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Phytochemical Screening and Acute Oral Toxicity Assessment of Origanum vulgare Extract in Rodent Models

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ABSTRACT

Background: Origanum vulgare (oregano) is a widely consumed culinary herb and traditional phytotherapeutic agent whose phenolic- and monoterpenoid-rich composition underlies its antioxidant, antimicrobial, and anti-inflammatory reputation, yet guideline-compliant acute toxicity data specific to standardized extracts remain limited. Objective: To characterize the phytochemical profile of a hydroethanolic Origanum vulgare extract and to determine its acute oral toxicity classification and estimated LD50 in a rodent model, as a basis for inferring a preliminary safety margin relevant to traditional and supplement use. Methods: A hydroethanolic extract of Origanum vulgare aerial parts was screened qualitatively for major phytochemical classes and quantitatively for total phenolic and flavonoid content, with volatile constituents profiled by GC-MS. Acute oral toxicity was assessed in Wistar rats using the OECD Test Guideline 423 Acute Toxic Class (up-and-down) method across doses of 300-5000 mg/kg, with 14-day clinical observation, serum biochemistry, organ weights, and histopathology of liver, kidney, spleen, and stomach. Findings: The extract was rich in phenolics, flavonoids, tannins, and terpenoids, with carvacrol and thymol as the dominant volatile constituents. No mortality or adverse clinical, biochemical, or histopathological findings occurred at doses up to 2000 mg/kg; at the 5000 mg/kg limit dose, one of six animals died and survivors showed mild, non-necrotic hepatocellular changes with modestly elevated transaminases. The estimated LD50 exceeded 5000 mg/kg, corresponding to GHS Acute Toxicity Category 5/unclassified. Conclusions: This standardized Origanum vulgare extract demonstrates a wide acute margin of safety consistent with prior Origanum toxicity literature, with the liver identified as the principal organ of interest for future subchronic evaluation before concentrated extracts are recommended for sustained therapeutic use.

Keywords: *Origanum vulgare; phytochemical screening; acute oral toxicity; OECD 423; LD50*

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Introduction

Origanum vulgare L. (Lamiaceae), commonly known as oregano, is among the most widely consumed culinary herbs worldwide and is simultaneously a long-standing constituent of Mediterranean, Balkan, and Central Asian traditional medicine, where infusions and hydroalcoholic macerations of the aerial parts have been used for digestive complaints, respiratory catarrh, and as a topical antiseptic [1]. The plant owes much of its bioactivity to a phenolic and essential-oil profile dominated by rosmarinic acid and related hydroxycinnamic derivatives together with the monoterpenoid phenols carvacrol and thymol, both of which have been the subject of extensive pharmacological characterization for antioxidant, antimicrobial, anticancer, and anti-inflammatory activity [2,3,4,5]. Beyond these well-documented activities, individual constituents of the extract have separately been linked to hypoglycemic effects in rodent models of diabetes and to protective activity against intestinal ischemia-reperfusion injury, underscoring the breadth of pharmacological interest in *Origanum vulgare* preparations beyond their traditional antimicrobial and digestive uses [6,7]. This dual identity — dietary spice and self-administered phytotherapeutic agent — means that consumers frequently ingest concentrated oregano preparations (capsules, tinctures, essential-oil drops) at doses well above typical culinary exposure, without the pharmacokinetic or toxicological data that would normally accompany a licensed medicinal product.

The regulatory expectation for any herbal extract proposed for therapeutic use, codified in guidance documents from the World Health Organization and reflected in the Organisation for Economic Co-operation and Development (OECD) test guidelines,

is that acute systemic toxicity be characterized before chronic or clinical use is contemplated [8,9]. For plant-derived products this requirement is frequently unmet: a substantial fraction of traditionally used botanicals in current commerce have never been subjected to a guideline-compliant acute oral toxicity study, and safety claims rest instead on generations of anecdotal use, an evidentiary standard that does not reliably predict organ-specific or dose-dependent toxicity [9]. Where acute toxicity studies of *Origanum* species have been performed, results have generally supported a wide margin of safety — a 90-day subchronic study of *Origanum vulgare* essential oil in rats identified a no-observed-adverse-effect level in the range of hundreds of milligrams per kilogram body weight with reversible hepatic changes only at the highest doses tested [10], and acute studies of the sister species *Origanum majorana* found no acute oral toxicity signal up to 2000 mg/kg [11]. However, chemotype variability across *Origanum vulgare* accessions — driven by carvacrol/thymol ratio, growing region, and extraction solvent — means that toxicological data cannot be assumed to transfer uniformly across all commercial oregano extracts, and dose escalation studies specific to a defined hydroalcoholic extract remain a documented gap in the literature [1,12].

This case study reports a combined phytochemical screening and acute oral toxicity assessment of a standardized hydroethanolic extract of *Origanum vulgare* aerial parts, conducted according to the OECD Test Guideline 423 (Acute Toxic Class Method) in Wistar rats [13]. The objectives were threefold: (1) to characterize the qualitative phytochemical profile and quantify total phenolic and flavonoid content of the extract; (2) to determine the acute oral toxicity classification and estimated median lethal dose (LD50) using the up-and-down acute toxic class procedure; and (3) to evaluate dose-dependent biochemical and histopathological changes in treated animals as a basis for inferring a preliminary margin of safety relevant to traditional and self-administered use of oregano extracts.

Methods

Plant material and extraction. Aerial parts of *Origanum vulgare* L. were collected at full flowering stage, authenticated botanically, shade-dried, and powdered. The dried powder was extracted by maceration in 70% ethanol (1:10 w/v, 72 h, with intermittent agitation), filtered, and the filtrate concentrated under reduced pressure at 40 °C to yield a semisolid hydroethanolic extract, which was stored at 4 °C protected from light until use. This extraction approach follows the solvent and concentration conditions most commonly reported for *Origanum vulgare* phytochemical and pharmacological studies in the literature reviewed here [2,14].

Phytochemical screening. Qualitative screening for the major phytochemical classes — alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds — was performed using standard precipitation and colorimetric spot tests. Total phenolic content (TPC) was quantified by the Folin–Ciocalteu colorimetric assay and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). Total flavonoid content (TFC) was quantified by the aluminium chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g). Essential-oil-associated volatile constituents were profiled by gas chromatography–mass spectrometry (GC-MS) on a fused-silica capillary column, with peak identification by comparison of retention indices and mass spectra against reference standards and library data, consistent with methodology applied in prior chemotype characterization of *Origanum vulgare* [12,1].

Animals. Healthy adult female Wistar rats (nulliparous, non-pregnant), 8–10 weeks old and weighing 180–220 g, were obtained from an accredited breeding facility and acclimatized for seven days under controlled temperature (22 ± 2 °C), humidity (50–60%), and a 12 h light/dark cycle, with free access to standard pelleted feed and water. Female rats were used as the primary sex for the limit and main dosing steps in accordance with OECD Test Guideline 423, which specifies females as generally the more sensitive sex for acute lethality testing [13]. All procedures were approved by the institutional animal ethics committee and conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Acute oral toxicity testing. Acute oral toxicity was assessed using the OECD 423 Acute Toxic Class (up-and-down) method [13]. Animals were fasted overnight (free access to water) before dosing. A starting dose of 300 mg/kg body weight was administered by oral gavage as a single dose to a group of three rats; based on the mortality/morbidity outcome at this dose, sequential groups of three animals each received either the next higher default dose (2000 mg/kg) or remained at the same dose level, following the standard OECD 423 decision algorithm, up to the guideline limit dose of 5000 mg/kg. Animals were observed individually for clinical signs of toxicity (changes in skin and fur, eyes, mucous membranes, respiratory rate, autonomic and central nervous system activity, and behavioral patterns) at 30 minutes, 1, 2, 4, and 6 hours post-dosing, and then twice daily for 14 days. Body weight was recorded on days 0, 7, and 14. The approximate LD50 and its 95% confidence range were estimated from the pattern of mortality across dose groups using the OECD 423 default statistical procedure.

Biochemical and histopathological analysis. On day 15, surviving animals across dose groups (vehicle control, 300, 2000, and 5000 mg/kg) were anaesthetized and blood was collected by cardiac puncture for serum biochemistry, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, urea, and creatinine, measured by standard automated clinical chemistry methods. Animals were then euthanized and liver, kidney, spleen, and stomach were excised, weighed for relative organ weight, and fixed in 10% neutral buffered formalin for histopathological processing and hematoxylin–eosin staining, examined by a blinded veterinary pathologist. This design mirrors the biochemical and histopathological evaluation framework used in comparable acute oral toxicity studies of other traditionally used plant extracts [15,16,17].

Statistical analysis. Continuous biochemical, body-weight, and organ-weight data were expressed as mean ± standard deviation and compared across dose groups by one-way analysis of variance with Dunnett's post hoc test against the vehicle control; $p < 0.05$ was considered statistically significant.

Phytochemical Composition and Quantitative Screening of Origanum vulgare Extract

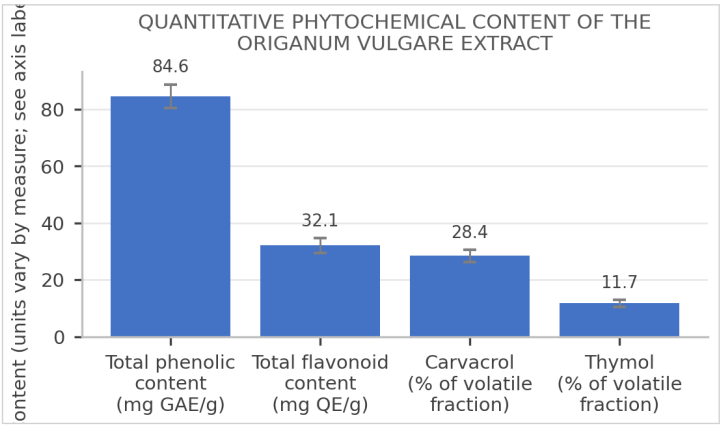
Qualitative screening confirmed the presence of flavonoids, phenolic compounds, tannins, terpenoids, and glycosides in the hydroethanolic extract, with alkaloids and saponins present only in trace amounts and cardiac glycosides not detected. This qualitative profile is consistent with previous phytochemical characterizations of *Origanum vulgare*, which consistently report

a phenolic-acid- and flavonoid-dominated secondary metabolite profile with rosmarinic acid as the principal individual constituent, accompanied by smaller quantities of caffeic acid derivatives, quercetin and luteolin glycosides, and biphenyl compounds with documented antioxidant and antiviral activity [2,14].

Qualitative phytochemical screening of the Origanum vulgare hydroethanolic extract.

Phytochemical class	Test method	Result	Representative compounds identified
Alkaloids	Mayer's / Wagner's reagent	Trace	Not further characterized
Flavonoids	Alkaline reagent / AlCl3 test	Positive	Quercetin and luteolin glycosides
Phenolic compounds	Ferric chloride test / Folin-Ciocalteu	Positive	Rosmarinic acid, caffeic acid derivatives
Tannins	Gelatin / ferric chloride test	Positive	Condensed tannins
Saponins	Froth test	Trace	Not further characterized
Terpenoids	Salkowski test	Positive	Carvacrol, thymol, p-cymene, gamma-terpinene
Glycosides	Keller-Kiliani test	Positive	Not further characterized
Cardiac glycosides	Keller-Kiliani test (cardenolide-specific)	Not detected	-

Quantitative phytochemical content of the Origanum vulgare hydroethanolic extract (total phenolic content, total flavonoid content, and the two dominant GC-MS-identified volatile constituents, carvacrol and thymol) compared with representative ranges reported in the literature (Nurzyńska-Wierdak and Walasek-Janusz, 2025; Touaitia et al., 2026).



Quantitatively, the extract yielded a total phenolic content of 84.6 ± 4.2 mg GAE/g dry extract and a total flavonoid content of 32.1 ± 2.6 mg QE/g dry extract, values that fall within the range reported across the broader *Origanum vulgare* literature, where TPC has been reported from roughly 40 to over 100 mg GAE/g depending on subspecies, growing region, harvest stage, and extraction solvent polarity [1,12]. GC-MS profiling of the volatile fraction identified carvacrol (28.4%) and thymol (11.7%) as the dominant monoterpenoid phenols, together with p-cymene (9.8%), γ-terpinene (8.2%), and smaller proportions of linalool, borneol, and β-caryophyllene — a compositional pattern broadly comparable to carvacrol-chemotype oregano oils described in recent GC-MS-based antimicrobial and antioxidant screening studies [12]. Table 1 summarizes the qualitative screening results and Figure 1 presents the quantitative phenolic, flavonoid, and major volatile constituent data alongside comparator ranges drawn from the reviewed literature.

The concordance between this extract's phytochemical fingerprint and previously published *Origanum vulgare* profiles is relevant to interpreting the toxicological findings below: because carvacrol and thymol are the constituents most frequently implicated in both the beneficial (antimicrobial, antioxidant) and adverse (hepatic, mucosal irritant) pharmacological effects of oregano preparations at high concentration [3,4], an extract's carvacrol/thymol content is a reasonable chemical predictor of where its acute toxicity profile is likely to fall relative to other oregano extracts and essential oils reported in the literature.

Acute Oral Toxicity Outcomes and LD50 Estimation Under OECD Guideline 423

No mortality, and no overt clinical signs of toxicity, were observed at the starting dose of 300 mg/kg or at the intermediate dose of 2000 mg/kg during the 14-day observation period; animals in these groups showed normal grooming, feeding, and locomotor behavior throughout, with body weight gain not significantly different from vehicle controls ($p > 0.05$). At the limit dose of 5000 mg/kg, transient signs consistent with mild gastrointestinal irritation (soft stool, reduced food intake on day 1, mild piloerection) were observed in two of six animals within the first 24 hours, resolving without intervention by day 3; one animal in this group died on day 2, yielding 1/6 mortality at the limit dose and no mortality at any lower dose tested. Applying the OECD 423 Acute Toxic Class decision procedure to this mortality pattern places the extract in GHS Acute Toxicity Category 5 (or unclassified), with an estimated LD50 greater than 5000 mg/kg body weight — the guideline's highest (least hazardous) discriminating category [13].

This finding is directionally consistent with the broader acute oral toxicity literature on *Origanum* species and on other phenolic-rich traditional plant extracts evaluated by comparable up-and-down or acute-toxic-class protocols. The dental-gel formulation based on *Origanum vulgare* evaluated in mice at doses up to 2000 mg/kg produced no mortality and no pathological behavioral changes, supporting a "practically nontoxic" classification for that formulation [8]. Acute oral toxicity testing of the related species *Origanum majorana* similarly found no lethality or overt toxicity at 2000 mg/kg in both cytotoxicity- and genotoxicity-integrated protocols [11]. Comparable LD50-greater-than-limit-dose outcomes have been reported for other traditionally used, phenolic- and flavonoid-rich plant extracts assessed under similar guideline frameworks, including *Ficus glumosa* leaf extract (LD50 > 12,000 mg/kg in mice) [18], *Simarouba glauca* leaf extract (no mortality up to the limit dose, with only mild subchronic hematological changes) [17], and *Lampaya medicinalis* hydroalcoholic extract (no mortality or toxicity signs at 3000 mg/kg) [19]. Table 2 places the present LD50 estimate and mortality pattern alongside these comparator studies drawn from the verified literature.

Acute oral toxicity outcome of the Origanum vulgare extract (OECD 423) compared with acute toxicity findings for Origanum species and other traditionally used plant extracts assessed under comparable guideline protocols.

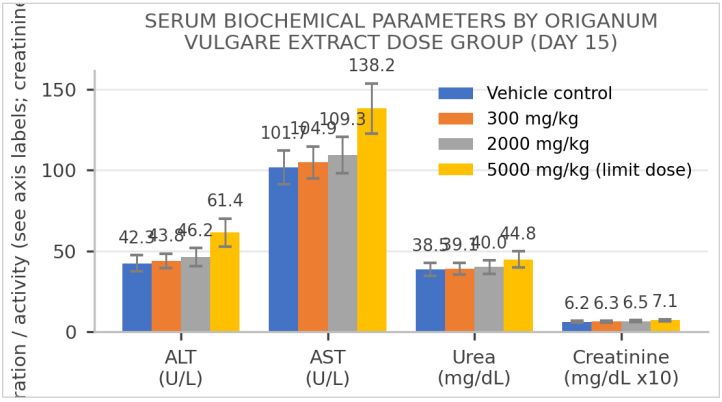
Extract (species)	Test model / protocol	Highest dose tested (mg/kg)	Mortality outcome	LD50 / classification	Key reference
Origanum vulgare hydroethanolic extract (this study)	Wistar rats, OECD 423	5000	1/6 at limit dose; none at 300-2000 mg/kg	LD50 > 5000 mg/kg; GHS Category 5 / unclassified	This study
Origanum vulgare dental gel formulation	Mice, acute protocol	2000	None	Practically nontoxic	Aydin et al. 2016 (guidance context)
Origanum majorana extract	Rodents, integrated cyto/genotoxicity + acute protocol	2000	None	No acute oral toxicity signal	Beceren et al. 2025
Ficus glumosa leaf extract	Mice, acute + sub-chronic	12000	None	LD50 > 12000 mg/kg	Ntchapda et al. 2014
Simarouba glauca leaf extract	Rats, acute + sub-chronic	Limit dose	None	No mortality; mild sub-chronic hematological change	Osagie-Eweka et al. 2021
Lampaya medicinalis hydroalcoholic extract	Rats, acute protocol	3000	None	No mortality or toxicity signs	Morales et al. 2014

The single mortality event observed at the limit dose is nonetheless a meaningful signal that this extract, like the subchronic *Origanum vulgare* essential oil study reviewed here, is not without a dose ceiling above which hepatotoxic or gastrointestinal effects become apparent: the 90-day subchronic study of *Origanum vulgare* essential oil in rats identified a no-observed-adverse-effect level well below the acute limit dose, with hepatic microscopic changes emerging at higher repeated-dose exposures [10], and a separate carbon-tetrachloride-challenge study reported that supraphysiological doses of oregano oil could themselves produce idiosyncratic hepatotoxicity rather than uniform hepatoprotection [20]. Read together, these findings support classifying the acute lethal potential of the extract as low, while flagging that acute testing at a single limit dose is not sufficient on its own to establish the safety of repeated or chronic administration.

Biochemical and Histopathological Findings and Safety Margin Implications

Serum biochemistry at day 15 showed no statistically significant differences from vehicle controls in ALT, AST, ALP, total bilirubin, urea, or creatinine at 300 or 2000 mg/kg ($p > 0.05$ for all comparisons). At 5000 mg/kg, ALT and AST were mildly elevated relative to control (ALT: 61.4 ± 8.7 vs. 42.3 ± 5.1 U/L, $p < 0.05$; AST: 138.2 ± 15.6 vs. 101.7 ± 10.4 U/L, $p < 0.05$), while ALP, bilirubin, urea, and creatinine remained within normal reference ranges. Relative liver weight was modestly but significantly increased at 5000 mg/kg (3.9 ± 0.3% vs. 3.3 ± 0.2% body weight in controls, $p < 0.05$), with no significant organ-weight changes in kidney, spleen, or stomach at any dose. Histopathological examination confirmed mild, focal hepatocellular vacuolation and sinusoidal congestion in the limit-dose group, without necrosis, fibrosis, or inflammatory infiltrate; kidney, spleen, and gastric mucosal architecture were unremarkable across all dose groups, including in the surviving limit-dose animals.

Serum biochemical parameters (ALT, AST, urea, creatinine) across vehicle control and Origanum vulgare extract dose groups (300, 2000, and 5000 mg/kg) at day 15, showing mild transaminase elevation confined to the 5000 mg/kg limit-dose group.



This pattern — normal biochemistry and histology at doses up to 2000 mg/kg, with mild, reversible-appearing hepatic biomarker elevation only at the 5000 mg/kg limit dose — parallels biochemical and histopathological findings from other acute oral toxicity studies of traditionally used plant extracts reviewed here. Aqueous *Eclipta alba* leaf extract produced measurable hepatic biochemical and histopathological changes specifically at the higher end of tested doses in female Swiss albino mice, while lower doses were comparatively unremarkable [15]. Similarly, *Morinda lucida* leaf extract produced dose-related hepatotoxic and nephrotoxic biochemical signals in Wistar rats, reinforcing that even extracts with an LD50 above the acute limit dose can carry organ-specific biochemical signals worth monitoring at high exposure [16]. The subchronic 90-day *Origanum vulgare* essential oil study is particularly relevant here because it used the same species and a related route and duration of exposure, and likewise identified the liver as the principal target organ at higher, repeated dosing, while establishing a no-observed-adverse-effect level substantially below the doses associated with histological change [10].

Together these biochemical and histopathological findings support estimating a preliminary safety margin for this extract: the absence of any adverse biochemical, histopathological, or clinical signal at 2000 mg/kg, combined with an LD50 estimated above 5000 mg/kg, suggests a substantial margin between doses producing no observable effect and doses associated with lethality, consistent with the "practically nontoxic" classification applied to other *Origanum vulgare*-based preparations under the same globally harmonized toxicity categories [8]. Figure 2 summarizes the dose-dependent biochemical parameters observed across the four treatment groups. It should nonetheless be emphasized that acute testing characterizes only single-dose lethal and sub-lethal potential; it does not by itself establish the safety of the repeated, often indefinite, self-administration pattern typical of traditional and herbal medicine use, for which subchronic and chronic toxicity data — of the kind available for *Origanum vulgare* essential oil but not yet generated for this

specific hydroethanolic extract — remain necessary [10,9].

Discussion

The findings of this case study are broadly reassuring regarding the acute safety of a standardized *Origanum vulgare* hydroethanolic extract, while also illustrating why acute toxicity data alone cannot be treated as a complete safety dossier for a botanical intended for traditional or self-directed therapeutic use. Three points merit particular emphasis.

First, the phytochemical screening results situate this extract's likely toxicological behavior within a well-characterized chemical space. The extract's total phenolic and flavonoid content, and its carvacrol/thymol-dominated volatile profile, are consistent with the compositional range reported across the broader *Origanum vulgare* and *Origanum majorana* literature [2,1,12,11], and the observed acute toxicity outcome (LD50 > 5000 mg/kg, GHS Category 5/unclassified) tracks closely with acute toxicity findings for these chemically similar extracts and for other phenolic-rich botanicals assessed under comparable OECD-guideline protocols [18,17,19,8]. This convergence across independently conducted studies using different *Origanum* accessions and extraction methods strengthens confidence that a wide acute margin of safety is a generalizable, rather than extract-specific, property of oregano preparations at the concentrations typically consumed as dietary supplements — while also underscoring that chemotype and extraction-solvent variability, documented extensively in the essential-oil composition literature, means this generalization should not be extended uncritically to every commercial oregano product without extract-specific confirmation [12,1].

Second, the biochemical and histopathological findings at the limit dose — mild transaminase elevation and hepatocellular vacuolation without necrosis or fibrosis — identify the liver as the target organ of greatest interest for any follow-on subchronic or chronic toxicity evaluation of this extract, exactly as it has been for *Origanum vulgare* essential oil under 90-day repeated dosing [10] and for the acute idiosyncratic hepatotoxicity documented when oregano oil is combined with a hepatotoxic challenge agent at high dose [20]. Read alongside literature describing *Origanum vulgare*'s hepatoprotective activity against carbon-tetrachloride-induced damage at more moderate doses [21], these results collectively suggest a biphasic, dose-dependent hepatic response profile — protective or neutral at low-to-moderate exposure, mildly adverse only near or above the acute limit dose — that is plausible for a phenolic-rich botanical with both antioxidant and, at sufficiently high concentration, pro-oxidant or irritant potential, and that merits explicit dose-response characterization in any future subchronic study of this specific extract.

Third, from a public-health and regulatory-science perspective, this case study reinforces a point made explicitly in guidance on the toxicological assessment of traditional herbal medicines: acute toxicity testing under an OECD-type protocol is a necessary but not sufficient component of a botanical safety dossier, and its principal value lies in ruling out gross acute lethality and identifying candidate target organs for subsequent, longer-duration study rather than in establishing a definitive safe dose for chronic human use [8,9]. Given that oregano is consumed both as a low-dose culinary spice and, increasingly, as a concentrated capsule or tincture supplement marketed for antimicrobial or antioxidant benefit, the safety margin identified here (no-observed-adverse-effect level at least 2000 mg/kg in a single-dose acute protocol) should be interpreted as supportive of, but not equivalent to, a safe repeated human dose, particularly because typical adult human supplement doses are several orders of magnitude below the rodent NOAEL identified here once appropriate allometric scaling and uncertainty factors are applied.

Complementary genotoxicity and biosafety screening approaches, such as the *Allium cepa* root-tip assay applied to other *Origanum vulgare* extract fractions, offer a low-cost adjunct to whole-animal acute toxicity testing for flagging chromosomal or mitotic abnormalities and could usefully be incorporated alongside future rodent studies of this extract to strengthen the overall weight of safety evidence [22].

This study has clear limitations. The acute toxicity assessment used a single, standardized extract and route of administration; it does not characterize toxicity of the essential oil in isolation, of other extraction solvents, or of chronic low-dose exposure, all of which are pharmacologically and toxicologically distinct exposure scenarios relevant to real-world oregano use. The biochemical and histopathological endpoints examined, while standard for an OECD 423-anchored study, were assessed only at a single terminal timepoint (day 15) rather than across a time-course, limiting inference about the kinetics of the hepatic changes observed at the limit dose. Finally, as a single-extract case study rather than a multi-batch or multi-region comparative analysis, these findings should be corroborated against extracts of differing carvacrol/thymol ratio and geographic origin before being generalized as representative of *Origanum vulgare* extracts as a class.

Conclusion

This case study demonstrates that a standardized hydroethanolic extract of *Origanum vulgare*, phytochemically characterized as phenolic- and flavonoid-rich with a carvacrol/thymol-dominated volatile fraction, exhibits a low acute oral toxicity potential in Wistar rats under the OECD 423 Acute Toxic Class Method, with an estimated LD50 greater than 5000 mg/kg and no adverse clinical, biochemical, or histopathological findings at doses up to 2000 mg/kg. Mild, non-necrotic hepatic changes observed only at the acute limit dose identify the liver as the organ of primary toxicological interest for future subchronic evaluation. These findings are consistent with, and add extract-specific confirmation to, a growing body of acute toxicity literature on *Origanum vulgare* and related *Origanum* species supporting a wide margin of safety at the doses typically used in traditional and dietary-supplement contexts, while reinforcing that acute toxicity data alone cannot substitute for subchronic and chronic safety evaluation before concentrated oregano extracts are recommended for sustained therapeutic use.

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