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Abstract

Background and Objective: Addiction with Cannabis represents one of the major spreading phenomena worldwide. The objective of this study is to evaluate the oxidative stress enzyme markers and lipid peroxidation marker in cannabis administered female rats via intraperitoneal injection. MATERIALS AND METHODS: Thirty female rats were randomly allocated into three groups. First group regarded as control, while the animals of the second and third groups were daily injected intraperitoneally with (5 and 10 mg Cannabis/kg b.w.) for seven successive days. Enzymatic activities of alkaline phosphatase (ALP), Xanthine Oxidase (XO), Glucose-6- phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) in serum were analyzed, and concentration of Malondialdehyde (MDA) was assayed. RESULTS: The activity of ALP, G6PDH and LDH significantly affected, whereas XO not affected. The lipid peroxidation marker; MDA significantly elevated in high dose treatment. CONCLUSION: This finding suggests that intraperitoneal injection of cannabis causes alterations in oxidation process

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ABSTRACT

Background and Objective: *Addiction with Cannabis represents one of the major spreading phenomena worldwide. The objective of this study is to evaluate the oxidative stress enzyme markers and lipid peroxidation marker in cannabis administered female rats via intraperitoneal injection.* ***MATERIALS AND METHODS:*** *Thirty female rats were randomly allocated into three groups. First group regarded as control, while the animals of the second and third groups were daily injected intraperitoneally with (5 and 10 mg Cannabis/kg b.w.) for seven successive days. Enzymatic activities of alkaline phosphatase (ALP), Xanthine Oxidase (XO), Glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) in serum were analyzed, and concentration of Malondialdehyde (MDA) was assayed.* ***RESULTS:*** *The activity of ALP, G6PDH and LDH significantly affected, whereas XO not affected. The lipid peroxidation marker; MDA significantly elevated in high dose treatment.* ***CONCLUSION:*** *This finding suggests that intraperitoneal injection of cannabis causes alterations in oxidation process.*

keyword: ***Cannabis, Oxidative stress, Female rats***

INTRODUCTION

Cannabis refers to the dried flowertops of the female plant of Cannabis. The preparations of this herbal product *Cannabis sativa L.* is also commonly known as marijuana, hashish, and dagga. The main way to administer cannabis is by smoking, which is also the way most medicinal users consume it(Mechoulam and Hanus, 2000). Although this term is used colloquially to describe a single entity, over 60 different compounds have been identified and collectively referred to as cannabinoids (Kumar et al., 2001).

According to the 2012 National Survey on Drug Use and Health, an estimated 2.7 million people 12 years of age and older met the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) criteria for dependence on marijuana, and 5.1 million people met the criteria for

dependence on any illicit drug (Abuse, 2014). Cannabis affects many organ systems like nervous, cardiovascular, immune and endocrine systems (Volkow et al., 2014). Pharmacological and physiological studies observed that the major effect of cannabinoids is like neuromodulators via activation of presynaptic cannabinoid-1 (CB1) receptors in the brain (Nagarkatti et al., 2009).

The vast majority of research on biologic effects of cannabinoids has addressed the neurologic and psychotropic activity of these compounds (Adams and Martin, 1996). However, (Sarafian et al., 1999) concluded that Marijuana smoke containing Δ^9-THC (delta-9-tetrahydrocannabinol or Δ9-tetrahydrocannabinol) is a potent source of cellular oxidative stress that could contribute significantly to cell injury and dysfunction in the lungs of smokers. While (Mandal and Das, 2010) showed that intraperitoneal injection of cannabis extract at low doses (total doses ranging from 40 mg to 60 mg per mouse) induced adverse effect on testes and oxidative stress.

This study is designed for better understanding of the underlying mechanisms of cannabis-induced toxicity through estimation the level of oxidative biomarker enzymes in female addicted rats.

MATERIALS AND METHODS

Cannabis preparation

The directorate of narcotics control in Erbil province-Iraq obtained cannabis extract. For preparing stock solution, 500mg of cannabis extract was dissolved in 1ml Tween 80 and then diluted to 50 ml of ethanol (20%), obtaining the final concentration (10mg/ml). 1kg of animals was i.p injected of the solution (10mg cannabis /1kg rat b.w.). Second dilution was prepared by suspending the stock solution in diluted ethanol solution.

Animals and housing

Eighteen adult female albino rats (*Rattus norvegicus*) were used in this study. The used rats were weighing about 235 - 280 gm and 7-9 weeks of age when the experiment started. The rats were housed in standard plastic cages bedded with wooden chips. They were housed under standard laboratory conditions, light/dark 12:12 photoperiod at 23 ± 2 ºC. The rats were given standard rat pellets and tap water *ad libitum*. The employed experimental animals were met the criteria of ethic rules.

Experimental Design

This experiment was planed to study the effects of two doses of cannabis on some hemodynamic and liver function measurements. The experimental rats were divided to three groups, each with six individuals and the treatments were continued for 7 days as the following:

The rats were given standard rat chow and tap water ad libitum. **Group 1:** The animals were injected with normal saline. **Group 2:** the animals were injected with cannabis (5 mg/kg, intraperitonial). **Group 3:** the animals were injected with cannabis 10 mg/kg, intraperitonial) .

Collection of blood samples

When the rats were anesthetized with ketamine hydrochloride (50 mg/kg),blood samples were obtained by cardiac puncture into plastic tubes and centrifuged at 3000 rpm for 20 minutes; then the sera were stored at -80C^0 until assay .

Serum enzymatic analysis

Blood samples were taken into glass bottles with rubber caps, labelled and centrifuged at 4000 g for 10 min. Serum enzyme (ALP), (XO), (G6PDH) and (LDH) were assayed using colorimetric specific kits for each enzyme.

Determination of serum malondialdehyde:

The assessment of the lipid peroxidation process is done by determination of the end product,Malondialdehyde (Muslih, 2002) .The level of serum MDA was determined spectrophotometer in brief , 150 µl of serum sample was mixed with 1ml trichloroacetic acid (TCA) 17.5% and 1ml of 0.66% thiobarbituric acid(TBA), then vortexed, incubated in boiling water for 15 minutes, and allowed to cool. After that one ml of 70% TCA was added. The mixture was allowed to sit at room temperature for 20 minutes. Then the sample centrifuged at 2000 rpm for 15 minutes, and the supernatant absorbency was read at 532nm wavelength.

Statistical analysis

All data are expressed as means ± standard error (SE) and statistical analysis was performed by statistical soft ware (SPSS version 15). Data analysis was made using one-way analysis of variance (ANOVA). The comparisons between groups were done by Duncan post hoc test analysis. The bar charts were made by Graph Pad Prism (Version5). P value <0.05 was taken as statistical significant.

RESULTS AND DISCUSSION

Effect of Cannabis on the serum enzymes activity

The current study results demonstrated that both concentrations of cannabis didn't significantly changed the XO levels with mean values (1.4±0.187) and (1.5±0.158) of cannabis 5mg/kg and 10mg/kg respectively, as compared to the control treatment (1.2±0.2) as shown in figure (1). Statistical analysis of XO results demonstrated that cannabis addiction significantly increased the activity of serum xanthine oxidase ($P<0.01$). The induction of ALP activity by cannabis was concentration dependent. The levels of ALP were (13.52± 0.65) and (16.80± 1.15) in rats treated with 5mg/kg and 10mg/kg cannabis respectively as illustrated n figure (2).

The results also showed the action of cannabis addiction on the activity of serum G6PDH and LDH. The activity of G6PDH was significantly decreased in both concentrations comparing to control rats. The mean values were (0.47±0.075) and (0.35±0.028) in rats treated with 5mg/kg and 10mg/kg cannabis respectively as compared to the control value (0.82± 0.14) as shown in figure (3). While significant induction of LDH activity were reported only in first low dose of cannabis treatments with means (644.2±133.16) as compared to control value which was (436.8±88.83) as clarified in figure (4).

The present results also showed that cannabis could affect enzymes related to free radical level and liver functions. Experimental data on the effects of cannabis in liver diseases are limited. However, studies observed that cannabis smoke may causes liver damage through free radical dependent process (Agarwal et al., 2012). Avraham et al., 2008, showed that Cannabinoids and Capsaicin improve liver function (ALT) after thioacetamide-induced acute injury in mice, while (Mukhta et al, 2011) suggests in her study that canabinoids increase the (ALP) activity in both injected rats and human smokers and this will increase with the increase of dose and time but the (ALT) and the (AST) increase at the beginning of consumption then will decrease with time. Recently (Quraishi et al., 2013) observed that serum levels of ALP and other liver function variables were raised in cannabis users. The two major cannabinoids from cannabis sativa are Δ9-THC and CBD. Δ9-THC is a CB1R and CB2R partial agonist, and produces a broad range of effects not mediated by either CB1R or CB2R, although in some systems it may behave as a CB2R inverse agonist (Pertwee, 2008). This is the most likely explanation of the discrepant effects of cannabis on glucose metabolism in short-term studies and after chronic exposure. Owolabi et al., (2017) showed in his study that the level of the enzymes cytochrome-6-oxidase and G6PDH in the test groups were significantly higher relative to the control group; this increase could be attributed to the stimulatory effects of cannabis which tend to increase metabolic activities generally in the body, this increase in activity would induce an increase in the utility of ATP and hydrolysis of glucose, thus the increase in cytochrome-6-oxidase and G6PDH observed.

Furthermore, it has been concluded that CB1 receptor activation is responsible for liver fibrosis progression (Mallat and Lotersztajn, 2010) and the results mostly due to oxidative stress (Parfieniuk and Flisiak).

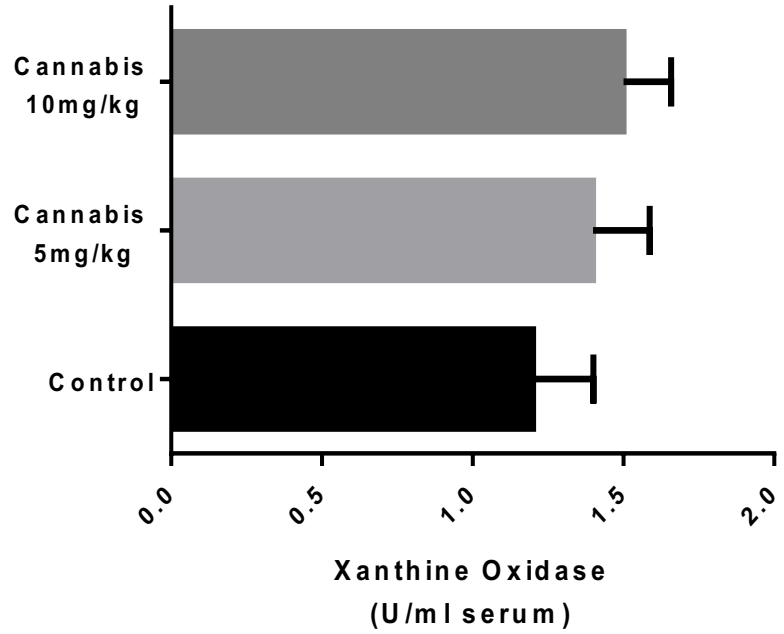

Figure (1): Serum XO activity in control and two doses of cannabis. No significant changes between the groups were observes. Two doses of Cannabis (5 and 10 mg\kg) were injected intraperitoneally for seven successive days. The data were presented as Mean and S.E.

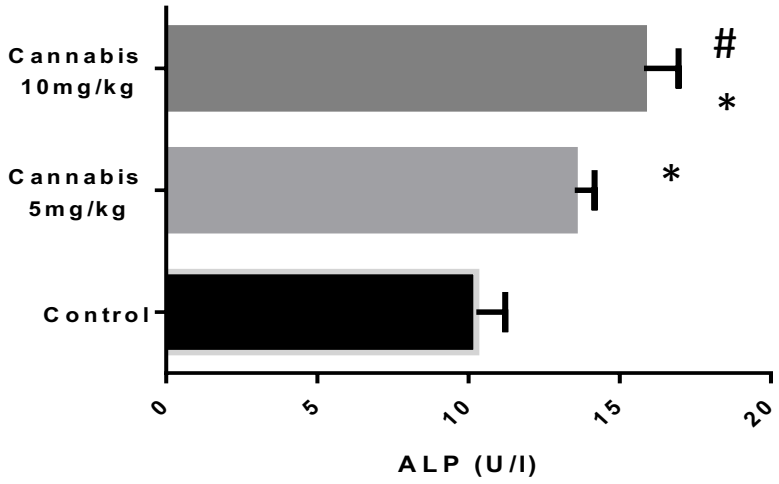

Figure (2): Elevation of serum ALP activity in a concentration dependent manner by using two doses of cannabis. (*) indicates the significant differences at level (P<0.05) between cannabis treated rats and control group. (#) indicates the significant differences at level (P<0.05) between the two doses. Two doses of cannabis (5 and 10 mg\kg) were injected intraperitoneally for seven successive days. The data were presented as Mean and S.E.

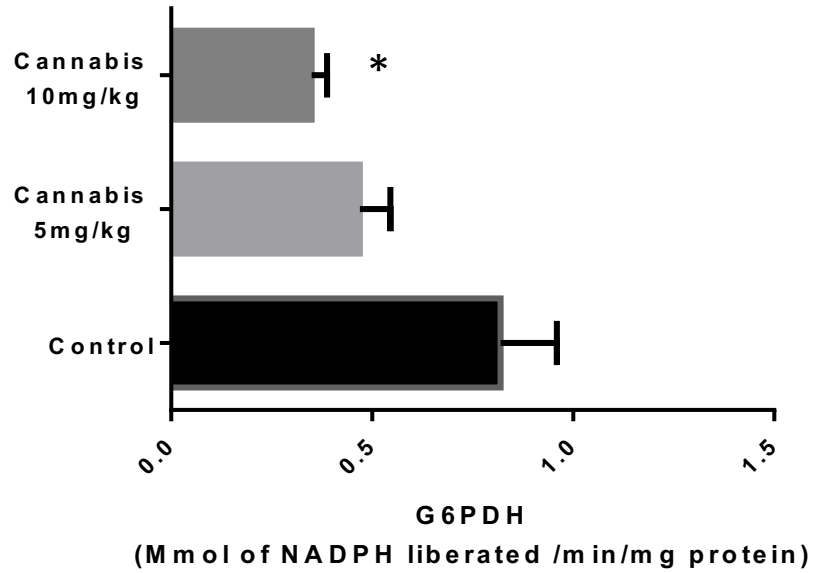

Figure (3): Elevation of serum G6PDH activity significantly in second concentration of cannabis. (*) indicates the significant differences at level (P<0.05) between cannabis treated rats and control group. Two doses of cannabis (5 and 10 mg\kg) were injected intraperitoneally for seven successive days. The data were presented as Mean and S.E.

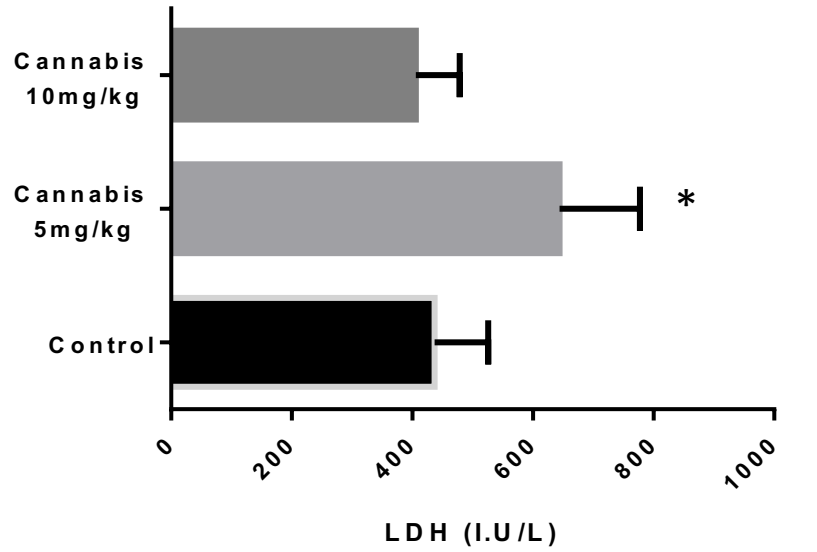

Figure (4): Elevation of serum LDH activity significantly in first low concentration of cannabis. (*) indicates the significant differences at level (P<0.05) between cannabis treated rats and control group. Two doses of cannabis (5 and 10 mg\kg) were injected intraperitoneally for seven successive days. The data were presented as Mean and S.E.

Effect of cannabis on the serum MDA activity

The marker of lipid peroxidation was significantly elevated in sera of rats treated with the high dose of cannabis (10mg/kg) with mean (4.23±0.62) as compared with control rats (1.92±0.30), while 5mg/kg cannabis didn't show significant effect (Figure 5). One possible mechanism for this elevation is that cannabis activates free radicals as measured from the current results as MDA levels in the serum. Also, (Athanasiou et al., 2007) confirmed that THC causes a marked increase in H_2O_2 production in the mitochondria.

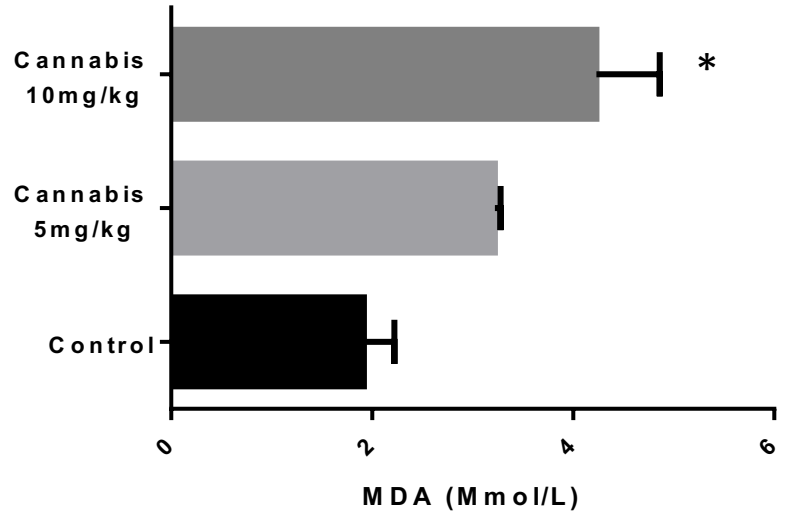

Figure (5): Elevation of serum MDA activity significantly in second concentration of cannabis. (*) indicates the significant differences at level (P<0.05) between cannabis treated rats and control group. Two doses of cannabis (5 and 10 mg\kg) were injected intraperitoneally for seven successive days. The data were presented as Mean and S.E.

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