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In vivo toxicological profiling of *Teucrium polium* L. fractions: indications of safety via oral and dermal routes

Original Article

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Abstract:

Teucrium polium L. is widely consumed as a traditional remedy across the Mediterranean basin and North Africa, yet its safety remains contentious due to reported cases of herb-induced liver injury in humans. This study examined the *in vivo* tolerability of polar and apolar fractions of *T. polium* following oral and dermal exposure in male and female Wistar rats. For the oral route, animals received 2000 mg/kg/day for 28 consecutive days, and safety was assessed using endpoints aligned with OECD TG 407, including daily behavioral observations, body-weight evolution, hematology, serum biochemistry with hepatic and renal biomarkers, and histopathology of major organs. In parallel, repeated-dose dermal exposure was conducted for 28 days under occlusive conditions in accordance with OECD TG 410, with systematic scoring of local dermal reactions and monitoring for systemic toxicity, complemented by histopathological evaluation of treated skin. Across both exposure routes, no treatment-related mortality or clinically meaningful adverse effects were detected. Hematological indices and serum biochemical markers remained within physiological ranges, and histopathological assessment revealed no extract-related lesions in the examined organs or skin. Collectively, these findings indicate good short-term tolerability of the tested *T. polium* fractions at 2000 mg/kg/day under the present experimental conditions. It is crucial to note that these conclusions are confined to the specific fractions tested and the 28-day exposure period. They cannot be extrapolated to other preparations, dosing regimens, or prolonged exposure scenarios. Given ongoing clinical concern regarding hepatotoxicity, longer-duration studies incorporating dose–response designs and mechanistic endpoints are warranted to more definitively characterize the risk.

Key words:
Teucrium polium; herbal safety; dermal exposure; OECD TG 407; OECD TG 410; hematology; serum biochemistry; histopathology; hepatotoxicity.

Apstrakt:

In vivo toksikološko profilisanje frakcija _Teucrium polium_ L.: indikacije bezbednosti pri oralnoj i dermalnoj primeni

Teucrium polium L. se koristi kao tradicionalni lek širom mediteranskog basena i Severne Afrike, ali njegova bezbednost ostaje predmet rasprave zbog prijavljenih slučajeva oštećenja jetre izazvanog biljnim preparatima kod ljudi. U ovoj studiji ispitivana je *in vivo* tolerancija polarnih i nepolarnih frakcija *T. polium* nakon oralne i dermalne primene kod mužjaka i ženki Wistar pacova. Kod oralne primene, životinje su dobijale dozu od 2000 mg/kg/dan tokom 28 uzastopnih dana, a bezbednost je procenjivana u skladu sa OECD smernicom TG 407, uključujući svakodnevna posmatranja ponašanja, praćenje telesne mase, hematološke analize, biohemijske analize seruma sa parametrima funkcije jetre i bubrega, kao i histopatološki pregled glavnih organa. Paralelno je sprovedena i dermalna primena ponovljenih doza tokom 28 dana pod okluzivnim uslovima, u skladu sa OECD smernicom TG 410. Sistematski su ocenjivane lokalne dermalne reakcije i praćeni znaci sistemske toksičnosti, uz histopatološku analizu tretirane kože.Pri oba načina primene nije zabeležen mortalitet povezan sa tretmanom niti klinički značajni neželjeni efekti. Hematološki parametri i biohemijski markeri u serumu ostali su u granicama fizioloških vrednosti, a histopatološka analiza nije pokazala lezije povezane sa ekstraktom u ispitivanim organima niti na koži. Ukupno posmatrano, rezultati ukazuju na dobru kratkoročnu podnošljivost ispitivanih frakcija *T. polium* pri dozi od 2000 mg/kg/dan u datim eksperimentalnim uslovima. Važno je naglasiti da se ovi zaključci odnose isključivo na konkretno ispitivane frakcije i period izloženosti od 28 dana. Ne mogu se ekstrapolirati na druge preparate, režime doziranja ili produžene periode izloženosti. Imajući u vidu stalnu kliničku zabrinutost u vezi sa hepatotoksičnošću, neophodne su dugotrajnije studije koje uključuju dizajn sa dozno-zavisnim odgovorom i mehanističke parametre kako bi se preciznije definisao rizik.

Ključne reči:
Teucrium polium; bezbednost biljnih preparata; dermalna izloženost; OECD TG 407; OECD TG 410; hematologija; biohemija seruma; histopatologija; hepatotoksičnost.

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Introduction

Medicinal-plant use remains deeply embedded in North African healthcare systems, including Algeria, where herbal preparations continue to be employed for primary care and symptom management. In this context, *Teucrium* species (*Lamiaceae*) are prominent in Mediterranean folk medicine and have attracted sustained attention for their phytochemical and pharmacological properties. A recent comprehensive review of *Teucrium* essential oils highlights the wide ethnomedical use of the genus and its broad spectrum of reported biological activities (Gagliano Candela et al., 2021).

Teucrium polium L. (felty germander) is a perennial aromatic plant distributed throughout the Mediterranean basin, including Algeria. Its aerial parts are commonly used in traditional preparations, yet the plant's phytochemical output is strongly influenced by geography and developmental stage. In fact, chemical and genetic differentiation has been documented between Mediterranean subspecies, including populations from western Algeria (Djabou et al., 2012), and phenological stage has been shown to modulate phenolic content and related in vitro activities (Sharifi-Rad et al., 2022).

Contemporary syntheses describe multiple bioactivities of *T. polium* extracts and fractions (e.g., antioxidant, anti-inflammatory, antimicrobial, and metabolic effects), with polyphenols and other specialized metabolites frequently implicated (Bahramikia et al., 2022). Importantly, studies on Algerian material support the relevance of local populations: an infusion extract of *T. polium* collected in Algeria was evaluated for acute and 28-day repeated-dose oral exposure alongside wound-healing activity (Meguellati et al., 2019), and topical formulations prepared from *T. polium* extracts have been reported to promote wound contraction with short-duration dermal tolerance observations (Chabane et al., 2021).

Despite this therapeutic potential, safety remains a key issue for *Teucrium*-based preparations. Herb-induced liver injury has been reported after *T. polium* consumption in clinical case reports (Savvidou et al., 2007). At the mechanistic level, neo-clerodane diterpenes (furanoid diterpenoids) found in *Teucrium* species have been investigated for hepatotoxic potential using hepatic cell models, including compounds isolated from *T. polium* (Fiorentino et al., 2011). These observations underscore the necessity of rigorous toxicological characterization that considers extract type, dosing regimen, and population-specific chemistry.

Accordingly, well-designed safety studies aligned with internationally recognized protocols are needed to support risk assessment and the responsible development of *Teucrium polium*-derived products. OECD Test Guideline 407 provides a standardized framework for 28-day repeated-dose oral toxicity studies in rodents (OECD, 2008), and OECD Test Guideline 402 addresses acute dermal toxicity evaluation (OECD, 2017). In this context, the present work investigates the acute and repeated-dose toxicity of Algerian *T. polium* using complementary extracts spanning a polarity gradient (petroleum ether (PE), ethyl acetate (EtOAc), and *n*-butanol (BuOH) fractions) administered via oral and/or dermal routes, with the objective of generating robust evidence to inform safe use and future pharmacological development.

Materials and Methods

Plant material

Aerial parts of *T. polium* were harvested in April 2025 from the Timgad area (Batna Province, northeastern Algeria). The plant material was authenticated by a botanist from the Department of Organism Biology, University of Batna 2 (Algeria), and a voucher specimen was deposited in the herbarium of the same institution under the reference number UB2-HB-TP-2024-001. The collected aerial parts were shade-dried at room temperature and then ground into a fine powder. The powder was stored at −4 °C until extraction and subsequent analyses.

Extraction

Extracts of *T. polium* were prepared by cold maceration. Briefly, 500 g of dried aerial-part powder was extracted with 1 L of solvent (distilled water, absolute ethanol, or hydroethanol 80:20, v/v) under continuous stirring at room temperature for 72 h. The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator.

For liquid–liquid fractionation, the crude hydroethanolic extract was re-dissolved in distilled water and successively partitioned with solvents of increasing polarity: petroleum ether, ethyl acetate, and *n*-butanol. Each partition step was performed in a separatory funnel using three successive extractions (3×) with the corresponding solvent. The combined organic layers were dried (if needed), filtered, and evaporated to dryness under reduced pressure to obtain the petroleum ether (PE), ethyl acetate (EtOAc), and *n*-butanol (BuOH) fractions. The remaining aqueous phase was freeze-dried to yield the aqueous fraction. All dried extracts/fractions were stored at −20 °C until further toxicological evaluation.

Animals

Adult male and female Wistar rats (7–8 weeks old; 200–250 g) were obtained from the animal facility of the Department of Organism Biology, University of Batna 2 (Batna, Algeria). Animals were housed in polypropylene cages with a density of n=5 per sex per cage under controlled environmental conditions (25 ± 3 °C; 55 ± 20% relative humidity) with a 12-hour light/dark cycle. Rats had *ad libitum* access to standard chow and drinking water. A two-week acclimatization period was allowed before the start of the experiment. Prior to treatment, rats were fasted for 12 hours with free access to water.

All experimental procedures were conducted in accordance with European Directive 2010/63/EU on the protection of animals used for scientific purposes and were reported following the ARRIVE guidelines 2.0 (Directive 2010/63/EU, 2010; Percie du Sert et al., 2020).

Oral toxicity study (28 days)

A 28-day repeated-dose oral toxicity study was conducted in accordance with OECD Test Guideline 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents). Rats were randomly assigned to treatment groups and received the test extracts/fractions by oral gavage once daily for 28 consecutive days (dose: 2000 mg/kg/day). Animals were observed at least once daily for mortality and clinical signs. Body weight (and, where applicable, food intake) was monitored throughout the study as recommended by the guideline.

Dermal toxicity study (28 days)

Repeated-dose dermal toxicity was assessed according to OECD Test Guideline 410 (Repeated Dose Dermal Toxicity: 21/28-day study). Prior to treatment, the dorsal area was carefully clipped/shaved (approximately >=10% of body surface) to avoid abrasion. Test preparations were applied once daily to the shaved area for 28 consecutive days. The application site was covered with a non-irritating occlusive dressing during exposure to ensure standardized contact; however, occlusion may increase percutaneous absorption compared with typical consumer use, so dermal findings should be interpreted as a controlled worst-case exposure scenario. Animals were monitored daily for systemic signs of toxicity and local skin reactions in line with the guideline.

Anaesthesia, blood sampling, necropsy, and tissue collection

At study termination, animals were deeply anesthetized using a ketamine/xylazine combination. Terminal blood was collected by cardiac puncture under deep general anesthesia, which is considered a terminal procedure and must not be performed in conscious animals. Blood was collected into EDTA tubes for hematology and into plain tubes for serum biochemistry. After exsanguination, animals were euthanized while still under deep anesthesia in accordance with the AVMA Guidelines for the Euthanasia of Animals (AVMA, 2020). A complete gross necropsy was performed, and major organs (liver and kidneys) as well as treated skin were excised, rinsed in cold saline, and fixed in 10% neutral buffered formalin for histopathological examination.

Hematology and serum biochemistry

Hematological parameters were measured from EDTA blood using an automated hematology analyzer. Serum was separated by centrifugation and used for biochemical analyses, including markers of hepatic and renal function (e.g., ALT, AST, urea, and creatinine) and selected metabolic indicators, consistent with repeated-dose toxicity evaluation.

Histopathology

Formalin-fixed tissues were processed by standard paraffin-embedding procedures, sectioned (≈4–5 µm), stained with hematoxylin–eosin (H&E), and examined by light microscopy.

Statistical analysis

Quantitative data are expressed as mean +/- SD. Assumptions for parametric testing were checked (normality and homogeneity of variances). Group comparisons were performed using one-way ANOVA followed by an appropriate post hoc multiple-comparison test (specify the test used). A p-value <0.05 was considered statistically significant.

General observations and body-weight evolution

Throughout the 28-day repeated exposure period, animals remained clinically stable, and all groups displayed the expected growth trajectory for Wistar rats. Body weight increased progressively from Day 0 to Day 28 in both the control and treated groups (**Tab. 1**). Baseline body weights were highly comparable (≈182–183 g) and remained closely aligned throughout the study, reaching ≈223–225 g at day 28. The magnitude of weight gain (≈22–23%) showed only minimal between-group variability, providing no evidence of compromised nutritional status or generalized systemic intolerance following repeated administration of the petroleum ether (PEF), ethyl acetate (EtOAcF), and *n*-butanol (n-BuOHF) fractions under the tested conditions **(Tab. 1)**.

Table 1. Body-weight evolution during the 28-day repeated-dose study (female Wistar rats; mean ± SD).

Time point	Control	PEF	EtOAcF	n-BuOHF
Day 0 (g)	182.6 ± 6.9	183.1 ± 7.2	181.8 ± 7.0	182.9 ± 6.6
Day 7 (g)	191.9 ± 7.4	192.2 ± 7.6	190.8 ± 7.5	191.6 ± 7.1
Day 14 (g)	202.4 ± 8.2	202.1 ± 8.3	201.0 ± 8.4	202.0 ± 7.8
Day 21 (g)	213.3 ± 9.0	213.0 ± 9.1	212.0 ± 9.2	213.1 ± 8.6
Day 28 (g)	224.6 ± 9.7	224.1 ± 9.6	223.2 ± 9.9	224.4 ± 9.2
Δ Day 0–28 (%)	23.0 ± 3.2	22.4 ± 3.4	22.8 ± 3.3	22.7 ± 3.1

Values are expressed as mean ± SD (n=5 per sex/group). No statistically significant differences were observed between the treated and control groups.

Hematological profile at Day 28

The hematological assessment at Day 28 did not reveal patterns indicative of treatment-related hematotoxicity **(Tab. 2)**. Erythrocyte-related parameters (RBC count, hemoglobin, hematocrit, and red-cell indices) remained comparable across groups, supporting preserved erythropoietic function and the absence of anemia-like trends. Likewise, total leukocyte counts and leukocyte distribution (neutrophils and lymphocytes) were similar between control and treated animals, providing no clear evidence of systemic inflammatory activation or immunotoxicity in peripheral blood. Platelet counts were also within a comparable range across groups, arguing against thrombocytopenic or reactive thrombocytotic responses in this dataset **(Tab. 2)**.

Serum biochemistry at Day 28: hepatic and renal biomarkers

Clinical biochemistry at Day 28 showed a conserved profile of hepatic and renal biomarkers across treatment groups **(Tab. 3)**. Enzymatic indicators of hepatocellular integrity (ALT, AST) and cholestasis-related markers (ALP, total bilirubin) remained close to control values, with no consistent trend suggestive of liver injury. Markers of renal function (urea and creatinine) were likewise comparable to controls, providing no biochemical indication of overt nephrotoxicity under the present dosing regimen. In addition, glucose, total protein, and albumin remained stable, consistent with preserved metabolic and protein homeostasis during repeated exposure **(Tab. 3)**. In accordance with repeated-dose toxicity best practices, these clinical pathology outcomes should be interpreted alongside organ weights and histopathology to exclude subclinical or focal lesions that may not translate into serum changes (OECD, 2008).

Dermal tolerance scoring

Dermal application was well tolerated, with no macroscopic evidence of irritation at the application site. Erythema and edema scores remained 0 at each recorded time point (1, 24, 48, and 72 h) in the control group and all treated groups, yielding a Primary Irritation Index (PII) of 0.00. According to the Draize-based evaluation framework described in OECD Test Guideline 404, this scoring pattern is consistent with a non-irritant response under the applied exposure conditions (OECD, 2015).

3.5. Histopathological assessment (liver, kidneys, and treated skin)

Histopathological evaluation was performed on tissues fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned, and stained with hematoxylin–eosin (H&E). Lesions (when present) were described and interpreted using harmonized nomenclature and standardized diagnostic criteria, in line with INHAND recommendations (Mann et al., 2012). In repeated-dose toxicity studies, histopathology is a critical line of evidence for assessing the presence or absence of target-organ injury and integrating clinical pathology findings within a coherent safety assessment framework (OECD, 2008).

In the representative micrographs, liver sections displayed preserved lobular architecture with regular hepatocyte plates and intact sinusoidal organization, without overt hepatocellular degeneration/necrosis, conspicuous inflammatory infiltrates, or bile duct injury **(Fig. 1, Fig. 2)**. Kidney sections similarly showed maintained cortico-medullary organization, with normal-appearing glomeruli and tubules and no evident tubulo-interstitial lesions, casts, or notable interstitial inflammation **(Fig. 3)**. Consistent with the absence of macroscopic skin reactions, treated-skin sections did not reveal histological changes suggestive of irritation, including epidermal necrosis/ulceration, marked dermal edema, or prominent inflammatory cell infiltrates **(Fig. 4).**

Table 2. Hematological parameters at Day 28 following repeated exposure (female Wistar rats; mean ± SD).

	Control	Control	PEF	PEF	EtOAcF	EtOAcF	n-BuOHF	n-BuOHF
	M	F	M	F	M	F	M	F
RBC (×10^6/µL)	7.70 ± 0.38	7.54 ± 0.39	7.66 ± 0.40	7.50 ± 0.41	7.63 ± 0.42	7.47 ± 0.43	7.68 ± 0.37	7.52 ± 0.38
Hb (g/dL)	14.4 ± 0.7	14.0 ± 0.6	14.3 ± 0.7	13.9 ± 0.7	14.2 ± 0.8	13.8 ± 0.8	14.5 ± 0.6	14.1 ± 0.6
Hct (%)	43.4 ± 2.0	41.8 ± 1.9	43.2 ± 2.1	41.6 ± 2.0	42.9 ± 2.2	41.3 ± 2.1	43.7 ± 1.9	42.1 ± 1.8
MCV (fL)	56.3 ± 2.2	55.5 ± 2.3	56.4 ± 2.1	55.6 ± 2.2	56.2 ± 2.3	55.4 ± 2.4	56.6 ± 2.0	55.8 ± 2.1
MCH (pg)	18.8 ± 0.8	18.4 ± 0.8	18.8 ± 0.8	18.4 ± 0.8	18.7 ± 0.9	18.3 ± 0.9	19.0 ± 0.7	18.6 ± 0.7
MCHC (g/dL)	33.4 ± 1.0	33.0 ± 1.0	33.3 ± 1.1	32.9 ± 1.1	33.2 ± 1.2	32.8 ± 1.2	33.5 ± 1.0	33.1 ± 1.0
WBC (×10^3/µL)	8.4 ± 1.2	8.0 ± 1.1	8.2 ± 1.1	7.8 ± 1.0	8.3 ± 1.2	7.9 ± 1.1	8.5 ± 1.3	8.1 ± 1.2
Neutrophils (%)	22.6 ± 4.0	21.0 ± 4.0	23.0 ± 3.9	21.4 ± 4.0	22.8 ± 4.2	21.2 ± 4.3	22.2 ± 4.1	20.8 ± 4.2
Lymphocytes (%)	71.8 ± 5.0	73.4 ± 5.0	71.2 ± 4.9	72.8 ± 5.0	71.6 ± 5.2	73.2 ± 5.3	72.3 ± 4.8	73.9 ± 4.9
Platelets (×10^3/µL)	770 ± 90	740 ± 92	762 ± 93	734 ± 95	756 ± 98	728 ± 100	775 ± 88	745 ± 90
RDW-CV (%)	13.6 ± 0.8	13.8 ± 0.9	13.7 ± 0.8	13.9 ± 0.9	13.7 ± 0.9	14.0 ± 1.0	13.6 ± 0.8	13.9 ± 0.9
RDW-SD (fL)	36.5 ± 2.6	37.0 ± 2.8	36.7 ± 2.7	37.2 ± 2.9	36.8 ± 2.8	37.4 ± 3.0	36.6 ± 2.6	37.1 ± 2.8
Reticulocytes (%)	2.2 ± 0.4	2.3 ± 0.4	2.2 ± 0.4	2.3 ± 0.4	2.2 ± 0.5	2.3 ± 0.5	2.2 ± 0.4	2.3 ± 0.4
Monocytes (%)	3.3 ± 0.8	3.4 ± 0.8	3.3 ± 0.7	3.4 ± 0.8	3.3 ± 0.8	3.4 ± 0.8	3.3 ± 0.8	3.4 ± 0.8
Eosinophils (%)	1.5 ± 0.6	1.6 ± 0.6	1.5 ± 0.6	1.6 ± 0.6	1.5 ± 0.6	1.6 ± 0.6	1.5 ± 0.6	1.6 ± 0.6
Basophils (%)	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
Neutrophils (×10^3/µL)	1.90 ± 0.45	1.68 ± 0.42	1.89 ± 0.43	1.67 ± 0.41	1.89 ± 0.46	1.68 ± 0.44	1.89 ± 0.48	1.69 ± 0.46
Lymphocytes (×10^3/µL)	6.03 ± 1.10	5.87 ± 1.05	5.84 ± 1.05	5.68 ± 1.00	5.95 ± 1.10	5.79 ± 1.05	6.15 ± 1.20	5.99 ± 1.15
Monocytes (×10^3/µL)	0.28 ± 0.10	0.27 ± 0.10	0.27 ± 0.09	0.27 ± 0.09	0.27 ± 0.10	0.27 ± 0.10	0.28 ± 0.10	0.28 ± 0.10
Eosinophils (×10^3/µL)	0.13 ± 0.07	0.13 ± 0.07	0.12 ± 0.07	0.12 ± 0.07	0.12 ± 0.07	0.13 ± 0.07	0.13 ± 0.07	0.13 ± 0.07
Basophils (×10^3/µL)	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.02 ± 0.02
MPV (fL)	7.7 ± 0.7	7.8 ± 0.7	7.7 ± 0.7	7.8 ± 0.7	7.7 ± 0.8	7.8 ± 0.8	7.7 ± 0.7	7.8 ± 0.7
PDW (%)	15.6 ± 1.4	15.8 ± 1.5	15.7 ± 1.4	15.9 ± 1.5	15.7 ± 1.5	16.0 ± 1.6	15.6 ± 1.4	15.8 ± 1.5
PCT (%)	0.59 ± 0.08	0.58 ± 0.08	0.59 ± 0.08	0.58 ± 0.08	0.58 ± 0.09	0.57 ± 0.09	0.60 ± 0.08	0.59 ± 0.08
P-LCR (%)	22.5 ± 4.0	23.0 ± 4.2	22.6 ± 4.1	23.1 ± 4.3	22.6 ± 4.2	23.2 ± 4.4	22.5 ± 4.0	23.0 ± 4.2

Values are expressed as mean ± SD (n=5 per sex/group). Abbreviations: RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells; RDW, red cell distribution width; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit; P-LCR, platelet large cell ratio. No statistically significant differences were observed between the treated and control groups

Table 3. Serum biochemical parameters in male (M) and female (F) rats after repeated exposure.

	Control	Control	PE	PE	EtOAc	EtOAc	BuOH	BuOH
	M	F	M	F	M	F	M	F
ALT (U/L)	42.5 ± 6.2	39.9 ± 6.3	41.6 ± 6.4	40.0 ± 6.5	41.1 ± 6.8	39.9 ± 6.7	43.2 ± 6.1	40.8 ± 6.2
AST (U/L)	116 ± 18	110 ± 17	113 ± 17	109 ± 16	112 ± 18	108 ± 17	118 ± 19	112 ± 18
ALP (U/L)	162 ± 27	154 ± 26	158 ± 28	152 ± 27	156 ± 29	150 ± 28	165 ± 26	155 ± 25
Total bilirubin (mg/dL)	0.23 ± 0.05	0.21 ± 0.05	0.23 ± 0.05	0.21 ± 0.05	0.24 ± 0.06	0.22 ± 0.06	0.22 ± 0.05	0.20 ± 0.05
Urea (mg/dL)	45.0 ± 5.5	42.6 ± 5.4	45.2 ± 5.7	42.8 ± 5.6	44.3 ± 5.9	41.9 ± 5.8	45.5 ± 5.4	42.9 ± 5.3
Creatinine (mg/dL)	0.60 ± 0.08	0.52 ± 0.07	0.61 ± 0.08	0.53 ± 0.07	0.59 ± 0.07	0.51 ± 0.06	0.60 ± 0.08	0.52 ± 0.07
Glucose (mg/dL)	107 ± 12	103 ± 11	106 ± 11	102 ± 11	105 ± 12	101 ± 12	108 ± 13	104 ± 12
Total protein (g/dL)	6.7 ± 0.4	6.5 ± 0.4	6.7 ± 0.4	6.5 ± 0.4	6.6 ± 0.5	6.4 ± 0.5	6.7 ± 0.4	6.5 ± 0.4
Albumin (g/dL)	3.9 ± 0.3	3.7 ± 0.3	3.9 ± 0.3	3.7 ± 0.3	3.9 ± 0.3	3.7 ± 0.3	4.0 ± 0.3	3.8 ± 0.3

Values are expressed as mean ± SD (n=5 per sex/group). Abbreviations: RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells; RDW, red cell distribution width; MPV, mean platelet volume; PDW, platelet distribution width; PCT, plateletcrit; P-LCR, platelet large cell ratio. No statistically significant differences were observed between the treated and control groups

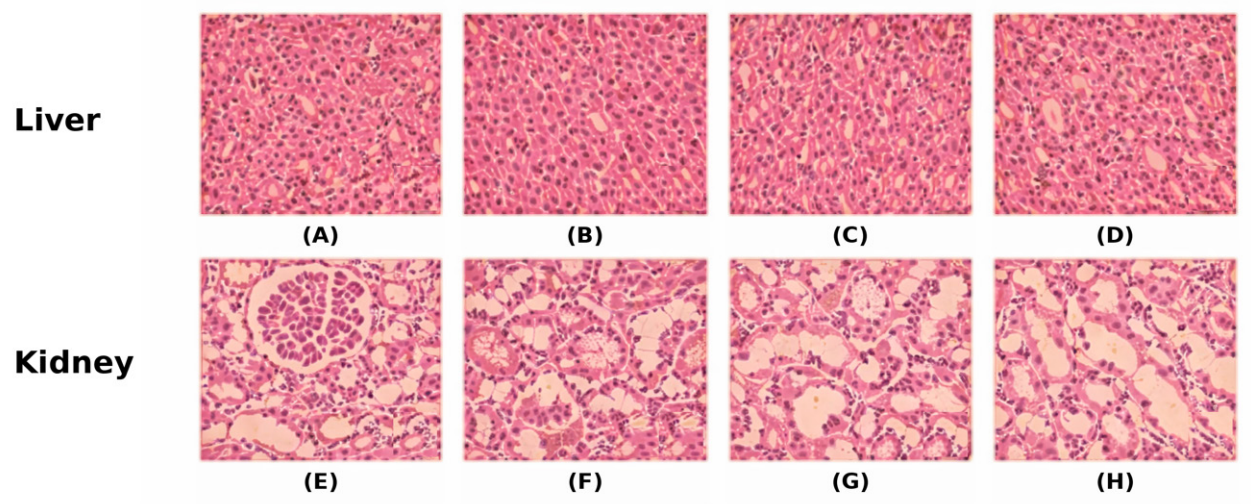


Fig. 1. Histological examination of liver (A–D), kidney (E–H) tissues from male rats following treatment: (A, E) Control group; (B, F) PE-treated group; (C, G) EtOAc-treated group; (D, H) BuOH-treated group. Sections were stained with hematoxylin and eosin (H&E). Scale bar: 100 μm; magnification: 100×.

Discussion

Herbal medicines are intrinsically complex, multi-constituent products, and their safety cannot be inferred from ethnomedical popularity alone. Contemporary toxicology emphasizes that botanical preparations may produce adverse effects through dose, duration, and preparation-dependent mechanisms, and they can also precipitate clinically relevant herb–drug interactions. In particular, herb-induced liver injury (HILI) is increasingly recognized as a major safety concern for natural products, with challenges related to product variability, patient underreporting, and limited biomarkers for early detection (Fan et al., 2025; Zhang et al., 2024). These considerations support the need for rigorous preclinical assessment and transparent reporting of botanical extract composition and exposure regimens.

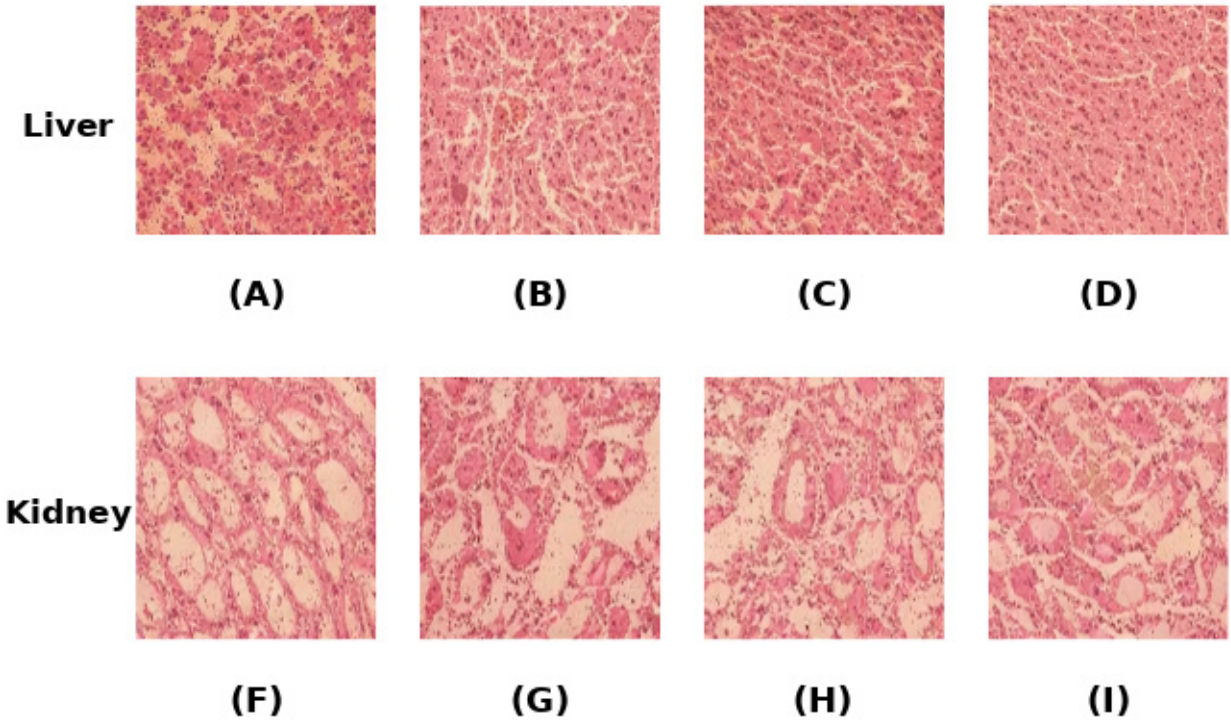


Fig. 2. Histological examination of liver (A–D), kidney (F–I) tissues from female rats following treatment: (A, F) Control group; (B, G) PE-treated group; (C, H) EtOAc-treated group; (D, I) BuOH-treated group. Sections were stained with hematoxylin and eosin (H&E). Scale bar: 100 μm; magnification: 100×.

T. polium (*Lamiaceae*) is widely used in the Mediterranean basin and North Africa for gastrointestinal complaints, inflammation, and metabolic disorders, and is also employed topically in folk practice. Phytochemical investigations indicate that the aerial parts are rich in phenolics (e.g., flavonoids and phenolic acids) and terpenoids, including neoclerodane diterpenes, which have been repeatedly highlighted as a potential liability for *Teucrium*-based products (Sharifi-Rad et al., 2022; Keller et al., 2023). Importantly, the toxicological profile of *Teucrium* preparations is not uniform: it can vary with plant chemotype/subspecies, harvesting conditions, and extraction solvent, all of which affect the qualitative and quantitative distribution of constituents (Sharifi-Rad et al., 2022).

In our study, we did not perform an acute oral limit test. Instead, we focused on repeatedexposure safety under the experimental conditions used to support *in vivo* pharmacological relevance. Specifically, we evaluated three solvent fractions: petroleum ether extract, ethyl acetate extract, and n-butanol extract administered orally and dermally for 14 and 28 days. This design is toxicologically meaningful because repeated dosing can reveal cumulative or targetorgan effects that may be missed after a single exposure, while dermal testing addresses safety under topical use scenarios. Moreover, solvent fractionation is not merely a technical step: it can concentrate different chemical families across fractions (e.g., enrichment of lipophilic terpenoids in nonpolar fractions versus enrichment of more polar phenolics in intermediate/ polar fractions), potentially shifting both efficacy and risk (Sharifi-Rad et al., 2022; Keller et al., 2023).

A central context for interpreting *Teucrium* safety is the clinical and mechanistic literature linking some *Teucrium* products to liver injury. Recent clinical reporting continues to add evidence that *Teucrium* polium exposure may be associated with serious adverse outcomes in susceptible settings, reinforcing the principle that botanicals may trigger clinically significant toxicity even when shortterm animal testing appears reassuring (Ghassa et al., 2024). Mechanistic toxicology further indicates that drug and herb-induced liver injury is strongly influenced by the hepatic microenvironment (interactions among hepatocytes, Kupffer cells, stellate cells, cholangiocytes, and sinusoidal endothelium), which can determine susceptibility and progression of injury (Zhang et al., 2024). Therefore, repeated-dose studies should be interpreted within this broader framework: the absence of toxicity signals over 14–28 days supports tolerability under the tested conditions, but it does not fully eliminate the possibility of delayed, idiosyncratic, or chemotype-specific hepatotoxicity.

Across our repeatedexposure protocols, animals showed no mortality, no treatmentrelated clinical deterioration, and no adverse effects on body weight,

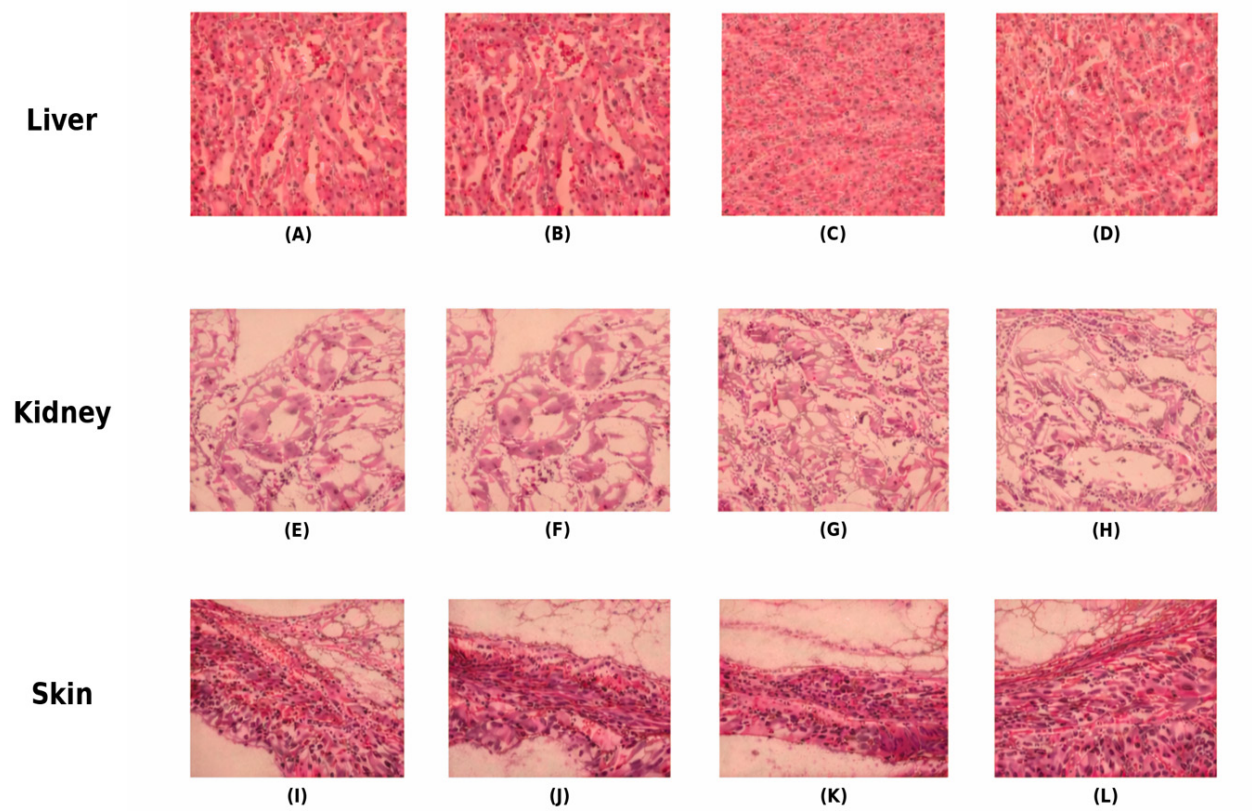


Fig. 3. Histological examination of liver (A–D), kidney (E–H), and treated skin (I–L) tissues from male rats following treatment: (A, E, I) Control group; (B, F, J) PE-treated group; (C, G, K) EtOAc-treated group; (D, H, L) BuOH-treated group. Sections were stained with hematoxylin and eosin (H&E). Scale bar: 100 μm; magnification: 100×.

suggesting the absence of systemic toxicity under the tested regimens. Organ weight evaluation (absolute and relative) is a sensitive integrative endpoint in toxicology, particularly for the liver and kidney, which are primary sites for xenobiotic metabolism and clearance. The absence of relevant organ weight changes in treated groups is consistent with a lack of overt targetorgan stress during the exposure window. However, because organ weights can remain unchanged in early or mild injury, convergent interpretation with clinical chemistry and histology is essential (Zhang et al., 2024; Fan et al., 2025).

Clinical biochemistry provides functional evidence for hepatic and renal integrity. Hepatocellular injury is commonly reflected by increased aminotransferases (ALT/AST), while ALP can provide complementary information related to biliary involvement. In our study, repeated oral and dermal administration of PE, EtOAc, and BuOH did not produce clinically meaningful enzyme elevations relative to controls, supporting the conclusion that hepatic function was preserved under the tested conditions. This is particularly relevant given that *Teucrium*-associated liver injury in humans has been reported, indicating that careful biochemical monitoring is warranted when extrapolating to realworld use (Ghassa et al., 2024). In parallel, renal safety was supported by stable urea and creatinine profiles, suggesting preserved renal clearance and no nephrotoxic signal across fractions during the study period.

Histopathological assessment remains a cornerstone endpoint because it directly evaluates tissue architecture and cellular integrity. The absence of treatment related microscopic lesions in liver and kidney sections corroborated the biochemical findings, strengthening the weight of evidence for a lack of target organ toxicity for PE, EtOAc, and BuOH under our exposure conditions. Hematological parameters further supported systemic safety, as no relevant deviations were observed in red cell indices, leukocyte counts, or platelet parameters. This convergence across clinical observations, body weight, organ weights, biochemistry, hematology, and histology increases confidence that the three fractions were tolerated during repeated oral and dermal exposure over 28 days.

Despite these reassuring findings, highlevel toxicology interpretation requires explicit consideration of uncertainties and productrelated

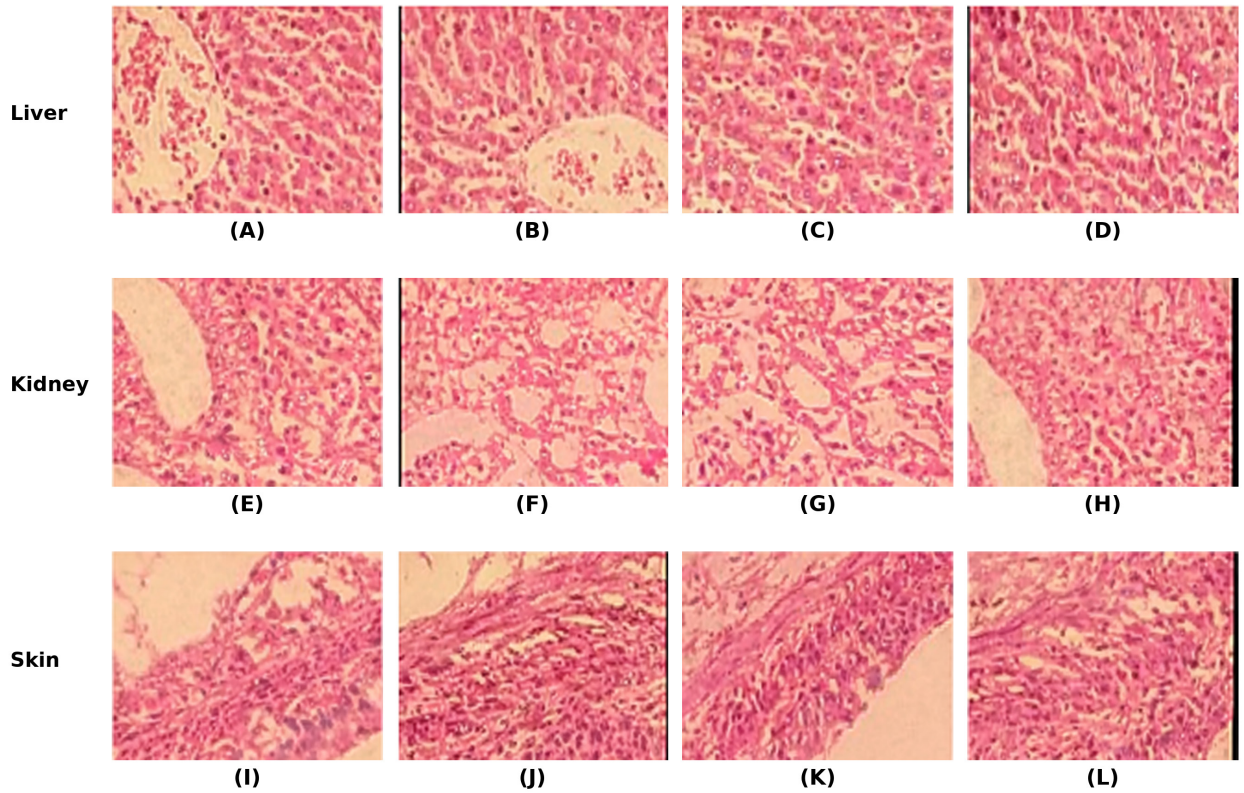


Fig. 4. Histological examination of liver (A–D), kidney (E–H), and treated skin (I–L) tissues from female rats following treatment: (A, E, I) Control group; (B, F, J) PE-treated group; (C, G, K) EtOAc-treated group; (D, H, L) BuOH-treated group. Sections were stained with hematoxylin and eosin (H&E). Scale bar: 100 µm; magnification: 100×.

risk drivers. First, *Teucrium* products are chemically variable, and the presence/abundance of neoclerodane diterpenes can differ across taxa and preparations, potentially modifying hepatotoxic risk (Keller et al., 2023; Sharifi-Rad et al., 2022). Second, HILI can be influenced by host susceptibility (e.g., immune status, comedications, metabolic polymorphisms) and diagnostic limitations that complicate translation from controlled animal studies to heterogeneous human exposures (Fan et al., 2025; Zhang et al., 2024). Third, our study window (28 days) may not capture delayed or lowincidence effects that can emerge with longer use.

Altogether, our repeated-dose data indicate that petroleum ether, ethyl acetate, and n-butanol fractions of *T. polium* were not associated with detectable toxicity signals under the applied oral and dermal regimens. These findings should be positioned as evidence of short-term tolerability within a defined exposure window and for well-described fractions, rather than as a blanket safety claim for all *Teucrium* products.

Future work should prioritize (i) robust chemical characterization and batch standardization (including marker-based quantification of relevant diterpenoids and phenolics), (ii) longer-term repeated-dose designs where justified by intended human use, and (iii) mechanistic integration using contemporary herb-induced liver injury frameworks to better connect composition, bioactivation potential, and susceptibility factors.

Conclusion

This study evaluated the tolerability of polar and apolar fractions of *Teucrium polium* following repeated oral and dermal exposure for 28 days at a dose of 2000 mg/kg/day. Under the defined experimental conditions, no treatment-related mortality or clinically relevant adverse effects were observed. Body-weight evolution, hematological parameters, serum biochemical markers of hepatic and renal function, and histopathological findings remained comparable to controls, with no evidence of target-organ injury. Dermal application was well tolerated, as indicated by the absence of macroscopic irritation (Primary Irritation Index, PII = 0.00) and no treatment-related microscopic skin alterations.

Collectively, these data support the short-term tolerability of the *T. polium* fractions within a 28-day repeated-dose framework. However, given

the known chemical variability of *Teucrium polium* preparations and existing clinical reports of herb-induced liver injury, these findings should not be extrapolated beyond the specific fractions, dose, and exposure duration evaluated. Comprehensive chemical characterization, longer-term exposure studies, dose–response analyses, and mechanistic investigations remain necessary to more fully delineate the safety profile of *Teucrium polium*-derived products.

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