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Nephroprotective effect of Cannabis sativa oil nanoemulsion in rats exposed to valproic acid during the intrauterine period

Efeito nefroprotetor da nanoemulsão de óleo de *Cannabis sativa* em ratos expostos ao ácido valpróico durante o período intrauterine

Efecto nefroprotector de la nanoemulsión de aceite de Cannabis sativa en ratas expuestas a ácido valproico durante el período intrauterine

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ABSTRACT

Objective: To evaluate the therapeutic effects of Cannabis sativa oil nanoemulsion (CBDON) on renal alterations induced by valproic acid (VPA) in rats. **Methods:** Pregnant female Wistar rats were exposed to VPA, and their offspring received treatment with CBD nanoemulsion at concentrations of 1% and 2%. After 60 days, evaluations were performed by means of body biometry, renal morphometry and oxidative stress analyses, with quantification of malondialdehyde (MDA) and antioxidant enzyme activity. Data were analyzed by descriptive and inferential statistics. **Results:** VPA caused a reduction in body weight and alterations in renal structures, such as decreased glomerular area and diameter. Treatment with CBD nanoemulsion, especially at a concentration of 2%, showed a positive effect on the recovery of renal morphometric parameters and on the reduction of oxidative stress, partially reversing the damage caused by VPA. An increase in the activity of antioxidant enzymes and a decrease in MDA were observed in the groups treated with CBD. **Conclusion:** The study suggests that CBD nanoemulsion may have a nephroprotective effect, mitigating the renal changes induced by VPA during the intrauterine period.

Keywords: Kidney, Oxidative stress, Nanoemulsion.

RESUMO

Objetivo: Avaliar os efeitos terapêuticos da nanoemulsão de óleo de Cannabis sativa (CBDON) nas alterações renais induzidas pelo ácido valproico (VPA) em ratos. **Métodos**: Fêmeas grávidas de ratas Wistar foram expostas ao VPA, e seus filhotes receberam tratamento com nanoemulsão de CBD nas concentrações de 1% e 2%. Após 60 dias, avaliações foram feitas por meio de biometria corporal, morfometria renal e análises de estresse oxidativo, com quantificação de malondialdeído (MDA) e atividade de enzimas antioxidantes. Os dados foram analisados por estatística descritiva e inferencial. **Resultados**: O VPA causou redução no peso corporal e alterações nas estruturas renais, como diminuição da área e diâmetro glomerular. O tratamento com a nanoemulsão de CBD, especialmente na concentração de 2%, mostrou um efeito positivo na recuperação dos parâmetros morfométricos renais e na redução do estresse oxidativo, revertendo

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parcialmente os danos causados pelo VPA. Foi observado aumento na atividade de enzimas antioxidantes e diminuição do MDA nos grupos tratados com CBD. **Conclusão**: O estudo sugere que a nanoemulsão de CBD pode ter um efeito nefroprotetor, mitigando as alterações renais induzidas pelo VPA durante durante o período intrauterino.

Palavras-chave: Rim, Estresse oxidativo, Nanoemulsão.

RESUMEN

Objetivo: Evaluar los efectos terapéuticos de la nanoemulsión de aceite de Cannabis sativa (CBDON) sobre las alteraciones renales inducidas por el ácido valproico (VPA) en ratas. **Métodos:** Ratas Wistar hembras preñadas fueron expuestas a VPA y sus crías recibieron tratamiento con nanoemulsión de CBD en concentraciones de 1% y 2%. Después de 60 días, se realizaron evaluaciones mediante biometría corporal, morfometría renal y análisis de estrés oxidativo, con cuantificación de la actividad de malondialdehído (MDA) y enzima antioxidante. Los datos fueron analizados mediante estadística descriptiva e inferencial. **Resultados:** El VPA provocó una reducción del peso corporal y cambios en las estructuras renales, como una disminución del área y el diámetro glomerular. El tratamiento con nanoemulsión de CBD, especialmente a una concentración del 2%, mostró un efecto positivo en la recuperación de los parámetros morfométricos renales y la reducción del estrés oxidativo, revirtiendo parcialmente el daño causado por el VPA. Se observó un aumento de la actividad de las enzimas antioxidantes y una disminución de MDA en los grupos tratados con CBD. **Conclusión:** El estudio sugiere que la nanoemulsión de CBD puede tener un efecto nefroprotector, mitigando los cambios renales inducidos por VPA durante el período intrauterino.

Palabras clave: Riñón, Estrés oxidativo, Nanoemulsión.

INTRODUCTION

Valproic acid (VPA), structurally corresponding to 2-propyl-pentanoic acid, is a short-chain branched fatty acid (ADEWOLE KE, et al., 2021), metabolized by conjugation with glucuronic acid, mitochondrial β-oxidation, and cytochrome P450-dependent ω-oxidation (TAN L, et al., 2010). Initially used in the industry as an organic solvent, its use was later expanded to medicine due to its therapeutic potential, especially in anticonvulsant treatment (BRACONNIER A, et al., 2018). VPA is a medication used for epilepsy and certain neurological and psychiatric conditions (ZHU MM, et al., 2017).

The use of VPA is generally well tolerated by individuals who take it (PERUCCA E, 2002). However, there are limitations with its prolonged administration, associated with the gradual loss of its efficacy and the occurrence of adverse effects, which may lead to restriction or discontinuation of treatment in some cases (GAYAM V, et al., 2018). Some side effects attributed to this drug include weight gain, hair loss, gastrointestinal disturbances, as well as its potential to induce teratogenicity, reproductive toxicity, nephrotoxicity, and hepatotoxicity (SAFDAR A e ISMAIL F, 2023). For example, the use of VPA in pregnant women must be carefully evaluated due to its teratogenic potential, which can cause toxicity and result in behavioral abnormalities and alterations in various organs, including the respiratory, cardiovascular, genitourinary, gastrointestinal, endocrine, and skeletal systems (ALSDORF R e WYSZYNSKI D, 2005). These effects vary depending on the stage of fetal development, the dose-effect relationship, and the maternal-fetal genotype (AGUILAR S, et al., 2016).

The nephrotoxic potential of valproic acid (VPA) is associated with oxidative stress, mitochondrial deficiencies, fibrosis, and inflammation in kidney tissue, according to experimental and clinical studies (HAMED SA, 2017). Observed effects include an increase in malondialdehyde (MDA), a marker of lipid peroxidation, as well as oxidative stress markers like xanthine oxidase and carbonylated protein, alongside a reduction in antioxidants such as glutathione. Mitochondrial deficiencies, including reduced succinate dehydrogenase (SDA) activity, low ATP levels, and increased reactive oxygen species (ROS), also contribute to kidney injury (HEIDARI R, et al., 2018). VPA increases inflammatory and apoptotic markers, such as IL-1β,

TNF-α, caspase-3, and NF-κB (GAD AM, 2018). It is also linked to genotoxic alterations and Fanconi syndrome in epileptic children (ADEWOLE KE, et al., 2021). However, the kidneys play a fundamental role in maintaining body homeostasis by excreting metabolic waste and preserving essential substances, such as proteins, electrolytes, and water. The use of nephrotoxic drugs can compromise their function, leading to renal failure. Therefore, it is crucial to monitor kidney function in patients using these medications, as they can cause acute or chronic kidney injury (MELLO PA, et al., 2021). Studies suggest that prolonged use of VPA rarely induces proximal tubular injury, but often causes mild proximal tubular involvement, with isolated tubular proteinuria, mainly N-acetyl-β-glucosaminidase, also being reported (ANGUISSOLA G, et al., 2023). Current data suggest that renal function can be modulated by the endocannabinoid system, which could be a viable therapeutic intervention route for kidney dysfunction (CHUA JT, et al., 2019).

In light of this, Cannabis sp. becomes a viable alternative as its therapeutic use is increasingly widespread (BARRETO LAA, 2002). The medicinal potential of Cannabis sp. is related to its chemical compounds, including phytocannabinoids, nitrogen compounds, amino acids, proteins, aldehydes, hydrocarbons, simple acids and fatty acids, glycosides, and vitamins (HONÓRIO KM, etal., 2006). Several studies have shown that CBD, a cannabis compound, has positive effects on various neurological and inflammatory disorders (URITS et al., 2020). However, CBD has low water solubility, resulting in low oral bioavailability (GASTON TE, et al., 2017). To improve bioavailability, the preparation of nanoemulsions provides enhanced solubility (CHEN O, et al., 2020). Given this, the present work aimed to analyze the benefits of a nanoemulsion based on Cannabis sp. oil and its therapeutic use in treating renal alterations caused by the use of valproic acid during the intrauterine period.

METHOD

Preparation of nanoemulsions

The experiments were performed at the *Universidade Federal Rural de Pernambuco*, Brazil. The preparation of CBD-rich corn oil nanoemulsion (CBDON) was carried out according to Silva et al. (2024) by the high-energy emulsification method.

Ethics approval

The protocol adopted is by the Brazilian guide for Animal Experimentation (COBEA), filed and approved by the Committee on Ethics in the Use of Animals (CEUA) from the Federal Rural University of Pernambuco under number 2881210120

Animals

Ten Wistar female rats of reproductive age (200±250 g), from the Animal Facility of the Department of Veterinary Morphology at the Federal Rural University of Pernambuco (DMV-UFRPE), were used. They were kept under standardized conditions of ambient temperature (23±2º C) and a light/dark cycle (12/12 hours daily). They were housed in collective polypropylene cages (maximum of five animals per cage) with food and water *ad libitum.*

The females were mated, and the pregnant ones were divided into two groups: the first group (saline; n=2) received a single intraperitoneal injection of saline solution on the 12.5th day of gestation, while the second group (VPA; n=8) received a single intraperitoneal injection of valproic acid (500 mg/kg) at the same time.

From the 21st postnatal day (PND), the offspring born to the VPA-treated females were divided into three groups (n=8): the VPA group (positive control), which received 1 ml of saline solution orally; the CBDON1 group, which received 1 ml of Cannabis sativa oil nanoemulsion rich in CBDON at 1% (10 mg/ml) orally; and the CBDON2 group, which received 1 ml of Cannabis sativa oil nanoemulsion rich in CBDON at 2% (20 mg/ml) orally. The offspring from the saline-treated females formed the negative control group and received 1 ml of saline solution orally. All animals were treated by gavage twice a day for a period of 60 days.

Collection of Material and Light Microscopy

24 hours after the last treatment administration, the animals were weighed and anesthetized with an intraperitoneal injection of ketamine (60 mg/kg) and xylazine (20 mg/kg). Once anesthesia was confirmed, the kidneys were collected and weighed. The right kidneys were dissected, weighed, and fixed by immersion in Karnovsky's fixative (4% glutaraldehyde + 2.5% paraformaldehyde in 0.01M sodium cacodylate buffer, pH 7.4), and prepared for histomorphometric and pathological evaluation. The left kidneys were frozen in liquid nitrogen and stored in a -80ºC freezer until oxidative stress analysis.

The material for histology was dehydrated in an ascending ethanol series and embedded in paraffin. Semi-serial 4 µm sections were obtained using a rotary microtome (RM 2255, Leica Biosystems, Nussloch, Germany), with at least a 40 µm interval between sections. The histological slides were stained with hematoxylin and eosin. For morphometric analysis, images were captured using a bright-field photomicroscope (Olympus BX-53, Tokyo, Japan) equipped with a digital camera (Olympus AX 70 TRF, Tokyo, Japan). All images were analyzed using the Image J® software (National Institutes of Health, USA).

Renal Morphometry

The volumetric density of renal components (glomerulus, capsule, renal tubule, and interstitium) was determined by observing histological fields, using a grid of 266 points over the images, in 10 fields per animal at 200x magnification. The volumetric proportion (%) of each evaluated component was calculated using the equation Vv = PP / PT * 100, where PP is the number of points over the structure of interest and PT is the total number of points in the histological area (CUPERTINO MC, et al., 2023). For the glomerulus, the glomerular diameter was calculated by measuring 20 glomeruli; the glomerular area, glomerular capsule area, and subcapsular space area were also calculated. Additionally, the number of glomeruli per area was calculated by analyzing 10 fields per animal, from which the glomerular density was obtained using the following formula: glomerular density = Number of glomeruli per field / field area.

Oxidative Stress

The samples were homogenized in potassium phosphate buffer (pH 7.4, 0.2M) containing 1M EDTA, using a homogenizer (OMNI), and centrifuged (13,800 x g at 4°C for 10 min). The supernatant was used to determine the concentrations of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST), total antioxidant activity, as well as the levels of nitric oxide (NO) and malondialdehyde (MDA), along with the total protein concentration. The biochemical data were normalized to the total protein levels in the supernatant. The analyses were performed in duplicate. All enzymatic activities were determined in duplicate using a spectrophotometer (UV-Mini 1240, Shimadzu) or an ELISA reader (Thermo Scientific, Waltham, MA, USA).

NO production was indirectly determined through the nitrite/nitrate content using the standard Griess reaction (RICART-JANÉ D, et al., 2002). CAT activity was assessed by measuring the rate of H2O2 decomposition (Aebi 1984), SOD activity was determined following Siddiqui IA, et al. (2005), and GST activity through the formation of 1-chloro-2,4-dinitrobenzene (CDNB) conjugate (HABIG WH, et al., 1974). The total antioxidant capacity (FRAP) was measured according to Benzie IF e Strain JJ (1996). MDA levels were measured following the protocol reported by Gutteridge and Halliwell (GUTTERIDGE JMC e HALLIWELL B, 1990). Total protein concentration was measured using the Lowry method (LOWRY OH, et al. 1951) and used to standardize the stress data.

Statistical Analysis

The results were assessed for normality using the Shapiro-Wilk test, followed by analysis of variance (ANOVA) and the Student-Newman-Keuls test. The STATISTICA for WINDOWS 3.11 software was used, with a significance level set at $p \leq 0.05$. All results were expressed as mean ± standard deviation.

Additionally, Principal Component Analysis (PCA) was performed to identify possible clusters, aiming to eliminate redundancies and define the most important variables for group separation. The data were normalized, and the importance level of each variable was determined by the eigenvector values

(MCGARIGAL K, et al., 2000). Substantial correlation values for each attribute in Principal Components (PC) 1 and 2 were reported. The importance level of each PC was determined using the Broken-stick method, where eigenvalues exceeding the expected value were retained for interpretation. The analyses were conducted using the Fitopac 2.1.2.85 program.

RESULTS

Body and Renal Biometric Analyses

In the present study, a significant variation in the weight of the evaluated animals was observed. All treated groups had lower body weights compared to the control group; however, the animals in the CBDON2 group had body weights closest to the control group (**Table 1**). This indicates that treatment with VPA reduced the animals' average body weight, while treatment with CBDON2 helped increase their body weight (**Table 1**).

Table 1 - Body and renal biometry of adult rats exposed to valproic acid during the gestational period and treated with nanoemulsion of CBDON-Rich oil in the postnatal period.

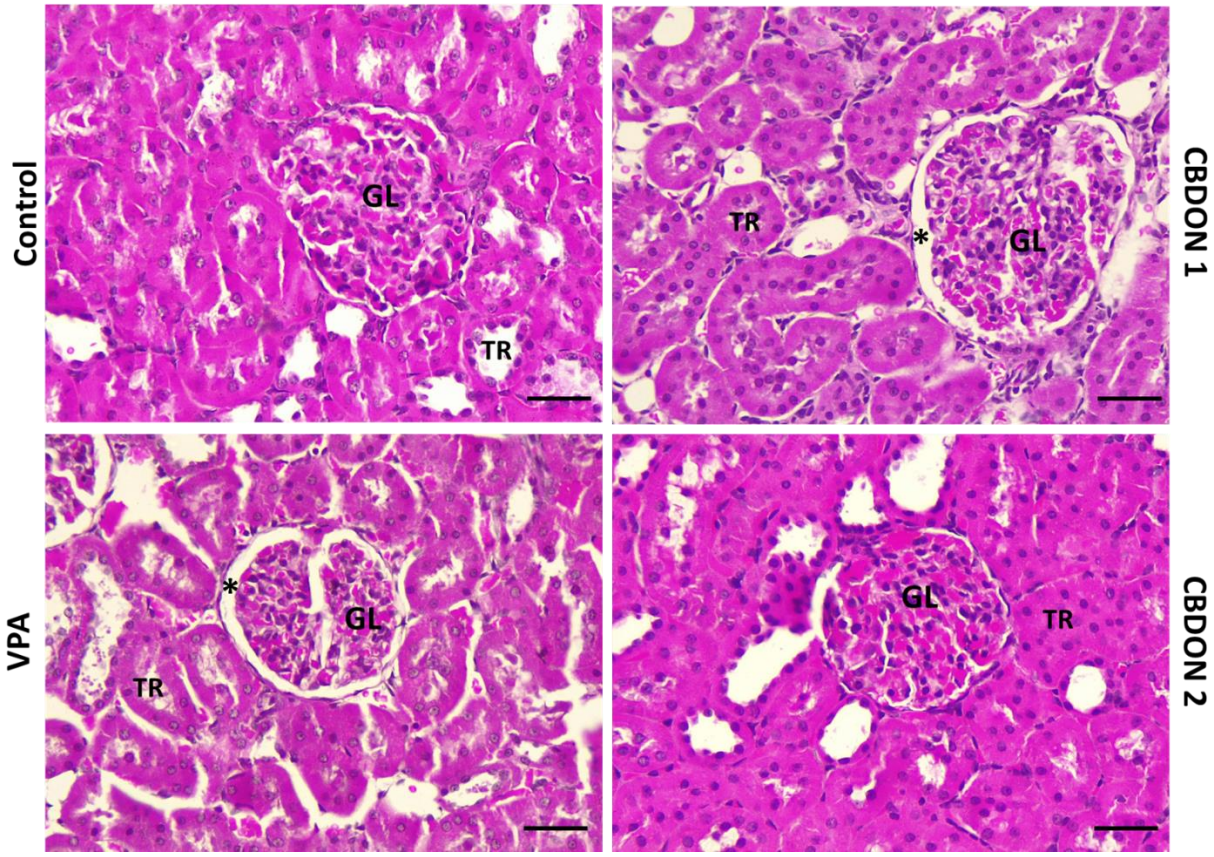
	Control	VPA	CBDON1	CBDON2
Body weight	369,66 ± 15,66[a]	287 ± 28,18[b]	300,33 ± 28 [b]	340,16 ± 18,20 [b]
Kidney weight	1,50 ± 0,12 [a]	1,21 ± 0,13 [b]	1,42 ± 0,22 [a]	1,80 ± 0,13 [b]
Relative weight	0,40 ± 0,040 [a]	0,42 ± 0,035 [a]	0,47 ± 0,054 [b]	0,52 ± 0,026 [b]

VPA – Group that received valproic acid; CBDON1 – Group treated with 1% Cannabis; CBDON2 – Group treated with 2% Cannabis. Data expressed as mean±SD. Different letters among treatments indicate significant differences (p≤0.05) (Student-Newman-Keuls test).
Source: Lopes MT, et al., 2025.

Renal Morphometry

In the morphometric analysis, it was observed that the glomerular diameter in the VPA group decreased by approximately 8.21% compared to the control group. When analyzing the treated groups, a normalization of these parameters was noted, indicating that Cannabis sp. aids in mitigating the alterations caused by VPA (**Figure 1** and **Table 2**). Additionally, a statistical difference was observed in the glomerular area, which was approximately 15.55% smaller in the VPA group compared to the control group. However, no statistical differences were found in the proportions of any of the analyzed structures (**Table 3**). There was no significant difference in the volume of the glomerulus, capsule, and renal tubule between the control group and the VPA group. Nonetheless, in the group treated with 2% Cannabis extract, an increase in the volume of these structures was observed (**Table 3**). No significant difference was found in the volume of the glomerulus, capsule, and renal tubule between the control and VPA groups, although the 2% treatment group showed an increase in the volume of these structures (**Table 3**).

Figure 1 - Photomicrograph of renal parenchyma stained with Hematoxylin-Eosin (HE) from adult Wistar rats exposed to valproic acid during the gestational period and treated with nanoemulsion of CBDON-rich oil in the postnatal period.



VPA – Group exposed to valproic acid; CBDON1 – Group treated with 1% Cannabis; CBDON2 – Group treated with 2% Cannabis. GL: Glomerulus. TR: Renal Tubule. *: Subcapsular space. Scale bar = 20 µm.L.
Source: Lopes MT, et al., 2025.

Table 2 - Renal morphometry of adult rats exposed to valproic acid during the gestational period and treated with nanoemulsion of CBDON-Rich oil in the postnatal period.

	Control	VPA	CBDON1	CBDON2
Glomerular Diameter	57,91 ± 3,80 [a]	53,16 ± 1,36 [b]	62,30 ± 4,61 [a]	60,90 ± 3,56 [a]
Glomerulus Area	3532,56 ± 398 [a]	2975,03 ± 177,50 [b]	3905 ± 575 [a]	3643,40 ± 381,60
Capsule Area	4304,18 ± 518,50 [a]	4306,60 ± 268,80 [a]	4898,61 ± 1065,16 [a]	4583, 70 ± 516,1 [a]
Subcapsular Space Area	771,63 ± 380,30 [a]	1331,55 ± 145,58 [a]	993,70 ± 515,36 [a]	940,33 ± 235,41 [a]
Glomerular Density	$6x10^{-6} \pm 1x10^{-6}$ [a]	$6x10^{-6} \pm 7x10^{-7}$ [a]	$6x10^{-6} \pm 7x10^{-7}$ [a]	$6x10^{-6} \pm 1x10^{-6}$ [a]

VPA – Group exposed to valproic acid; CBDON1 – Group treated with 1% Cannabis; CBDON2 – Group treated with 2% Cannabis. Data expressed as mean±SD. Different letters among treatments indicate significant differences (p≤0.05) (Student-Newman-Keuls test).
Source: Lopes MT, et al., 2025.

Table 3 - Proportion and renal volume of adult rats exposed to valproic acid during the gestational period and treated with nanoemulsion of CBDON-Rich oil in the postnatal period.

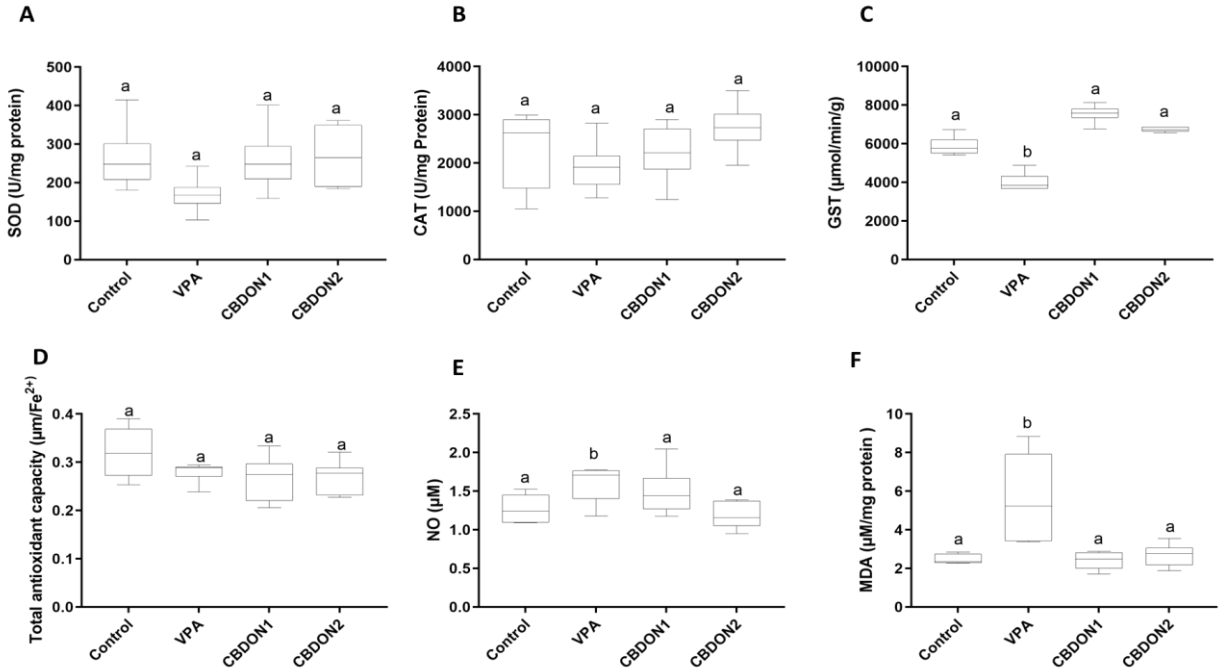
		Control	VPA	CBDON1	CBDON2
Glomerulus	**V.**	0,19 ± 0,033 [a]	0,16 ± 0,041 [a]	0,20 ± 0,056 [a]	0,24 ± 0,050 [a]
	P.	12,73 ± 2,90 [a]	13,01 ± 2,12 [a]	13,88 ± 3,22 [a]	13,63 ± 2,31 [a]
Capsule	**V.**	0,073 ± 0,013 [a]	0,070 ± 0,017 [a]	0,078 ± 0,016 [a]	0,10 ± 0,019 [b]
	P.	4,94 ± 0,95 [a]	5,72 ± 0,88 [a]	5,53 ± 0,78 [a]	5,91 ± 0,86 [a]
Tubule	**V.**	1,22 ± 0,14 [a]	0,96 ± 0,080 [a]	1,13 ± 0,19 [a]	1,14 ± 0,10 [b]
	P.	81,45 ± 3,22 [a]	79,92 ± 3,11 [a]	79,87 ± 4,75 [a]	80,20 ± 3,10 [a]
Interstitial space	**V.**	1,50 ± 0,12 [a]	1,20 ± 0,13 [a]	1,41 ± 0,22 [a]	1,78 ± 0,13 [b]
	P.	99,12 ± 0,72 [a]	98,65 ± 0,64 [a]	99,27 ± 1,09 [a]	99,74 ± 0,27 [a]
Subcapsulaspace	**V.**	0,0036 ± 0,0031 [a]	0,0057 ± 0,0027 [a]	0,0033 ± 0,0049 [a]	0,0013 ± 0,0013 [a]
	P.	0,88 ± 0,72 [a]	1,35 ± 0,64 [a]	0,73 ± 1,09 [a]	0,26 ± 0,27 [a]

V. – Volume; P. – Proportion; VPA – Group exposed to valproic acid; CBDON1 – Group treated with 1% Cannabis; CBDON2 – Group treated with 2% Cannabis. Data expressed as mean±SD. Different letters among treatments indicate significant differences (p≤0.05) (Student-Newman-Keuls test).
Source: Lopes MT, et al., 2025.

Oxidative Stress Indicators

SOD levels did not show significant changes in any of the evaluated groups, as was also observed in the analysis of CAT and total antioxidant capacity (**Figures 2A, 2B,** and **2D**, respectively).On the other hand, GST levels in the VPA group were lower compared to the control group, while the CBDON1 and CBDON2 groups had values that were statistically similar to the control group (**Figure 2C**). This indicates lipid peroxidation occurred in the untreated VPA (autistic) group, but treatment with Cannabis reversed this process. The nitric oxide (NO) results showed a similar trend (**Figure 2E**). The results from the malondialdehyde (MDA) analyses showed a significant difference in this biomarker in the VPA group compared to the control group. In contrast, the treated groups had values that were statistically similar to the control group (**Figure 2F**).

Figure 2 - Effect of CBDON treatment on the oxidative status of rats with autism induced by valproic acid.

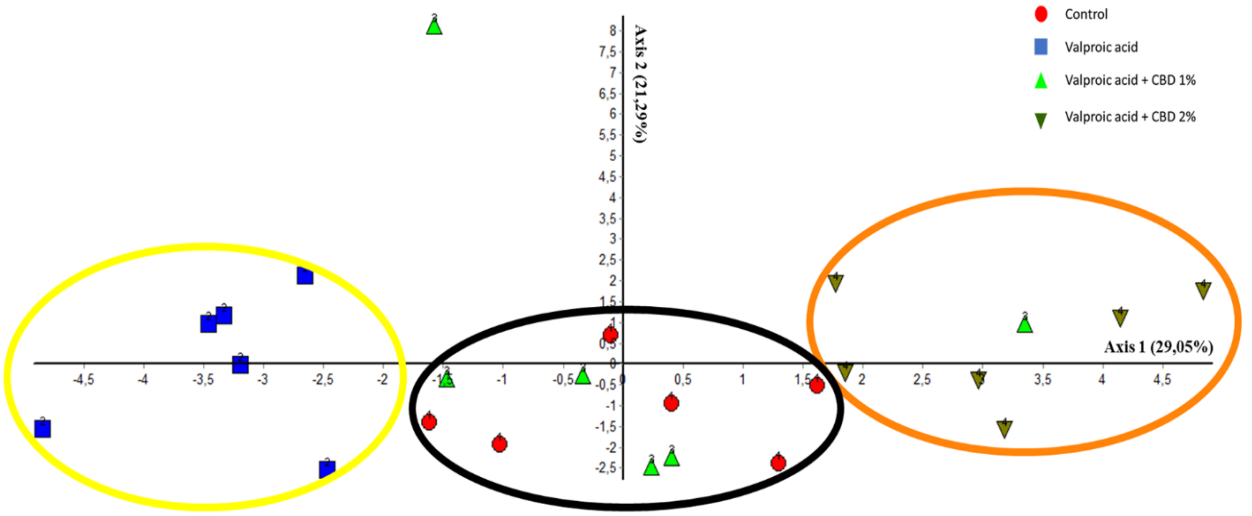


A: SOD – Superoxide dismutase; B: CAT – Catalase; C: GST – Glutathione; D: Total antioxidant capacity; E: NO – Nitric oxide; F: MDA – Malondialdehyde. Different letters among treatments indicate significant differences (p≤0.05) (Student-Newman-Keuls test). **Source:** Lopes MT, et al., 2025.

Principal Component Analysis

The total variation of the data was 50.34%, with the most important attributes for distinguishing the groups having correlation values > 0.6 (**Figure 3**). For PC1 (horizontal axis), the most relevant attributes and their respective correlation values were GST (0.2216); MDA (-0.2008); NO (-0.2565); PC (0.2066); Kidney weight (0.3341); Relative weight (0.2080); Interstitial percentage (0.2538); Subcapsular space percentage (-0.2538); Glomerular volume (0.2344); Capsule volume (0.2700); Renal tubule volume (0.3006); Interstitial volume (0.3203); Subcapsular space volume (-0.2491).

Figure 3 - Principal Component Analysis (PCA).



In PC2 (vertical axis), the treatments were primarily separated by Capsule area (0.3373); Subcapsular space area (0.2950); Glomerular percentage (0.3535); Capsule percentage (0.2440); Renal tubule percentage (-0.3770); Glomerular volume (0.3115). This resulted in three distinct groups: the group that received valproic acid without treatment, the group that received valproic acid and was treated with CBD 1%, and the negative control group, along with a third group formed by the group that received valproic acid and was treated with CBD 2%.
Source: Lopes MT, et al., 2025.

DISCUSSION

Our data indicate that intrauterine exposure to valproic acid was able to cause a reduction in body weight, kidney weight, relative renal weight, as well as a reduction in glomerular diameter and area, in addition to causing oxidative and nitrosative stress. However, treatment with the cannabis nanoemulsion was able to reverse this condition at a 1% dose, although body and organ weights had not returned to normal within this treatment period.

Valproic acid, classified as an antiepileptic drug, is widely recognized for its association with weight gain, contributing to overweight or obesity (BITON V, 2003). However, in groups exposed to valproic acid during the intrauterine period, a reduction in body weight was observed. These findings are consistent with the data reported by Chen O, et al. (2020), demonstrating that experimental use of valproic acid (VPA) induces alterations in gut microbiota, potentially leading to weight loss. Similar results have been documented in studies analyzing VPA exposure during both the prenatal and postnatal periods (ORNOY A, et al., 2019; MATTOS BS, et al., 2020), further supporting the notion that experimental VPA exposure may induce weight loss. Although groups receiving CBD nanoemulsion at concentrations of 1% or 2% exhibited partial body weight recovery, this recovery was insufficient to fully restore normal weight in these animals.

The reduction in kidney weight observed in the VPA group may indicate a response associated with pathophysiological mechanisms of progressive renal injury (REMUZZI G, et al., 2006). The primary renal disease associated with kidney shrinkage is chronic kidney disease (CKD). Polzin DJ (2010) reports that as

CKD progresses, nephron loss occurs, meaning that the more advanced the disease, the smaller the kidney dimensions. Hall AM, et al. (2014) reported that VPA can induce renal injury, typically manifesting as proximal tubular dysfunction. However, the underlying pathophysiological mechanisms of this damage remain incompletely understood. Furthermore, Heidari R, et al. (2018) observed that animals treated with valproic acid exhibited biochemical markers consistent with renal injury, in addition to histopathological alterations such as interstitial nephritis, tissue necrosis, and atrophy. In contrast, the CBDON2 group showed an increase in kidney weight, suggesting that cannabis may play a nephroprotective role. Regarding relative kidney weight—an index that represents the relationship between body weight and kidney weight—no significant changes were observed in the VPA group due to a proportional reduction in both body and kidney weight, thereby maintaining a constant index. However, differences were noted in the groups treated with CBD. In the CBDON1 group, an increase was observed only in body weight, whereas in the CBDON2 group, the increase was specifically in kidney weight.

Regarding glomerular analyses, a reduction in both glomerular area and diameter was observed. These changes may indicate a decrease in the glomerular filtration rate (GFR), a crucial parameter for evaluating renal function and staging chronic kidney disease (CKD) (PECOITS-FILHO R, 2004). The glomerulus, a highly specialized filtration structure within the kidney, is composed of various cell types, including glomerular endothelial cells, mesangial cells, podocytes, and parietal epithelial cells. The increase in capsule volume, tubule size, and interstitial space observed in the CBDON2 group may be associated with potential nephroprotective effects of cannabis. This suggests a physiological response of renal tissue to the presence of active CBD compounds, potentially promoting structural modifications in the kidney as an adaptive mechanism to counteract previous damage induced by VPA.

One potential factor contributing to these alterations is the increased production of reactive oxygen and nitrogen species. Although these molecules are continuously generated by cells as part of their metabolic processes (BHATTACHARYYA L, et al., 2014), an imbalance in their production can lead to oxidative and nitrosative stress, respectively. In the study conducted by Heidari R, et al. (2018), alterations in renal oxidative stress markers were observed in animals treated with VPA, indicating that VPA induced oxidative and nitrosative stress. This was supported by the observed increase in nitric oxide (NO) and malondialdehyde (MDA) levels. Elevated NO levels serve as an early indicator of oxidative stress and/or cell death (MONCADA S, 1991), as NO modulates inflammatory or anti-inflammatory responses depending on the cell type and stimulus (ADAMS HR, 1996). The increase in MDA levels is associated with lipid peroxidation, given that malondialdehyde is a byproduct of lipid peroxidation. Its accumulation can lead to tissue damage and is implicated in the pathogenesis of various diseases (GILLHAM B, et al., 1997; URSO ML, et al., 2003). This oxidative stress profile was observed exclusively in the VPA group, whereas the other groups did not exhibit similar patterns, indicating that treatment with CBD nanoemulsion was effective in mitigating the damage induced by VPA.

Regarding the activity of antioxidant enzymes and non-enzymatic antioxidants, a reduction in glutathione (GSH) activity was observed exclusively in the VPA group. This indicates that the first line of defense was insufficient to counteract the increase in reactive species, leading to the activation of secondary defense mechanisms. Glutathione is closely associated with detoxification processes, as it enhances the solubility of xenobiotics, facilitating their elimination (HABIG WH, 1974).

Glutathione plays a crucial role in preventing the accumulation of hydrogen peroxide (H_2O_2), a function of significant physiological importance. This is because H_2O_2, through Fenton and Haber-Weiss reactions involving iron and copper ions, leads to the formation of the hydroxyl radical (OH•), against which there is no enzymatic defense system (FERREIRA ALA, 1997; SCHNEIDER CD, 2004). Treatment with CBDON1 and CBDON2 did not alter the levels of superoxide dismutase (SOD), catalase (CAT), or glutathione S-transferase (GST), suggesting that an increase in antioxidant activity was unnecessary. This implies that CBD likely prevented tissue damage, maintaining redox homeostasis without requiring additional antioxidant response activation.

CONCLUSION

We conclude that intrauterine exposure to valproic acid (VPA) induces renal oxidative and nitrosative stress, leading to morphometric alterations such as a reduction in glomerular diameter and area, as well as a decrease in body and kidney weight. However, treatment with CBD-rich oil nanoemulsion demonstrated a nephroprotective effect, mitigating the structural and oxidative status alterations induced by VPA. The groups treated with CBD, particularly at a 2% concentration, exhibited partial recovery of body and kidney weight, along with normalization of oxidative stress markers, without the need for increased antioxidant enzyme activity. Thus, these findings suggest that CBD nanoemulsion may represent a promising therapeutic strategy for alleviating renal damage caused by VPA exposure.

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