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Efficacy And Safety of Cannabidiol on Juvenile Diabetes Outcomes in Male Rats

By

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DEDICATION

To my loving husband Cezar

ACKNOWLEDGMENT

This research was made possible with the help and support of many people in my life. First, I would like to express my gratitude to my supervisor Dr. Yolande Saab. I am extremely grateful for her support throughout the journey. Thanks go to my colleagues who made the journey a memorable one.

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Efficacy And Safety of Cannabidiol on Juvenile Diabetes Outcomes in Male Rats

Nai Yaacoub Awkar

ABSTRACT

Background: Cannabidiol (CBD) is a major non-psychotomimetic cannabinoid found in the Cannabis sativa plant with several pharmacological effects. Juvenile Diabetes is a major chronic illness that affects the life of patients. CBD presents desirable effects on hyperglycemia by protecting against oxidative stress and inflammation, having neuroprotective properties, and acting on receptors linked to glycemia. Thus, CBD has the potential to decrease HbA1C in diabetic patients (Diabetes Mellitus type 1) and improve their lives.

Objective: The current research aims to explore the pharmacological effect of CBD on Juvenile Diabetes in rat models, as well as the safety profile of CBD including toxicology studies on the liver and the pancreas.

Methods: The research was done by conducting experiments on young adult male Wistar rats. The rats were given streptozotocin (STZ) for Juvenile Diabetes induction. The rats were randomly assigned to four experiments for a total duration of 30 days. Each experiment contained a vehicle control group alongside several groups of different

dosage regimens of CBD. Each Experiment consisted of a specific and required number of animals (according to the standardized toxicity tests for rodents).

Results: The animal data and findings from this study offer the opportunity for a safe and effective oral drug for the treatment of Juvenile Diabetes to be considered for further research and clinical trials.

Conclusion: The animal data from this study confirmed the safety of CBD when administered in different dosing regimens per body weight with NOAEL (the no-observed-adverse-effect level) = 150mg/kg. In addition, the results gave promising insights for considering CBD as an oral treatment option for juvenile diabetes as dose-dependent. The CBD dose of 50mg/kg when administered for 14 days was able to control blood glycemia values, body weights and produce insulin concentration levels that are close to normal values.

Keywords: Juvenile Diabetes, Cannabidiol, Hyperglycemia, HbA1c, Insulin, Toxicology, Hepatology.

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CHAPTER ONE
INTRODUCTION

1.1 Overview

There has been a growing interest in the study and use of cannabinoids (CBD) for treating medicinal illnesses such as anxiety. [1] A new medicinal illness has a possible promise with CBD usage: Juvenile Diabetes Mellitus. [1] Type 1 diabetes mellitus, also called Juvenile Diabetes, is an autoimmune disease resulting in the immune destruction of beta cells in the islets of Langerhans in the pancreas. [2] This autoimmune destruction causes insulin shortage in the body. [2] Insulin is a well-known and important hormone that stimulates the uptake of glucose by muscles and adipose cells. [2] Without the effect of insulin in the body, life-threatening diabetic ketoacidosis (DKA) occurs. [3] DKA is characterized by the build-up of glucose, acids, and ketones, known as the triad of hyperglycemia, acidemia, and ketonemia.[3] DKA is the major cause of death in children diagnosed with Juvenile Diabetes with a mortality rate of 0.15-0.3%.[3] Moreover, children under the age of 5, with infection, and/or belonging to the lower socioeconomic status are at higher risk of developing DKA complications. [3] Thus, patients with Juvenile Diabetes must have access to medical treatment and are often bound to insulin replacement therapy for their lifetime.

1.2 Epidemiology

Juvenile Diabetes is a common chronic illness that occurs mainly in children (ages 4-6 and early puberty). [3] Juvenile Diabetes's occurrence has increased steadily to reach around 5-10% of total diabetic patients. [2] In the United States, approximately 1.24 million people are diagnosed with Juvenile Diabetes, and by 2050 around 5 million people are expected to be suffering from this chronic illness in the States. [2] However, the occurrence of

Juvenile Diabetes worldwide varies from one region to another. [2] For instance, the highest rate of Juvenile Diabetes occurrence is in Northern European countries, especially Finland, and this rate is 400 times greater than the rate of the disease occurrence in China or Venezuela, which are regarded as the countries with the lowest occurrence rates. [2]

1.3 Pathophysiology of Juvenile Diabetes

Homeostasis of glucose is well regulated by insulin. [4] Insulin is the well-known hormone that is secreted by pancreatic beta cells and is responsible for the uptake, use, and storage of glucose. [4] Thus, insulin is a vital hormone in glucose homeostasis in the body. [4] The loss of insulin (secretion or action) causes persistent hyperglycemia, which causes diabetes. [4] In Juvenile Diabetes, it starts with insulitis: infiltration of immune cells in and around islets. [4]

Certain $CD4^+$ T cell subsets, such as helper type 1 (Th1) and 2 (Th2), Th17, and T regulatory (Treg) cells, participate in the adaptive immune response since they secrete specific cytokines. [4] Clearly, an increase in Th1/Th2 and differentiation of Naïve $CD4^+$ to Th17 occur in Juvenile Diabetes. [4] Both Th1 and Th17 T cells secrete pro-inflammatory cytokines (IFNγ, IL17, TNFα) that stimulate intra-islet inflammation, resulting in the differentiation of $CD8^+$ T cells to cytotoxic T lymphocytes (CTL). [4] In turn, CTLs penetrate the islets and provoke beta cell death. [4]

The presence of circulating islet cell cytoplasmic antibodies (ICA), antibodies to insulin (IAA), glutamic acid decarboxylase (GAD65), insulinoma-associated 2, or protein tyrosine phosphatase antibodies (IA-2), and zinc transporter8 (ZnT8) indicate that the person is at risk or has already Juvenile Diabetes. [2(p2)]

Juvenile Diabetes occurs in 3 stages: asymptomatic, asymptomatic with abnormal glucose levels, and symptomatic with hyperglycemia and autoantibodies (pancreatic). [2(p2)] Stage 1, the asymptomatic stage, has normal glucose levels, and the existence of 2

pancreatic autoantibodies. [2(p2)] In stage 2, the glucose levels are abnormal with impaired glucose tolerance and the existence of 2 pancreatic autoantibodies. [2(p2)] In the final stage, the patients develop symptoms of diabetes or hyperglycemia, with the existence of 2 or more pancreatic autoantibodies. [2(p2)]

Symptoms of Juvenile Diabetes can occur suddenly and are mainly the symptoms of hyperglycemia such as polyuria and polydipsia. [2(p2)] Hyperglycemia causes osmotic diuresis, which leads to polyuria. [2(p2)] Then, polyuria causes dehydration and hyperosmolality, leading to polydipsia. [2(p2)] Also, weight loss might be common in children. [2(p2)] However, if the symptoms are not recognized and managed immediately, a medical emergency may occur with cases of DKA. [2(p2,3)]

1.4 Available Drugs for Juvenile Diabetes and Its Management

Since Juvenile Diabetes leads to lack of insulin, patients diagnosed with Juvenile Diabetes need insulin replacement therapy. [5(p2453)] Insulin analogs with different onsets and durations of action are given subcutaneously as an injection (pen or needle) to treat Juvenile Diabetes. [5(p2453)] The only non-insulin-approved medication is pramlintide. [5(p2453)] Thus, the medication treatment is limited mainly to insulin.

Continuous glucose monitoring, glycemic intake control, and controlling HbA1c (below 7.5%) are also important factors for managing Juvenile Diabetes. [5(p2455)]

CHAPTER TWO
LITERATURE REVIEW

2.1 CBD and Juvenile Diabetes

In previous literature, CBD was studied on autoimmune diseases such as rheumatoid arthritis in animal models. [6] Results showed that CBD can restrain the cell-mediated autoimmune destruction of the joints. [6] Since Juvenile diabetes is also an autoimmune disease, research was conducted to examine CBD effects on NOD mice (non-obese diabetic mice). [7] Results showed that CBD treated NOD mice had a significant decrease in diabetes incidence compared to the control group (30% in CBD treated mice/ 86% in control mice). [7] In addition, CBD treatment was also given to NOD mice in a latent diabetes stage or with initial diabetes, and results showed that the disease symptoms were improved by CBD treatment. [7]

In a study conducted by Weiss et al. (2008), results showed that treatment with CBD for NOD mice at an early or latent stage of Juvenile Diabetes caused constant inhibition of insulitis. [7] It was observed that CBD treatment decreased diabetes from 86% to 32%. [7] Results of the histological examination of the pancreas further supported the absence of insulitis in 77% of the islets of the CBD-treated mice, in comparison to only 13% absence in the pancreas of the untreated groups. [7] Weiss et al. (2008) were also able to show the CBD blockage of TNF-α production, which plays a key role in induced β-cell damage as previously discussed. [7]

2.2 Mechanism of Action of CBD

The endogenous signaling system known as the endocannabinoid system consists of the cannabinoid CB1 receptor, an abundant G-protein-coupled receptor found in the central

nervous system, and the CB2 receptor found in abundance in several immune cells and tissues. [6] CB1 receptors are involved in controlling energy homeostasis, and CB2 receptors are involved in controlling cytokines release and function. [6]

The molecular mechanism of CBD is greatly related to the human endocannabinoid system. [6] CBD binds weakly to the cannabinoid receptors CB1 and CB2. [6] Its effects are mainly due to the pharmacological inhibition of adenosine uptake, antioxidant activity, and action on 5-HT receptors. [6]

CBD is an effective anti-inflammatory agent. [7] It suppresses IFN-γ and TNF-α production and development of Th1-mediated autoimmune response by blocking T cell proliferation. [7]

2.3 Benefits of CBD

Treatment with Cannabidiol in vivo has presented promising effects in controlling diabetes. [1] Preclinical data have identified the properties of CBD as antioxidant, anti-inflammatory, immunological stimulator, neurological, and cardiovascular protective. [1] Hence, this pharmacological profile of CBD might help in controlling diabetes and its complications. [1] In addition, CBD acts on other non-cannabinoid receptors such as 5-HT1A and GPR55 which have been linked to controlling blood sugar levels. [8]

In this article, we will investigate the effects of CBD on controlling Juvenile diabetes and decreasing its complications in young adult male rats.

2.4 Toxicity Studies of CBD

A CBD drug “Epidyolex” has gained FDA approval for the management of myoclonic epilepsy in children, Dravet Syndrome (DS). [9] The design of the toxicity studies was illustrated by the oral administration of CBD doses: 15, 50, and 150mg/kg/day. [9] The no-observed-adverse-effect level (NOAEL) was found to be 150mg/kg/day for Wistar rats. [9]

These CBD doses were found to be pharmacologically effective for seizures, and without severe or life-threatening side effects. [9] The doses were safe in terms of fertility and embryonic development, no carcinogenic potential was detected, and negative genotoxicity results. [9]

On another hand, these toxicity studies revealed the possible adverse reactions from CBD. [9] For instance, high doses of CBD (150mg/kg/day and higher) were found to lead to an increase in liver weight, increase in alkaline phosphatase (ALT), and an increase in the mean alanine amino transaminase/alanine aminotransferase (ALT) activities, which is usually accompanied by thyroid follicular hypertrophy. [9] Possible hormonal dysregulation, and drug abuse/ dependence. In terms of cardiovascular function and safety, no adverse effects were observed. [9]

2.5 Objective

The literature is limited when it comes to understanding the effects of CBD on Juvenile Diabetes and whether CBD can be considered a safe oral treatment option. Moreover, the properties of CBD as an antioxidant, anti-inflammatory, and immunological stimulator might help in controlling diabetes and its complications. Hence, this study aims to assess the CBD effect on reducing the markers of inflammation in a Type 1 diabetes rat model as well as exploring the safety of CBD with Juvenile Diabetes.

CHAPTER THREE

METHODOLOGY

3.1 Animals

Experiments were conducted with young adult male Wistar rats (aged between 7-10 weeks) weighing 90-230 grams with the age of the rats being aligned with a similar study conducted by Chaves et al. (2021); [10] animals were supplied from the Lebanese American University Animal Center. The animals were housed in 4 experiments (n_{total}= 114 rats) under controlled conditions: temperature (22 ± 1 °C) and an alternating light/dark cycle (12h/12h) for 2 weeks before the experiments. [10] Animals were fed a standard chow diet and water was provided as needed. [10] Efforts were made to reduce the number of animals used and minimize their suffering. [10] The experimental procedures align with the ethical principles of the Lebanese American University and the total number of animals used was 114.

3.2 Induction of Juvenile Diabetes (DM Type 1) by Streptozotocin

The rats were fasted for 12 h (6 PM to 6 AM), then Streptozotocin (STZ) (purchased from Ibra. Hadad et Fils, Lebanon) was dissolved in 10 mM citrate buffer (pH 4.5) and given intraperitoneal (I.P) as a single administration of 60mg/kg dose. STZ induces diabetes type 1 in animals. [10] Glycemia levels were assessed from blood drops taken from the tails of the rats (5µL) using the On Call Plus blood glucose test strips (Acon Biotech). Animals were regarded as diabetic with glycemia values greater than 250 mg/dl three days after STZ injection. [5,10]

STZ handling: stored at -20°C to prevent degradation; the microcentrifuge tube containing STZ was protected from light by aluminum foil coverage; STZ was mixed with

the citrate buffer immediately before injection and was given within 5 mins of preparations (STZ decomposes in citrate buffer in 15-20 mins). [11]

3.3 Drugs

Synthetic pure Cannabidiol (CBD) tablets were diluted in 2% Tween 80 (2.5ml) and 100% NaCl solution (45ml). [8] The CBD tablets were purchased from drug stores since the tablets are already in the market for the treatment of epilepsy. A vehicle composed of 2% Tween 80 and 98% saline solution (5ml/kg according to the recommended volumes of substances administered orally to laboratory animals). [8,12] The animals were randomly assigned to receive a daily dose (administered through the drinking bottles) of vehicle or CBD (according to their groups). [8] The minimum and ascending doses of CBD were backed by literature showing their safety. [9]

It is noteworthy to mention that the vehicle of Tween and saline solution does not interfere with the values of diabetes in animals.

3.4 Experimental Design

Young-adult aged rats were injected with STZ (60mg/kg) after prior fasting of 12h, and their glycemia level was taken after 3 days. The diabetic rats were randomly divided into 4 Experiments: with each Experiment consisting of diverse groups. The target organs for toxicity monitoring are the liver and pancreas (organ enlargement). NOAEL= 150mg/kg/day. The design of the experiments, the groups, and the dosing regimen were done based on the literature and prior safety data of the approved cannabidiol product in the market: Epidyolex. In the Epidyolex assessment report, the toxicity studies conducted on Wistar rats of both sexes consisted of three dosing regimens: 15, 50, and 150 mg/kg/day. [9] Also, we conducted a 4-Day experiment on eight rats taking CBD

50mg/kg/day PO for 4 days only to test if CBD 50mg/kg/day treatment taken for less than a week is effective or not.

Thus, in this study, we adapted this dosing regimen to all experiments. [9] In addition, we added a mid-range dose of 30mg/kg/day. The treatment of CBD or vehicle was given via the rats' drinking bottles in cages containing a maximum of five rats per cage. The Organization for Economic Cooperation and Development (OECD) advises that if the substance under study is to be given via drinking water bottles, then it is necessary to make sure that the amount of the test substances do not affect the normal water balance of the animals.[13] In our study, the rats took the treatment via their drinking bottles, and their appropriate daily amount of drinking water was provided.

Experiment 1 = Testing and monitoring of acute toxicity: (5 rats per group, 4 groups, and a total of 20 rats for Experiment 1). Epidiolex experiments were already available for toxicity studies, but we decided to investigate toxicity with DM1 and CBD.

- Group 1= Control group of hyperglycemic induced animals; treated with vehicle PO for 24 hours.
- Group2= Hyperglycemic induced animals; treated with CBD 15mg/kg PO once per 24 hours.
- Group3= Hyperglycemic induced animals; treated with CBD 50mg/kg PO OD once per 24 hours.
- Group4= Hyperglycemic induced animals; treated with CBD 150mg/kg PO OD once per 24 hours.

Experiment 2 = Testing and monitoring of sub-chronic toxicity: (10 rats per group, 5 groups, and a total of 50 rats).

- Group1= Control group of hyperglycemic induced animals; treated with vehicle PO for 14 days.
- Group2= standard group of hyperglycemic induced animals; treated with CBD 15mg/kg PO once a day for 14 days. Here, we considered 15mg as a standard and safe dose with local tolerance based on previous studies.
- Group3= Hyperglycemic induced animals; treated with CBD 30mg/kg PO OD for 14 days.
- Group4= Hyperglycemic induced animals; treated with CBD 50mg/kg PO OD for 14 days.
- Group5= Hyperglycemic induced animals; treated with CBD 150mg/kg PO OD for 14 days.

Experiment 3 = Testing and monitoring of chronic toxicity: (18 rats per group, 3 groups, and a total of 54 rats).

- Goup1= Control group of hyperglycemic induced animals; treated with vehicle PO for 90 days but was stopped on Day 30 of treatment due to lack of effect.
- Group2= Hyperglycemic induced animals; treated with CBD 15mg/kg PO once a day for 90 days but was stopped on Day 30 of treatment due to lack of effect.
- Group3= Hyperglycemic induced animals; should be treated with CBD 150mg/kg PO OD for 90 days, but in Experiment 2 the 150mg/kg dose was toxic, hence we disregarded this group. No experimental process was carried out for this dosing regimen.

4-Day experiment = Testing and controlling our procedure: (8 rats in one group).

- Group= Hyperglycemic induced animals; treated with CBD 50mg/kg PO once a day for 4 days.

Animals were monitored closely for adverse events and/or mortality. Glycemia and body weight values of the animals were recorded once per week (total measurements 5 times with the initial pretreatment assessment, and for the whole 5 weeks of the study). At the end of the study, rats were euthanized, liver and pancreas were examined for organ growth/abnormalities, and blood was extracted for blood tests (insulin and glucose levels).

3.5 Measuring Plasma Levels of Insulin and Glucose

Blood samples were collected after the euthanasia of the animals in specific tubes to prevent glycolysis: glucose-specific tube (Glucomedics) containing NaF/KOx, citrate, and EDTA (having a purple-top, purchased from IVLAB- Lebanon).[14] Plasma was separated by centrifugation at 2500 rpm (15 min) at 4 °C and stored at -20 °C until use. [14] Measuring plasma levels of insulin was done based on the manufacturer´s instructions by Enzyme-Linked Immunosorbent Assay Rat Insulin ELISA kit (colorimetric) purchased from NOVUS Biologicals. [14]

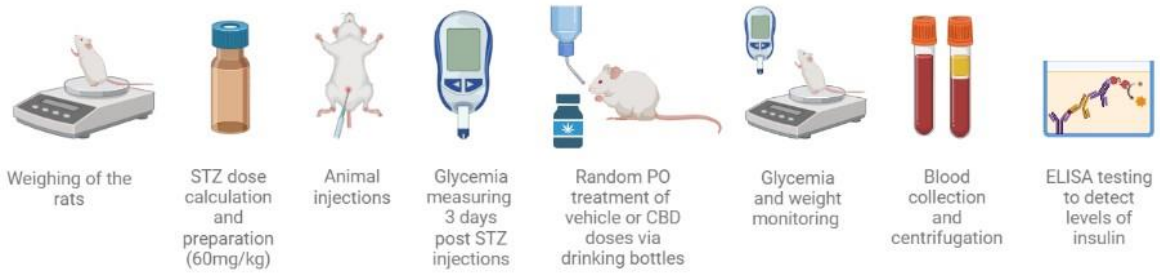

Figure 1. Time course of experimental design. STZ: streptozotocin; CBD: cannabidiol. Here, all steps done in the research are summarized: starting with weighing the animals to administer the appropriate STZ dose, then checking their glycemia values after 3 days, the initiation of PO treatment with vehicle or CBD, and until the sacrifice of the animals and ELISA testing.

CHAPTER FOUR

FINDINGS

4.1 Mortality of Rats Following STZ Injection

STZ injection was done three times, with each time targeting a specific experiment (acute, sub-chronic, or chronic). For the acute experiment, 20 rats were injected with STZ, and all 20 of them were diabetic and assigned randomly to their respective treatment group. In the sub-chronic experiment, 50 rats were injected with STZ. After 3 days, diabetes was confirmed in all of them, and they were assigned randomly to a different treatment group. For the chronic experiment, 40 rats were injected with STZ in a 60mg/kg dose. The rats were monitored closely for any adverse effects. However, 3 days following the STZ injection, a total number of 4 rats deceased, and a remaining 36 hyperglycemic-induced rats remained for the experiment. These 36 rats were divided randomly and equally into 2 groups with each group having a total of 18 rats.

4.2 Experiment 1 Findings and Discussion

Fasting glycemia values were taken and recorded for each experiment and each group. The average glycemia values (table.1) and average weight values (table.2) were obtained and analyzed. Fasting glycemia values between 70–180 mg/dl are considered in the euglycemic range, less than 70mg/dl are considered hypoglycemia, and above 180mg/dl are labeled as hyperglycemia. [15]

Table 1. Average Glycemia Values of the Rats per Experimental Group for the Treatment Duration

Average Glycemia of rats per experiment per week								
Experiment #	Group #	CBD Dose/ Vehicle	Treatment Duration	Average Glycemia Pre-treatment initiation (mg/dl): Day 0	Average Glycemia (mg/dl): Week1	Average Glycemia (mg/dl): Week2	Average Glycemia (mg/dl): Week3	Average Glycemia (mg/dl): Week4
1 (Acute)	1	Vehicle	24hours	472.2	370.6			
1 (Acute)	2	15mg/kg	24hours	414	403.8			
1 (Acute)	3	50mg/kg	24hours	381.8	232.2			
1 (Acute)	4	150mg/kg	24hours	405.2	351.2			
2 (Sub-chronic)	1	Vehicle	14Days	457.2	303.9	317		
2 (Sub-chronic)	2	15mg/kg	14Days	428.8	177.8	194.7		
2 (Sub-chronic)	3	30mg/kg	14Days	487.2	285.2	270.25		
2 (Sub-chronic)	4	50mg/kg	14Days	486.4	459.6	120.6		
2 (Sub-chronic)	5	150mg/kg	14Days	403.6	193.1	278		
3 (Chronic)	1	Vehicle	30Days	436.2	339.2	297.3	344.5	384.8
3 (Chronic)	2	15mg/kg	30Days	495.5	358.7	193.3	324.8	363.9
Validation (8 rats)		50mg/kg	4Days	610	600			

Table 2. Average Weight Values of the Rats per Experimental Group for the Treatment Duration

Average weight of rats per experiment per week (after treatment initiation)								
Experiment #	Group #	CBD Dose/ Vehicle	Treatment Duration	Average Weight Pre-treatment initiation (g): Day 0	Average Weight (g): Week1	Average Weight (g): Week2	Average Weight (g): Week3	Average Weight (g): Week4
1 (Acute)	1	Vehicle	24hours	184.4	184.2			
1 (Acute)	2	15mg/kg	24hours	150.8	150.4			
1 (Acute)	3	50mg/kg	24hours	158.6	160.2			
1 (Acute)	4	150mg/kg	24hours	189	189			
2 (Sub-chronic)	1	Vehicle	14Days	121.6	121	171		
2 (Sub-chronic)	2	15mg/kg	14Days	136.8	133.9	143.5		
2 (Sub-chronic)	3	30mg/kg	14Days	118.1	124.8	141.5		
2 (Sub-chronic)	4	50mg/kg	14Days	146	142	143.3		
2 (Sub-chronic)	5	150mg/kg	14Days	132.8	129.7	144.4		
3 (Chronic)	1	Vehicle	30Days	156.6	150.2	125.7	145.5	155.4
3 (Chronic)	2	15mg/kg	30Days	142.6	133.7	121.3	139.2	161.7
Validation (8 rats)		50mg/kg	4Days	143.2	133.6			

The raw data for each rat in each specific group and experiment is archived and available in the annex.

For experiment 1 (acute treatment for 24 hours only), glycemia values were obtained 24 hours after treatment initiation and before the sacrifice of rats.

- For the 1st group in this experiment (vehicle), the glycemia results were almost the same before and after treatment initiation. The body weight of the rats remained constant.
- For group 2 in experiment 1 (15mg/kg of CBD), glycemia results and weights recorded were also stable before and after treatment. All rats remained in the hyperglycemia range.
- As for the 3rd group in experiment 1 (50mg/kg), there is a slight decrease in the glycemia values of the rats after 24 hours of taking CBD 50mg/kg. The rats were still in the hyperglycemic range. The weights of the rats remained constant.
- The last group in this experiment is group 4 where the rats took once a single dose of 150mg CBD orally. The glycemia values obtained after 24 hours of the CBD dose show a minimal decrease. However, all rats remained in the hyperglycemia range as well. The weights remained the same as the weight initially obtained before the STZ injection.

4.3 Experiment 2 Findings and Discussion

For experiment 2 (sub-chronic treatment for 14 days), glycemia values were acquired after the induction of the treatment, weekly (a total of two times).

- The first group in this experiment took the vehicle for 14 days. 1 out of the remaining 3 rats who survived for 2 weeks had a normal glycemia value. The high mortality rate here could be linked to the fact that the rats are diabetic and not

taking any treatment for 14 days. Thus, they might have died secondary to diabetes complications. As for the weight, the rats had a slight decline in their body weight after 1 week of treatment and followed by a minor increase in the body weight after 2 weeks of treatment.

- For group 2, the rats took CBD 15mg/kg dose daily for 14 days. Out of the 6 rats that survived till the end of the experiment duration, 4 had hypoglycemia values, while the remaining 2 rats were still hyperglycemic. The mortality rate here is 4/10=40%. Rat 2 and rat 5: had a low weight (<100 g) after STZ injection with an extremely high glycemia level (>600 mg/dl). The death of these two rats might be explained by the low body weight and the high glycemia value which the rats could not manage. Rat 1 might have died due to a low body weight of only 77 grams on week 1 of treatment. Finally, rat 8 had a hypoglycemic episode on week 1 where his glycemia level was 22mg/dl and he couldn't survive this.
- For the 3rd group in experiment 2, the rats took CBD daily dose of 30mg for 14 days. Here, only 1 rat (rat 2) had a normal glycemia value out of the remaining 4 rats in week 2. Thus, the CBD dose of 30mg/kg was not sufficient to maintain the glycemia of diabetic rats in the normal range. In addition, the high mortality rate (60%) might be linked to low body weight coupled with hyperglycemia. For instance, rats 1, 3, 6, 9, and 10 all had low body weights (97, 97, 99, 102, and 104g) coupled with extremely high glycemia values (589, 590, 610, 610, 610 mg/dl). As for rat 4, his weight decreased to reach 91g after 1 week of treatment, so he might have become weaker and couldn't handle the diabetic state. As for the weights, there was a slight decrease in weight ranges after 1 week of treatment, and it is followed by stable weight ranges for the 2nd week and final week of treatment.

- The 4th group in experiment 2 was on a 50mg/kg dose of CBD. In this group, there was a noticeable improvement in the fasting glycemia values of the rats from week 1 post-treatment initiation until the end of week 2. For instance, the lowest glycemic value for this group in week 1 was 307mg/dl, the highest value was >600mg/dl, and all other values obtained were in the hyperglycemia range with an average of 458.5mg/dl. Whereas, in week 2 (7 rats) the lowest fasting glycemia value was 37mg/dl (hypoglycemia), the highest value was 261mg/dl (hyperglycemia), but the remaining 5 glycemia values of the 5 rats were all in the euglycemic range: 97, 117, 70, 146, and 116mg/dl with an average of 119.5mg/dl. If we calculate the percentage of the decrease between the 2 weeks= (458.5 – 119.5)/458.5= 339/458.5= 0.73936750272628≈ 74% (calculated on Microsoft Excel). Thus, for this dosing regimen of 50mg/kg CBD, results start to significantly improve (≈74%) following a minimum of 14 days of treatment. In addition, the survival rate in this group was also considered good since we had 3 rats deceased out of the 10 initial rats (70% survival rate). This could be justified by saying that the 50mg dose was adequate to protect the rats from severe complications of juvenile diabetes while maintaining a normal glycemia level for most of the rats. The 3 rats who died had hyperglycemia values of 590mg/dl and values above 600mg/dl. Thus, they suffered from severe diabetes complications from which they could not survive. Moving on to their weights, the weights remained stable after 1 week of treatment with few declines in the body weights. Then, in week 2 the weights remained stable with few decreases in the body weights. Hence, the 50mg dose of CBD was also able to maintain the body weights of all the rats.

- On the other hand, the 150mg/kg dosing taken for 14 days (experiment 2, group 5) did not show adequate response to treatment in terms of blood glycemia control where only 42.8% of rats had glycemia values within the normal range. Changes in glycemia values: in week 1 post-treatment with CBD, we have decreases in the glycemia values, followed by increases in the glycemia values in the 2nd week with only 3 rats being in the euglycemic range. We could interpret these results by considering the high dose of CBD and its effect on cortisol in the body. A study conducted in 2017 by Carol et al. showed that CBD might have a significant impact on controlling the release of cortisol in the body in a dose-dependent manner. [16] Cortisol is a glucocorticoid that acts on the liver, muscle, fat tissue and pancreas to elevate gluconeogenesis and decline glycogen synthesis. [17] This metabolic pathway leads to the production of glucose to release energy, and thus, the blood sugar level will be elevated. [17] Therefore, the 150mg/kg dose of CBD was toxic and ineffective to treat hyperglycemia.

4.4 Experiment 3 Findings and Discussion

For experiment 3 (chronic treatment for 90 days, but was stopped on Day 30), glycemia values were taken once per week (a total of 5 times) throughout the treatment period.

- For the first group the rats were taking vehicle treatment, so the results are as expected. Almost all rats had hyperglycemia values except for 2 rats who had values in the euglycemic range. These 2 rats had high body weights (211g and 226g) which might be the reason why they were able to overcome juvenile diabetes. Meaning the STZ dose given did trigger diabetes, but it had a minimal effect. As for the body weights, the weights were controlled throughout the treatment period with few variations of loss and gain. For weeks 1 and 2 of the

post-treatment initiation, the rats had a noticeable decrease in their body weight. This decrease is then followed by an increase in the body weights for almost all of the rats for the rest of the treatment duration. Here, the mortality rate was low where only 4 rats died out of the 18 initial ones, enabling us to obtain larger data on the rats taking the vehicle for the whole treatment duration.

- For group 2 on 15mg/kg CBD, there was a minor improvement in the glycemia values: 1st week: 1 rat is in the euglycemic range; 2nd week: 3 rats are considered in the euglycemic range, but here we have 6 rats with hypoglycemic episodes; 3rd week: 4 rats are stable in the euglycemic range; but for the 4th week: 3 rats are stable in the euglycemic range and it appears that 1 rat (rat 13) previously in the euglycemic range had a hyperglycemic episode of 402mg/dl. This comes alongside his weight gain of +26g (from 97g to 123g) in the same period. This finding might be related to the increased appetite and food consumption of this rat which deteriorated his blood glucose control. Clearly, the CBD 15mg/kg dose was not enough to control the glycemia values for this diabetic group. For the weights of the rats, there was a slight decrease in their body weight after 1 week of treatment that continues to fall for the 2nd week of treatment as well. However, the rats' body weight rises again in the 3rd week post-treatment with CBD and continues to increase with 1 rat reaching a maximum weight of 273g in week 4. The mortality rate here was acceptable with 7 rats deceased out of the initial 18 ones (approx. 38.9% mortality rate) throughout the treatment duration. Rat 3, rat 4, and rat 9 all had low body weights accompanied by hyperglycemia. This finding might be the cause of their death. Whereas rat 5, rat 10, rat 12, and rat 15, all had hypoglycemic episodes from which they weren't able to survive.

4.5 Mortality of Rats and Adverse Events Following Treatment Induction (CBD/vehicle)

In the 4-Day experiment, all 8 rats remained alive with no complications on treatment with CBD 50mg/kg/day PO for 4 days.

In experiment 1 (acute treatment), no rats died or suffered from any complications within 24 hours of treatment.

In experiment 2 (sub-chronic treatment), the rats that deceased are listed below with their specific group and the specific duration of death after treatment initiation. After exactly 1 week post-treatment initiation, all the rats of group 3 (CBD 30mg/kg/day) suffered from diarrhea for 1 day. In group 5 (150mg/kg/day), 1 rat suffered from blindness but did not die.

- Group 1 (Vehicle):
    - 2 rats died after 2 days of vehicle treatment (5 days post-STZ).
    - 1 rat died after 3 days of vehicle treatment (6 days post-STZ).
    - 2 rats died after 8 days of vehicle treatment (11 days post-STZ).
    - 2 rats died after 10 days of vehicle treatment (13 days post-STZ).
- Group 2 (CBD 15mg/kg/day):
    - 2 rats died after 3 days of CBD treatment (6 days post-STZ).
    - 1 rat died after 8 days of CBD treatment (11 days post-STZ).
    - 1 rat died after 11 days of CBD treatment (14 days post-STZ).
- Group 3 (CBD 30mg/kg/day):
    - 3 rats died after 2 days of CBD treatment (5 days post-STZ).
    - 2 rats died after 3 days of CBD treatment (6 days post-STZ).
    - 1 rat died after 9 days of CBD treatment (12 days post-STZ).
- Group 4 (CBD 50mg/kg/day):

- 1 rat died after 4 days of CBD treatment (7 days post-STZ).
- 2 rats died after 12 days of CBD treatment (15 days post-STZ).

Thus, 7 rats remained alive and healthy out of the 10 rats for the 14 days: 30% mortality rate.

- In group 5 (CBD 150mg/kg/day), the rats were monitored for toxicity. Three rats were blind starting day 2 of CBD treatment. Two rats suffered from severe diarrhea after 5 days of starting treatment. Four rats were blind after 8 days of treatment with CBD. Thus, we considered CBD 150mg as toxic for the caused side effects.
  - 1 rat died after 2 days of CBD treatment (5 days post-STZ).
  - 2 rats died after 8 days of CBD treatment (11 days post-STZ).

In experiment 3 (chronic treatment), the rats that died are recorded with details on the time of death after treatment initiation. In this experiment, the mortality rate was high, and the well-being of the rats was at risk. Hence, for the sake of the rats' well-being, the experiment was terminated on day 30 of vehicle/CBD treatment. The rats were sacrificed, and the blood was collected. As for the side effects, in group 1 (vehicle), Rat #2 suffered from blindness 7 days following treatment initiation with the vehicle and then died on day 8.

- Group 1 (vehicle daily):
  - 2 rats died after 8 days of vehicle treatment (11 days post-STZ).
  - 1 rat died after 11 days of vehicle treatment (14 days post-STZ).
  - 1 rat died after 18 days of vehicle treatment (21 days post-STZ).
- Group 2 (CBD 15mg/kg/day):
  - 1 rat died after 8 days of treatment with CBD (11 days post-STZ).

- 1 rat died after 11 days of treatment with CBD (14 days post-STZ).
- 2 rats died after 15 days of treatment with CBD (18 days post-STZ).
- 1 rat died after 19 days of treatment with CBD (22 days post-STZ).
- 1 rat died after 23 days of treatment with CBD (26 days post-STZ).
- 1 rat died after 24 days of treatment with CBD (27 days post-STZ).

For toxicity assessment of the liver and the pancreas, we observed and assessed these organs after the euthanasia of the animals. All of the rats in this research study maintained a normal liver and pancreas size. Thus, the doses of CBD given with their corresponding duration of treatment were safe and did not cause liver/pancreas organ enlargement.

4.6 Insulin Plasma Quantification

The blood samples that were collected from the rats after sacrifice were centrifuged at 2500 rpm for 15 min at 4 °C and stored at -20 °C (as previously mentioned). Then, we measured the plasma levels of insulin as per the manufacturer´s instructions for the Enzyme-Linked Immunosorbent Assay Rat Insulin ELISA kit (colorimetric).

4.7 Data Analysis

The data recorded from the 4 experiments (acute, sub-chronic, 4-day, and chronic) were analyzed by one-way analysis of variance (ANOVA) on IBM SPSS statistics 23 to detect any statistical significance. The ANOVA test was done for each experiment separately to compare the test groups with the vehicle group of each experiment, respectively. For us to consider the tests as statistically significant, the p-value should be <0.05.

In experiment 1 (acute groups), we compared the vehicle group's glycemia post-treatment results to each test group's glycemia post-treatment results. For this experiment, the ANOVA results for the 3 treatment groups compared with the vehicle group had a p-value > 0.05. Thus, there is no statistically significant difference between the vehicle and

the treatment groups. This concludes that a single treatment for 24 hours with CBD, despite the dose given, is not sufficient to normalize fasting glycemia values in the blood.

In experiment 2, the sub-chronic groups, data were also analyzed using one-way ANOVA to test whether the glycemia values post-treatment with vehicle (results at the end of the 14 days) compared to post-treatment with CBD are statistically significant or not. Results from group 4, the rats that took 50mg CBD treatment for 14 days, had a p-value <0.05, which means the results here are statistically significant compared to the vehicle group. Thus, the dosing of 50mg/kg CBD for 14 days was effective to decrease the fasting glycemia values of these rats.

In experiment 3, the chronic groups, the glycemia results obtained at the end of the experiment (day 30) from the 2 groups: vehicle and 15mg/kg CBD were analyzed by one-way ANOVA as well. The p-value obtained is less than 0.05. Hence, the 15mg/kg CBD dose was not enough to control glycemia values of juvenile diabetes despite being given for a lengthy time.

Finally, the 4-Day experiment conducted consisted of only 1 treatment group with 50mg/kg CBD. This was done to compare the effects of this dosing regimen across different periods of treatment. Hence, here we will compare the results obtained from the groups of rats that took 50mg/kg across all the experiments (experiment 1: group 3, experiment 2: group 4, and the 4-Day experiment group). The results are illustrated below (Fig.2) for visual differentiation.

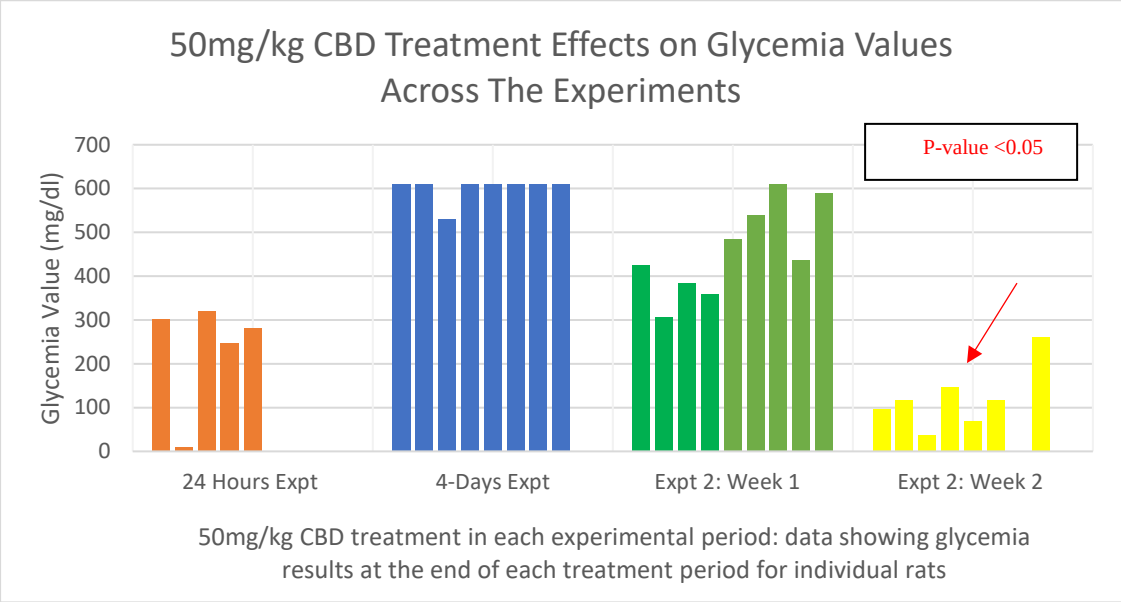

Figure 2. Chart displaying the glycemia values obtained at the end of each experiment for the groups of rats that took 50mg/kg CBD treatment. The colored columns refer to individual rats. The first group of rats "Day 1 after treatment initiation" belongs to Experiment 1 (24 hours only), the "Day 4 after treatment initiation" group of rats are those of Experiment 4, whereas the remaining groups of rats for week 1 and week 2 are those who took CBD 50mg/kg for 14 days and the results displayed show their improvement from the 1st week to the 2nd week of treatment. Expt: Experiment.

In addition, we run the one-way ANOVA to test the glycemia values of the first recording (post-treatment) for all groups on 50mg/kg (experiment 1: group 3, experiment 2: group 4, and the 4-Day experiment group). The null hypothesis here is that all groups have the same glycemia values, which means that taking 50mg/kg CBD for 1 day, 4 days or a week should have the same result. However, the ANOVA test shows a p-value <0.05, and we will reject the null hypothesis. Therefore, there is a significant difference between the groups' glycemia results. This can be seen in Fig.2 where the glycemia values begin to drop after 1 week of treatment with CBD, then values are well controlled within 2 weeks of treatment.

The Insulin ELISA analysis results are displayed in the chart below (Fig.3) where the mean of insulin concentration (pmol/L) is displayed per the corresponding experimental group. The normal concentration of the insulin protein in the plasma of healthy Wistar rats is 93.54 ± 2 pmol/L. [18] The results below clearly show that the highest mean of insulin

concentration (pmol/L) is found in experiment 2: group 4 (2.4) taking CBD 50mg/kg treatment for 14 days. This result aligns with the previously obtained data showing the improvement and control of the fasting glycemia levels and body weights of the same rats. Thus, these controlled diabetes symptoms with the increased insulin concentration suggest that the CBD 50mg/kg oral dosage is effective in this rat model.

One-way ANOVA was done to examine the statistical significance of the insulin concentration for each experiment and each group. The test showed significance (p-value <0.05) for each experimental group when compared to the corresponding control group.

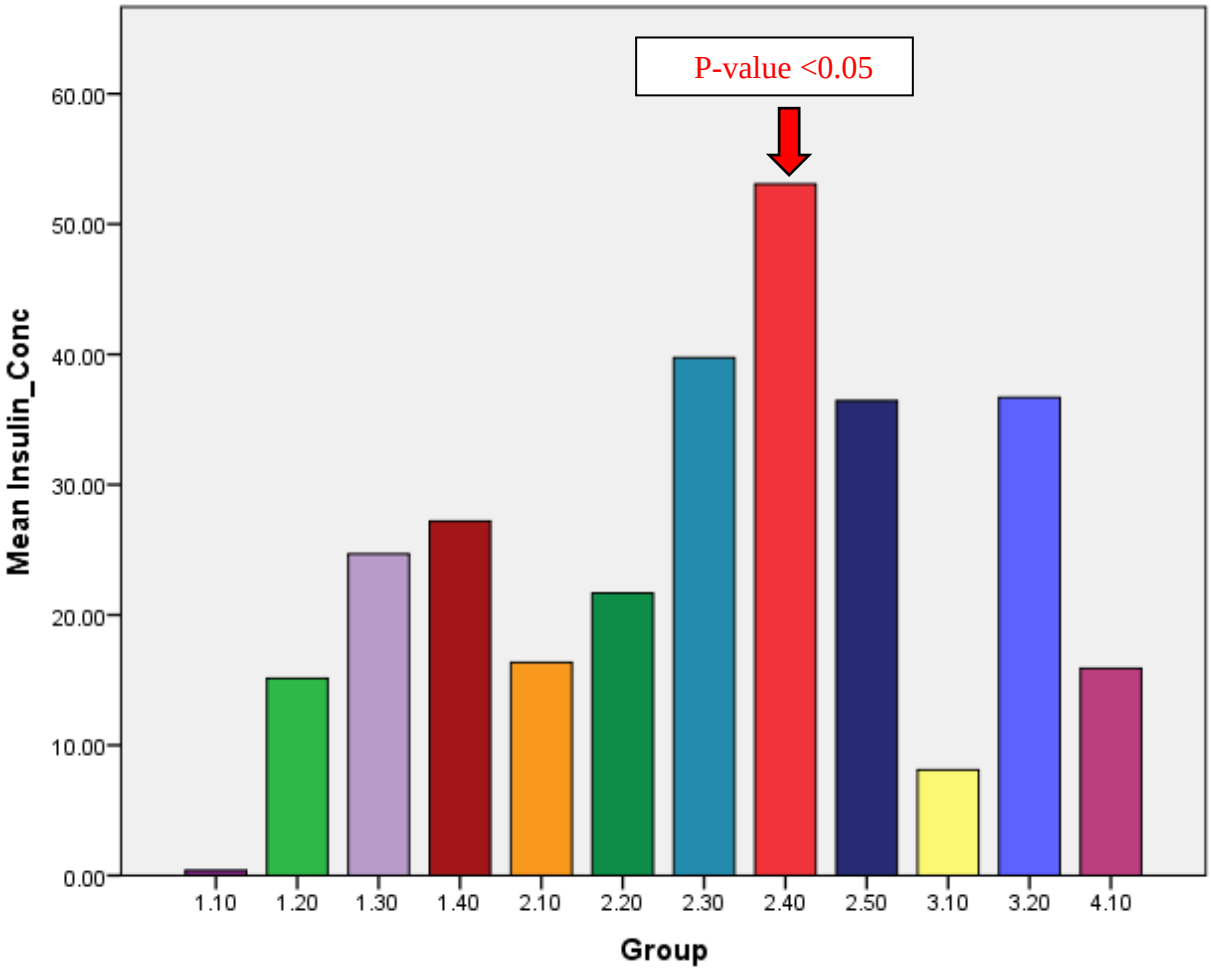

Figure 3. Mean of plasma insulin concentration (pmol/L) per experiment: per group. Groups of Experiment 1 (1, 2, 3 & 4) are labeled as "1.1, 1.2, 1.3 & 1.4" in the above chart respectively. Groups of Experiment 2 (1, 2, 3, 4, & 5) are labeled as "2.1, 2.2, 2.3, 2.4 & 2.5) correspondingly. In the same order, the groups of Experiment 3 are labeled "3.1 & 3.2", and the group of Experiment 4 is noted as "4.1". Here, the p-value <0.05 corresponds to the statistical significance when the insulin concentration was compared for the test groups with their respective control group. The highest insulin concentration is clearly seen in group 4 of Experiment 2 "2.4" (50mg/kg for 14 days).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

It is important to examine the effects of CBD as a treatment option for juvenile diabetes. In this study, the toxicity results of CBD were consistent with previous research indicating its safety and tolerability. In addition, the NOAEL dose of CBD= 150mg/kg was also consistent with previous literature indicating increased adverse events with this dose. In this study, the rats of experiment 2, group 5 were taking CBD 150mg/kg and they presented severe diarrhea and blindness as adverse events related to CBD 150mg dosing. Now, for the efficacy of CBD in treating diabetes it is important to note that there were no similar studies conducted to be able to compare the results obtained. However, from the results, we found that the CBD 50mg/kg dose taken for 14 days (experiment 2, group 4) was adequate in controlling the blood glycemia values with approximately 71.5% of the rats having their blood glycemia values in the normal range. Moreover, the 50mg/kg dosing taken for 14 days was also effective in controlling the rats' weights and reducing the progressive weight loss that was seen in other groups. It was important to note that the 50mg/kg CBD dose started to be effective after a minimum of 1 week of treatment with the best result after 2 weeks of treatment. Treatment with 50mg/kg CBD for 1 or 4 days was not effective. The insulin concentration results from the ELISA experiment align with our findings where the group that took 50mg/kg CBD for 14 days had a mean insulin concentration of 53.07 pmol/L which is the highest and closest to the normal range of insulin in healthy Wistar rats. A similar study conducted in 2017 on the effects of Aloe Vera extract treatment for STZ-induced diabetic Wistar rats showed similar plasma insulin

results.[19] The insulin secretion started to increase from the 2nd week of treatment with Aloe Vera with blood-glucose-lowering effects suggesting pancreatic islet cell rejuvenation.[19]

On the other hand, the 150mg/kg dosing taken for 14 days (experiment 2, group 5) did not show adequate response to treatment in terms of blood glycemia control where only 42.8% of rats had glycemia values within the normal range which might be linked to high CBD effect on cortisol release in the body. Also, the mean insulin concentration (=36.45 pmol/L) that was found in the plasma of these rats was not significant to enable the rats to control diabetes. Therefore, the 150mg/kg dose of CBD was toxic and ineffective to treat hyperglycemia.

Now, the 15mg/kg dose that was given to rats across the 3 experiments (acute for 24 hours, sub-chronic for 14 days, and chronic for 30 days) did not show any significant effect in controlling hyperglycemia of the rats across the different treatment periods, nor did it show an improved insulin concentration in the plasma. However, it did show some control in the weights of the rats across the different treatment periods. Hence, this dosing regimen is not sufficient in controlling hyperglycemia for type 1 diabetes and should not be considered for future studies.

The last dosing regimen that was given to the rats was 30mg/kg in experiment 2, group 3 for a total period of 14 days. This treatment did not control hyperglycemia in the rats, but it did maintain the weights of the rats and slowed down the weight loss. This dosing regimen had the second-highest mean insulin concentration level of 39.74 pmol/L. However, it was not sufficient to control diabetes in this rat group.

5.2 Conclusion

This study confirmed the safety of CBD when administered in different dosing regimens per body weight with NOAEL= 150mg/kg. The results we obtained gave

promising data for considering CBD as an oral nutraceutical supplement for juvenile diabetes at a recommended dose of 50mg/kg which is dose-dependent.

Further research is recommended to reinforce the therapeutic potential of 50mg/kg of CBD treatment. For instance, a long period of treatment for this dose should be studied containing a larger group to test if the insulin concentrations will be closer to normal when taken for a longer duration. In addition, it is recommended for future studies to test for CBD 100mg/kg efficacy. In this study, we did not test for the CBD 100mg/kg dose since this dose was already studied for and is in the market for epilepsy. Moreover, it is also recommended not to limit to male rats and to include females to study the hormonal interferences. In this study, only male rats were included to be able to study the efficacy of the drug without any risk of hormonal interference from the female rats. Finally, future studies should be designed to answer the following question: Do we need to reach the normal insulin level for diabetic patients or is the insulin level reached by the CBD 50mg/kg dose enough for their survival and well-being?

5.3 Limitation

In this study, we were not able to give the bottled treatment of CBD or vehicle to each rat alone. However, it is recommended that the treatment given via drinking bottles be administered to each rat separately to avoid any loss of treatment or vehicle doses. For instance, individual rats must be placed in a single cage with their respective treatment given via their drinking bottles. This way, each rat will completely drink the entire dose of treatment.

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ANNEX

ANNEX: RAW DATA

The following tables represent the raw data measured from all rats of the research study. The black segments correspond to deceased rats.

Table 3. Raw Data for Rats in Experiment 1 Group 1

Experiment 1: Group 1: Vehicle; Treatment Period: 24hours					
	Weights (g)			**Glycemia (mg/dl)**	
Rat Number	**Weight pre-STZ injection**	**Day 0 (3 days post-STZ injection**	**Day 1 after treatment initiation**	**Day 0 (3 days post-STZ injection**	**Day 1 after treatment initiation**
Rat 1	201	199	200	610	610
Rat 2	188	182	180	401	368
Rat 3	207	205	203	378	387
Rat 4	190	189	191	462	40
Rat 5	148	147	147	510	458

Table 4. Raw Data for Rats in Experiment 1 Group 2

Experiment 1: Group 2: CBD 15mg/kg; Treatment Period: 24hours					
	Weights (g)			**Glycemia (mg/dl)**	
Rat Number	**Weight pre-STZ injection**	**Day 0 (3 days post-STZ injection**	**Day 1 after treatment initiation**	**Day 0 (3 days post-STZ injection)**	**Day 1 after treatment initiation**
Rat 1	174	172	170	550	522
Rat 2	161	160	158	376	375
Rat 3	149	149	150	431	421
Rat 4	152	150	153	288	283
Rat 5	125	123	121	425	418

Table 5. Raw Data for Rats in Experiment 1 Group 3

Experiment 1: Group 3: CBD 50mg/kg; Treatment Period: 24hours					
	Weights (g)			**Glycemia (mg/dl)**	
Rat Number	**Weight pre-STZ injection**	**Day 0 (3 days psot-STZ injection**	**Day 1 after treatment initiation**	**Day 0 (3 days post-STZ injection)**	**24 Hours Expt**
Rat 1	140	137	135	389	302
Rat 2	200	199	198	472	9
Rat 3	204	203	204	360	321
Rat 4	135	133	136	301	247
Rat 5	122	121	128	387	281

Table 6. Raw Data for Rats in Experiment 1 Group 4

Experiment 1: Group 4: CBD 150mg/kg; Treatment Period: 24hours					
	Weights (g)			**Glycemia (mg/dl)**	
Rat Number	**Weight pre-STZ injection**	**Day 0 (3 days psot-STZ injection**	**Day 1 after treatment initiation**	**Day 0 (3 days psot-STZ injection**	**Day 1 after treatment initiation**
Rat 1	200	202	200	379	358
Rat 2	205	209	211	288	224
Rat 3	180	180	176	407	380
Rat 4	170	172	175	398	359
Rat 5	180	182	183	554	435

Table 7. Raw Data for Rats in Experiment 2 Group 1

Experiment 2: Group 1: Vehicle; Treatment Period: 14 days							
	Weights (g)				**Glycemia (mg/dl)**		
Rat Number	**Weight pre-STZ injection**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**
Rat 1	206	204	187	258	308	74	117
Rat 2	123	122	116	146	405	378	377
Rat 3	110	108	100	109	441	347	457
Rat 4	102	102	86		483	462	
Rat 5	103	99			610		
Rat 6	120	118	109		340	227	
Rat 7	133	134	123		397	382	
Rat 8	131	130	126		368	257	
Rat 9	100	98			610		
Rat 10	102	101			610		

Table 8. Raw Data for Rats in Experiment 2 Group 2

Experiment 2: Group 2: CBD 15mg/kg; Treatment Period: 14 days							
	Weights (g)				**Glycemia (mg/dl)**		
Rat Number	**Weight pre-STZ injection**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**
Rat 1	100	97	77		300	254	
Rat 2	100	98			610		
Rat 3	154	153	145	148	352	313	452
Rat 4	158	157	142	144	501	399	497
Rat 5	102	99			610		
Rat 6	162	160	145	139	515	25	60
Rat 7	134	134	120	113	302	138	56
Rat 8	151	150	137		489	22	
Rat 9	160	158	154	160	312	60	57
Rat 10	162	162	151	157	297	212	46

Table 9. Raw Data for Rats in Experiment 2 Group 3

Experiment 2: Group 3: CBD 30mg/kg; Treatment Period: 14 days							
	Weights (g)				**Glycemia (mg/dl)**		
Rat Number	**Weight pre-STZ injection**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**
Rat 1	105	104			610		
Rat 2	180	180	174	201	300	100	113
Rat 3	100	97			610		
Rat 4	119	118	91		348	303	
Rat 5	138	138	127	134	292	248	233
Rat 6	105	102			589		
Rat 7	113	110	104	106	510	459	508
Rat 8	138	136	128	125	413	316	227
Rat 9	102	99			590		
Rat 10	102	97			610		

Table 10. Raw Data for Rats in Experiment 2 Group 4

Experiment 2: Group 4: CBD 50mg/kg; Treatment Period: 14 days							
	Weights (g)				**Glycemia (mg/dl)**		
Rat Number	**Weight pre-STZ injection**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**
Rat 1	205	202	195	195	450	425	97
Rat 2	123	122	119	114	312	307	116
Rat 3	122	120	113	96	400	385	37
Rat 4	160	158	152	143	366	359	146
Rat 5	201	197	180	190	490	485	70
Rat 6	123	122	116	105	580	540	117
Rat 7	115	115	105		591	610	
Rat 8	178	177	169	160	455	436	261
Rat 9	148	146	128		610	590	
Rat 10	103	101			610		

Table 11. Raw Data for Rats in Experiment 2 Group 5

Experiment 2: Group 5: CBD 150mg/kg; Treatment Period: 14 days							
	Weights (g)				**Glycemia (mg/dl)**		
Rat Number	**Weight pre-STZ injection**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**
Rat 1	133	130	124		366	44	
Rat 2	113	112	108	110	356	206	300
Rat 3	120	120	115	112	410	295	395
Rat 4	130	129	127	127	340	70	124
Rat 5	164	163	157	183	362	73	116
Rat 6	105	102			589		
Rat 7	135	133	125	110	388	324	410
Rat 8	142	139	132	126	502	425	489
Rat 9	193	190	184	243	355	131	112
Rat 10	110	110	95		368	170	

Table 12. Raw Data for Rats in Experiment 3 Group 1

Experiment 3: Group 1: Vehicle; Treatment Period: 30 days											
	Weights (g)						Glycemia (mg/dl)				
Rat Number	**Weight pre-STZ injection**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 3 after treatment initiation**	**Week 4 after treatment initiation**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 3 after treatment initiation**	**Week 4 after treatment initiation**
Rat 1	182	180	178	133	138	151	580	518	473	415	361
Rat 2	132	130	121	63			443	406	9		
Rat 3	192	190	184	136	141	158	378	49	318	107	427
Rat 4	188	185	163	150	184	186	411	343	116	149	380
Rat 5	103	102	98				578	410			
Rat 6	122	120	116				511	372			
Rat 7	211	210	204	160	187	182	501	430	53	75	142
Rat 8	150	149	144	129	149	157	460	362	262	301	358
Rat 9	160	158	144	127	139	112	332	259	610	610	591
Rat 10	192	190	185	153	165	203	610	550	501	429	322
Rat 11	226	224	220	202	220	226	341	284	72	104	115
Rat 12	104	102	100				610	468			
Rat 13	132	129	120	91	105	140	425	387	377	432	390
Rat 14	145	145	144	90	100	104	388	294	543	445	502
Rat 15	162	160	159	132	140	160	353	289	344	401	389
Rat 16	153	152	147	109	135	146	351	344	322	368	330
Rat 17	160	160	154	122	138	153	279	121	61	415	512
Rat 18	135	133	122	89	96	98	302	220	398	572	568

Table 13. Raw Data for Rats in Experiment 3 Group 2

Experiment 3: Group 2: CBD 15mg/kg; Treatment Period: 30 days											
				Weights (g)				Glycemia (mg/dl)			
Rat Number	**Weight pre-STZ injection**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 3 after treatment initiation**	**Week 4 after treatment initiation**	**Week 0 (3 days post-STZ injection)**	**Week 1 after treatment initiation**	**Week 2 after treatment initiation**	**Week 3 after treatment initiation**	**Week 4 after treatment initiation**
Rat 1	158	155	146	115	150	171	412	360	90	136	116
Rat 2	116	112	99	76	87	96	361	246	210	367	361
Rat 3	100	98	74				580	472			
Rat 4	100	90	65				587	441			
Rat 5	123	122	117	95			540	90	36		
Rat 6	127	125	120	84	87	115	501	21	487	481	294
Rat 7	160	160	155	118	138	152	416	9	339	443	576
Rat 8	128	126	120	81	105	143	455	426	413	570	610
Rat 9	120	119	112	81	107		550	507	456	610	
Rat 10	193	190	184	220			610	610	32		
Rat 11	140	138	132	112	137	153	610	588	297	440	328
Rat 12	128	126	120	104	128		610	610	384	9	
Rat 13	140	135	127	97	97	123	400	77	67	135	402
Rat 14	210	209	205	170	175	195	456	436	63	315	431
Rat 15	130	128	120	99			441	240	36		
Rat 16	164	162	155	147	160	186	410	400	63	180	189
Rat 17	180	180	175	158	170	172	480	437	49	435	610
Rat 18	193	191	180	184	268	273	500	486	70	102	86

Table 14. Raw Data for Rats in Experiment 4 Group 1

Experiment 4: Group 1: 50mg/Kg/Day; Treatment Period: 4 days					
	Weights (g)			**Glycemia (mg/dl)**	
Validation 8 rats: 50mg/kg	**Weight pre-STZ injection**	**Day 0 (3 days psot-STZ injection**	**Day 4 after treatment initiation**	**Day 0 (3 days psot-STZ injection**	**Day 4 after treatment initiation**
Rat 1	130	126	115	610	610
Rat 2	190	188	169	610	610
Rat 3	195	190	185	610	531
Rat 4	120	115	108	610	610
Rat 5	136	133	125	610	610
Rat 6	120	116	109	610	610
Rat 7	136	133	121	610	610
Rat 8	148	144	137	610	610