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# Hemodynamic ,thyroid and immunomodulater effects of cannabinoid in rats

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**Abstract**

The aims of the present study are to investigate the cannabinoid effects on serum monocyte chemoattractant protein-1 (MCP-1), Troponin-1 ,liver and renal test parameters. The experimental rats were divided into three groups, the first group served as a control, and the animals were injected with normal saline. In the second group, animals were injected with cannabis (5 mg/kg, intraperitonial), while in the third group animals were injected with cannabis (10 mg/kg). After the course of the experiment, systolic blood pressure (SBP) tended to reduce significantly in the second dose of cannabis administered rats. Serum nitric oxide (NO) level slightly increased in dose dependent manner but it did not reach the level of significance. Serum level of malondialdehyde (MDA), troponin-T, MCP-1 and serum total bilirubin were significantly increased in the second dose of cannabis administration as compared to control rats. Neither triiodothyronin ($T_3$) nor tetraiodothyronin ($T_4$) hormones had statistically changed in cannabis group comparing with the control rats. Furthermore, serum uric acid reduced in cannabis group as compared with control. **In conclusions**, for the first time, our findings suggested that cannabis could affect immune system through MCP-1 elevation. As well as, cannabis may affect cardiac and liver functions via increasing troponin-T and bilirubin levels.

## Introduction

Cannabis has been used in medicinal aspects in the recent years. It is extracted from Cannabis sativa. Cannabis is also known as hemp. On the other hand, Marijuana is the dried cannabis flowers and leaves, while hashish is the blocks of the plant resin that can be eaten [13]. Cannabis affects many organ systems like nervous, cardiovascular, immune and endocrine systems [5]. 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 [18].

It has been found that the CB1 receptor and its endogenous ligand play a major role in arterial tone regulation by the influx of calcium modulation through L-type calcium channels [10]. Also, [24] concluded that vascular and myocardial CB1 receptors participates in blood pressure and heart rate modulations .

There is now evidence that tetrahydrocannabinol (THC) may has an antihypertensive effects through vasodilatation [14]. Although previous study recorded that in anesthetized animals, administered cannabinoids can reduce blood pressure and heart rate. However, recently [19] reported that THC significantly can increase heart rate. On the other hand, cannabidiol's activity is firmly related with a reduction in the prostaglandins , lipid peroxide and NO [5]. In addition, [18] observed that cannabinoids can down regulate chemokine production as a mechanism to reduce inflammatory reactions. It is also interesting to note that chronic THC reduces CB-1 and CB-2 receptor levels and attenuates the expression of MCP-1 (24). MCP-1 is a potent chemo attractant which can promote monocyte recruitment into an inflammatory site and monocytes and macrophages activation [14]. There is a few information about the effects of THC on thyroid hormones. Recently,[4] showed that the levels of T3 and T4 hormones did not correlate with THC levels in serum. Because of little known about hemodynamic, immunologic and liver effects of cannabis and few studies have yet been conducted to test the effects of cannabis on blood pressure, electrolytes, liver and renal function tests in rats. Therefore, the aims of the present study are to investigate the cannabis effects on serum MCP-1, Troponin-1 and other liver and renal test parameters.

## 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 of the supervising committee of College of Science.

### Experimental Design

This experiment was planed to study the effects of two doses of cannabis on some hemodynamic, thyroid and renal 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:

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

### Blood pressure measurement

Systolic BP and heart rate were measured by the tail-cuff method in all groups by power Lab (AD Instruments, 2/25). The animals were trained to become accustomed to the BP measurements. Animals were placed in a restraining chamber and they were warmed to an ambient temperature of approximately 37C$^0$. Five readings were taken for each animal, the highest and lowest readings were discarded. The average readings were taken to obtain a value for a given rat .

### Biochemical determination

### Serum nitric oxide measurement

Serum NO was estimated using NO non –enzymatic assay kit (US Biological, USA).

### Determination of serum $T_3$, $T_4$ and Troponin T

Serum $T_3$, $T_4$ and Troponin T were determined by electrochemiluminescnce immunoassay "ECLIA" using Elecsy and Cobas immunoassay analyzers.

### Determination of serum sodium, potassium and chloride ion concentrations

Serum $Na^+$, $K^+$ and $Cl^-$ ion concentrations were determined by using automated electrolyte analyzer (ELITE, USA)

### Serum total calcium ion determination

$Ca^{2+}$ Kit enables colorimetric determination of total calcium without deproteinization. In serum, the calcium kit reacts with methylthymol blue indicator (MTB) in an alkaline medium. The intensity of the complex was measured at 612 nm. The kit was obtained from BIOLABO.SA, France.

### Determination of renal function test parameters

Serum creatinine, urea and uric acid levels were determined by colorimetric method kit (BIOLABO . SA, France).

### Determination of serum total protein and albumin

Serum total protein and albumin were also determined by colorimetric test kit (Biolab, France)

### Determination of serum malondialdehyde (MDA)

The MDA was determined spectrophotometrically with thiobarbituric acid (TBA) solution. 150µl serum was added to 1ml trichloroacetic acid 17.5 %, 1ml of 0.66 % thiobarbituric acid (TBA), then they mixed by vortex, incubate it in boiling water for fifteen minutes, then allowed it to cool in room temperature. After that 1ml of 70 % TCA was added and the mixture was let to stand at room temperature for twenty minutes, centrifuged at 2000 rpm for 15 minutes, & take out the supernatant for scanning spectrophotometrically

### Determination of serum total bilirubin

Serum total bilirubin was determined by sulfanilic acid method ((BIOLABO . SA, France)

### Determination of serum inorganic phosphate

Serum inorganic phosphate was determined by ultra violet method . The absorbance measured at 340 nm is proportional to phosphate ions in the specimen ((BIOLABO . SA, France)

### 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

After the course of the experiment, SBP tended to reduce significantly ($P<0.05$) from 115.5 ± 1.765 mmHg in control rats to 98.66 ± 3.809 in the second dose of cannabis administered rats( Figure 1). On the other hand, results from table 1 shows that serum NO level slightly increased in dose dependent manner but it did not reach the level of significance. MDA was measured as a marker for lipid peroxidation , thus according to the present result serum level of MDA was significantly increased in the second dose of cannabis administration (4.230 ± 0.620 µmol/L) as compared to control rats(1.920 ± 0.310 µmol/L)( Figure 2).

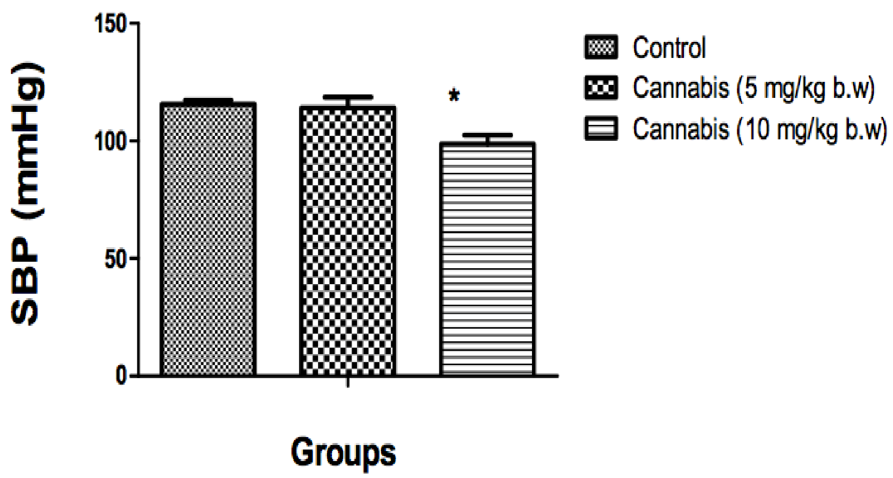


**Figure 1: Effects of cannabis on SBP in rats**

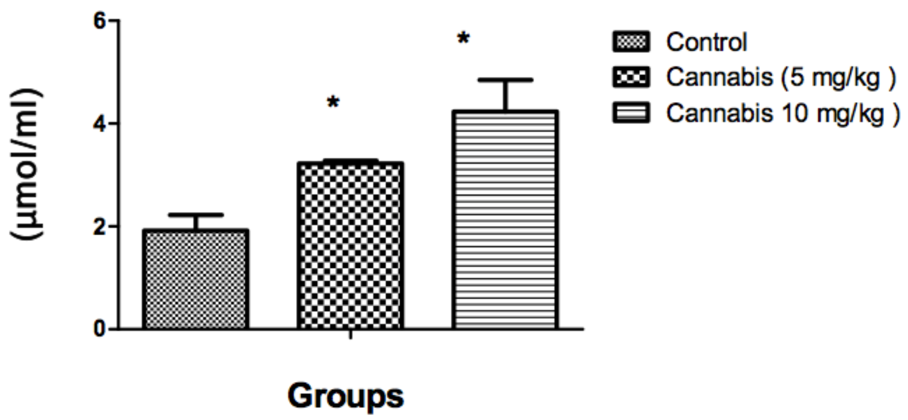


**Figure 2: Effects of cannabis on serum MDA in rats**

Table 1, shows that there was a slight effect of cannabis (second dose) on serum $Na^+$ ,but a marked effect on serum $K^+$ levels compared with the control group. The decreased serum $K^+$ was observed in the second dose ( 4.260 ± 0.186 meq/L) compared with control group ( 5.06 ± 0.140 meq/L). Also, serum $Cl^-$, and $Ca^{+2}$ were non significantly (P>0.05) decreased ,whereas, serum $PO^{-4}$ was slightly increased in the second dose of cannabis administration.

**Table 1 Effects of cannabis on serum electrolytes ,calcium , phosphate and nitric oxide levels in albino rats**

| Groups<br>Parameters | Control | Cannabis<br>(5 mg/Kg b.w) | Cannabis<br>(10 mg/Kg b.w) ) |
|---|---|---|---|
| Serum $Na^{+\ *}$ (meq/L) | 163.80±1.593$^a$ | 158.40±12.905$^a$ | 142.00±5.1575$^a$ |
| Serum $K^{+\ *}$ (meq/L) | 5.060±0.1400$^a$ | 4.9800±0.3382$^{ab}$ | 4.2600±0.1860$^b$ |
| Serum $Cl^-$ ( meq/L) | 124.00±1.378$^a$ | 129.60±10.819$^a$ | 109.00±4.24264$^a$ |
| Serum $Ca^{+2}$ (mg/dL) | 10.952±0.363$^a$ | 10.523±0.4221$^a$ | 10.142±0.5513$^a$ |
| Serum $PO_4^-$ (mg/dL) | 9.0930±1.439$^a$ | 9.5543±1.1089$^a$ | 10.600±1.1806$^a$ |
| Serum NO (nmol/L) | 10.96±1.2608$^a$ | 11.156±0.6862$^a$ | 11.8922±0.8015$^a$ |

**Data are expressed mean ± standard error (mean ± SEM). $^*$P<0.05 .One way ANOVA and post-hoc Duncan were used.**

As shown in figure 3, serum Troponin-T was significantly increased in the second dose of cannabis group (1.722 ± 0.496 ng/ml) as compared to control group ( 0.637 ± 0.096 ng/ml). Neither T3 nor T4 hormones had statistically changed (P>0.05) in cannabis group from the control rats (Table ,2).

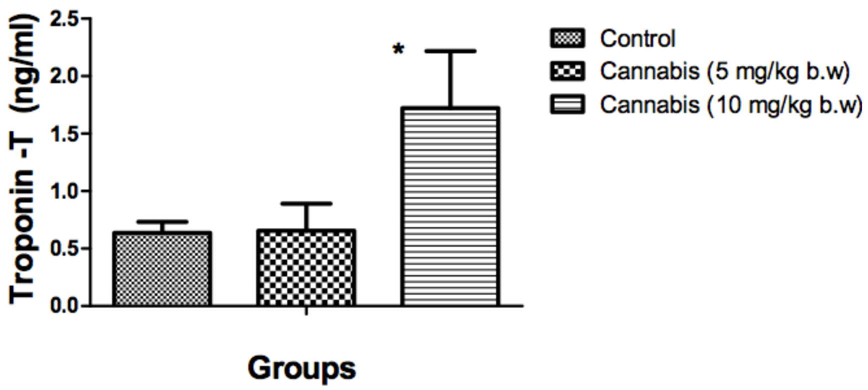


**Figure 3: Effects of cannabis on Troponin-T in rats**

The present results as illustrated in figure 4, showed that serum MCP-1 was significantly elevated in the second dose of cannabis group (14.66±0.4225 ng/ml)as compared with control group (7.919 ± 1.270 ng/ml ). Table 2, shows that both serum total protein and albumin were slightly increased but they did not reach significant level. However, cannabis administration (second dose) elevated serum total bilirubin (0.2544 ± 0.0513 mg/dL) as compared with control ( 0.1193± 0.0102 mg/dL). Serum creatinine and urea were measured for the studied groups. Both parameters did not show significant differences after cannabis administration as compared with control. However, serum uric acid tended to decrease ($P<0.05$) by first and second dose of cannabis ( 5.5614 ± 0.3574 mg/dL and 6.4035 ± 0.4225 mg/dL, respectively) compared with the control rats ( 10.00 ± 1.5598 mg/dL)(Table 2).

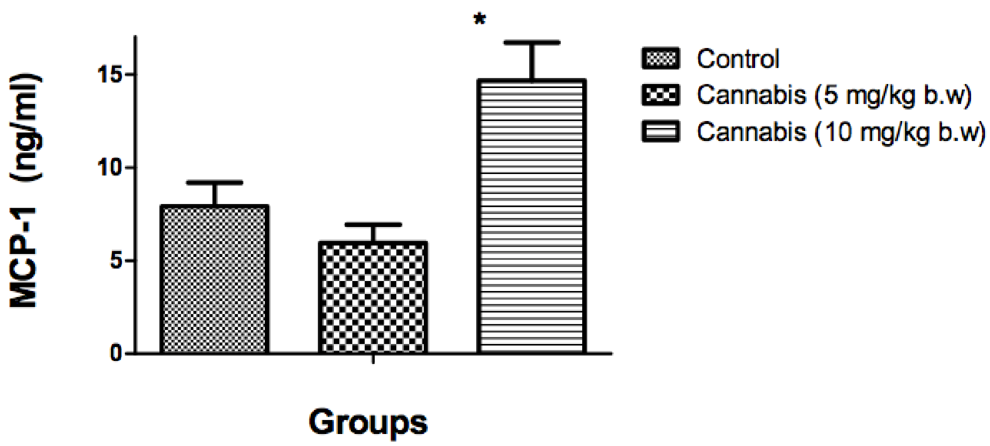


**Figure 4: Effects of cannabis on MCP-1 in rats**

**Table 2 :Effects of cannabis on some liver , renal function test parameters , T3 and T4 in albino rats**

| Groups<br>Parameters | Control | Cannabis<br>(5 mg/Kg b.w ) | Cannabis<br>(10 mg/Kg b.w ) |
|---|---|---|---|
| Serum total protein gm/dL | 8.3120±0.2589a | 8.0200±0.5292a | 9.2133±0.4216a |
| Serum albumin (gm/dL) | 3.4736±0.0994[a] | 3.3672±0.1126[a] | 3.7552±0.1024[a] |
| Serum bilirubin (mg/dL) * | 0.1193±0.0102[a] | 0.1749±0.0106[ab] | 0.2544±0.0513[b] |
| Serum creatinine (mg/dL) | 0.8803±0.0967[a] | 0.8120±0.1677[a] | 0.8962±0.0554[a] |
| Serum urea (mg/dL) | 19.13±5.0612[a] | 21.82±4.0588[a] | 19.69±2.1341[a] |
| Serum uric acid (mg/dL)* | 10.00±1.55981[a] | 4.5614±0.3574[b] | 6.4035±0.4225[b] |
| $T_3$ (nmol/L) | 1.5833±0.0384[a] | 1.3860±0.0276[a] | 1.4880±0.1266[a] |
| $T_4$(nmol/L) | 51.01±1.2560[a] | 48.85±5.0126[a] | 53.68±4.2599[a] |

**Data are expressed mean ± standard error (mean ± SEM). * P<0.05 .One way ANOVA and post-hoc Duncan were used.**

## Discussion

The present results showed that SBP was decreased significantly in cannabis administered rats. The possible hypothesis of BP reduction would be through calcium influx modulation via calcium channels [10]. [19] concluded that cannabidiol-induced aortic vasorelaxation may be due to calcium channel inhibition. As well as CB1 receptor from the myocardial cells may participate in BP regulation [15]. Another possible mechanism for antihypertensive effects of cannabis may be due to THC, an active compound in cannabis, which can reduce BP through vasodilatation [19]. The present result is consistent with [11],observed that THC lowers over the six days in male daily cannabis smokers. As presented from the current result, serum NO level was slightly increased in dose dependent manner, such elevation in NO level may be a cause of a significant decrease in BP. Prostaglandin pathway modulation by cannabis also may participate in such reduction of BP [22]. It is well known that potassium has an important role for BP modulation [12]. Research related to the effects of cannabis on troponin-T are very limited. As shown in figure 3, serum troponin-T significantly elevated by cannabis administration.

It is well known that cardiac ischemia is mainly associated with troponin-T elevation [1]. Interestingly, the level of serum MDA as obtained from the present result also increased by the cannabis administration. [8] recorded that cannabidiol caused significant elevations of serum ALT and hepatic MDA levels associated with significant decrease in hepatic reduced glutathione. The elevated free radicals may be due to THC as confirmed by [17] who suggested that THC increases lipid peroxidation and they also found that it lowered antioxidant enzyme such as catalase, superoxide dismutase and glutathione peroxidase. However, [16] resulted that cannabidiol significantly reduces the elevations of serum troponin T in of doxorubicin-treated rats, but according to our knowledge ,this is the first study shows the possibility of cannabis to produce cardiac failure through rising free radicals followed by serum troponin-T elevation.

As shown in table 2, cannabis administered rats did not show alteration in serum T3 and T4. This finding is consistent with [4] who showed that the level of both hormones did not associate with the level of THC in the serum. Also, [2] indicated that CB1 receptors can modulate T3 and T4 release in the rat's thyroid gland and they returned the results to the active substance present in cannabis named as cannabinoids. Interestingly, and for the first time, the present result shown in figure 4,recorded that cannabis administration caused a significant rise in serum MCP-1 levels as compared with the control. As mentioned in the review,MCP-1 is a potent chemoattractant capable for monocyte and macrophage activation [14]. As well as ,MCP-1 plays key roles in atherosclerosis processes [26]. Although [25] showed that chronic THC attenuated the expression of MCP-1. However, it has not been known yet how cannabis elevates its expression or release. Even though, on possible mechanism for this elevation is that cannabis activates free radicals as measured from the current results as MDA levels in the serum. Also, [3] confirmed that THC causes a marked increase in $H_2O_2$ production in the mitochondria. Consequently, apoptosis or necrosis from the tissue injury may occur and such ischemia may stimulate MCP-1 expression [9]. However, the exact mechanism for this new finding as far as we know, is not understood yet.

The present results also showed that cannabis could affect liver functions. It significantly increased serum total bilirubin (Table 2). 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 [22]. Also, recently [23] observed that serum levels of bilirubin, AST and ALT were raised in cannabis users. Furthermore, it has been concluded that CB1 receptor activation is responsible for liver fibrosis progression [24] and the results mostly due to hepatic stellate cells stimulation [25]. The current results shown in table 3 indicated that serum uric acid tended to reduce in cannabis group as compared with control. Although, very little is known about he effect of cannabis on uric acid, but very recently studies showed that cannabinoids may use as therapy for arthritis, because it has ability to reduce cartilage breakdown [26]. Therefore, we can conclude that cannabis act as anti-arthritic agent at least through reduction of uric acid levels.

In conclusions, the results suggest that the hypotensive effects of cannabis may be returned to its nitric oxide and potassium modulation. For the first time, the present results suggested that cannabis could affect immune system through increasing MCP-1 levels in the blood. As well as cannabis may affect cardiac and liver functions via increasing oxidative stress marker like MDA, troponin-T and bilirubin levels.

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