, in turn, can elevate transforming growth factor-β1 (TGF-β1) and the latter can cause excessive extracellular matrix (ECM) and hepatic fibrogenesis as was previously reported by **Teixeira-Clerc et al. (2006)**. The significant elevation in serum GGT, ALP and serum glutamic pyruvic transaminase (SGPT) mean activities and the significant reduction in AChE mean activity together with elevations in the mean bile acids and bilirubin levels in the blood sera of marijuana smokers than those of the non-smokers confirm the excessive liver damage accompanying inhalation of marijuana smoke. The results of the present study also confirm those of **Mukhta et al. (2011)** who found that**,** cannabinoids increase the ALP activity in both injected rats and human smokers with the increase of dose and time but the activities of ALT and AST were increased at the beginning of consumption then decreased with time. Also, the results of the present study confirm the conclusion of **Hézode et al. (2005)** who showed that daily cannabis smoking is significantly associated with fibrosis. The mechanism of the latter liver damage may involve activation of CB1 receptors as was

shown by **Tam et al. (2011)** who illustrated that such receptors is activated in various liver diseases and contributes to the underlying pathologies. The last outhers added that, the activation of CB1 receptors located on hepatic stellate cells of the liver; which are the major participators in ECM synthesis, contributes to fatty liver, insulin resistance, to the vasodilated state observed in liver affected patients and to the cardiomyopathy. Unfortunately, the levels of any of the ECM components were not evaluated in the present study but the disturbances of the parameters of liver functions, as before, can be used as indirect evidences on the occurrence of liver damage.

It has frequently been reported that abnormally low values of serum AChE were found in sera of patients suffering from liver disease. The results of the present study also confirm that of **Hernández *et al.* (2006)** who found a very slight impairment of the liver function with a concomitant depression in AChE activity by a greater than 25% from the baseline activity of the enzyme. Simultaneously, the activity of AST was increased. The results of the present study support the latter two findings. In the present study, only 11.3% of AChE activity was reduced compared to those of the control group.

Bile acids play an important role in normal digestive processes, and they also have been used as therapeutic agents during recent decades. They may also function as immunosuppressive agents and are able to induce apoptosis and carcinogenesis **(Debruine et al., 2001).** Also, apoptosis induced by hydrophobic bile acids is thought to contribute to liver injury during cholestasis **(Raimondi et al., 2008).** In the present study, the increase in GGT (121.7%) and ALP (86.6%) activities compared with those of the control group confirm the occurrence of hepatobiliary damage of the liver tissues of marijuana smokers. The results of the present study also confirm the role of bile acids in the regulation of hepatic microcirculation which has been recently suggested by **Rust et al. (2009)**. Moreover, TGR5 are bile acids receptor and is localized on the plasma membrane of sinusoidal endothelial cells of rat liver. Stimulation of such receptors increased cAMP levels, activated protein kinase A and lead to a serine phosphorylation of endothelial NO synthetase and subsequent elevation of NO production **(Keitel et al., 2007).** Therefore, the elevation in the mean serum levels of bile acids in sera of marijuana smokers (39.2% higher than that of the control), in the present study, may be a cause of NO elevation. This is because, the NO levels was 210.8% higher than that of the nonsmokers.

Hypotension, low systemic vascular resistance, and a reduced sensitivity to vasoconstrictors are features of cirrhosis. These cardiovascular changes might be the result of the increase in the synthesis of the potent vasodilator nitric oxide (NO). Such molecule is synthesized in and released from peripheral blood vessels in man. Studies in animals indicated that bacterial endotoxin and cytokines induce NO synthase expression in the walls of the blood vessels, with sustained NO release and consequent hypotension. Thus, the dramatic increase in the mean level of NO in sera of marijuana smokers, in this study, may be a consequent of endotoxaemia which is a common feature of liver damage including cirrhosis and may also be a cause of hypotension which coexist with liver damage as was previously reported by **Vallance and Moncada (1991)**.

In 2003 Serracino-Inglott et al. found that during the reperfusion period after hepatic ischemia, endothelial nitric oxide synthase is down regulated while inducible nitric oxide synthase is expressed in both hepatocytes and inflammatory cells. This may lead one to conclude that marijuana smoke can cause increase in the inflammatory cells and hepatocytes damage. **Don and John (1998)** showed that sinusoidal endothelial cells (SECs) are an important source of endothelial cell NO synthase (ecNOS) in the liver. These data have important implications for the pathogenesis of intrahepatic portal hypertension as well as other disorders in which endothelia may be damaged. Therefore, on can suggest that SECs damage may contribute to the elevation in NO levels in sera of marijuana smokers and such damage, in turn, reflects liver involvement.

Hepatic ischemia/reperfusion injury (I/R) is a significant clinical problem involved in the liver failure associated with circulatory shock, hepatic surgery, and liver transplantation. Such injury is characterized by Kupffer cell activation and PMN cell infiltration and activation as well as inflammatory cytokine responses. The first step in the pathophysiology of this injury is the priming and recruitment of neutrophils into the liver vasculature upon reperfusion by inflammatory mediators **(Pacher et al., 2007)**. This may reflect, the reduction in granulocytic count, in this study. After recruitment of neutrophils into the liver vasculature, the second step comprises endothelial cell activation, which promotes the attachment and activation of inflammatory cells resulting in endothelial damage and liver dysfunction. Next, adherent inflammatory cells transmigrate through the damaged endothelium, attach to hepatocytes, and become fully activated to release oxidants, proinflammatory cytokines, free radicals and large amounts of NO and proteolytic enzymes,

which in turn trigger intracellular oxidative stress and mitochondrial dysfunction in hepatocytes, eventually culminating in cell death **(Pacher et al., 2007)**. The reduction of GSH, the elevation of MDA and the reduction of the TAC capacity confirm the involvement of the previous mechanisms in hepatic damage as a result of marijuana smoking. Hepatic ischemia/reperfusion (I/R) also leads to significant reduction of endothelial NO synthase activity in sinusoidal endothelial cells**.** This results in an imbalance between sinusoidal vasoconstrictors and vasodilators (NO) in the liver, creating a situation favoring sinusoidal vasoconstriction during reperfusion. In the present study, the total MDA levels in sera of marijuana smokers were increased but the total GSH and TAC were simultaneously decreased indicating the presence of a state of oxidative stress. The reduction in the antioxidants mechanisms in the blood of marijuana smokers may favors the reaction of NO with the excessive free radicals, including superoxide anion **(Pacher et al., 2007)** forming peroxynitrite causing decreased NO bioavailability **(Pacher et al., 2007).** The latter events lead to further activation of endothelial cells, neutrophils, and hepatocytes, resulting in amplified reactive oxygen and nitrogen species which aggravate the liver injury **(Pacher and Gao, 2008).** The mechanism of liver damage by the marijuana smoke, in this study may also involve crossing biological membranes by the excessively formed peroxynitrite which diffuse one to two cell diameters as was described by **Denicola et al. (1998).** The latter diffusion allows significant interactions with lipids, DNA, and proteins causing more organs damage which may include, liver, gonads and pituitary glands **(Hogg and Kalyanaraman 1999 and Pryor and Squadrito, 1995)**. **Sarafian et al. (1999)** showed that marijuana (MJ) smoking produces inflammation, edema, and cell injury in the tracheobronchial mucosa of smokers and may be a risk factor for lung cancer via oxidative stress mediated mechanism. The latter authors added that the brief exposure to smoke from 3.95% MJ cigarettes stimulated the formation of reactive oxygen species (ROS) by 80% over control levels and lowered intracellular glutathione levels by 81%. Also, the smoke-induced ROS generation in a dose- and time-dependent manner. They also added that, MJ smoke passed through a Cambridge filter that removed particulate matter was 3.4-fold more active in ROS production compared with unfiltered smoke, suggesting that most of the oxidative effects are produced by the gaseous phase and concluded that Delta9-THC that present in the smoke is a potent source of cellular oxidative stress that could also contribute significantly to cell injury and dysfunction in the lungs of smokers.

Also, it was found that a control group smoking a single marijuana cigarette every other day for a year had a white-blood-cell count that was 39 percent lower than normal, thus damaging the immune system and making the user more susceptible to infection and sickness **(National Institute of Drug Abuse, 1997 and Klein et al., 2003).** In the present study, reduction of the count of total white blood cells and disturbances in the granulocytes/lymphocytes ratio as well as the percentage of the mixed leucocytes in both marijuana smokers and the non-smokers control confirm the disturbances in the immune system function and the immune response the smoke inhalation.

In the present study, a significant decrease in the mean level of testosterone (48.7 %) was found in sera of marijuana smokers compared with the non-smoking controls. Also, a concomitant and highly significant decrease in LH (but not in FSH mean level) was also found in the blood sera of the smoking individuals compared with that of control. This may be due to damage of tissues due to oxidative stress including gonads and pituitary glands tissues (**Denicola et al. 1998 and Pacher et al., 2007**). The significant reduction in testosterone and LH but not in FSH may be due to the fact that LH stimulates the tests to produce testosterone stimulate testicular spermatogenesis **(Champ et al, 2005). Also,** negative correlations were found between testosterone and ALP, GGT and NO with a stronger negative correlation between the latter and testosterone. On the other hand, positive correlations were found between NO and both GGT and ALP with the strongest one between the latter and NO. Also, a positive correlation was found between GGT and ALP. The latter negative and positive correlations may lead one to conclude that marijuana smoke may participate in both testicular and liver damage via NO and its related radicals-dependent mechanisms.

Corresponding author:
Dr/ El-Shahat A. Toson
Chemistry department
Mansoura University
Damietta Branch
Damietta, Egypt
Mobile: 002 0100 3899211

REFERANCES

Adams IB and Martin BR (1996):"Cannabis: Pharmacology and Toxicology in Animals and Humans". Addiction, 91: 1585-1614.

Bátkai S, Járai Z, Wagner JA, Goparaju SK, Varga K, Liu J, Wang L, Mirshahi F, Khanolkar AD, Makriyannis A, Urbaschek R, Garcia N Jr, Sanyal AJ and Kunos G. (2001): Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. Nat Med, 7(7):827-32.

Benarroch and Eduardo (2010): Acetylcholine in the cerebral cortex: Effects and clinical implications. Neurology,75:659-665.

Berkels, R., S. Purol-Schnabel, and R. Roesen, (2004) :*Measurement of nitric oxide by reconversion of nitrate/nitrite to NO.* Methods Mol Biol,. **279**: p. 1-8

Beutler E, Duron O and Kelly B (1963): Improved method for determination of blood glutathione. J. Lab. Clin. Med., 61:882-890.

Champe PC, Harvey RA and Ferrier DE (2005): Lippincott Illustrated Reviews, (3rd edn). Lippincott Williams & Wilkins. 351 West Camden Street, Baltimore, MD 21201. P 238

Debruine PR, Bruineel EA, Li X, Zimber A, Gespach C and Mareel MM (2001): The role of bile acids in carcinogenesis. Mutat Res, 480-81: 359-369.

Denicola A, Souza JM and Radi R (1998): Diffusion of peroxynitrite across erythrocyte membranes. Proc Natl Acad Sci (USA), 95:3566 - 3571.

Dobson J (1999): Marijuana Can Cause Great Harm. Washington Times, February 23.

Don C. Rocky and John J. Chung (1998): Reduced nitric oxide production by endothelial cells in cirrhotic rat liver: Endothelial dysfunction in portal hypertension. Gastroenterology, 114:344-351.

Doumas BT, Watson WA and Biggs HG (1971): Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta.,31:87-93.

Hasselmo ME and Sarter M (2011): Modes and models of forebrain cholinergic neuromodulation of cognition. Neuropsychopharmacology. 36 (1) : 52-73

Hernández AF, Amparo Gómez M, Pérez V, García-Lario JV, Pena G, Gil F, López O, Rodrigo L, Pino G and Pla A (2006): Influence of exposure to pesticides on serum components and enzyme activities of cytotoxicity among intensive agriculture farmers. Environ Res, 102(1):70-6.

Hézode C, Roudot-Thoraval F, Nguyen S, Grenard P, Julien B, Zafrani ES, Pawlotsky JM, Dhumeaux D, Lotersztajn S and Mallat A (2005): Daily cannabis smoking as a risk factor for progression of fibrosis in chronic hepatitis C. Hepatology, 42(1):63-71.

Hogg N and Kalyanaraman B (1999): Nitric oxide and lipid peroxidation (1999): Biochim Biophys Acta,1411:378–384.

Hon WM, Lee KH and Khoo HE (2002): Nitric oxide in liver diseases: friend, foe, or just passerby? Ann N Y Acad Sci, 962:275-295.

Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, Dong B, Huang X, and Moore DD (2006): Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science, 312: 233-236.

Keitel V, Kubitz R and Häussinge D (2008): Endocrine and paracrine role of bile acids. World J Gastroenterol, 14(37): 5620-5629.

Keitel V, Reinehr R, Gatsios P, Rupprecht C, Gorg B, Selbach O, Häussinger D and Kubitz R (2007): The G-protein coupled bile salt receptor TGR5 is expressed in liver sinusoidal endothelial cells. Hepatology, 45: 695-704

Klein TW, Newton C, Larsen K, Lu L, Perkins I, Nong L and Friedman H. (2003): The cannabinoid system and immune modulation. J Leukoc Biol,74(4):486-96.

Knedel M, Bottger R. (1967) [A kinetic method for determination of the activity of pseudocholinesterase (acylcholine acyl-hydrolase 3.1.1.8.)]. *Klin Wochenschr.*;**45**:325-7.

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. (2001): Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol.*;**54**:356-61.

Lefer AM and Lefer DJ (1996): The role of nitric oxide and cell adhesion molecules on the microcirculation in ischemia-reperfusion. Cardiovascular Res, 32(4):743-51.

Mallat A and Lotersztajn S (2006): Endocannabinoids as novel mediators of liver diseases. J Endocrinol Invest., 29 (3 Suppl):58-65.

Mallat A, Teixeira-Clerc F, Deveaux V and Lotersztajn S (2007): Cannabinoid receptors as new targets of antifibrosing strategies during chronic liver diseases. Exp Opin Ther Targets, 11:403- 409.

Marashall J.C. (1975): Clinics in endocrinology and metabolism. Investigative procedures. Clin Endocrinol Metab. 4: 545

Mashinge F, Tanaka N, Maki A, Kamei S, Yamanaka M. (1981). Diresct spectrophotometry of total bile acids in serum. Clincal Chemistry. 27: 1352-1365

Mukhta AH, Elbagir NM and Gubara AA (2011): The effect of Cannabis sativa on certain enzymes of clinical significance in rats and men. Journal of Pharmacognosy, 2 (1):10-13.

National Institute of Drug Abuse (1997): Smoking any substance raises risk of lung infections. NIDA Notes, **12 (1).**

Noto H, Matsushita M, Koike M, Takahashi M, Matsue H, Kimura J and Todo S (1998): Effect of high concentrations of bile acids on cultured hepatocytes. Artif Organs 22:300-307.

Otsuji S, Shibata H, Umeda M (1982): Turbidimetric immunoassay of serum C-reactive protein. Clin Chem, 28: 2121-4

Pacher P and Gao B (2008): Endocannabinoids and Liver Disease. III. Endocannabinoid effects on immune cells: implications for inflammatory liver diseases. Am J Physiol Gastrointest Liver Physiol, 294(4): G850–G854.

Pacher P, Beckman JS and Liaudet L (2007): Nitric oxide and peroxynitrite in health and disease. Physiol Rev, 87:315–424.

Pryor WA and Squadrito GL(1995): The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol Lung Cell Mol Physiol., 268:L699-L722.

Raimondi F, Santoro P, Barone MV, Pappacoda S, Barretta ML, Nanayakkara M, Apicella C, Capasso L, and Paludetto R (2008): Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. Am J Physiol Gastrointest Liver Physiol, 294: G906-G913.

Reitman S and Frankel S (1975): Determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28: 56 – 61.

Rust C, Wild N , Bernt C, Vennegeerts T, Wimmer F and Beuers U (2009): Bile Acid-induced apoptosis in hepatocytes is caspase-6-dependent.The Journal of Biological Chemistry, 284:2908-2916.

Sarafian, T. A, Magallanes, J.A.,Shau,H,Tashkin, D.Roth, M. D (1999): Oxidative stress produced by marijuana smoke. An adverse effect enhanced by cannabinoids, 20 (6) 1286-93

Serracino-Inglott F, Virlos IT, Habib NA, Williamson RCand Mathie RT (2003): Differential nitric oxide synthase expression during hepatic ischemia-reperfusion. Am J Surg, 185(6):589-95.

Siegmund SV and Schwabe RF (2008): Endocannabinoids and liver disease. II. Endocannabinoids in the pathogenesis and treatment of liver fibrosis. Am J Physiol Gastrointest Liver Physiol, 294(2):G357-62. smoking marijuana as compared with tobacco. N Engl J Med, 318:347–351.

Stocks J and Donnandy T (1971): The autoxidation of human red cell lipids induced by hydrogen peroxide. Br. J. Haematol., 20:95-111.

Szasz G., Rosenthal P. and Fritzsche W (1969): Gamma glutamyl transpeptidase activity in the serum in hepatobiliary diseases. Dtsch Med Wochenschr. 94 (38): 1911-7.

Tam J, Liu J, Mukhopadhyay B, Cinar R, Godlewski G and Kunos G. (2011): Endocannabinoids in liver disease. Hepatology, 53(1):346-55.

Teixeira-Clerc F, Julien B, Grenard P, Tran Van Nhieu J, Deveaux V, Li L, Serriere-Lanneau V, Ledent C, Mallat A and Lotersztajn S (2006): CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. Nat Med, 12(6):671-6.

Uotila M., Ruoslahti E. and Engvall E. (1981). Two-site sandwich enzyeme immunoassay with monoclonal antibodies to human alpha-fetoprotein, J immunol Methods. 42: 11-15

Vallance P and Moncada S (1991): Hyperdynamic circulation in cirrhosis: a role for nitric oxide? The LANCET, 337, (Issue 874**e**4): 776 -778.

Wu TC, Tashkin DP, Rose JE, Djahed B. (1988). Influence of marijuana potency and amount of cigarette consumed on marijuana smoking pattern. *J Psychoactive Drugs*.;**20**:43-6.

11/11/2011