



Article Info

Received: 12th November 2025Revised: 10th January 2026Accepted: 23rd January 2026Department of Medical Laboratory
Science, University of Benin, Nigeria

*Corresponding author's email:

bolaji.odigie@uniben.edu

ORCID IDs:

-E. B. Odigie: 0000-0002-1233-0491

-B. E. Ogeyemhe: 0009-0002-9244-2131

Cite this: *CaJoST*, 2026, 1, 55-62

Short-term Hepatic Effects of *Euphorbia hirta* Aqueous Extract in Rats: Biochemical and Histological Assessment

Blessing E. Ogeyemhe and Efosa B. Odigie

A subacute experimental study was conducted to evaluate the short-term hepatic effects of aqueous *Euphorbia hirta* extract in adult male albino rats. Twenty rats were randomly assigned into four groups (n = 5): an untreated control group and three treatment groups administered 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight of the extract orally for fourteen consecutive days. Serum activities of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and bilirubin levels were determined using standard biochemical methods. Liver tissues were processed using routine histological techniques, stained with hematoxylin and eosin, and examined using a semi-quantitative histological scoring system. Statistical analysis was performed using one-way analysis of variance, with significance set at $p < 0.05$. The results showed no statistically significant differences ($p > 0.05$) in serum liver enzyme activities or bilirubin concentrations between treated groups and the control. Histological evaluation revealed preserved hepatic architecture across all groups, with only mild vascular congestion observed at the highest dose. These findings indicate that short-term oral administration of aqueous *Euphorbia hirta* extract at the tested doses does not induce biochemical or histopathological evidence of liver injury. Further studies involving longer exposure periods and additional mechanistic markers are recommended to clarify its hepatic safety profile.

Keywords: *Euphorbia hirta*; hepatotoxicity; liver enzymes; subacute toxicity.

1. Introduction

Medicinal plants remain a major component of primary healthcare systems worldwide, particularly in low- and middle-income countries where access to conventional medicines may be limited. According to the World Health Organization, the use of traditional, complementary, and integrative medicine continues to expand globally, with herbal remedies playing a central role in disease prevention and management (World Health Organization, 2025). While these products are often perceived as safe due to their natural origin, increasing evidence indicates that some herbal preparations may exert adverse effects, particularly when used repeatedly or without standardized dosing (Baba *et al.*, 2025).

Euphorbia hirta Linn., commonly referred to as asthma weed, is a widely distributed medicinal plant traditionally used for the management of respiratory, gastrointestinal, and inflammatory conditions. Ethnomedicinal and pharmacological reviews describe a broad range of reported biological activities, including antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects (Anshu, 2024; Pavithra *et al.*, 2024; Sharma *et al.*, 2025).

Phytochemical investigations have shown that the plant contains flavonoids, tannins, phenolic compounds, and other secondary metabolites that may contribute to its therapeutic potential (Meda *et al.*, 2023; Subramani *et al.*, 2024; Costa *et al.*, 2025). Owing to these properties, *E. hirta* continues to attract scientific interest, as reflected by the growing volume of experimental and review studies over the past three decades (Elisha *et al.*, 2023).

Despite its extensive traditional use, concerns remain regarding the hepatic safety of *E. hirta*, particularly under repeated exposure. The liver plays a central role in xenobiotic metabolism and is therefore highly susceptible to injury from bioactive plant constituents (Kgosiemang *et al.*, 2025). Several experimental studies have reported hepatoprotective effects of *E. hirta* extracts in chemically induced liver injury models, where normalization of liver enzymes and improved histological features were observed following extract administration (Akinboboye *et al.*, 2025; Imtiaz *et al.*, 2025; Senniappan *et al.*, 2024). In contrast, toxicological investigations have shown that high doses or prolonged administration of plant extracts

may produce biochemical or histological alterations suggestive of hepatic stress (Baba *et al.*, 2025; Mwalongo *et al.*, 2025).

These contrasting findings highlight the dualistic nature of many medicinal plants, in which biological effects are influenced by dose, extraction method, duration of exposure, and experimental context. Reviews focusing on *Euphorbia* species have emphasized that while certain constituents may confer therapeutic benefits, others may pose toxicological risks when consumed repeatedly or at elevated concentrations (Kgosiemang *et al.*, 2025; Sakharkar *et al.*, 2024). Importantly, many available studies on *E. hirta* have relied on disease-induced models or non-standardized dosing regimens, making it difficult to draw conclusions regarding its baseline hepatic safety in healthy systems (Akinboboye *et al.*, 2025; Baba *et al.*, 2025).

Furthermore, there remains a relative scarcity of controlled experimental studies that simultaneously evaluate biochemical liver function indices and histopathological features following short term administration of aqueous *E. hirta* extract. This gap is notable given that aqueous preparations are commonly used in traditional practice and may differ in toxicological profile from organic solvent extracts. Establishing whether repeated oral exposure to aqueous *E. hirta* extract induces hepatotoxic changes under controlled conditions is therefore essential for evidence-based risk assessment.

In this context, the present study was designed to investigate the hepatic effects of short-term oral administration of aqueous *E. hirta* extract in albino rats. Serum liver enzymes and bilirubin levels were assessed alongside histological examination of hepatic tissue to evaluate potential biochemical and structural alterations. It was hypothesized that repeated administration of aqueous *E. hirta* extract for fourteen days would not produce hepatotoxic changes in healthy rats.

2. Materials and Methods

2.1 Experimental Animals

Twenty healthy adult male albino rats (*Rattus norvegicus*), weighing between 100 and 150 g, were used for this study. The animals were obtained from the institutional Animal House of the Department of Anatomy and housed in standard laboratory cages under controlled environmental conditions, including a temperature of 25 ± 2 °C, relative humidity of 50–60%, and a 12 h light and dark cycle. The rats were allowed a two-week acclimatization period prior to the commencement of the experiment and were provided with standard laboratory feed and clean drinking water ad libitum.

Ethical approval for the study was obtained from the Institutional Animal Ethics Committee (Ref. No. UNIBEN/ANA/ETH/2025/01; approved January 2025), and all experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

2.2 Collection and Authentication of Plant Material

Fresh leaves of *E. hirta* Linn. were collected from the university environment and authenticated by a qualified botanist. The leaves were thoroughly washed with clean water, air dried under shade, and ground into a fine powder using a mechanical grinder. The powdered plant material was stored in an airtight container at room temperature until extraction.

2.3 Preparation of Aqueous Extract and Yield Determination

A measured quantity of the powdered leaves was soaked in distilled water and allowed to stand for 48 h with intermittent stirring. The mixture was filtered using standard filter paper, and the filtrate was concentrated to dryness using a rotary evaporator at reduced temperature. The resulting crude extract was stored at 4 °C until use. The percentage yield of the aqueous extract was 12.8% w/w following solvent evaporation. Fresh solutions of the extract were prepared daily prior to administration.

2.4 Experimental Design and Treatment

The study followed a subacute experimental design in line with the principles of the OECD Guideline 407 for repeated dose oral toxicity, adapted for a fourteen-day exposure period (OECD, 2025). The animals were randomly assigned into four groups of five rats each. Group A served as the untreated control and received distilled water only, which also served as the extraction solvent and vehicle control. Groups B, C, and D received aqueous *E. hirta* extract orally at doses of 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight, respectively. All treatments were administered once daily by oral gavage for 14 consecutive days. The selected doses fall within ranges commonly employed in repeated dose toxicity studies of medicinal plants and were chosen to evaluate potential dose dependent hepatic responses (Baba *et al.*, 2025). Investigators involved in biochemical and histopathological analyses were blinded to group allocation during sample evaluation.

2.5 Sample Collection

Twenty-four hours after the final administration, the animals were fasted overnight and euthanized using an approved humane method. Blood samples were

collected by cardiac puncture into plain sample bottles and allowed to clot. The samples were centrifuged at 3000 rpm for 10 min to obtain serum for biochemical analysis. The liver was carefully excised, rinsed in normal saline, and fixed in 10% buffered formalin for histological examination.

2.6 Biochemical Analysis

Serum activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were determined using standard colorimetric assay kits according to the manufacturers' instructions. Absorbance measurements were obtained using a Jenway 6305 UV-Visible spectrophotometer (Jenway, Bibby Scientific Ltd., United Kingdom), which was calibrated prior to use in accordance with the manufacturer's guidelines. Alanine aminotransferase and aspartate aminotransferase activities were measured at a wavelength of 340 nm, alkaline phosphatase activity was measured at 405 nm, and total and direct bilirubin concentrations were determined at 546 nm, following established analytical protocols (Klauke *et al.*, 2018; Sonntag and Lippi, 2025).

2.7 Histopathological Examination and Scoring

Fixed liver tissues were processed using routine histological techniques. The tissues were dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin wax. Sections of approximately 5 μ m thickness were cut using a rotary microtome and stained with hematoxylin and eosin. The stained sections were examined under a light microscope for hepatocellular integrity, sinusoidal architecture, vascular changes, and inflammatory cell infiltration, following standard histological assessment principles (Sigmund *et al.*, 2025).

For objective assessment, a semi-quantitative scoring system was applied to evaluate hepatic

changes based on vascular congestion and inflammatory cell presence. Scores were assigned as follows: 0 = normal hepatic architecture, 1 = mild vascular congestion, and 2 = moderate inflammatory changes. Histological evaluation and scoring were performed by an investigator blinded to treatment groups.

2.8 Statistical Analysis

All data were expressed as mean \pm standard error of the mean. Prior to inferential analysis, data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests, respectively. Statistical comparisons among groups were performed using one way analysis of variance. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

3.1 Results

3.1.1 Effects of *Euphorbia hirta* Extract on Liver Enzymes and Bilirubin Levels

Table 1 presents the effects of fourteen-day oral administration of aqueous *E. hirta* extract on serum liver enzyme activities and bilirubin levels in albino rats. One way analysis of variance revealed no statistically significant differences among the control and treated groups for alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, or direct bilirubin ($p > 0.05$ for all parameters). Overall group comparisons indicated no significant treatment effect on hepatic biochemical indices ($F(3,16) < 1.0$ for all measured variables). Mean values for all biochemical parameters remained within comparable ranges across groups, with only minor variations that did not reach statistical significance. These findings indicate that short term oral administration of aqueous *E. hirta* extract at doses of 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight did not result in measurable alterations in serum markers of hepatocellular integrity or biliary function.

Table 1. Effects of aqueous *E. hirta* extract on serum liver enzyme activities and bilirubin levels in albino rats (mean \pm SEM, $n = 5$).

Parameter	Control	200 mg/kg	600 mg/kg	1000 mg/kg	p-value
ALT (U/L)	34.20 \pm 2.11	35.40 \pm 2.56	33.85 \pm 1.98	36.10 \pm 2.42	0.74
AST (U/L)	92.45 \pm 5.13	93.60 \pm 4.80	90.85 \pm 6.01	94.20 \pm 5.54	0.82
ALP (U/L)	105.80 \pm 8.30	108.50 \pm 7.92	104.10 \pm 8.74	110.60 \pm 7.88	0.77
Total Bilirubin (mg/dL)	0.82 \pm 0.06	0.84 \pm 0.05	0.81 \pm 0.07	0.86 \pm 0.06	0.69
Direct Bilirubin (mg/dL)	0.21 \pm 0.02	0.23 \pm 0.02	0.22 \pm 0.03	0.24 \pm 0.02	0.73

No statistically significant differences were observed among groups (one way ANOVA, $p > 0.05$).

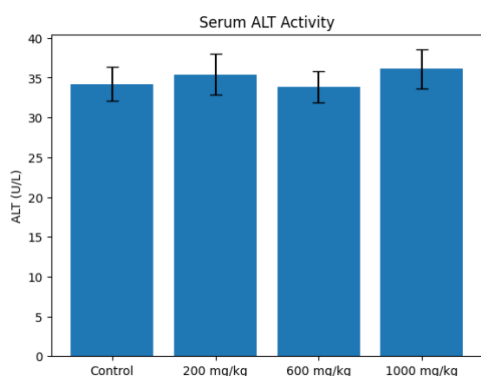


Figure 1. Effect of aqueous *E. hirta* extract on serum alanine aminotransferase (ALT) activity in albino rats.

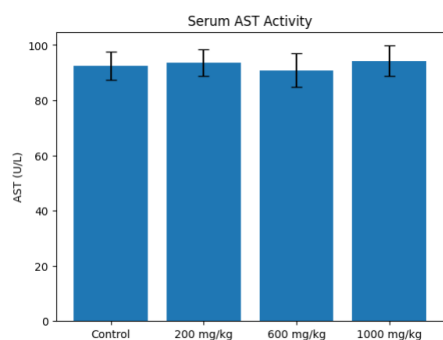


Figure 2. Effect of aqueous *E. hirta* extract on serum aspartate aminotransferase (AST) activity in albino rats

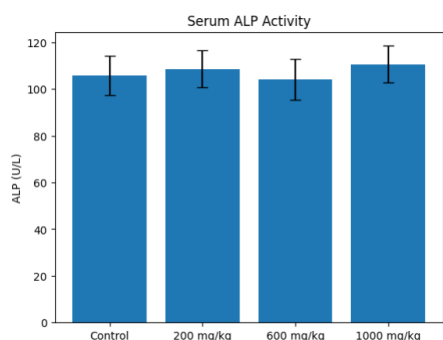


Figure 3. Effect of aqueous *E. hirta* extract on serum alkaline phosphatase (ALP) activity in albino rats

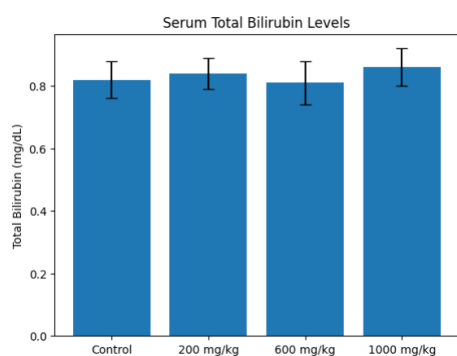


Figure 4. Effect of aqueous *E. hirta* extract on serum total bilirubin levels in albino rats

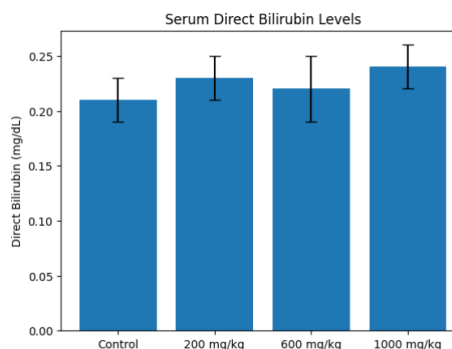


Figure 5. Effect of aqueous *E. hirta* extract on serum direct bilirubin levels in albino rats.

Mean serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as total and direct bilirubin concentrations, in albino rats following fourteen-day oral administration of aqueous *Euphorbia hirta* extract. Values are expressed as mean \pm standard error of the mean (SEM) for the control group and treatment groups administered 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight ($n = 5$ per group). No statistically significant differences were observed among groups (one-way ANOVA, $p > 0.05$).

3.1.2 Histopathological Findings

Histological examination of liver sections from both control and treated rats revealed generally preserved hepatic architecture across all groups. Hepatocytes were arranged in cords around central veins, with intact sinusoidal spaces. No evidence of hepatocellular necrosis, fatty change, or architectural distortion was observed in any group.

Semi-quantitative histological scoring showed that liver sections from the control, 200 mg/kg, and 600 mg/kg groups were predominantly scored as normal (score 0). Occasional sections from the 1000 mg/kg group demonstrated mild vascular congestion, corresponding to a score of 1. No sections from any group exhibited moderate inflammatory changes or higher pathological scores.

Representative photomicrographs illustrating liver histology across groups are presented in Plate 1 (A-D) at $\times 400$ magnification.

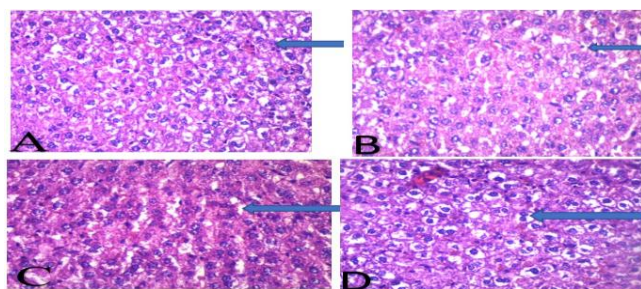


Plate 1A-D. Representative photomicrographs of liver sections from rats administered aqueous *E. hirta* extract for 14 days (H&E stain, $\times 400$; scale bar = 50 μm).

(A) Control group showing normal hepatic architecture.

(B) 200 mg/kg group showing preserved hepatocyte arrangement and sinusoids.

(C) 600 mg/kg group showing comparable hepatic architecture to control.

(D) 1000 mg/kg group showing preserved structure with occasional mild vascular congestion.

3.2 Discussion

The present study investigated the short-term hepatic effects of aqueous *E. hirta* extract following fourteen-day oral administration in albino rats, using biochemical indices and histopathological evaluation. As shown in Table 1 and illustrated in Figures 1-5, administration of the extract at doses of 200 mg/kg, 600 mg/kg, and 1000 mg/kg body weight did not result in statistically significant alterations in serum liver enzyme activities or bilirubin concentrations. In addition, histological examination of liver sections (Plate 1A-D) demonstrated preserved hepatic architecture across all treatment groups. These findings collectively suggest that short-term exposure to aqueous *E. hirta* extract does not elicit overt hepatotoxic effects under the experimental conditions employed.

Serum activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase remained comparable across all experimental groups (Table 1; Figures 1-3). These enzymes are widely recognized indicators of hepatocellular integrity and hepatobiliary function, and their elevation is typically associated with membrane disruption, cellular leakage, or cholestasis (Baba *et al.*, 2025; Kgosiemang *et al.*, 2025). The absence of significant changes in these parameters suggests that hepatocyte membranes remained intact and that biliary function was not impaired. Similar biochemical stability has been reported in acute and subacute toxicity studies of *E. hirta*, where aqueous extracts administered within controlled dose ranges did not induce enzyme elevations in rodents (Baba *et al.*, 2025). Comparable findings have also been observed in hepatoprotective studies using paracetamol-induced liver injury models, where normalization of liver enzymes following *E. hirta* administration indirectly supports its lack of intrinsic hepatotoxicity at moderate doses (Akinboboye *et al.*, 2025; Senniappan *et al.*, 2024).

Bilirubin metabolism provides additional insight into hepatic and biliary integrity. As presented in Table 1 and Figures 4 and 5, total and direct bilirubin concentrations were not significantly different between the control and treated groups. Because bilirubin conjugation and excretion depend on intact hepatocellular transport systems and bile flow, these findings indicate preserved hepatic clearance mechanisms. The observed stability aligns with analytical and clinical considerations that emphasize bilirubin as a sensitive marker of hepatobiliary dysfunction (Klauke *et al.*, 2018; Sonntag and Lippi, 2025). Similar maintenance of bilirubin levels has been reported in short-term toxicity assessments of medicinal plant extracts, including *E. hirta*, when administered within non-toxic dose ranges (Baba *et al.*, 2025).

Histopathological evaluation further corroborated the biochemical findings. Examination of liver sections revealed normal lobular architecture, intact hepatocyte cords, and preserved sinusoidal spaces across all groups, as shown in Plate 1A-C. Semi-quantitative scoring demonstrated that the majority of sections from the control, 200 mg/kg, and 600 mg/kg groups were classified as normal. Occasional mild vascular congestion was observed in sections from the 1000 mg/kg group (Plate 1D), corresponding to a low histological score. Importantly, no necrosis, fatty degeneration, or inflammatory lesions were detected. Because these mild vascular changes were infrequent and not accompanied by biochemical alterations (Table 1; Figures 1-5), they were not statistically validated as adverse effects. Such minor vascular responses have been described as adaptive or transient changes associated with increased hepatic blood flow during xenobiotic metabolism rather than indicators of structural injury (Sabucedo-Suárez *et al.*, 2025).

Mechanistically, the absence of biochemical and structural liver injury may reflect stabilization of hepatocyte membranes and attenuation of oxidative stress. Phytochemical investigations of *E. hirta* have identified flavonoids, phenolic acids, and other bioactive compounds with antioxidant and cytoprotective properties (Meda *et al.*, 2023; Costa *et al.*, 2025; Subramani *et al.*, 2024). These compounds are known to modulate oxidative pathways, reduce lipid peroxidation, and support cellular redox balance. Although oxidative stress biomarkers were not measured in this study, the stable enzyme and bilirubin profiles (Figures 1-5) are consistent with indirect preservation of oxidative homeostasis. *In silico* and *in vivo* evidence further suggests that flavonoid-rich extracts of *E. hirta* may influence pathways associated with fibrosis and cellular stress responses, supporting a non-hepatotoxic profile at moderate exposure levels (Imtiaz *et al.*, 2025).

Differences in hepatic responses reported across the literature may be attributed to variation in extraction solvents, formulation complexity, dose magnitude, and exposure duration. Ethanolic and polyherbal formulations have been shown to concentrate lipophilic constituents, which may increase both pharmacological potency and toxicological risk during repeated exposure (Mwalongo *et al.*, 2025; Sakharkar *et al.*, 2024). In contrast, aqueous extracts, such as the preparation used in the present study, more closely resemble traditional usage patterns and may contain lower concentrations of potentially hepatotoxic lipophilic compounds (Anshu, 2024; Pavithra *et al.*, 2024; Kgosiemang *et al.*, 2025). This distinction likely contributes to the favorable hepatic safety profile observed.

3.2.1 Limitations of the Study

Despite the consistency between biochemical and histological findings, several limitations should be considered. The sample size was modest, and the exposure duration was limited to fourteen days, which may not capture delayed or cumulative toxic effects. Oxidative stress markers and molecular indicators of hepatic injury were not assessed, limiting mechanistic resolution. In addition, the study employed only male rats, precluding assessment of sex-related differences in hepatic response.

3.2.2 Future Research Directions

Future investigations should extend exposure periods to evaluate subchronic and chronic toxicity, incorporate oxidative and inflammatory biomarkers, and examine sex-specific hepatic responses. Comparative studies involving aqueous and organic solvent extracts would further clarify dose standardization and solvent-dependent safety profiles. Such efforts are essential for establishing evidence-based guidelines for the safe medicinal use of *E. hirta*.

4. Conclusion

This study demonstrates that fourteen-day oral administration of aqueous *Euphorbia hirta* extract at doses of 200-1000 mg/kg body weight does not induce biochemical or histopathological liver injury in rats, establishing 1000 mg/kg as a no-observed-adverse-effect level under short-term exposure. These findings support the short-term hepatic safety of aqueous *E. hirta* preparations when used within controlled dosage limits. However, further subchronic and chronic evaluations incorporating oxidative stress biomarkers, DNA damage assessment, and cytochrome P450 modulation assays are required to define comprehensive safety margins and guide its rational use in traditional and complementary medicine.

Authors' Contributions

The authors contributed collectively to the conception and design of the study. Data collection, laboratory analysis, and histological evaluation were performed by the authors. Data analysis and interpretation were carried out jointly. All authors participated in drafting and revising the manuscript and approved the final version for submission.

Conflict of Interest

The authors declare no conflict of interest.

References

- Akinboboye, T. E., Olaniyi, T. D., Adeleke, G. E., Aborisade, A. B., and Adetutu, A. (2025). The hepatoprotective effects of *Euphorbia hirta* in ameliorating paracetamol-induced liver injury in rats. *Asian Journal of Research in Biosciences*, 7(1), 75–85. <https://doi.org/10.56557/ajorib/2025/v7i1112>
- Anshu, R. (2024). A review on pharmacological potential of *Euphorbia hirta* Linn. *International Journal for Modern Trends in Science and Technology*, 10(3), 530-534. http://ijmtst.com/volume10/issue03/83IJMTS_T1003083.pdf
- Baba, R. T., Oluboyo, B. O., Egbegi, A. H., Aladodo, M., Soliu, M. A., AbdulRahman, A. O., Babatunde, S. K., Muhammed, T. O., and Yakubu, F. (2025). Acute and sub-acute toxicity studies of *Euphorbia hirta* on mice and Wistar rats. *International Journal of Current Science Research and Review*, 8(7), 3213-3220. <https://doi.org/10.47191/ijcsrr/V8-i7-07>
- Costa, J. A. D., Marinho, A. O., Alves, R. R. V., de Barros, M. C., Nova, I. C. V., de Oliveira, S. A., Alves, J. V. O., Silva, V. F., Ferreira, M. R. A., de Oliveira, A. M., Soares, L. A. L., Maia, C. S., Tenório, F. D. C. Â. M., Lorena, V. M. B., Sá, R. A., Napoleão, T. H., Patriota, L. L. S., Macedo, M. L. R., and Paiva, P. M. G. (2025). Phytochemical profile, toxicological screening, antitumor activity, and immunomodulatory response of saline extract from *Euphorbia hirta* L. leaves. *Molecules*, 30(15), 3105. <https://doi.org/10.3390/molecules30153105>
- Elisha, I. L., Gotep, J. G., Makoshi, M. S., Usman, J. G., Shok, B. Z., Oyebade, K. F., Muhammad, Z., Akogwu, I. E., Tondo, K. B., Joseph, H., Akpojosevbe, E. J., Yakubu, B., and Muhammad, M. (2023). Trends in *Euphorbia hirta* research: A 30-year bibliometric appraisal of the present realities. *Journal of Complementary*

- Medicine Research*, 14(1), 81-92.
<https://doi.org/10.5455/jcmr.2023.14.01.16>
- Imtiaz, A., Shah, S. M. A., and Ayaz, S. (2025). Therapeutic potential of *Euphorbia hirta* and *Lepidium sativum*, and their phytochemicals in mitigating liver fibrosis: An in silico and in vivo study. *TPM – Testing, Psychometrics, Methodology in Applied Psychology*, 32(S7), 1244–1252.
<https://tpmap.org/submission/index.php/tpm/article/view/2335>
- Kgosiemang, I. K. R., Lefojane, R., Adegoke, A. M., Ogunyemi, O., Mashele, S. S., and Sekhoacha, M. P. (2025). Pharmacological significance, medicinal use, and toxicity of extracted and isolated compounds from *Euphorbia* species found in Southern Africa: A review. *Plants*, 14(3), 469.
<https://doi.org/10.3390/plants14030469>
- Klauke, R., Kytzia, H.-J., Weber, F., Grote-Koska, D., Brand, K., and Schumann, G. (2018). Reference measurement procedure for total bilirubin in serum re-evaluated and measurement uncertainty determined. *Clinica Chimica Acta*, 481, 115–120.
<https://doi.org/10.1016/j.cca.2018.02.037>
- Meda, R., Kam, S., Kagambega, W., Zongo, E., Ouedraogo, C., Segda, A., Koama, B., Somda, F., Zongo, E., and Ouedraogo, G. (2023). A review on bioactive compounds isolated from *Euphorbia hirta* L. *American Journal of Plant Sciences*, 14, 710–726.
<https://doi.org/10.4236/ajps.2023.146048>
- Mwalongo, O., Kisula, L., and Sauli, E. (2025). Phytochemical composition, toxicity, and in vivo antimicrobial properties of *Euphorbia hirta* and *Plectranthus barbatus* ethanolic extracts' polyherbal formulation. *Natural Product Communications*, 20(10), 1-14.
<https://doi.org/10.1177/1934578X251388330>
- Organisation for Economic Co-operation and Development. (2025). *Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents*. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing.
<https://doi.org/10.1787/9789264070684-en>
- Pavithra, T., Noorain, S., Vishwas, G., and Yogesha, J. S. (2024). A comprehensive review on plant of *Euphorbia hirta* Linn. *International Journal of Pharmacognosy*, 11(9), 457–468.
[https://doi.org/10.13040/IJPSR.0975-8232.IJP.11\(9\).457-68](https://doi.org/10.13040/IJPSR.0975-8232.IJP.11(9).457-68)
- Sabucedo-Suárez, A., Pérez, X., López, M., and García, P. (2025). Histological and immunohistochemical methods in normal and osteoarthritis cartilage in rat models: A systematic review of techniques. *International Journal of Molecular Sciences*, 26(21), 10300.
<https://doi.org/10.3390/ijms262110300>
- Sakharkar, S. R., Solake, U. D., Sonone, Y. P., Jadhao, A. G., Zore, M. M., and Sanap, J. B. (2024). Formulation evolution and pharmacological properties of *Euphorbia hirta*. *Journal of Pharmacognosy and Phytochemistry*, 13(4), 7–13.
<https://doi.org/10.22271/phyto.2024.v13.i4a.14989>
- Senniappan, P., Kolli, S., and Mandava, V. B. R. (2024). The antiulcer and hepatoprotective effects of *Euphorbia hirta* petroleum ether, chloroform and ethanolic extracts on paracetamol-induced ulcerated Wistar rats. *Letters in Drug Design and Discovery*, 21(10), 1793-1800.
<https://doi.org/10.2174/1570180820666230505122331>
- Sharma, A., Chaturvedi, M., and Nandha, A. (2025). *Euphorbia hirta* Linn. (Asthma plant): A comprehensive review on its phytochemical constituents and pharmacological properties. *Journal of Pharmacognosy and Phytochemistry*, 14(3), 388-395.
<https://doi.org/10.22271/phyto.2025.v14.i3e.15392>
- Sigmund, I. K., Abolghasemian, M., Bostrom, M., Bue, M., Burr, R., Jensen, L. K., Nace, J., Randelli, P. S., Rosso, F., Sabater-Martos, M., Sierra, R. J., Stefánsdóttir, A., Sukhonthamarn, K., and Yirong, Z. (2025). 2025 ICM: Diagnostic Techniques: Histology. *The Journal of Arthroplasty*, 41(1S1), S402–S406.
<https://doi.org/10.1016/j.arth.2025.08.038>
- Sonntag, O., and Lippi, G. (2025). Clinical implications of conjugated vs. direct bilirubin testing: analytical limits and clinical consequences. *Diagnosis (Berlin, Germany)*, 10.1515/dx-2025-0068. Advance online publication.
<https://doi.org/10.1515/dx-2025-0068>
- Subramani, P., Murugappan, S., Ganesh, G., Jones, S., and Mani, D. (2024). Determination of phytochemical properties of “Amman Pacharisi” (*Euphorbia hirta* Linn.). *Journal of Pharmacy and Bioallied Sciences*, 16(Suppl

5), S4697-S4700.
https://doi.org/10.4103/jpbs.jpbs_822_24

World Health Organization. (2025). *WHO global report on traditional, complementary and integrative medicine 2024*. WHO Global Centre for Traditional Medicine. <https://www.who.int/publications/i/item/9789240111387>