



An assessment safety of acute and chronic oral toxicity studies of high cannabidiol (CBD) from female inflorescence of *Cannabis sativa* L. (Hemp) extract in Sprague-Dawley rats

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ABSTRACT

Cannabis sativa L. contains cannabidiol (CBD), a non-psychoactive cannabinoid with potential therapeutic effects including anxiolytic, hypnotic, and analgesic properties. With increasing global use of CBD-containing products, comprehensive evaluation of their long-term safety is essential. Although pharmacological studies support its benefits, data on the chronic toxicity of high-CBD hemp extracts remain limited. This study aimed to evaluate both the acute and chronic toxicities of high-CBD hemp extract derived from the feminized *Candida* (CD-1) hemp strain, containing 50.6 ± 1.2 % w/w CBD, in accordance OECD guidelines. Acute toxicity was evaluated according to OECD Test Guideline 423 by administering a single dose of 5,000 mg/kg to female rats, which were then observed for 14 days to estimate the LD₅₀. Chronic toxicity was evaluated under OECD Test Guideline 452 in male and female Sprague-Dawley rats administered daily oral doses of 0, 10, 20, and 40 mg/kg b.w./day for 180 days. Hematological, clinical chemistry and urinalysis parameters remained within normal ranges. No mortality or severe toxicological signs were observed during the study period. However, significant reductions in body weight and feed consumption were observed in the medium- and high-dose groups of females as well as the high-dose group of the males, which may be associated with the pharmacological activity of CBD. Centrilobular hepatocyte hypertrophy was observed in both sexes, accompanied by hypertrophy of the pars distalis of the pituitary gland and follicular cell hypertrophy in males. These findings are regarded as adaptive and

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non-adverse. Additional minimal to slight changes corresponded to spontaneous background alterations. These findings suggest that the high-CBD hemp extract exhibits low acute toxicity and does not induce significant chronic toxicity at doses up to 40 mg/kg/day in rats.

Keywords: *Cannabis sativa* L., cannabidiol, hemp, chronic toxicity, acute toxicity

1. Introduction

Medicinal plants are essential to global healthcare, with the World Health Organization (WHO) estimating that approximately 80% of the world's population relies on them for primary care. With over 390,000 identified plant species used in traditional medicine, their therapeutic potential continues to be a subject of extensive research.¹ In Thailand, herbal medicine has a long-standing history, with over 1,800 local species recognized for their therapeutic properties. Among these, *Cannabis sativa* L. has gained significant prominence in recent years.

Cannabis sativa L. (commonly referred to as hemp and locally known as “Kan-Chong” in Thailand) belongs to the Cannabaceae family and has been cultivated for over 10,000 years in Central Asia.² Historically, this species has been used extensively in traditional medicine across different cultures, and its diverse therapeutic potential continues to attract scientific attention.³ Hemp contains a broad spectrum of bioactive compounds, including terpenes, flavonoids, alkaloids, and over 90 identified cannabinoids, most notably tetrahydro-cannabinol (THC) and cannabidiol (CBD).⁴⁻⁶

Hemp primarily contains THC as its main constituent, with most of its properties being psychoactive, whereas, CBD is non-psychoactive and has attracted increasing scientific interest for its pharmacological effects.⁷ In the 1940s, Adam and colleagues in the UK first separated CBD from cannabis.⁸ In hemp, the CBD content typically ranges between 30% and 90%, often exceeding that of THC.⁹ CBD interacts with the endocannabinoid system primarily via CB1 and CB2 receptors, although it exhibits low affinity for both.¹⁰ The inflorescence has the highest CBD content (15.8-20.4%),

followed by leaves (>2%), stem (1-8%), and seeds (<0.02%).¹¹⁻¹³ Essential oils in female inflorescences can reach up to 24.9%.¹⁴

Numerous studies have explored the therapeutic benefits of CBD, including its potential for pain relief,¹⁵⁻¹⁷ insomnia,¹⁸ anxiety,¹⁹⁻²¹ and its antioxidant and anti-inflammatory properties.²²⁻²⁵ Previous sub-chronic toxicity studies have supported the non-toxic nature of hemp extract. Marx et al. (2018) found no toxic effects in a sub-chronic study of a hemp aerial component (96% CBD, <1% THC).²⁶ Similarly, Dziwenka et al. established No-Observed-Adverse-Effect Levels (NOAELs) of 400 mg/kg/day in male rats and 800 mg/kg/day in female rats over 90 days.²⁷ However, a significant gap remains in the scientific literature regarding the comprehensive safety assessment of a high-CBD extract from the inflorescence, particularly over a long-term exposure period exceeding 90 days. Most prior studies have focused on pure CBD isolate, which may not adequately represent the complex phytochemical composition and potential interactions among cannabinoids, terpenes, and other bioactive constituents present in full-spectrum hemp extracts. Therefore, a comprehensive long-term toxicity assessment of a high-CBD inflorescence extract is crucial to ensure its safety for widespread use.

This study is the first to evaluate the acute and chronic oral toxicities (180 days) of this extract in rats, utilizing standard methodology recognized by the Organization for Economic Co-operation and Development (OECD) to generate robust and internationally recognized safety data. The findings provide essential information for regulatory bodies and support the safe development of medicinal and dietary supplement products containing high-CBD hemp extracts.

2. Materials and Methods

2.1 Preparation of standardized high-CBD hemp extract.

Female inflorescences of *Cannabis sativa* L. (Hemp) were obtained from the High Research and Development Institute (Public Organization) in Chiang Mai, Thailand. Briefly, the plant was dried at 50°C for 24 hours and then grounded into a fine powder. Ethanol extraction was performed at -40°C using an Ultra Low Temperature Extraction (GRD Tech Co., Ltd, Thailand) with a material-to-solvent ratio of 1:5 (w/v). The extraction procedure was repeated four times to maximize yield. The combined extract contained CBD at a concentration of 50.58 ± 1.20 %w/w, as determined by validated analytical methods.

2.2 Animals and housing conditions

Male and female Sprague-Dawley rats (6-8 weeks old) were obtained from Nomura Siam International, Co., Ltd. (Thailand) and acclimatized for 7 days at the Center for Animal Research Naresuan University (NUCAR), Phitsanulok, Thailand. Animals were housed under controlled environmental conditions (22.0 ± 1.0 °C, $55 \pm 10\%$ relative humidity, and a 12:12 h light/dark cycle) with ad libitum access to a standard diet and reverse osmosis (RO) water.

2.3 Acute toxicity study

Acute oral toxicity was evaluated in accordance with the OECD Test Guideline No.423.²⁸ Female Sprague-Dawley rats (n=3) were administered a single oral dose of 5000 mg/kg body weight of high-CBD hemp extract, a limit dose anticipated to elicit signs of toxicity or mortality. Animals were observed individually at 30 minutes post-dosing and periodically during the first 24 hours, with special attention during the first 4 hours. Subsequent clinical observations were conducted daily for 14 days. Body weights were recorded on days 1, 3, 7, and 14. Clinical signs of toxicity and mortality were monitored throughout the observation period.

2.4 Chronic toxicity study

2.4.1 Dose selection

Human- equivalent CBD dosages were calculated based on therapeutic equivalence, yielding dosage levels of 10, 20 and 40 mg/kg. Dose selection was derived from the human Health-Based Guidance Value (HBGV), which in turn was established from the Lowest Observed Adverse Effect Level (LOAEL), representing the upper limit of acceptable daily intake.²⁹

2.4.2 Experimental design

Chronic Toxicity was evaluated following OECD Test Guideline No. 452.³⁰ Animals were divided into four groups, with 10 males and 10 females per group and two recovery groups consisting of 5 animals as follows:

Group I: Control group receiving corn oil

Group II: Low dose (LCBD), receiving 10 mg/kg high-CBD hemp extract

Group III: Medium dose (MCBD), receiving 20 mg/kg high-CBD hemp extract

Group IV: High dose (HCBd), receiving 40 mg/kg high-CBD hemp extract

Group V: Control recovery group (CS), receiving corn oil

Group VI: High dose recovery (HCBDS), receiving 40 mg/kg high-CBD hemp extract

All treatments were administered once daily via oral gavage for 180 days. Following the treatment period, animals in satellite groups were observed for an additional 28 days without treatment to assess the reversibility or persistence of any potential toxicology effects. At the end of the experimental period, all animals were fasted overnight before euthanasia.

2.4.3 Body weight and consumption monitoring

Body weight, food consumption, and water intake were recorded weekly throughout the duration of the study. At the end of the treatment or recovery period, all animals were fasted overnight (approximately 12-16 hours)

prior to euthanasia and subsequent sample collection for toxicological analysis.

2.4.4 Clinical observations

Clinical signs were monitored once daily following high-CBD hemp extract administration. Animals were observed for general appearance and behavior to evaluate potential toxic effects. Parameters assessed included eye condition, respiratory patterns, pain responses, locomotor activity, behavioral changes, tremors, convulsions, salivation, diarrhea, lethargy, and coma. All observations were conducted in accordance with standard toxicological assessment protocols.

2.4.5 Hematological and biochemistry analysis

Blood samples were collected from the abdominal aorta under deep anesthesia. For hematological parameters, blood was collected in EDTA-coated tubes, while samples for biochemical analysis were collected in lithium heparin tubes. All samples were centrifuged at 3,000 rpm (approximately 1,000 x g) for 10 minutes at 4°C to separate plasma. Processed samples were transported under controlled temperature conditions to the Biolab Clinic (Phitsanulok, Thailand) for comprehensive analysis.

Hematological analysis included red blood cell count (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution (RDW), white blood cell count (WBC), and differential leukocyte counts (neutrophil, lymphocyte, and eosinophil).

Serum biochemical analysis included blood urea nitrogen (BUN), creatinine, total protein, albumin, globulin, total bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), electrolytes (calcium, sodium, potassium, chloride) and carbon dioxide (CO₂).

2.4.6 Organ weight assessment

Following euthanasia, major organs including the brain, heart, lungs, liver, spleen, kidneys, adrenal glands, thymus, and reproductive organs (testis, epididymis,

prostate, penis in males; ovary in females) were excised, rinsed with normal saline, and weighed. Absolute organ weights were recorded, and relative organ weights were calculated using the following formula:

Relative organ weight (%) = (absolute organ weight (g) / final body weight (g)) x 100

2.4.7 Urinalysis

Fresh urine samples were collected from each animal prior to euthanasia. Urinalysis was performed using test strips in conjunction with a semi-automatic urine analyzer (DIRUI H-500, China). The parameters evaluated included pH, specific gravity, urobilinogen, bilirubin, ketones, blood, protein, nitrite, leukocytes, and glucose.

2.4.8 Histopathological examination

Tissue samples were fixed in 10% neutral buffered formalin (Epredia, USA) for 24 hours. The samples were then processed, embedded in paraffin wax, and sectioned into 3 µm slices using a microtome (Leica, USA). Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus, Japan) for histopathological evaluation.

2.4.9 Statistical analysis

Results are presented as the means ± standard deviation (SD). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for multiple comparisons. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using Graph Pad Prism version 9.0 (GraphPad Software, San Diego, USA).

3. Results

3.1 Acute toxicity of high-CBD hemp extract

Human equivalent acute toxicity assessment of high-CBD hemp extract was performed at a single high dose of 5,000 mg/kg bodyweight in rats. No mortality occurred over the 14-day observation period; however, treated animals exhibited transient lethargy between 15 minutes and 3 hours post-administration. Body weight gain, as well as food and water intake, remained

within normal ranges throughout the study. Necropsy at study termination revealed no gross abnormalities in any organ. Thus, the oral LD₅₀ of CBD extract was determined to be greater than 5,000 mg/kg.

3.2 Chronic toxicity of high-CBD hemp extract

3.2.1 Clinical signs

Throughout the 180-day treatment period, no mortality or signs of treatment-related clinical toxicity (e.g., changes in behavior, neurological function, or general appearance) were observed in any of the CBD-treated groups.

3.2.2 Body weight and feed consumption

Chronic high-CBD hemp extract administration induced dose-dependent effects on body weight in both sexes. Male rats receiving the high dose (40 mg/kg/day) exhibited a statistically significant reduction in body weight compared to the vehicle control group ($p < 0.05$; Fig. 1A). In female rats, significant decreases were observed in both medium (20 mg/kg/day) and high dose (40 mg/kg/day) CBD groups relative to controls ($p < 0.05$ and $p < 0.01$; Fig. 1B). Consistent with these weight changes, feed consumption was significantly reduced in the high-dose male group ($p < 0.001$; Fig. 2A) and in both the medium and high-dose female groups ($p < 0.01$ and $p < 0.001$; Fig. B).

The extent of reduced food intake was positively correlated with the degree of weight loss observed across the treatment groups. In contrast, water consumption remained comparable across all groups, with no statistically significant difference observed at any dose level or in either sex throughout the 180-day treatment period.

3.2.3 Hematology and clinical chemistry analysis

Hematological parameters generally remained unchanged in both male and female rats across all extract dose groups (10, 20, and 40 mg/kg/day) after 180 days of treatment, with the exception of male rats. Specifically, MCHC showed a statistically significant increase in low and medium dose male rats ($p < 0.05$) (Table 1). Clinical biochemistry results revealed several notable alterations: in male rats, high-CBD hemp extract (40 mg/kg) caused a significant reduction in serum creatinine ($p < 0.001$) and total bilirubin levels ($p < 0.05$) (Table 3). Importantly, no statistically significant alterations in either hematological or biochemical parameters were observed in the recovery group for either sex, suggesting reversibility or absence of persistent toxicity following the 28-day cessation of treatment.

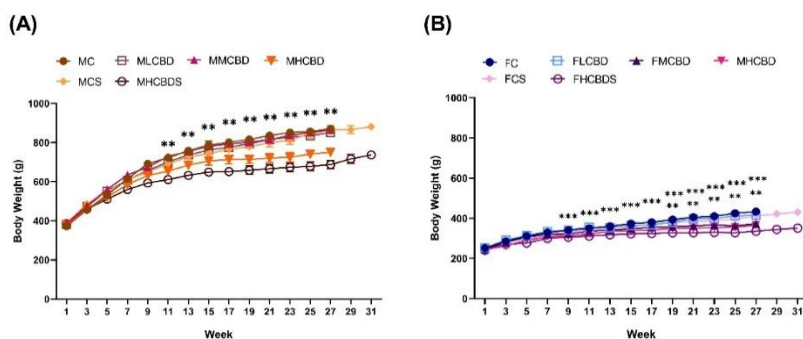


Fig. 1. Body weight changes in male (A) and female (B) rats treated with high-CBD hemp extract for 180 days. Data are expressed as mean $\pm$ standard deviation ($n = 10$ per sex per group).

** $p < 0.01$ and *** $p < 0.001$ denote significant changes when compared with the control group.

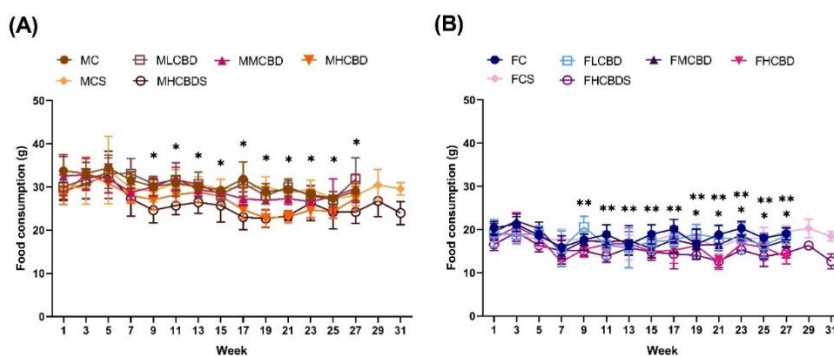


Fig. 2. Food consumption in male (A) and female (B) rats treated with high-CBD hemp extract for 180 days. Data are expressed as mean $\pm$ standard deviation (n = 10 per sex per group).

** p < 0.01 and *** p < 0.001 denote significant changes when compared with the control group.

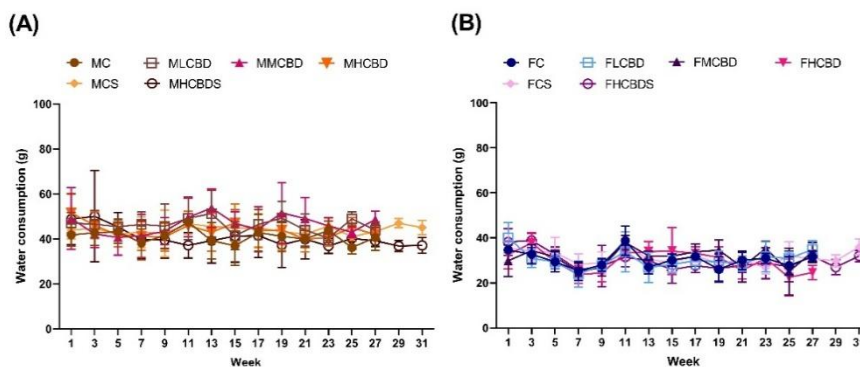


Fig. 3. Water consumption in male (A) and female (B) rats treated with high-CBD hemp extract for 180 days. Data are expressed as mean $\pm$ standard deviation (n = 10 per sex per group).

** p < 0.01 and *** p < 0.001 denote significant changes when compared with the control group.

3.2.4 Urinary analysis

Urinalysis results revealed no significant alterations in any assessed parameters, including pH, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrites, leukocytes, and urobilinogen, in either sex at any dose level when compared to the vehicle control group. These findings suggest the absence of renal or urinary tract toxicity associated with high-CBD hemp extract administration.

3.2.5 Relative organ weight

The relative organ weights of male and female rats following 180-day repeated oral administration of high-CBD hemp extract are presented in Table 5. In male rats, there were no statistically significant changes in the relative weights of most organs among

the control and treatment groups, except for the spleen. The high-dose group (40 mg/kg) showed a significant decrease in relative spleen weight (p < 0.05) and increased left testis weight (p < 0.001) when compared to the control group. The increase in testis weight was observed only in relative measurement, whereas absolute testis weight remained comparable to the control group. No significant alterations were observed in the recovery group, suggesting reversibility of the spleen effect.

In female rats, a significant, dose-dependent increase in brain weight was observed in the medium and high-dose groups compared to the control group (p < 0.05 and p < 0.01, respectively). However, other organ weights, including

liver, kidney, lung, and reproductive organs, did not show statistically significant differences among groups. Although relative brain weight was increased, no significant difference in absolute brain weight was observed when compared with the control group.

3.2.6 Histopathology

Histopathological observations revealed

a significant increase in centrilobular hepatocyte hypertrophy incidence and severity in both sexes (Fig. 4). In addition, increases in hypertrophy of the pars distalis of the pituitary gland in males (Fig. 5) and hypertrophy of follicular cells of the thyroid gland in males (Fig. 6) in severity and/or incidence were observed.

Table 1. Hematological parameters of male rats administered with high-CBD hemp extract 0, 10, 20 and 40 mg/kg/day for 180 days, followed by a 28-day recovery period.

Parameters	Control	Treatment group			Recovery group	
		10 mg/kg	20 mg/kg	40 mg/kg	Control	40 mg/kg
WBC ($10^3/\mu\text{L}$)	7.16 $\pm$ 1.12	7.79 $\pm$ 2.27	8.65 $\pm$ 1.97	7.16 $\pm$ 1.09	5.72 $\pm$ 1.10	6.44 $\pm$ 0.98
RBC ($10^6/\mu\text{L}$)	8.65 $\pm$ 0.41	8.77 $\pm$ 0.31	8.79 $\pm$ 0.61	8.62 $\pm$ 0.37	8.38 $\pm$ 0.41	8.70 $\pm$ 0.30
MCH (pg)	17.32 $\pm$ 0.23	17.18 $\pm$ 0.43	17.36 $\pm$ 0.59	17.36 $\pm$ 0.65	17.36 $\pm$ 0.50	17.44 $\pm$ 0.53
MCHC (g/dl)	33.71 $\pm$ 0.31	34.13 $\pm$ 0.38*	34.14 $\pm$ 0.38*	34.09 $\pm$ 0.39	33.84 $\pm$ 0.49	33.88 $\pm$ 0.38
MCV (fl)	51.40 $\pm$ 0.84	50.20 $\pm$ 1.03	50.90 $\pm$ 1.52	50.90 $\pm$ 1.85	51.60 $\pm$ 1.67	51.40 $\pm$ 1.14
Hb (g/dl)	14.99 $\pm$ 0.76	15.03 $\pm$ 0.30	15.24 $\pm$ 0.72	14.94 $\pm$ 0.43	14.56 $\pm$ 0.44	15.18 $\pm$ 0.56
Hct (%)	44.30 $\pm$ 2.31	44.20 $\pm$ 1.03	44.70 $\pm$ 2.26	44.00 $\pm$ 1.41	43.20 $\pm$ 1.92	44.80 $\pm$ 1.79
RDW (%)	12.91 $\pm$ 0.43	13.08 $\pm$ 0.44	12.91 $\pm$ 0.66	12.92 $\pm$ 0.62	13.70 $\pm$ 1.20	13.06 $\pm$ 0.69
Neutrophil (%)	26.90 $\pm$ 14.79	31.10 $\pm$ 8.94	30.10 $\pm$ 8.48	26.10 $\pm$ 6.89	28.00 $\pm$ 13.29	18.20 $\pm$ 6.83
Lymphocyte (%)	69.20 $\pm$ 15.53	65.40 $\pm$ 8.04	67.40 $\pm$ 8.34	70.50 $\pm$ 7.49	68.20 $\pm$ 14.46	79.20 $\pm$ 6.83
Eosinophil (%)	4.10 $\pm$ 2.08	2.90 $\pm$ 1.60	2.40 $\pm$ 1.26	3.67 $\pm$ 1.58	3.60 $\pm$ 1.82	3.25 $\pm$ 1.71

Values are expressed as mean $\pm$ standard deviation (one-way ANOVA followed by Dunnet's post-hoc test).

*P < 0.05 compared to the control group.

Table 2. Hematological parameters of female rats administered with high-CBD hemp extract 0, 10, 20 and 40 mg/kg/day for 180 days, followed by a 28-day recovery period.

Parameters	Control	Treatment group			Recovery group	
		10 mg/kg	20 mg/kg	40 mg/kg	Control	40 mg/kg
WBC ($10^3/\mu\text{L}$)	3.81 $\pm$ 1.54	3.78 $\pm$ 1.30	4.03 $\pm$ 1.74	4.12 $\pm$ 6.29	3.50 $\pm$ 0.70	4.04 $\pm$ 0.48
RBC ($10^6/\mu\text{L}$)	7.96 $\pm$ 0.52	7.61 $\pm$ 0.46	7.66 $\pm$ 0.47	8.18 $\pm$ 0.62	7.56 $\pm$ 0.39	7.58 $\pm$ 0.47
MCH (pg)	18.41 $\pm$ 0.65	18.73 $\pm$ 0.72	18.50 $\pm$ 0.48	18.30 $\pm$ 0.46	19.18 $\pm$ 0.37	18.46 $\pm$ 0.52
MCHC (g/dl)	34.79 $\pm$ 0.35	34.76 $\pm$ 0.37	34.67 $\pm$ 0.60	34.74 $\pm$ 0.38	35.10 $\pm$ 0.21	34.98 $\pm$ 0.44
MCV (fl)	53.10 $\pm$ 1.85	53.90 $\pm$ 1.66	53.50 $\pm$ 1.43	52.70 $\pm$ 1.16	54.60 $\pm$ 1.14	53.20 $\pm$ 1.48
Hb (g/dl)	14.64 $\pm$ 0.76	14.24 $\pm$ 0.89	14.16 $\pm$ 0.95	14.95 $\pm$ 0.96	14.48 $\pm$ 0.97	14.02 $\pm$ 0.90
Hct (%)	42.30 $\pm$ 2.16	41.10 $\pm$ 2.73	40.90 $\pm$ 2.96	43.00 $\pm$ 2.83	41.40 $\pm$ 2.88	40.20 $\pm$ 2.68
RDW (%)	11.72 $\pm$ 0.71	11.68 $\pm$ 0.48	11.64 $\pm$ 0.59	11.61 $\pm$ 0.51	11.58 $\pm$ 0.43	11.98 $\pm$ 0.53
Neutrophil (%)	29.40 $\pm$ 16.36	22.60 $\pm$ 8.19	21.30 $\pm$ 6.55	19.70 $\pm$ 8.25	30.20 $\pm$ 16.87	21.00 $\pm$ 4.12
Lymphocyte (%)	67.20 $\pm$ 15.40	72.20 $\pm$ 7.55	75.30 $\pm$ 7.09	77.60 $\pm$ 8.38	65.20 $\pm$ 17.91	74.60 $\pm$ 3.78
Eosinophil (%)	3.22 $\pm$ 1.86	3.44 $\pm$ 1.51	3.20 $\pm$ 1.55	2.78 $\pm$ 0.83	3.40 $\pm$ 1.14	4.00 $\pm$ 2.12

Values are expressed as mean $\pm$ standard deviation (one-way ANOVA followed by Dunnet's post-hoc test).

Table 3. Biochemical parameters of male rats administered with high-CBD hemp extract 0, 10, 20 and 40 mg/kg/day for 180 days, followed by a 28-day recovery period.

Parameters	Treatment group				Recovery group	
	Control	10 mg/kg	20 mg/kg	40 mg/kg	Control	40 mg/kg
BUN (mg/dl)	16.77 ± 2.22	15.49 ± 2.08	14.69 ± 2.63	14.92 ± 2.42	16.45 ± 1.70	17.54 ± 0.83
Creatinine (mg/dl)	0.38 ± 0.06	0.32 ± 0.08	0.35 ± 0.07	0.29 ± 0.02**	0.33 ± 0.05	0.31 ± 0.06
Total protein (g/dl)	5.15 ± 0.16	5.20 ± 0.27	5.13 ± 0.24	5.21 ± 0.19	5.25 ± 0.28	5.20 ± 0.20
Albumin (g/dl)	3.15 ± 0.17	3.17 ± 0.18	3.15 ± 0.16	3.21 ± 0.16	3.12 ± 0.11	3.16 ± 0.10
Globulin (g/dl)	2.01 ± 0.07	2.04 ± 0.12	2.00 ± 0.13	2.00 ± 0.15	2.14 ± 0.20	2.05 ± 0.14
Total Bilirubin (mg/dl)	0.18 ± 0.04	0.17 ± 0.02	0.17 ± 0.02	0.16 ± 0.01*	0.18 ± 0.02	0.17 ± 0.02
AST (U/L)	99.20 ± 32.99	94.70 ± 28.98	92.40 ± 22.44	75.50 ± 19.06	90.70 ± 11.50	89.00 ± 12.77
ALT (U/L)	37.20 ± 5.31	37.70 ± 5.31	38.80 ± 6.58	33.00 ± 7.07	39.60 ± 3.69	35.70 ± 6.31
ALP (U/L)	54.40 ± 4.81	54.10 ± 6.71	52.40 ± 5.42	55.10 ± 5.38	57.20 ± 4.73	52.60 ± 6.11
Calcium (mg/dl)	9.80 ± 0.14	9.85 ± 0.21	9.67 ± 0.30	9.78 ± 0.21	9.77 ± 0.35	9.74 ± 0.26
Sodium (mEq/L)	139.40 ± 0.97	139.60 ± 0.97	139.40 ± 1.17	139.60 ± 0.84	138.20 ± 1.23	138.80 ± 1.40
Potassium (mEq/L)	3.04 ± 0.40	2.97 ± 0.20	3.07 ± 0.14	3.00 ± 0.26	3.25 ± 0.24	3.15 ± 0.26
Chloride (mEq/L)	98.70 ± 2.50	99.80 ± 2.20	100.00 ± 2.16	100.00 ± 1.83	99.40 ± 1.90	100.20 ± 1.93

Values are expressed as mean ± standard deviation (one-way ANOVA followed by Dunnet's post-hoc test).

Table 4. Biochemical parameters of female rats administered with high-CBD hemp extract 0, 10, 20 and 40 mg/kg/day for 180 days, followed by a 28-day recovery period.

Parameters	Treatment group				Recovery group	
	Control	10 mg/kg	20 mg/kg	40 mg/kg	Control	40 mg/kg
BUN (mg/dl)	16.43 ± 3.42	16.16 ± 4.30	16.55 ± 3.74	16.96 ± 2.34	15.67 ± 2.07	17.53 ± 2.40
Creatinine (mg/dl)	0.41 ± 0.09	0.42 ± 0.08	0.37 ± 0.05	0.36 ± 0.06	0.39 ± 0.08	0.40 ± 0.08
Total protein (g/dl)	6.60 ± 0.61	6.58 ± 0.48	6.77 ± 0.61	6.53 ± 0.51	6.54 ± 0.48	6.46 ± 0.32
Albumin (g/dl)	4.78 ± 0.35	4.83 ± 0.41	5.07 ± 0.45	4.75 ± 0.30	4.76 ± 0.33	4.97 ± 0.35
Globulin (g/dl)	1.81 ± 0.37	1.74 ± 0.27	1.70 ± 0.21	1.77 ± 0.29	1.79 ± 0.27	1.49 ± 0.19
Total Bilirubin (mg/dl)	0.17 ± 0.02	0.18 ± 0.03	0.19 ± 0.04	0.19 ± 0.03	0.19 ± 0.02	0.20 ± 0.03
AST (U/L)	87.00 ± 18.01	85.10 ± 27.43	79.80 ± 23.94	72.30 ± 10.60	86.50 ± 28.04	82.50 ± 16.21
ALT (U/L)	41.40 ± 4.30	38.20 ± 8.78	36.70 ± 8.29	40.20 ± 5.73	41.40 ± 3.57	40.00 ± 5.03
ALP (U/L)	17.90 ± 7.74	19.30 ± 3.86	18.70 ± 5.48	18.10 ± 3.31	18.40 ± 3.84	18.00 ± 2.83
Calcium (mg/dl)	10.32 ± 0.59	10.37 ± 0.57	10.81 ± 0.89	10.77 ± 0.68	10.15 ± 0.33	10.10 ± 0.30
Sodium (mEq/L)	140.14 ± 2.13	140.10 ± 2.02	139.40 ± 1.17	138.80 ± 2.70	139.00 ± 1.56	138.90 ± 1.10
Potassium (mEq/L)	2.94 ± 0.63	3.39 ± 1.22	3.12 ± 0.78	4.27 ± 2.00	2.87 ± 0.32	2.83 ± 0.41
Chloride (mEq/L)	100.00 ± 4.19	101.70 ± 2.91	100.90 ± 2.56	102.40 ± 2.76	95.50 ± 2.07	97.00 ± 2.45

Values are expressed as mean ± standard deviation (one-way ANOVA followed by Dunnet's post-hoc test).

Table 5. Relative organ weights (g/100 g body weight) of male rats administered with high-CBD hemp extract 0, 10, 20 and 40 mg/kg/day for 180 days, followed by a 28-day recovery period.

Parameters	Treatment group				Recovery group	
	Control	10 mg/kg	20 mg/kg	40 mg/kg	Control	40 mg/kg
Brain	0.30 ± 0.02	0.29 ± 0.01	0.30 ± 0.02	0.33 ± 0.03	0.28 ± 0.04	0.33 ± 0.02
Heart	0.25 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	0.24 ± 0.03	0.21 ± 0.01	0.22 ± 0.02
Lung	0.42 ± 0.03	0.39 ± 0.03	0.41 ± 0.03	0.44 ± 0.05	0.31 ± 0.15	0.42 ± 0.04
Liver	2.41 ± 0.30	2.24 ± 0.19	2.29 ± 0.23	2.23 ± 0.12	2.49 ± 0.22	2.50 ± 0.18
Spleen	0.13 ± 0.02	0.11 ± 0.01	0.12 ± 0.02	0.10 ± 0.04*	0.12 ± 0.02	0.13 ± 0.02
Kidney Lt.	0.21 ± 0.08	0.23 ± 0.02	0.23 ± 0.02	0.22 ± 0.08	0.21 ± 0.03	0.25 ± 0.03
Kidney Rt.	0.22 ± 0.08	0.23 ± 0.02	0.24 ± 0.01	0.21 ± 0.09	0.21 ± 0.03	0.24 ± 0.03
Adrenal Lt.	0.03 ± 0.07	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.06	0.003 ± 0.001	0.004 ± 0.001
Adrenal Rt.	0.03 ± 0.07	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.06	0.003 ± 0.001	0.004 ± 0.001
Penis	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.08 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Testis Lt.	0.25 ± 0.03	0.24 ± 0.02	0.24 ± 0.04	0.30 ± 0.03***	0.21 ± 0.03	0.22 ± 0.06
Testis Rt.	0.24 ± 0.04	0.29 ± 0.10	0.25 ± 0.04	0.30 ± 0.02	0.22 ± 0.03	0.22 ± 0.07
Epididymis Lt.	0.11 ± 0.03	0.10 ± 0.01	0.11 ± 0.03	0.11 ± 0.02	0.10 ± 0.01	0.11 ± 0.03
Epididymis Lt.	0.13 ± 0.03	0.13 ± 0.04	0.12 ± 0.02	0.15 ± 0.05	0.11 ± 0.02	0.12 ± 0.04
Ovary	0.35 ± 0.09	0.45 ± 0.25	0.43 ± 0.12	0.47 ± 0.14	0.38 ± 0.06	0.34 ± 0.09

Values are expressed as mean ± standard deviation (one-way ANOVA followed by Dunnet's post-hoc test).

Table 6. Relative organ weights (g/100 g body weight) of female rats administered with high-CBD hemp extract 0, 10, 20 and 40 mg/kg/day for 180 days, followed by a 28-day recovery period

Parameters	Treatment group				Recovery group	
	Control	10 mg/kg	20 mg/kg	40 mg/kg	Control	40 mg/kg
Brain	0.51 ± 0.06	0.54 ± 0.06	0.59 ± 0.07*	0.62 ± 0.07**	0.51 ± 0.08	0.59 ± 0.06
Heart	0.28 ± 0.03	0.31 ± 0.03	0.31 ± 0.04	0.31 ± 0.02	0.33 ± 0.09	0.31 ± 0.02
Lung	0.56 ± 0.12	0.55 ± 0.05	0.59 ± 0.10	0.59 ± 0.06	0.52 ± 0.06	0.59 ± 0.07
Liver	2.69 ± 0.23	2.67 ± 0.21	2.81 ± 0.22	2.79 ± 0.22	2.69 ± 0.15	2.69 ± 0.27
Spleen	0.14 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.16 ± 0.01*	0.16 ± 0.02	0.17 ± 0.03
Thymus	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.01	0.08 ± 0.02
Kidney Lt.	0.24 ± 0.02	0.27 ± 0.02	0.24 ± 0.09	0.28 ± 0.03	0.24 ± 0.02	0.27 ± 0.03
Kidney Rt.	0.25 ± 0.02	0.26 ± 0.01	0.25 ± 0.08	0.29 ± 0.03	0.24 ± 0.02	0.27 ± 0.03
Adrenal Lt.	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.09	0.01 ± 0.00	0.009 ± 0.001	0.010 ± 0.002
Adrenal Rt.	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.09	0.01 ± 0.00	0.008 ± 0.002	0.009 ± 0.002

Values are expressed as mean ± standard deviation (one-way ANOVA followed by Dunnet's post-hoc test).

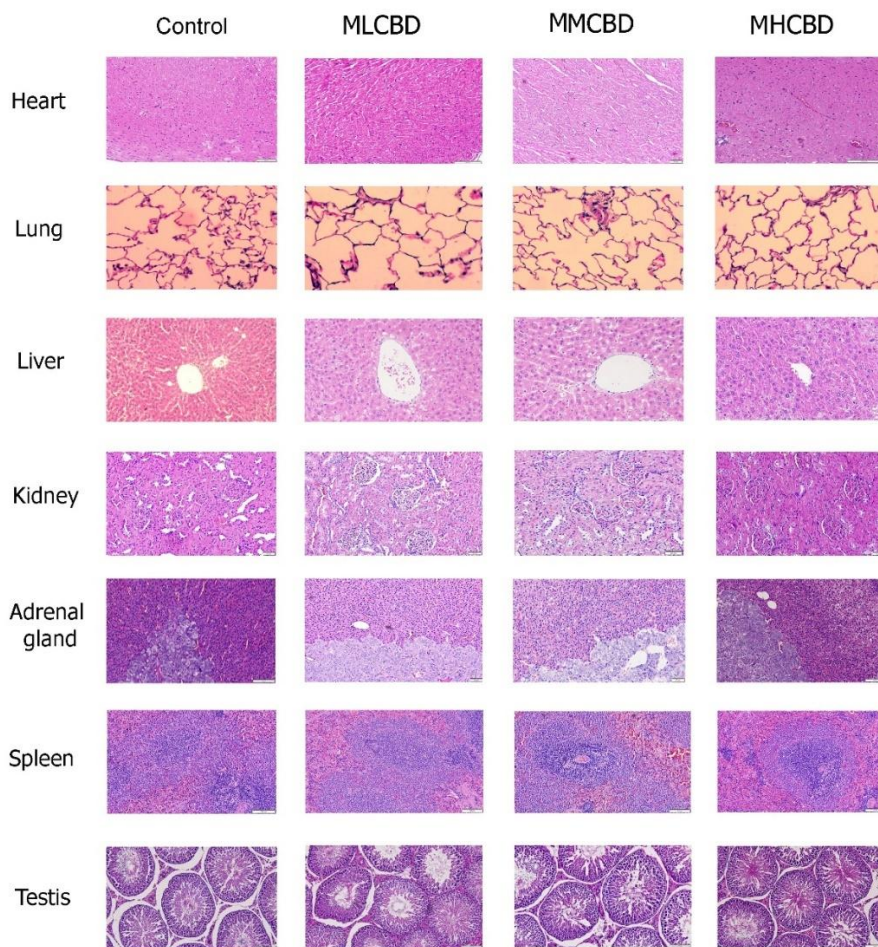


Fig. 4 Histological sections of selected internal organs from male rats treated with high-CBD hemp extract (H&E staining, 20x magnification).

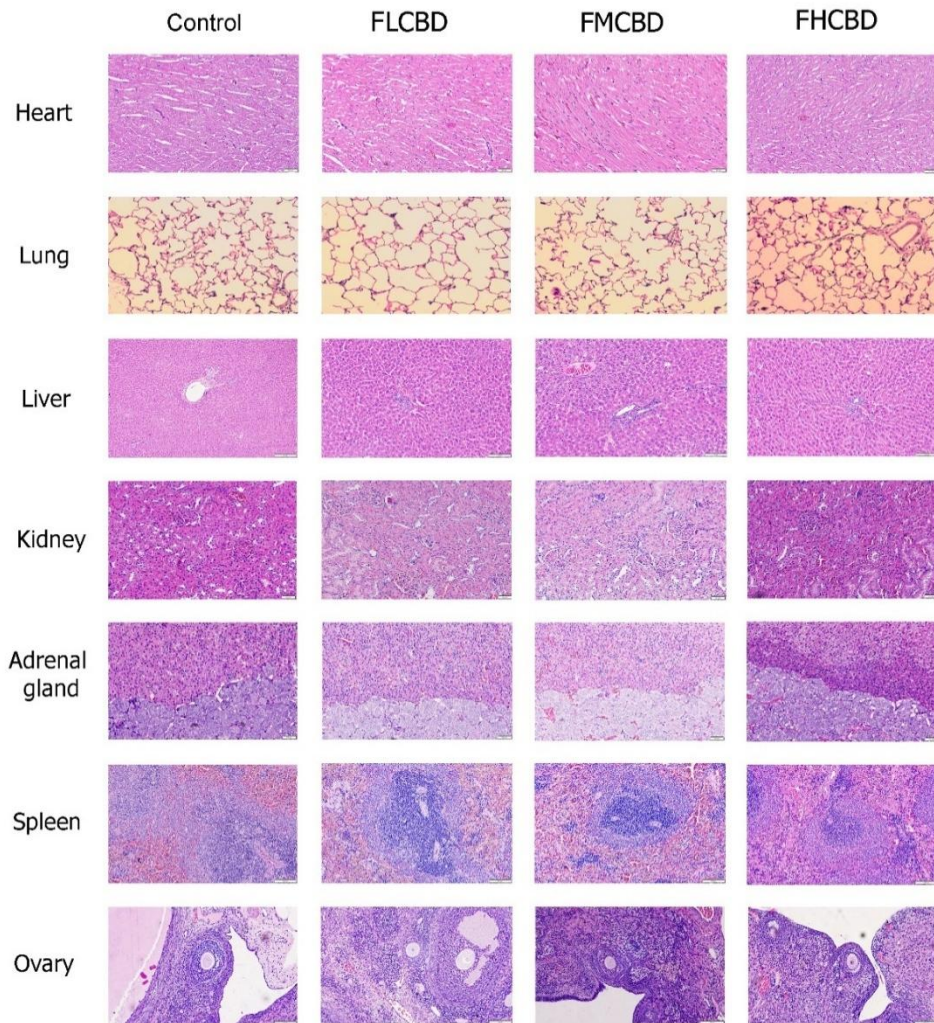


Fig. 5 Histological sections of selected internal organs from female rats treated with high-CBD hemp extract (H&E staining, 20x magnification).

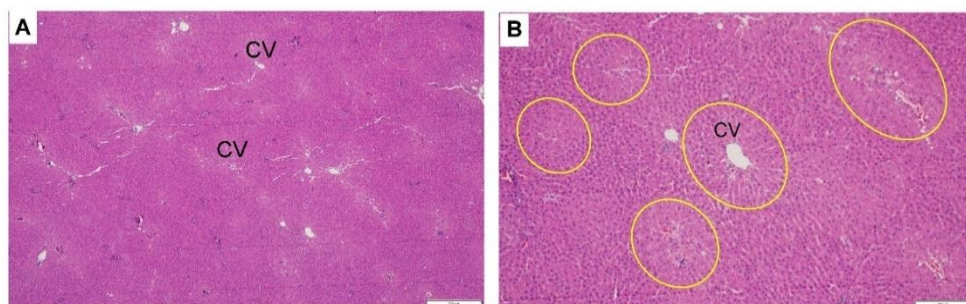


Fig. 6 Representative liver sections showing centrilobular hepatocyte hypertrophy. (A) Low-magnification view demonstrating diffuse pale-staining hepatocytes surrounding the central veins (CV). (B) Higher magnification of centrilobular hepatocyte hypertrophy (yellow circles). H&E staining. Scale bar = 500 μ m (A) and 200 μ m (B).

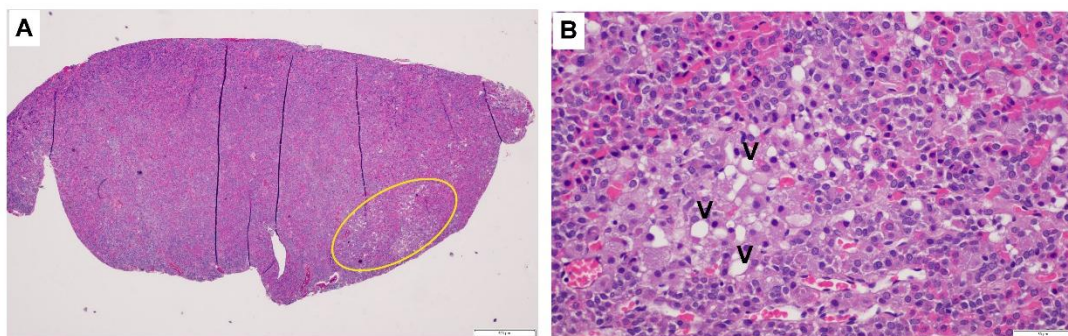


Fig. 7 Representative pituitary gland sections showing hypertrophy of pars distalis in a male rat. (A) Low- magnification view of the affected area (yellow circle). (B) Higher magnification demonstrating enlarged cytoplasm with occasional cytoplasmic vacuolation (V). H&E staining. Scale bar = 500 μm (A) and 50 μm (B).

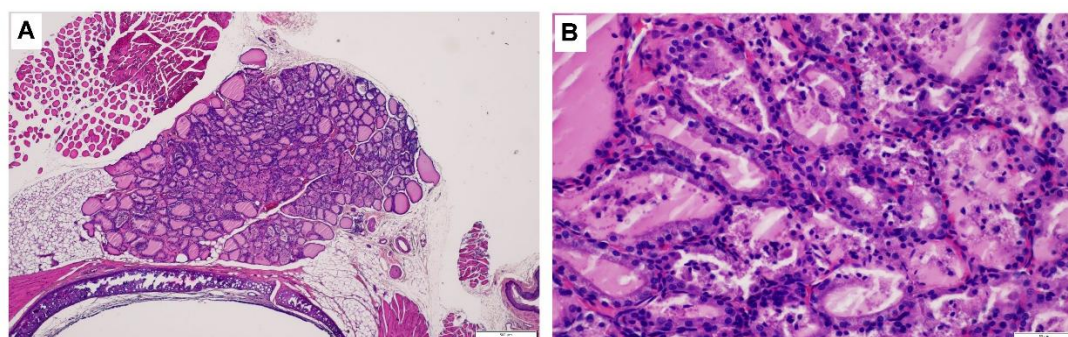


Fig. 8 Representative thyroid gland sections showing follicular hypertrophy. (A) Low- magnification view of the affected thyroid tissue. (B) Higher magnification demonstrating enlarged follicular cells. H&E staining. Scale bar = 500 μm (A) and 50 μm (B).

4. Discussion

The increasing scientific interest in *Cannabis sativa* L. stems from its natural origin and its rich profile of bioactive compounds. This phytochemical diversity strongly supports the plant's potential for multifaceted applications within both the food and pharmaceutical sectors, thereby necessitating comprehensive toxicological evaluation.³¹ Since 2015, the toxicological evaluation of hemp extracts has received substantial research attention. Within the complex profile of hemp constituents, CBD stands out as the most pharmacologically relevant compound, particularly given its recognized potential in pain management.

This therapeutic utility underscores the critical need for detailed safety and toxicity assessments.³² To date, a significant gap remains in the literature regarding a comprehensive, long-term toxicological assessment of standardized hemp extract for safety evaluation. This deficiency underscores the rationale for the present study, which was specifically designed to investigate the compound's toxicological profile. Accordingly, both 14-day acute and 180-day chronic oral toxicities were meticulously evaluated using a rat model.

The acute toxicity study of the standardized hemp extract administered at a concentration of 5,000 mg/kg body weight in rats revealed that the rats exhibited a decrease

in body weight within 24-48 hours,²⁶ accompanied by several clinical signs and behavioral changes. No mortality was observed during the 14-day observation period. The fact that no mortality was observed at the highest tested dose of 5,000 mg/kg indicates that the acute oral LD₅₀ of high-CBD hemp extract in rats is likely greater than this value.²⁷ These findings contrast with several previous studies, which have generally reported no significant clinical signs or behavioral changes following acute exposure to hemp-derived products. For example, Costa et al. reported that acute administration of a full-spectrum *Cannabis sativa* extract did not result in significant behavioral or physiological changes in rodents and rabbits, with only a transient reduction in motor activity observed at the highest dose level.³³ Similarly, Maneesai et al. demonstrated that acute exposure to hemp leaf oil did not produce noticeable neurobehavioral abnormalities or physiological disturbances in rats.³⁴

In addition, other studies have shown that even at high doses (up to 5,000 mg/kg), *Cannabis sativa* extracts did not induce significant clinical signs of toxicity or alterations in general behavior.

In the chronic toxicity study, standardized hemp extract was administered to rats at doses of 10, 20, and 40 mg/kg/day, consistent with the Health-Based Guidance Value (HBGV) for CBD.

Importantly, the extract did not induce any observable clinical signs of toxicity across all dose groups, including altered somatic activity, stereotypy, tremors, convulsions, diarrhea or coma. While initial body weights were comparable, a dose-dependent reduction in final body weight was evident, with the highest dose group exhibiting the lowest weight. This effect was notably more pronounced in male rats, suggesting a potential sex-related sensitivity to the extract at the highest exposure level.³⁵

The significant reductions in body weight gain and feed consumption observed

in the medium- and high-dose groups may reflect pharmacological effects associated with CBD administration rather than overt systemic toxicity. CBD has been reported to cross the blood-brain barrier and modulate central nervous system activity through cannabinoid and serotonergic pathways.³⁶⁻³⁷ At high doses, CBD may induce transient sedative effects or reduced locomotor activity, which could contribute to decreased feed intake and subsequent body weight reduction. Since no mortality, severe clinical signs, or treatment-related histopathological abnormalities were observed, these findings were considered mild and not indicative of severe systemic toxicity.³⁸

High doses of CBD have been associated with transient sedative effects, lethargy, and reduced locomotor activity in both experimental animal and humans. However, it is important to distinguish pharmacological sedation from severe acute CNS depression. In the present study, although reductions in feed consumption and body weight gain were observed in the medium- and high-dose groups, no severe neurological signs, respiratory distress, convulsion, loss of arousability, or treatment-related histopathological abnormalities were detected. The findings suggest that the observed effects were more consistent with mild pharmacological sedation rather than a pathologically compromised CNS or direct neurotoxicity.³⁹

The long-term repeated oral administration of high-dose CBD or hemp extract, generally demonstrated a good safety profile concerning relative organ weight, with no significant changes observed in critical organs like the liver, kidneys, or lungs in either sex. The few significant alterations, a decreased relative spleen weight and increased relative testis weight in males, and an increased relative brain weight in females, were notably reversible or not associated with histopathological changes, supporting the overall low-toxicity profile of the extract. In male rats, the significant decrease in

relative spleen weight in the high-dose group is a noteworthy observation. The spleen is a major secondary lymphoid organ involved in immune surveillance and hematopoiesis. Changes in spleen weight can reflect immune modulation or alterations in blood volume or hematological parameters.⁴⁰ Although a reduction in relative spleen weight was observed in high-dose males, no corresponding hematological abnormalities or histopathological lesions were detected. Therefore, the toxicological significance of this finding was considered limited and may reflect mild immunomodulatory effects associated with cannabinoid exposure rather than overt immunotoxicity.⁴¹ In contrast, the secondary relative testis weight observed in the high-dose male group was likely secondary to the reduction in terminal body weight, as absolute testis weight remained comparable to the control group. In addition, no treatment-related histopathological abnormalities were detected in the testes. Therefore, the observed change was considered non-adverse and not indicative of direct reproductive toxicity.⁴²

In female rats, the increase in relative brain weight was observed only in relative measurements, whereas absolute brain weights remained comparable to the control group. Therefore, the increase in relative brain weight was considered secondary to the reduction in terminal body weight rather than a direct effect on brain tissue. Interpretation of relative organ weight changes should be performed in conjunction with terminal body weight and histopathological findings, particularly when body weight reduction is present.⁴²⁻⁴³ Furthermore, no treatment-related histopathological abnormalities or neurological signs were observed, indicating that the findings were not suggestive of direct neurotoxicity.

Considered in their entirety, the absence of changes in the relative weights of major detoxification organs (liver and kidney) and the reversibility of the observed splenic change strongly support the conclusion that long-term oral administration

of the test substance up to 40 mg/kg does not induce systemic organ toxicity. The minor organ weight fluctuations in the spleen and brain are likely transient, adaptive, or physiological effects rather than signs of serious adverse toxicity, especially given the rapid recovery observed in the recovery group.

The hematological analysis revealed a statistically significant increase in the mean corpuscular hemoglobin concentration (MCHC). MCHC is a critical parameter representing the average concentration of hemoglobin within a red blood cell. Interpretation of MCHC is made in conjunction with other complete blood count (CBC) parameters, including red blood cell count (RBC), hemoglobin (Hb) content, and mean corpuscular volume (MCV). The reference range for MCHC in male Sprague-Dawley (SD) rats is reported to be 25.4-80.6 g/dL.⁴⁴ This finding aligns with previously reported hematological effects of *Cannabis sativa* extracts. Oluwasola et al. observed a significant elevation in MCHC at higher extract doses (4 and 6 mg/kg), whereas the 2 mg/kg group remained comparable to the controls.⁴⁵ Likewise, the report has demonstrated that high-dose *C. sativa* extracts increase MCHC without concomitant alterations in MCH or hemoglobin concentration.⁴⁶ Despite the statistically significant findings in males, it is important to note that the mean MCHC values in both the control and treatment groups remained within the established normal reference range. When considered alongside the other CBC parameters, the observed elevation was determined not to constitute an adverse effect. The increased MCHC values observed in the low- and medium-dose male groups were considered incidental findings because the changes lacked dose-dependency, were not observed in the high-dose group, and were not associated with related hematological abnormalities or histopathological lesions. The reductions in creatinine and bilirubin levels were not considered toxicologically

adverse, as decreases in these parameters are generally less indicative of renal or hepatic injury than increases. In particular, the decreased creatinine levels observed in high-dose male may be associated with reduced body weight and muscle mass secondary to decreased feed intake rather than renal toxicity.⁴⁷ Furthermore, no treatment-related histopathological abnormalities were observed in the liver or kidney, supporting the limited toxicological significance of these findings.

The present study investigated the long-term effects of a standardized high- CBD hemp extract on the systemic biochemistry of male rats over 180 days. Our primary findings suggest that chronic administration of the extract is well-tolerated and safe, as indicated by the absence of significant changes in key indicators of hepatic and renal damage, including ALT, AST, ALP, BUN, total protein and electrolytes. Furthermore, no clinical symptoms or histopathological abnormalities related to liver or kidney function were observed.

A particularly noteworthy finding was the significant reduction in plasma creatinine and total bilirubin levels in the high-dose group compared to the control group. This suggests that the hemp extract, likely rich in CBD and other non-psychoactive components, may exert nephroprotective and hepatoprotective effects under normal physiological conditions over an extended period.

Creatinine is a conventional biomarker for assessing renal filtration efficiency. A reduction in serum creatinine, points toward an improvement in the estimated glomerular filtration rate (eGFR). This finding aligns with mounting evidence demonstrating the protective properties of cannabinoids on kidney health. For instance, chronic CBD treatment has been shown to improve renal function and increase eGFR in rat models of metabolic syndrome.⁴⁸ The high-dosed CBD treatment also caused a significant reduction in total bilirubin levels. Bilirubin is primarily

metabolized and cleared by the liver. An increase in bilirubin often signals hepatic injury (hepatotoxicity) or bile duct obstruction (cholestasis). In contrast, the observed reduction, coupled with the absence of significant changes in liver transaminases (ALT, AST), strongly indicates that the dose was not hepatotoxic.⁴⁹ Instead, the decrease in total bilirubin might reflect an enhancement of hepatic clearance mechanism, such as the induction or modulation of hepatic uridine 5'-diphospho-glucuronosyltransferases (UGT enzyme) responsible for bilirubin conjugation.⁵⁰ The high-dose CBD treatment (40 mg/kg) demonstrated transient, reversible effects in male rats, notably enhancing renal filtration efficiency (creatinine reduction) and hepatic clearance (total bilirubin reduction) without inducing any signs of persistent toxicity. The complete return to normal physiological parameters following a 28-day washout period provides strong evidence for the safety and reversibility of chronic CBD consumption at this dosage in the rat model.

Increases in centrilobular hepatocyte hypertrophy in both sexes, hypertrophy of pars distalis of the pituitary gland in males, and hypertrophy of follicular cells of the thyroid gland in males observed in the test item-treated groups were considered test item-related because the findings showed dose-response relationships. The changes were considered non-adverse because they did not induce any damage to the tissues as adaptive responses. It is well known that hepatocellular hypertrophy, secondary to increases in microsomal enzymes, often occurs with a zonal or specific lobular pattern and commonly occurs following exposure to enzyme-inducing xenobiotics. There is enlargement of the hepatocyte cytoplasm secondary to increases in cytosolic protein or number of organelles (e.g., smooth endoplasmic reticulum, peroxisomes, mitochondria). Hepatocellular hypertrophy following enzyme induction is considered an adaptive response to chemical stress. While typically an adaptive response, excessive hypertrophy

from enzyme induction can lead to hepatocellular degeneration and necrosis. Hepatocellular hypertrophy may be associated with an increase in absolute liver weight.⁵¹

Hypertrophy of the pars distalis in the pituitary gland is well known and usually not due to a direct effect on the pituitary gland, but is related to toxicity or decreased function of the target endocrine organs or increased metabolism and/or excretion of the target organ hormones. The resulting reduction in circulating hormone concentrations removes the negative feedback mechanism to the hypothalamic-hypophyseal axis and causes stimulation of specific cell types in the pars distalis.⁵²

It is well known that follicular cell hypertrophy is a common change seen in toxicity studies in rats induced with a variety of chemicals, such as hepatic microsomal enzymes inducers, which is often associated with hepatocyte hypertrophy.⁵²

5. Conclusion

The findings support the safety of high-CBD hemp extract for long-term oral consumption at the tested dosage. This study, provides valuable toxicological data, including a robust demonstration of the reversibility of biochemical and organ weight changes, which can inform regulatory decisions and support the safe use of CBD-rich *Cannabis sativa* extracts in pharmaceutical and supplement development. Further mechanistic studies are warranted to elucidate the specific molecular pathways underlying the observed sex-dimorphic effects on brain weight and the modulating effects on creatinine and bilirubin clearance.

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Conflicts of Interest

The author declares no conflicts of interest.

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