

## Assessment of the Possible Oxidative Hepatotoxicity Induced by Cannabis and Pregabalin in Rats

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### Abstract:

Recently, great of consideration has been rewarded to Cannabis and Pregabalin because of the important role of their uses, by their presence in food, oil and fiber for humans and animals, and therapeutics also for their addiction effects. The present study pointed to scrutinize oxidative stress and histopathological changes in liver of rats caused by cannabis as well as Pregabalin. In this study, Thirty Wistar male mice were clustered into three group. First group kept as negative control, second group was cannabis group (administered Cannabis by gastric tube at a dose of 3 mg/kg b.wt.) and third group was the pregabalin group, (administered pregabalin by gastric tube on a dosage of 30 mg/kg b.wt.) every day for eight weeks. Oxidative stress parameters (MDA and GSH) were done and analyzed in serum. Histopathological examination of the liver sections was done. Results revealed that, cannabis cluster showed significant increase while, pregabalin showed significant decrease in MDA activity compared with control cluster; whereas Cannabis resulted in substantial reduction in the level of GSH while, pregabalin showed substantial increase in GSH level compared with control group. Along with, there were some pathological changes in liver tissue.

**Keywords:** Cannabis, Pregabalin, oxidative stress, Histopathology, liver.

### Introduction

Cannabis is used for recreational purposes in some geographic areas, and at the same time it's distributed as a drug of abuse with strict restrictions in utmost countries.

A dilemma girding cannabis safety and implicit remedial effectiveness arises among researchers because ultimate of the systemic reviews on cannabis use reported that cannabis

has dangerous issues and showed the clinical features of acute cannabis ingestion among children and grown-ups that include anxiety, respiratory torture, dropped situations of knowledge, confusion and intoxication, psychiatric symptoms (*Prattetal., 2019 and Champagne et al., 2020*).

Pregabalin, sold under the brand name Lyrica® among others, is an

anticonvulsant, analgesic and anxiolytic medicine used to treat epilepsy, neuropathic pain, fibromyalgia, restless leg pattern, opioid retirement and generalized anxiety complaint (GAD). Pregabalin is one of the new medicine for epilepsy drugs, which is a lipophilic analogue of gamma-amino butyric acid (GABA) designed to drawn-out through the blood brain barricade. (Evoyetal., 2017).

Meanwhile, the entrance of calcium into the neurons is the core experience that stimulates vesicle conflation with the cell membrane, the effective gradient of Pregabalin at the channel of calcium of the neurons outcomes in reticence of the release of calcium dependently of the excitatory neurotransmitters convoluted in pathways of pain sensation, containing calcitonin gene-related peptides, glutamate and noradrenaline (Stahl, 2004; Martiinotti et al., 2013; and deGuglielmo et al., 2013).

Antioxidants hinder the process of the biomolecules oxidation, and help the destruction of cell. Currently, a great interest has been focused on the road to the documentation of the antioxidants with natural origin that could be needed in the utilization for mortal intake. Cannabis recently has attainment significance for its powerful antioxidant exertion. Current studies have exposed that the active matters pulled from cannabis have pharmacological

analogous as antioxidant and antimicrobial effects (Tang. H. et al., 2009).

The present study pointed to examine oxidative stress and histopathological changes in liver of rats caused by cannabis as well as Pregabalin.

#### Materials and methods

Experiment were conducted on Wistar rats administered the Pregabalin or cannabis by gastric tube for a period of 8 weeks to descry their deposit in serum and hair.

#### Drugs

Pregabalin was used in this study under the trade name Pregavalex®, each capsule contains 150 mg pregabalin base. It's produced by Eva Pharma Company, Egypt. Cannabis: was bought from Alibaba online chemical store.(500mg).

#### Experimental Animals

Thirty Wistar male rats clinically healthy, weighting from 120 to 130 gm. Rats were obtained from the house of the lab animals, Faculty of veterinary Medicine, Suez Canal University, Ismailia. There was an adaptation period for 3 weeks before the experiment start. Animal were kept on standard ration.

#### Experimental design

Animals were weighted and aimlessly allocated one of the treatment groups to have nearly the same original body weights. The experimental animals were

Commented [m1]: Please mention the concentration of the extract

classified into 3 Groups (n = 10/ group).

**Study groups**

Cannabis group :10 rats received a daily dose of ½ ml of water containing 3mg extract /kg body weight (by stomach tube) (*NAHAS Gabriel, 1972*)

Pregablin group: 10 rats administered a daily dose of ½ ml of water contains 30mg pregablin/kg by stomach tube (*Sarah Hamed N. et al.,4 2020*)

Control group: 10 rats received blank tab water without medications.

**Histopathological examination**

At the end of the experiment, 5 rats of each group (15 rats) were sacrificed for histopathological examination, livers were removed and fixed in 10% formalin and embedded in paraffin. Sections of 3µm consistence from each block were succumbed, attached to glass slide, stained by eosin and hematoxylin (H&E). (*Bancroft and Gamble, 2008*)

**Oxidative stress labels tests**

Serum of each rat were collected from blood samples kept frozen at

**Table (1):** The effect of daily oral administration of cannabis and pregablin for 8 weeks on oxidative biomarkers malondialdehyde (MDA) and glutathione (GSH) in rat’s serum.

Biochemical marker	Experimental groups (n=5)		
	Control	Cannabis	Pregabalin
MDA (nmol/ml)	35.60±2.84 <sup>b</sup>	47.20±3.61 <sup>a</sup>	26.00±2.12 <sup>c</sup>
GSH (mg/dL)	1.52±0.18 <sup>b</sup>	0.76±0.08 <sup>c</sup>	3.80±0.37 <sup>a</sup>

Values are presented as means ± SEM. In the same row different letters indicate significances at P ≤ 0.05

the temperature of -20 for the process of recognition the MDA and GSH, using commercially available diagnostic kits.

Bio Vision’s Apo GSH TM Colorimetric Assay Kit, USA)

**Statistical analysis**

Attained outcomes were statistically signified as mean ± Standard error using statistical software package SPSS for Windows (Version 21.0;SPSS Inc., Chicago, IL, USA) statistics were examined by one-way ANOVA followed by Duncan’s post hoc test for comparisons of multiple group.

**Results**

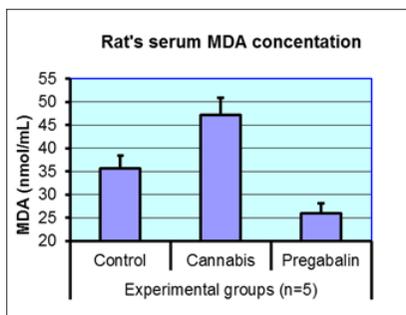
**Serum malondialdehyde (MDA) & glutathione (GSH) activities:**

As shown in table 1 & figure 1&2, cannabis group showed significant increase, while pregablin showed significant decrease in MDA activity compared with control group Serum.

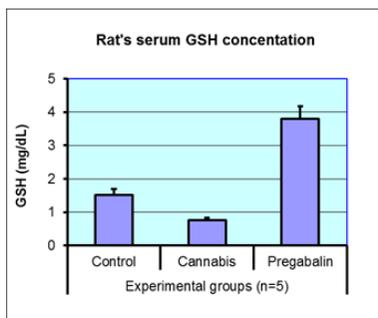
Cannabis results showed a significant decrease in GSH level, while pregablin showed a significant increase in GSH level with the comparison of control group.

**Table (2):** The outcome of daily oral administration of cannabis and pregabalin for 8 weeks on histopathological changes in rat's liver.

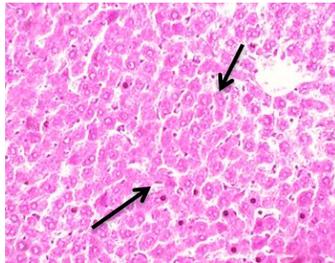
Groups	Control	Pregabalin	Cannabis
histopathological changes and grading	No pathological changes	Staging=0 Grading= 1 "Lytic necrosis" Mildly dilated sinusoids, some congestion in CV with coagulative necrosis in hepatocytes	Staging=0 Grading= 2 "Lytic necrosis" Significantly dilated sinusoids vacuolated cytoplasm in hepatocytes



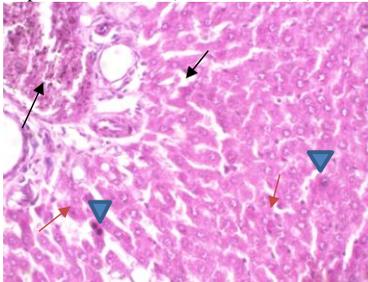
**Fig.1:** Effects of daily oral administration of cannabis and pregabalin for 8 weeks on serum malondialdehyde (MDA) in rats.



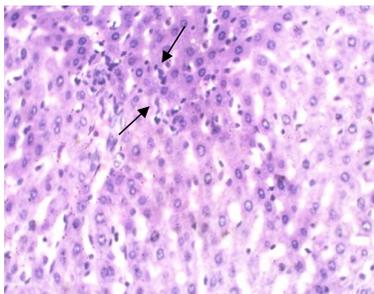
**Fig.2:** Effect of daily oral administration of cannabis and pregabalin for 8 weeks on glutathione (GSH) in rat's serum.



**Fig.3:** Liver of control group at week 8 shows Higher showing even hepatocytes arranged in plates with patent sinusoids (Black arrows) (H&E, 400x)



**Fig.4:** Liver of cannabis-treated group at week 8 of cannabis oral administration shows congested vessels in portal tract (Black arrows). There are dilated sinusoids (Red arrows). There are foci of lytic necrosis (Arrowheads). Hepatocytes show vacuolated cytoplasm (H&E, 400x).



**Fig.5:** Liver of pregabalin-treated group at week 8 of pregabalin oral administration shows coagulative necrosis of hepatocytes with mildly dilated sinusoids and a focus of lobular inflammation (Black arrows) (H&E, 400x).

## Discussion

Cannabis and pregabalin effects on oxidative status and histopathology of rat's liver were evaluated after daily drug administration for 8 weeks in Wistar male rats.

Malondialdehyde (MDA) is one of the end products of peroxidation in the cells. A rise in free revolutionaries' reasons over manufacture of MDA. Malondialdehyde position is generally recognized as a marker of oxidative stress and the antioxidant grade in cancerous cases (*Gawell. et al., 2004*).

Considering the essential part of glutathione in the cellular defense, our study also measured its situations after cannabis and pregabalin treatment. The attained results showed that cannabis treatment induced a significant drop in GSH levels while pregabalin induced a significant increase in GSH level in comparison to the control group. While, cannabis-treated group showed significant increase, and pregabalin showed significant drop in MDA levels compared with control group.

Those results were matching to *Nevenka et al. (2019)* who demonstrated that GSH levels in cannabis treated rats slightly lowered compared to controls. Those results also came in accordance with *Jaiyeola Abiola. et al. (2022)* who stated that the control group had a significantly advanced GSH level in Cannabis treated rats with slight decrease in

SOD activity.

*Kubiliene, A. et al. (2021)* reported that the GSH level was inferior ( $p > 0.05$ ) in the serum of the rats that had Cannabis, whereas in rats had Cannabis, MDA absorption in was just ( $p > 0.05$ ) more superior than the control group, also this result came in on agreement with the results in our study that showed an increase in the MDA level of the cannabis treated groups orally while there was decrease in the GSH level also in cannabis treated groups orally compare to control group.

The results of *Omar et al. (2018)* came matching to our findings as the reported that MDA levels significantly increased at oral administrated cannabis groups as compared to the saline control group, also they supported our results in GSH levels as they demonstrated that in cannabis treated rats, the levels of GSH decreased compared to control groups.

*Patryk et al. (2020)* results came in agreement with our results as they mentioned that products of lipid peroxidation increased in the Cannabis orally treated rats as (MDA increased +82%).

The levels of GSH were higher in the Pregabalin treated group versus control group (*Salem et al., 2020*), this support the result of GSH level in our study as GSH level increased compared to control group.

*Darwish and Dessouky (2015)* study confirmed those findings as oral administration of Pregabalin

also elevated GSH level and decreased the level of MDA level compared to control group, based on the present results of this study, Pregabalin treatment for 8 weeks decreased the concentrations of serum malondialdehyde (MDA). **Moustafa et al. (2018)** showed same results in decreasing MDA level after Pregabalin oral treatment; Malondialdehyde (MDA) is an indicator of lipid peroxidation and is generally used for this purpose.

**Heba et al. (2010)** showed in their histopathological findings of the liver in the cannabis treated group that there were areas of congestion of blood vessels, Most of the hepatocytes show vacuolated or ratified or hyalinized cytoplasm which came with accordance in our study that showed congested vessels in portal tract and hepatocytes show vacuolated cytoplasm, those results were supported by **Omar et al. (2018)** who reported that In the circumstance of animals treated with Cannabis ,there were tiny degeneration and karyolysis of vacuoles of hepatocytes plus widened, congestion of the portal vein which agreed with ore histopathological findings in liver. Histopathological findings in our study where liver in Pregablin group showed coagulative necrosis of hepatocytes at 8<sup>th</sup> week of pregabalin oral administration, similar results reported by **Ghaleb Sherien et al. (2021)** who reported that histopathological observations

in the liver of pregabalin treated group showed coagulative necrosis of hepatocytes and hemorrhages.

**Ismail et al. (2022)** mentioned that there were histopathological changes in liver sinusoids separating the hepatocytes also dilated central vein (CV) and it was congested with red blood cells and there was also cellular infiltration, which came in accordance with our histopathological findings in that showed mildly dilated sinusoids, some congestion in CV hepatocytes at 8<sup>th</sup> week of pregablin oral administration.

#### Conclusion

1. Cannabis revealed oxidative stress effects as it significantly increased MDA and significantly decreased GSH, while pregabalin showed the inverse results.
2. Both cannabis and pregabalin produced different pathological lesions in liver.

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### الملخص العربي

التسمم الكبدى التأكسدي الناتج عن القنب و البريجابالين  
شيرين عباس، شيرين محمد محمود<sup>2</sup>، كوثر عبدالواحد الهادي<sup>3</sup>،  
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أجريت الدراسة الحالية على ثلاثين من ذكور الجرذان السليمة، وزنها حوالي 120-130 جم. تم الحصول على الفئران من بيت الحيوان بكلية الطب البيطري جامعة قناة السويس بالإسماعية. تركت الحيوانات ثلاثة أسابيع للتكيف قبل الدراسة. تم فحص الحيوانات سريريًا على وجه الخصوص من أجل جلدتها وشعرها وكان جميعهم على ما يبدو بصحة جيدة ولديهم بشرة وشعر طبيعي. تم إيواء الحيوانات والمحافظة عليها على عليفة قياسية مكونة من (72٪ ذرة، 27٪ فول صويا و 1٪ مسحوق سمك) وتم توفير العلف والماء حسب الرغبة.

تم تقسيم الحيوانات إلى ثلاث مجموعات. كل مجموعة تحتوي على عشرة فئران، المجموعة الأولى هي مجموعة ضابطه، المجموعة الثانية هي مجموعة القنب والمجموعة الثالثة هي مجموعة البريجابالين.

- المجموعة الأولى من الجرذان كمجموعة ضابطه سلبية تلقت ماء فارغًا بدون أدوية.  
- المجموعة الثانية من الجرذان تناولت جرعة يومية من خلاصة القنب مقدارها (3 ملغ مخففة بـ 1٪ DMSO / كغ من وزن الجسم)-عن طريق أنبوب المعدة- لمدة ثمانية أسابيع.  
المجموعة الثالثة من الفئران تلقت البريجابالين يوميًا عن طريق أنبوب المعدة، بجرعة 30 مجم / كجم لمدة ثمانية أسابيع.

بعد اسبوعين من بداية الدراسة، تم جمع عينات من المصل من خمسة جرذان لفحص الإجهاد التأكسدي على عينات المصل.

بعد ثمانية أسابيع تم أخذ نفس العينات مرة أخرى من الخمسة جرذان المتبقية من كل مجموعة. تم أخذ عينات من الكبد للفحص النسيجي

مؤشرات الإجهاد التأكسدي:

(أ) (malondialdehyde MDA):

أظهرت مجموعة القنب زيادة معنوية بينما أظهرت مجموعة البريجابالين انخفاضًا معنويًا في نشاط MDA مقارنة بالمجموعة الضابطه.

(ب) الجلوتاثيون (GSH):

نتج عن القنب انخفاض معنوي في مستوى GSH بينما أظهر البريجابالين زيادة معنوية في مستوى GSH مقارنة بالمجموعة الضابطه.

نتائج الفحص النسيجي:

(أ) أنسجة الكبد: بفحص أنسجة الكبد وجد تغيرات سرطانية أو خلل نسيجي.