Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 4, https://carcinogenesis.com/

Acute Oral Toxicity Test Of Brazilin (From Caesalpinia Sappan l) On Female Wistar Rats

Nia Kurnia Sari[1,2], Sriwidodo[3] , Ajeng Diantini[4], Diki Prayugo W[2]

[1]Doctoral Programme, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia

[2]Sekolah Tinggi Farmasi Indonesia, Bandung, Indonesia

[3]Department of Pharmaceutical, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia

[4]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia

E-mail: nia22005@mail.unpad.ac.id

ABSTRACT

The development of novel anticancer therapeutics from natural sources requires comprehensive safety evaluation. The main bioactive component of sappan wood (Caesalpinia sappan L.), brazilin, has shown encouraging anticancer qualities, such as inducing apoptosis and inhibiting the multiplication of malignant cells, and so merits toxicological evaluation for clinical development. The purpose of this study is to assess the acute oral toxicity of brazilin as a potential anticancer agent and determine the LD_{50} values and target organ effects in female Wistar rats to establish safety parameters for anticancer drug development. The OECD 425 Up and Down Procedure was employed to evaluate acute oral toxicity. Female Wistar rats were administered oral dosages of 2000 mg/kg of body weight. Clinical symptoms, mortality rates, body weight changes, and histological alteration in the stomach, liver, kidneys, and spleen were observed during a period of 14 days. The data analysis using AOT425StatPgm software. No mortality or acute toxicity symptoms were observed at a dosage of 2000 mg/kg body weight, showing that brazilin is non-toxic with an LD_{50} exceeding 2000 mg/kg. Histopathological examination indicated mild, reversible alterations, such as gastric mucosal erosion and localized hepatic hydropic degeneration, without significant organ damage. The findings suggest adequate safety margins for the development of anticancer therapies. These findings provide acceptable safety margins for the development of anticancer therapies. Brazilin exhibits an excellent acute toxicity profile appropriate for anticancer drug development, with an LD_{50} approaching 2000 mg/kg body weight. The identified minor histological alterations offer significant safety implications for continuous dosage protocols in cancer therapy. Conclusions encourage the progression of brazilin to efficacy studies in cancer models and the assessment of chronic toxicity for clinical development.

Keywords: *Brazilin, Anticancer Agent, Acute Toxicity, OECD-425, LD50*

How to Cite: Nia Kurnia Sari1,2, Sriwidodo3 , Ajeng Diantini4, Diki Prayugo W2, (2025) Acute Oral Toxicity Test Of Brazilin (From Caesalpinia Sappan l) On Female Wistar Rats, *Journal of Carcinogenesis, Vol.24, No.4,* 185-192.

1. INTRODUCTION

Caesalpinia sappan L. (sappan wood) is a medicinal plant traditionally used in Indonesia. Beyond its use as a natural dye, it contains brazilin, a flavonoid that has recently emerged as a promising candidate for anticancer therapy. Numerous *in vitro* and *in vivo* studies have demonstrated that brazilin exerts potent anticancer effects through multiple mechanisms, including induction of apoptosis, inhibition of proliferation, cell cycle arrest, and suppression of metastasis in various cancer cell lines (Navarro-Tito, 2022; Pattananandecha et al., 2022).

The translation of a promising anticancer compound from the laboratory to the clinic is a multistep process that critically depends on establishing a comprehensive safety profile. While efficacy defines a drug's desired action, safety determines its therapeutic window and potential for human use (Shegokar, 2020). Acute oral toxicity testing is the fundamental first step in this safety assessment, providing initial data on the intrinsic toxicity of a substance after a single administration, identifying target organs, and determining the lethal dose 50 (LD_{50}) (Meng et al., 2024). This information is indispensable for calculating safe starting doses for subsequent repeated-dose and efficacy studies in animal models of cancer (Wang et al., 2019).

However, despite the growing body of evidence on the anticancer properties of brazilin, data on its systemic toxicity remains scarce (Hernández-Moreno et al., 2023). A compound intended for chronic administration in a potentially vulnerable population (cancer patients) requires a rigorous evaluation of its risk-benefit ratio, beginning with its acute effects (Singaraju et al., 2020).

Therefore, toxicity testing is required to evaluate the security of a drug, or a substance used as a supplement. The acute toxicity test aims to determine the short-term effects and appropriate duration and dosage to protect the public from possible detrimental effects (Sari et al., 2024).

The objectives were to determine its LD_{50} value, observe any clinical signs of toxicity, and examine histopathological alterations in vital organs. The findings from this study will provide the essential preliminary safety data required to de-risk future preclinical development of brazilin as a novel anticancer agent and to design appropriate dosing regimens for its antitumor efficacy studies.

2. METHODS

The brazilin compound used in this study was obtained from the Indonesian College of Pharmacy laboratory. Ethical approval was obtained from the Research Ethics Commission of Health, Padjadjaran University, Bandung, with letter number: 274/UN6.KEP/EC/2024.

Female Wistar rats weighing 197-246 grams were obtained from the Wistor Laboratory. Six test animals were used: one normal rat and five test rats for the limit test. After acclimatization for seven days, the rats were deemed suitable for research as their maximum weight remained within OECD (2022) guidelines of 250 grams. Physical and behavioral observations confirmed good health status with clear red eyes, normal fur, normal behavior, non-pregnant status, and no previous births. The brazilin compound was suspended in 0.5% Na-CMC due to its insolubility in water.

Limit Test Procedure
The experimental animals were divided into two groups: test group and normal control (0.5% NaCMC suspension). The initial dose used for the limit test was 2000 mg/kg body weight. Rats were fasted for 12 hours but provided sufficient water. Administration was performed using an oral probe.

Toxicity observations were made every 30 minutes for the first 4 hours, with 48-hour intervals. If rats died in the first test, the main test was immediately continued. If rats remained alive after 48 hours, they were administered the same dose with repeated observations. The test was stopped if 3 out of 5 rats survived or if 3 out of 5 rats died, requiring continuation of the main test.

Main Test Procedure
Rats were fasted for 12 hours but provided adequate water. Test animals were divided into normal control group (administered 0.5% NaCMC suspension) and test group with doses starting from 175 mg/kg body weight. Test animals were administered doses at 48-hour intervals, with observations conducted for 14 days.

If rats survived after 48 hours, the dose was increased from the previous dose; if rats died or were moribund, the dose was reduced following the OECD 425 sequence: 1.75, 5.5, 17.5, 55, 175, 550, 1750, and 5000 mg/kg body weight.
The test was stopped when meeting one of the following criteria:

1. Three consecutive animals survived above the test dose limit
2. Five repetitions occurred in every 6 animals tested consecutively
3. Three deaths were found at the same 4 concentrations

Clinical symptoms of toxic effects were monitored, including hair standing, eye clarity, pain, trembling, seizures, salivation, diarrhea, hyperactivity, respiratory system changes, and death. Body weight changes and organ weights (stomach, liver, spleen, kidneys) were recorded.

After 14 days of observation, all animals were sacrificed, and organs were surgically removed for macroscopic and microscopic examination. Histopathological preparation was conducted at Hasan Sadikin Hospital Laboratory, Bandung.
LD_{50} values were calculated using AOT425StatPgm (Acute Oral Toxicity Guideline 425 Statistical Program) software (Ganapathi et al., 2018).

3. RESULTS

Five female wistar rats were used. After seven days of acclimatization, body weights increased appropriately, confirming suitability for research. Physical and behavioral observations indicated good health status with normal characteristics.

Acute Oral Toxicity Test Results
The limit test was conducted with a dose of 2000 mg/kg body weight. Before treatment, rats were fasted for 14-18 hours but provided water. Animals were weighed and orally administered using an oral tube.

Body weight observations from days 1 to 14 showed continued weight increases in both normal and test rats. This weight change is normal, as detrimental body weight changes due to substance administration occur when there is a significant decrease of 10% from initial weight (Punger et al., 2024).

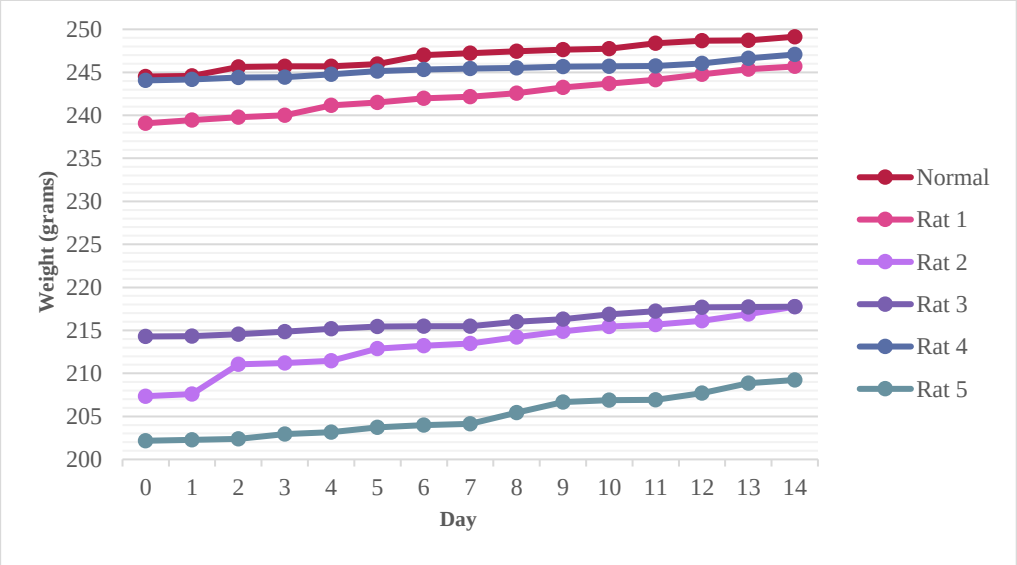

Figure 1. Graph of rats body weight after treatment

Clinical Observations and Mortality
No mortality was observed in any of the test animals during the 14-day observation period ollowing a single oral administration of brazilin at the limit dose of 2000 mg/kg body weight. Furthermore, careful monitoring revealed no signs of acute toxicity. As detailed in Table 1, all rats, including those in the control group, exhibited normal behavior. Parameters such as fur condition, eyes, motor activity, tremors, convulsions, salivation, diarrhea, and respiratory patterns showed no abnormalities compared to the control group.

Table 1. Observation of toxicity signs in rats after oral administration of brazilin

Sign toxicity	Control Rat		Rat 1		Rat 2		Rat 3		Rat 4		Rat 5	
	Day											
	1-2	3-14	1-2	3-14	1-2	3-14	1-2	3-14	1-2	3-14	1-2	3-14
Hair	-	-	-	-	-	-	-	-	-	-	-	-
Eye	-	-	-	-	-	-	-	-	-	-	-	-
Painful	-	-	-	-	-	-	-	-	-	-	-	-
Trembling	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-
Hyperactive	-	-	-	-	-	-	-	-	-	-	-	-
Breathing	-	-	-	-	-	-	-	-	-	-	-	-
Death	-	-	-	-	-	-	-	-	-	-	-	-

(-) = no toxic effect; (±) = slight toxic effect; (+) = toxic effect occurs

LD_{50} Value Determination

The LD_{50} value was analyzed using AOT425 StatPgm software version 1.0. After administering 2000 mg/kg body weight, no deaths were found in either short-term (48 hours) or long-term (14 days) observations. The test was stopped after meeting OECD termination criteria, with no deaths found in test animals at 2000 mg/kg body weight.

Results from AOT425StatPgm showed that brazilin compound administered at 2000 mg/kg body weight did not cause any test animal deaths. When no test animal deaths are found at the highest dose, the test preparation is classified as non-toxic (Sianturi S et al., 2020).

Macropathological Observations

After 14 days of observation, all rats were sacrificed, and organ surgery was performed to determine relative weights of stomach, liver, spleen, and kidneys for macroscopic and microscopic examination (Jimoh et al., 2023).

Visual observations of test animal organs (stomach, liver, spleen, kidneys) showed normal characteristics. Stomach organs appeared pink with smooth surfaces, chewy consistency, and no ulcers after brazilin compound administration. Kidney organs were reddish-brown with slippery surfaces and chewy consistency. Liver organs were reddish-brown with smooth surfaces and elastic consistency, matching normal liver characteristics (Singh et al., 2020). Spleen organs were dark reddish-brown with smooth surfaces and elastic consistency, consistent with normal spleen characteristics (Chaudhry et al., 2019).

Based on organ weight graphs, differences in liver organs were attributed to different mouse body weights when administered 2000 mg/kg body weight of brazilin compound. Visual observations showed that color, surface, and consistency did not show significant differences from normal rats.

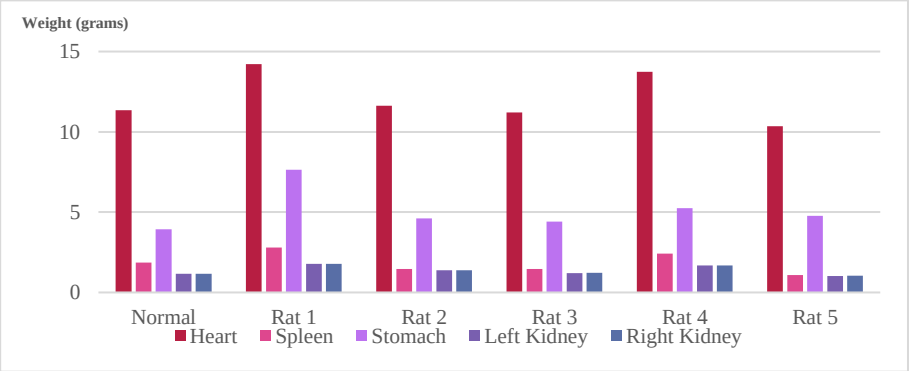

Figure 2. Graph of relative organ weights of rats treated with brazilin (2000 mg/kg bw)

Histopathological Observations

Histopathological observation involves examining sample tissue under a microscope which aims to strengthen the results of signs of toxicity in the brazilin compound. Microscopic examination parameters were used to observe damaged cells or cellular changes due to test materials (Camilla, 2018). Preparation of preparations for histopathological observation was carried out at the Hasan Sadikin Hospital Laboratory, Bandung.

Stomach Histopathology

Histopathological stomach observations showed test rats had mucosal erosion damage in all observed fields. Superficial erosion is caused by mucosal cell erosion due to decreased mucus secretion or epithelial layer disconnection of gastric mucosa. Erosive gastritis results from gastric mucosal defense damage, commonly caused by NSAIDs, alcohol, stress, and other factors (Islam et al., 2024).

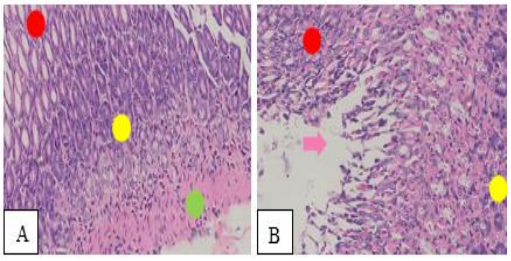

Figure 3. Histopathology of stomach (HE, 200x)
(A) Normal; (B) Rat Test

- Epithelium
- Lumina propria
- Tunica muscularis
- Desquamation
- Superficial Erosion
- Ulcer on the upper 1/3 of the mucosa

Average histopathology scoring showed brazilin compound administration affected stomach histopathology of test rats, which is reversible, meaning damage can return to normal with exposure cessation (Zanotelli et al., 2020). Superficial erosion remains in the normal category as it is reversible (Lacy & Ito, 2024).

Liver Histopathology

Liver histopathological observations showed test rats had hepatocyte hydropic degeneration damage, appearing increasingly shrunken, blackened, and disappeared, with parenchymal damage in certain spots merging without nucleus and filled with inflammatory cells (Mutlak & Mnati, n.d.). Hydropic degeneration is the mildest degeneration form, meaning damage returns to normal with exposure cessation (Azminida et al., 2024).

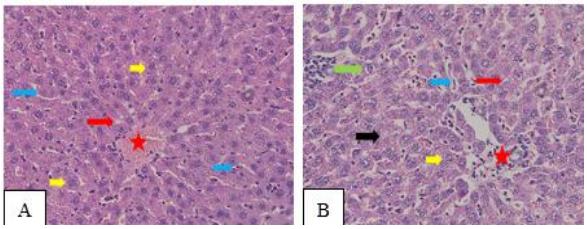

Figure 4. Histopathology of liver (HE, 200x)
(A) Normal ; (B) Rat Test

- Central Vein
- Sinusoid
- Normal liver cells / hepatocytes
- Hepatocytes undergoing hydropic degeneration
- Hepatocytes undergoing fatty degeneration
- Necrosis

Average liver histopathology scoring showed brazilin compound administration affected liver histopathology of test rats. In acute toxicity testing, brazilin compounds cause liver organ damage, namely irreversible fatty degeneration. Damage found in liver histopathology includes hydropic degeneration and necrosis. However, the percentage of damage based on scoring parameters and liver histopathological damage remains in the normal category (Susetyarini et al., 2022).

Spleen Histopathology

Spleen histopathological observations showed brazilin compound administration resulted in mild bleeding, whereas normal control spleens experienced normal bleeding; however, after brazilin compound administration, spleens experienced mild focal bleeding and necrosis, but still focal (mild necrosis) (Taveepanich et al., 2024).

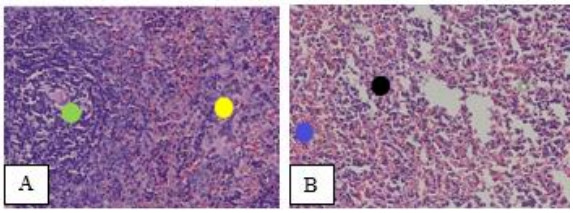

Figure 5 Histopathology of the spleen (HE, 200x)
(A) Normal ; (B) Rat Test

- Red Pulp
- White Pulp
- Bleeding
- Necrosis

Average spleen histopathological scoring showed brazilin compound administration affected spleen histopathology of test rats. Histopathological examination revealed mild necrosis and bleeding in spleen organs, namely mild focal bleeding. Both bleeding and necrosis remained in mild categories (Akat & Cakici, 2023).

Kidney Histopathology

Microscopic kidney observations showed test rats experienced hydropic degeneration damage (swollen cells). Hydropic degeneration, the mildest degeneration form, means damage returns to normal with exposure cessation (Wang et al., 2023).

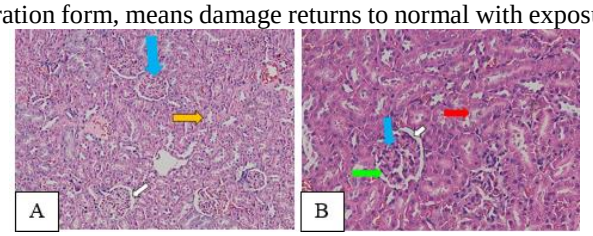

Figure 6. Histopathology of kidney (HE, 200x)
(A) Normal; (B) Rat Test

Bowman's capsule
Normal Glomerulus
Glomerular Edema
Inflammation around the glomerulus
Glomerular Atrophy
Normal Tubule
Hydropic degenerate tubules

Average kidney histopathology scoring showed brazilin compound administration influenced renal histopathology of test rats, with hydropic degeneration damage (cell swelling). Cell swelling, hydropic changes, and vacuolar degeneration are initial reversible injuries, meaning damage can return to normal. The main kidney function is maintaining ion and water content balance in blood (osmoregulation) (Tsaniya et al., 2024); therefore, kidneys maintain ion balance by filtering blood, excreting metabolic waste products, and reabsorbing small molecules (glucose, fatty acids, amino acids, peptides), ions, and water (John & Pasha, 2024).

4. DISCUSSION

The acute oral toxicity assessment of brazilin indicates a positive safety profile for anticancer drug development, with an LD_{50} surpassing 2000 mg/kg body weight. This safety margin is advantageous compared to numerous established anticancer drugs that demonstrate significant toxicity at therapeutic doses, offering a promising basis for further development (Arzuk et al., 2022).

The observed histopathological alterations, although mild and reversible, offer significant insights for the advancement of anticancer therapies (Aljehani et al., 2023). Gastric mucosal erosion and hepatic hydropic degeneration may attain clinical significance with chronic dosing regimens necessary for cancer therapy (He et al., 2023). The findings underscore the necessity of monitoring hepatic function during clinical development, given that hepatotoxicity is a prevalent issue in anticancer drug development (Frey, 2025).

The mild inflammatory responses noted in various organ systems may enhance anticancer efficacy via hormetic mechanisms, where regulated cellular stress improves immune surveillance, potentially complementing the direct anticancer effects of brazilin (Raptania et al., 2024). The liver findings are significant due to the organ's essential function in drug metabolism and its potential for drug-drug interactions in combination cancer therapies (DeLeve et al., 2004).

The alterations in the spleen, such as mild focal bleeding and necrosis, may indicate the immunomodulatory effects of brazilin pertinent to anticancer mechanisms. The spleen's involvement in immune surveillance and its interaction with tumor cells renders these findings significant for anticancer development (Wei et al., 2024).

The non-toxic classification establishes a basis for progressing to chronic toxicity studies and efficacy assessments in cancer models. The established safety margin facilitates dose escalation studies and the exploration of combination therapies with current anticancer agents (Prior et al., 2024). Future research must establish the relationship between observed histopathological changes and the anticancer mechanisms of brazilin, alongside pharmacokinetic characterization and long-term toxicity evaluation, which are essential for clinical development.

The study's limitations include the use of single-dose administration and short-term observation period, which may not reflect the chronic dosing regimens required for cancer treatment. Long-term toxicity studies will be essential to evaluate the cumulative effects of repeated brazilin administration and to identify any delayed-onset toxicities that could impact its clinical development trajectory.

5. CONCLUSION

Brazilin's acute oral toxicity profile is critical to its preclinical development as an anticancer drug. Brazilin is classified as non-toxic, with an LD_{50} surpassing 2000 mg/kg body weight, ensuring a secure basis for cancer model efficacy investigations and chronic toxicity evaluations. The absence of immediate mortality and severe clinical signs suggests a safe preliminary safety profile for anticancer medication development.

The observed histopathological changes, including gastric mucosal erosion and hepatic hydropic degeneration, reveal brazilin's potential target organ effects that must be considered in anticancer therapy development. In future preclinical and clinical trials, these data will guide safety monitoring and dose optimization. The multi-organ distribution of minor impacts suggests systemic activity that may affect cancer treatment efficacy and safety.

The safety margin allows brazilin's anticancer mechanisms and dose-response correlations to be studied in cancer models. Brazilin should be studied for longterm toxicity, pharmacokinetics, and effectiveness in diverse cancer types to progress clinical development. Investigation of potential synergistic effects with existing anticancer medicines may discover combination therapy techniques that improve treatment outcomes while maintaining safety.

Brazilin transitions from a traditional medicinal chemical to a scientifically confirmed anticancer medication candidate thanks to its toxicological base, adding to oncology's natural product-derived therapeutic armament. Brazilin's antitumor efficacy and low acute toxicity make it an attractive cancer drug candidate.

REFERENCE

[1] Akat, E., & Cakici, O. (2023). Evaluation the Histopathological Changes Accompanied for the Toxic Effects of Diazinon on the Spleen of Rats. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*. https://doi.org/10.18016/ksutarimdoga.vi.1096018

[2] Aljehani, M. R., Alamri, F. H., Elyas, M. E. K., Almohammadi, A. S., Alanazi, A. S. A., & Alharbi, M. A. (2023). The importance of histopathological evaluation in cancer diagnosis and treatment. *International Journal of Health Sciences*. https://doi.org/10.53730/ijhs.v7ns1.15270

[3] Arzuk, E., Ergüç, A., & Karakus, F. (2022). *Repurposing drugs for cancer therapy*. *5*(1), 41. https://doi.org/10.26650/jarhs2021-1133474

[4] Azminida, D. S., Firdaus, I. W. A. K., Utami, J. P., Oktiani, B. W., & Wydiamala, E. (2024). TOXICITY TEST OF Eusideroxylon zwageri BARK EXTRACT BASED ON LIVER HISTOPATHOLOGY HYDROPIC DEGENERATION AND NECROSIS. *Dentino Jurnal Kedokteran Gigi*, *9*(2), 170. https://doi.org/10.20527/dentino.v9i2.20400

[5] Camilla, R. (2018). *Microscopic Examination of Biological Tissues: Study of Microscopic Changes*. *3*(2), 1–3. https://doi.org/10.4172/2472-4971.1000156

[6] Chaudhry, S. R., Luskin, V., & Panuganti, K. K. (2019). *Anatomy, Abdomen and Pelvis, Spleen*. https://pubmed.ncbi.nlm.nih.gov/29489170/

[7] DeLeve, L. D., Hoyumpa, A. M., & Schenker, S. (2004). *Drugs and the Liver* (pp. 145–169). Current Medicine Group, London. https://doi.org/10.1007/978-1-4615-6502-4_6

[8] Frey, C. (2025). Hepatobiliary Adverse Events Associated With the KRAS p.G12C Inhibitor Sotorasib. *Pharmacoepidemiology and Drug Safety*, *34*(2). https://doi.org/10.1002/pds.70104

[9] Ganapathi, S. C., Holla, R., Ym, S. S., & Mundugaru, R. (2018). Acute oral toxicity study of ethanolic extract of actinoscirpus grossus (l.f.) goetgh. and d.a. simpson. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(7), 321–323. https://doi.org/10.22159/AJPCR.2018.V11I7.21688

[10] He, X., Wang, Y. Y., & Li, J. N. (2023). *Mechanism and management of cancer chemotherapy-induced gastrointestinal mucosa damage*. *62*(11), 1353–1357. https://doi.org/10.3760/cma.j.cn112138-20221118-00867

[11] Hernández-Moreno, A., Nava-Tapia, D. A., Bello-Martínez, J., Olea-Flores, M., Hernández-Moreno, T., Zuñiga-Eulogio, M. D., & Navarro-Tito, N. (2023). The isoflavonoid brazilin inhibits viability and cell migration in breast cancer cells. *bioRxiv*. https://doi.org/10.1101/2023.08.17.553723

[12] Islam, H., Siddiqui, A., Islam, R., Islam, T., Ahmed, S. K., Fahim, M., Khalid, M., Malik, G. M. A., & Imtiaz, H. (2024). NSAID-induced Gastric Ulcer Disease: A Deleterious Connection. *Discovery Medicine*, *36*(188), 1789. https://doi.org/10.24976/discov.med.202436188.165

[13] Jimoh, A., Maiha, B. B., Chindo, B. A., Ejiofor, J. I., & Yakubu, M. T. (2023). Effects of sub-chronic oral administration of hydromethanolic stem extract of Costus afer Ker Gawl. (Costaceae) on body weight, relative organ weight (ROW) and histopathology of selected organs in rats. *Journal of Current Biomedical Research*, *3*(4, July-August). https://doi.org/10.54117/jcbr.v3i4.1

[14] John, A., & Pasha, T. (2024). *Laboratory tests of renal function*. https://doi.org/10.1016/j.mpaic.2024.03.002

[15] Lacy, E. R., & Ito, S. (2024). *Mechanisms of Rapid Epithelial Restitution of the Superficial Rat Gastric Mucosa After Ethanol Injury*. 65–81. https://doi.org/10.1201/9781003574712-8

[16] Meng, L., Han, R., Li, J. K.-J., Wu, W., & Gu, J. (2024). Research Progress in Acute Oral Toxicity Testing Methods. *International Journal of Biology and Life Sciences*, *6*(1), 19–22. https://doi.org/10.54097/nv9van65

[17] Mutlak, B. H., & Mnati, I. M. (n.d.). *Histopathological Changes in Liver Induced by Piroxicam Administration in Adult Male Albino Rats Mus Musculus*. https://doi.org/10.37506/ijfmt.v14i3.10630

[18] Navarro-Tito, N. (2022). Brazilin: Biological activities and therapeutic potential in chronic degenerative diseases and cancer. *Pharmacological Research*, *175*, 106023. https://doi.org/10.1016/j.phrs.2021.106023

[19] Pattananandecha, T., Apichai, S., Julsrigival, J., Ogata, F., Kawasaki, N., & Saenjum, C. (2022). Antibacterial Activity against Foodborne Pathogens and Inhibitory Effect on Anti-Inflammatory Mediators' Production of Brazilin-Enriched Extract from Caesalpinia sappan Linn. *Plants*, *11*(13), 1698. https://doi.org/10.3390/plants11131698

[20] Prior, H., Baldrick, P., Clarke, D. N., Passini, E., Sewell, F., & van der Meer, P. (2024). Exploring Greater Flexibility for Chronic Toxicity Study Designs to Support Human Safety Assessment While Balancing 3Rs Considerations. *International Journal of Toxicology*. https://doi.org/10.1177/10915818241255885

[21] Punger, E. M., Norris, S. L., Stevens, S. W., Santos, K. H., & Christy, A. C. (2024). Investigating the Effect of Enterally Administered Capromorelin on Body Weight in Rats (Mus musculus). *Comparative Medicine*. https://doi.org/10.30802/aalas-cm-24-031

[22] Raptania, C. N., Zakia, S., Fahira, A. I., & Amalia, R. (2024). Article review: Brazilin as potential anticancer agent. *Frontiers in Pharmacology*. https://doi.org/10.3389/fphar.2024.1355533

[23] Sari, N. K., Fauzi, N. I., Diantini, A., & W, D. P. (2024). Acute toxicity test of tablets containing α-mangosteen, piperine, curcumin, methyl cinnamate and vitamin C in female wistar rats. *Pharmacy Education*. https://doi.org/10.46542/pe.2024.242.158162

[24] Shegokar, R. (2020). *Preclinical testing—Understanding the basics first* (pp. 19–32). Elsevier. https://doi.org/10.1016/B978-0-12-821222-6.00002-6

[25] Singaraju, M., Palaian, S., Shankar, P. R., & Shrestha, S. (2020). Safety Profile and Toxicity Amelioration Strategies of Common Adverse Effects Associated with Anticancer Medications. *Journal of Pharmaceutical Research*, 18–30. https://doi.org/10.9734/JPRI/2020/V32I1130499

[26] Singh, G., Joshi, H., Parmar, N., Saran, D., & Meshram, B. (2020). Macroscopic analysis on the kidneys of guinea fowl (Numida meleagris). *Journal of Entomology and Zoology Studies*, *8*(2), 938–940. https://www.entomoljournal.com/archives/?year=2020&vol=8&issue=2&ArticleId=6548

[27] Susetyarini, E., Ainiah, F., & Permana, F. H. (2022). Histopathological condition of male rat liver induced by condensed tannin extract of beluntas leave. *Jurnal Biosilampari*, *5*(1), 90–97. https://doi.org/10.31540/biosilampari.v5i1.1537

[28] Taveepanich, S., Chayajarus, K., Jittimanee, J., Phusri, N., Thongdee, P., Sawatdee, K., Kamsri, P., Punkvang, A., Suttisintong, K., Pungpo, P., Suwannaloet, W., Thongrung, R., & Pangjit, K. (2024). Iron chelating, antioxidant, and anti-inflammatory properties of brazilin from Caesalpinia sappan Linn. *Heliyon*, *10*(19), e38213. https://doi.org/10.1016/j.heliyon.2024.e38213

[29] Tsaniya, G. E. N., Firdaus, I. W. A. K., Dewi, N., Utami, J. P., & Setiawan, B. (2024). TOXICITY TEST OF Eusideroxylon zwageri BARK EXTRACT ON KIDNEY HISTOPATHOLOGY GLOMERULAR HYPERTROPHY AND HYDROPIC DEGENERATION. *Dentino Jurnal Kedokteran Gigi*, *9*(2), 188. https://doi.org/10.20527/dentino.v9i2.20403

[30] Wang, H., Piao, Z., Ma, H., Cao, L. P., Liu, J. J., & Wu, J. (2023). Short-term exposure to gossypol causes reversible reproductive toxicity and nephrotoxicity in rats. *Journal of Southern Medical University*, *43 2*(2), 251–256. https://doi.org/10.12122/j.issn.1673-4254.2023.02.13

[31] Wang, J., Dong, H., Liu, J., Zheng, N., Xie, X., & Jia, L. (2019). The Evaluation of Animal Models in the Development of Anticancer Agents: From Preclinical to Clinical Tests. *Current Cancer Drug Targets*, *19*(4), 277–284. https://doi.org/10.2174/1568009618666180817095331

[32] Wei, D., Wang, G., Li, S., Wang, S., Li, W., Liu, H., Liang, Y., Zhou, Z., He, X., Guo, W., Yuan, J., Chai, Y., Geng, J., & Li, Z. (2024). *Noninvasive focused ultrasound non-specifically activating spleen immunological function to suppress tumor proliferation: a new strategy for cancer immunotherapy*. https://doi.org/10.21203/rs.3.rs-4639146/v1

[33] Zanotelli, P., Locateli, G., Dalla Vecchia, C. A., Gomes, D. B., Oliveira, B. M. M., Lutinski, J., Predebom, A. J., Miorando, D., Zanatta, M. E. de C., Steffler, A. M., Carteri, C. S., Santos, M. de F. C., Barison, A., Silva, L. M. da, de Andrade, S. F., & Roman Junior, W. A. (2020). Gastroprotective Potential of the Hydroalcoholic Extract from Jacaranda puberula in Rats. *Revista Brasileira De Farmacognosia-Brazilian Journal of Pharmacognosy*, *30*(6), 838–843. https://doi.org/10.1007/S43450-020-00117-2