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EFEITO PROTETOR DA *CURCUMA LONGA* NA TOXICIDADE INDUZIDA POR DOCETAXEL: UMA ANÁLISE EXPERIMENTAL

Umuarama
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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal com Ênfase em Produtos Bioativos da Universidade Paranaense como parte dos requisitos para obtenção do título de Doutor em Ciência Animal com área de concentração em Saúde Única.

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Trabalho de conclusão do Programa de Pós-Graduação em Ciência Animal com Ênfase em Produtos Bioativos aprovado como requisito para obtenção do título de Doutor em Ciência Animal com Ênfase em Produtos Bioativos pela Universidade Paranaense – UNIPAR, pela seguinte banca examinadora:

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Umuarama, 30 de Agosto de 2025.

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Isabella Morais Tavares Huber

“Nenhum vencedor acredita no acaso". (Friedrich Nietzsche)

HUBER, Isabella Morais Tavares. **Efeito protetor da *Curcuma longa* na toxicidade induzida por Docetaxel: uma análise experimental**. Orientador: Emerson Luiz Botelho Lourenço. Co-orientador: Leonardo Garcia Velasquez. 2025. xx f. *(*f. = *número de folhas da tese)* Tese (Doutorado em Ciência Animal com Ênfase em Produtos Bioativos) - Universidade Paranaense, Umuarama, 2025.

RESUMO

A quimioterapia citotóxica permanece como eixo central do tratamento de diversas neoplasias sólidas, embora seu uso esteja frequentemente limitado por eventos adversos hematológicos e sistêmicos de alta morbidade. Nesse contexto, estratégias adjuvantes capazes de atenuar a toxicidade induzida por agentes como o docetaxel têm ganhado relevância, especialmente aquelas baseadas em compostos naturais com propriedades antioxidantes e imunomoduladoras. A *Curcuma longa*, rica em curcuminoides, emerge como uma candidata promissora. O presente estudo teve como objetivo investigar, em modelo experimental com ratos Wistar, os efeitos protetores da *Curcuma longa* frente à toxicidade hematológica e sistêmica induzida por docetaxel. O trabalho foi desenvolvido em dois eixos principais. No primeiro artigo, avaliou-se o impacto da *Curcuma longa* nos parâmetros hematológicos e no peso relativo do baço, em três tempos experimentais (7, 14 e 21 dias), com 5 subgrupos por tempo (n=7): placebo, docetaxel isolado, e docetaxel associado a *Curcuma longa* nas doses de 25, 50 ou 500 mg/kg/dia. O docetaxel promoveu reduções progressivas de hemácias, hemoglobina, hematócrito, leucócitos e plaquetas, com pico de mielossupressão no dia 21. A coadministração de *Curcuma longa* atenuou essas citopenias de forma tempo- e dose-dependente, com melhores resultados nas doses intermediárias (25–50 mg/kg). Observou-se também esplenomegalia significativa no grupo 500 mg/kg, sugerindo atividade hematopoiética extramedular ou ativação imune. A análise da largura de distribuição dos eritrócitos (RDW) e dos índices hematimétricos reforçou o papel regulador da *Curcuma longa* na homeostase hematológica. No segundo artigo, a ênfase foi dada à toxicidade sistêmica, com foco em marcadores bioquímicos hepáticos (AST, ALT, bilirrubinas) e renais (ureia, creatinina), eletrólitos séricos (sódio e potássio), além do peso relativo dos órgãos-alvo. O docetaxel promoveu elevação significativa de AST, ALT e bilirrubinas, assim como de ureia e creatinina, especialmente no dia 14. Houve também redução de potássio, hipertrofia hepática e pulmonar, hipotrofia renal e cardíaca, além de aumento intestinal específico em doses

intermediárias. A *Curcuma longa* promoveu reversão significativa desses efeitos adversos, especialmente nas doses de 50 e 500 mg/kg, restabelecendo valores bioquímicos à normalidade ao final de 21 dias. Os mecanismos envolvidos incluem modulação das vias Nrf2, NF-κB, MAPK e PI3K/Akt, redução de citocinas inflamatórias (IL-6, TNF-α) e aumento de proteínas antiapoptóticas (Bcl-2). Os dados obtidos sustentam o uso da *Curcuma longa* como adjuvante fitoterápico promissor na oncologia experimental, com benefícios hematológicos, hepatorrenais e sistêmicos. Estes achados justificam futuras investigações clínicas e moleculares, visando sua integração segura e eficaz aos protocolos quimioterápicos.
Palavras-chave: Fitoterapia; Quimioterapia; Terapia adjuvante; Toxicidade sistêmica; Hematopoiese.

HUBER, Isabella Morais Tavares. Thesis title. Protective effect of *Curcuma longa* on Docetaxel-induced toxicity: an experimental analysis. Advisor: Emerson Luiz Botelho Lourenço. Co-advisor: Leonardo Garcia Velasquez. 2025. xx p. (p. = number of pages of the dissertation/thesis). Thesis (Doctorate degree in Animal Science with Emphasis on Bioactive Products) - Universidade Paranaense, Umuarama, 2025.

ABSTRACT

Cytotoxic chemotherapy remains a cornerstone in the treatment of various solid tumors; however, its use is often limited by hematological and systemic adverse events with high morbidity. In this context, adjuvant strategies capable of attenuating the toxicity induced by agents such as docetaxel have gained increasing relevance, especially those based on natural compounds with antioxidant and immunomodulatory properties. *Curcuma longa*, rich in curcuminoids, emerges as a promising candidate. This study aimed to investigate, in an experimental Wistar rat model, the protective effects of *Curcuma longa* against docetaxel-induced hematological and systemic toxicity. The study was structured around two main investigative axes. In the first article, the effects of *Curcuma longa* on hematological parameters and spleen relative weight were evaluated across three experimental durations (7, 14, and 21 days), with five subgroups per time point (n=7): placebo, docetaxel only, and docetaxel combined with *Curcuma longa* at 25, 50, or 500 mg/kg/day. Docetaxel induced progressive reductions in red blood cells, hemoglobin, hematocrit, leukocytes, and platelets, with a peak of myelosuppression on day 21. Co-administration of *Curcuma longa* attenuated these cytopenias in a time- and dose-dependent manner, with the best outcomes observed at intermediate doses (25–50 mg/kg). Significant splenomegaly was also noted in the 500 mg/kg group, suggesting extramedullary hematopoiesis or immune activation. Analysis of red cell distribution width (RDW) and hematimetric indices further supported the regulatory role of *Curcuma longa* in hematopoietic homeostasis. In the second article, emphasis was placed on systemic toxicity, focusing on biochemical markers of hepatic (AST, ALT, bilirubins) and renal (urea, creatinine) function, serum electrolytes (sodium and potassium), and the relative weight of target organs. Docetaxel significantly increased AST, ALT, bilirubins, urea, and creatinine—particularly on day 14—and reduced potassium levels. It also led to hepatic and pulmonary hypertrophy, renal and cardiac hypotrophy, and specific intestinal mass increase at intermediate doses. *Curcuma longa* significantly reversed these adverse effects, particularly at 50 and 500 mg/kg, restoring biochemical parameters to near-normal values by day 21. The

mechanisms involved include modulation of the Nrf2, NF-κB, MAPK, and PI3K/Akt pathways, reduction of inflammatory cytokines (IL-6, TNF-α), and upregulation of anti-apoptotic proteins (Bcl-2). The data support the use of *Curcuma longa* as a promising phytotherapeutic adjuvant in experimental oncology, offering hematological, hepatorenal, and systemic benefits. These findings justify future clinical and molecular investigations to ensure its safe and effective integration into chemotherapy protocols.

Keywords: Phytotherapy; Chemotherapy; Adjuvant therapy; Systemic toxicity; Hematopoiesis.

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LISTA DE SIGLAS

CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
COPG	Coordenadoria de Pós-Graduação
UNIPAR	Universidade Paranaense
WHO	World Health Organization
RBC	Red Blood Cell (count)
Hb	Hemoglobin
Ht	Hematocrit
WBC	White Blood Cell (count)
PLT	Platelet (count)
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	Red Cell Distribution Width
ROS	Reactive Oxygen Species
Nrf2	Nuclear factor erythroid 2–related factor 2
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
IL	Interleukin
TNF-α	Tumor Necrosis Factor alpha
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
NLR	Neutrophil-to-Lymphocyte Ratio
IACUC	Institutional Animal Care and Use Committee
CONCEA	Conselho Nacional de Controle de Experimentação Animal
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ARE	Antioxidant Response Element
Bcl-2	B-cell Lymphoma 2
Bax	Bcl-2-associated X protein

CYP	Cytochrome P450
HO-1	Heme Oxygenase-1
IKK	IκB Kinase
iNOS	Inducible Nitric Oxide Synthase
MAPK	Mitogen-Activated Protein Kinase
NQO1	NAD(P)H Quinone Dehydrogenase 1
PI3K	Phosphoinositide 3-Kinase
ROS	Reactive Oxygen Species
TGF-β	Transforming Growth Factor Beta
TNF-α	Tumor Necrosis Factor Alpha

LISTA DE SÍMBOLOS

(Elemento opcional e deve ser realizado em ordem alfabética de abreviaturas usadas no texto e escrita por extenso)

@	Arroba
Ca^{++}	Íon cálcio
%	Porcentagem
µM	Micromolar (micromoles por litro)
µg	Micrograma
↑	Aumento ou elevação de valores
↓	Redução ou diminuição de valores
±	Mais ou menos (variação em torno da média)
×	Vezes; utilizado para expressar multiplicação (ex.: 2,5 mg/kg × peso)
Na^{+}	Íon sódio
K^{+}	Íon potássio
mg/kg	Miligrama por quilograma
U/L	Unidade por litro (utilizada para enzimas como AST e ALT)
mL/kg	Mililitro por quilograma (volume de administração)

SUMÁRIO

CAPÍTULO 1

REVISÃO DA LITERATURA

CÂNCER: ASPECTOS CLÍNICOS E TRATAMENTO

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1. Introdução

Segundo dados atualizados da Agência Internacional para Pesquisa sobre o Câncer (IARC), vinculada à Organização Mundial da Saúde (OMS), o câncer permanece como uma das principais causas de mortalidade global em 2024, refletindo uma carga progressivamente crescente da doença em escala mundial. No Brasil, o Instituto Nacional de Câncer (INCA) estima, para o triênio 2023–2025, a ocorrência de aproximadamente 704 mil novos casos anuais de câncer. Desconsiderando os tumores cutâneos não melanoma, os tipos mais prevalentes incluem câncer de mama feminina, próstata, cólon e reto, pulmão e estômago.

O arsenal terapêutico atual contra o câncer compreende intervenções como cirurgia, quimioterapia, radioterapia, hormonioterapia, terapias-alvo e imunoterapia. A escolha do tratamento é individualizada, considerando a localização e o estadiamento da neoplasia, além das condições clínicas e funcionais do paciente. Paralelamente, ensaios clínicos seguem em curso na investigação de novas abordagens terapêuticas experimentais, com o objetivo de ampliar a eficácia dos tratamentos, reduzir toxicidades e promover melhor qualidade de vida aos pacientes oncológicos.

Estima-se que, ao longo da vida, duas em cada cinco pessoas desenvolverão algum tipo de câncer. A compreensão sobre a biologia tumoral tem avançado de forma substancial nas últimas décadas, impulsionada pela elucidação de vias moleculares críticas envolvidas na carcinogênese. Esses progressos científicos têm contribuído para o desenvolvimento de terapias mais específicas e eficazes, com impacto positivo nas taxas de sobrevida e nos desfechos clínicos dos pacientes (SCHULZ et al., 2003; CARNERO et al., 2008; STEELMAN et al., 2008).

Nos últimos anos, tem-se observado um crescimento expressivo no interesse científico pela fitoterapia como estratégia terapêutica adjuvante no tratamento de diversas patologias, incluindo neoplasias. Produtos naturais derivados de frutas, vegetais, chás e especiarias têm despertado atenção na comunidade científica internacional devido ao seu potencial quimiopreventivo e terapêutico (AMINUDIN et al., 2023; MURUGAN, 2019). Entre esses compostos, destaca-se a curcumina, um polifenol extraído do rizoma da planta *Curcuma*

longa, tradicionalmente utilizado como condimento na culinária asiática e amplamente empregado na indústria alimentícia.

A curcumina, devido às suas propriedades farmacológicas multifacetadas, tem sido amplamente investigada como um adjuvante em esquemas de quimioterapia. Essa abordagem terapêutica inovadora tem demonstrado notável potencial em diversos tipos de neoplasias, evidenciando melhora da eficácia antitumoral ao mesmo tempo em que atenua os efeitos tóxicos associados ao tratamento convencional. Entre os mecanismos moleculares envolvidos, destaca-se sua capacidade de modular o estresse oxidativo — um dos principais gatilhos da carcinogênese (SOSA et al., 2013).

Desde a primeira demonstração de seu potencial antitumoral em 1985 (STEFANI et al., 2021), uma ampla gama de estudos in vitro e in vivo foi conduzida, revelando seus múltiplos efeitos biológicos sobre diferentes linhagens tumorais humanas e modelos animais experimentais (ZHANG et al., 2017). A curcumina apresenta um perfil farmacológico favorável, sendo reconhecida por sua baixa toxicidade e segurança mesmo em doses elevadas (TAMADDONI et al., 2020). Contudo, suas limitações farmacocinéticas — como baixa biodisponibilidade oral, rápida metabolização e reduzida solubilidade em água — têm motivado o desenvolvimento de estratégias alternativas, incluindo a síntese de análogos estruturais, formulações de derivados e sistemas de liberação baseados em nanotecnologia (LI et al., 2015; QIANG et al., 2019; VALLÉE; LECARPENTIER, 2018).

Diante desse contexto, a presente tese propõe uma análise experimental integrada à revisão da literatura recente, com o objetivo de investigar as propriedades adjuvantes da curcumina em associação ao quimioterápico Docetaxel. O foco principal recai sobre a elucidação dos mecanismos envolvidos na ação citotóxica da terapia combinada, bem como na avaliação do potencial modulador da curcumina na mitigação de eventos adversos e na atenuação da toxicidade induzida pelo agente citotóxico.

1.2 Revisão da Literatura

1.2.1 *Curcuma longa*

A planta *Curcuma longa* L., pertencente à família Zingiberaceae, é originária da Ásia meridional, especialmente da Índia, sendo amplamente conhecida e utilizada em diferentes culturas por suas propriedades medicinais e alimentícias. No Brasil, é popularmente

denominada “açafrão-da-terra” e pode ser encontrada tanto em cultivos direcionados quanto em forma subespontânea, conforme registrado pela Farmacopeia Brasileira (BRASIL, 2015).

Os rizomas de *C. longa*, partes mais utilizadas da planta, apresentam morfologia variável. Os rizomas principais podem ser ovalados, oblongos ou arredondados, com até 12 cm de comprimento e 5 cm de diâmetro. Já os rizomas laterais são predominantemente cilíndricos e alongados, variando de 6 a 15 cm de comprimento e de 1 a 4 cm de diâmetro, frequentemente exibindo ramificações nas extremidades. A coloração da epiderme externa varia entre tons de amarelo-pardo e amarelo-acastanhado, e sua superfície apresenta cicatrizes morfológicas características, como as anelares (decorrentes das bainhas foliares), irregulares (relacionadas às ramificações) e arredondadas (originadas pelas raízes laterais). Essas raízes, de coloração amarronzada, apresentam textura paleácea e estriada, e emergem lateralmente dos rizomas. Também são observáveis, sob magnificação, a presença de pelos longos e bainhas fibrosas aderidas ao rizoma principal (BRASIL, 2015).

A fratura dos rizomas é bem definida, com superfície interna de coloração amarelo-alaranjada a alaranjada, apresentando aspecto gelatinoso e pontos claros que correspondem aos feixes vasculares. A análise anatômica transversal revela duas zonas morfológicas distintas — córtex e cilindro central — separadas pela endoderme. O córtex é uma faixa periférica mais clara e estreita, enquanto a medula, bem desenvolvida, apresenta coloração intensamente alaranjada. A composição bioquímica dos rizomas inclui cerca de 8% de proteínas, conteúdo comparável ao observado em grãos de arroz, e até 14%, similar ao encontrado no trigo (CHAN et al., 2009; BRASIL, 2015).

1.2.2. Ação farmacológica da *C. longa*

Há muitos anos o rizoma da cúrcuma tem sido empregado de diversas formas, como conservante de alimentos, temperos, corante e até mesmo para tratar doenças em virtude de suas ações antissépticas e antinflamatórias (VOLPE *et al.*, 2009; ZHOU *et al.*, 2011). Outras atividades farmacológicas também têm sido atribuídas a ela, como por exemplo:

antiparasitária, antioxidante, antiviral, antifúngica, hipoglicemiante, anti-inflamatória, cicatrizante, neuroprotetora, imunomoduladora e anticâncer (BRASIL, 2015). Devido a sua variedade de alvos moleculares, há dificuldade em definir sua função bioquímica (SCIBERRAS et al., 2015), por isso, hoje busca-se compreender melhor de que forma os compostos bioativos da planta agem em fatores de transcrição celular, assim como em genes relacionados com a resposta inflamatória, alvos estes que são de fundamental importância na patogênese do câncer e que ainda necessitam serem melhores compreendidos pela comunidade científica que trabalha com a C. longa (JURENKA, 2009; DI PIERRO et al., 2013).

Conforme informação disponibilizada pelo Ministério da Saúde, há um extenso número de citações das principais atividades in vitro demonstradas para derivados de C. longa, bem como para substâncias isoladas da planta e produtos comerciais, conforme figura abaixo:

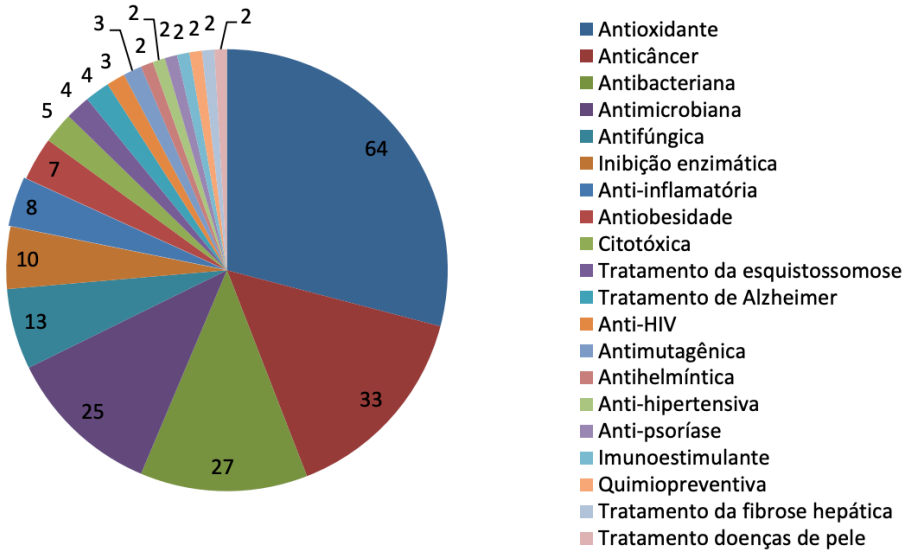

Fig.1 - Número de citações das principais atividades in vitro demonstradas para derivados de C. Longa. Fonte: Ministério da Saúde

1.2.3. Curcumina e seus mecanismos de ação

A curcumina é um componente derivado da planta cúrcuma (C. longa L.), caracterizado como um polifenol natural de baixo peso molecular. Isolada por Vogel em 1815, a curcumina [1,7-bis (4-hidroxi-3 metoxifenol) -1,6 heptadiene-3,5-diona] foi estruturalmente

caracterizada em 1910 por Lampe e Milobedeska, e é considerado o principal componente extraído da cúrcuma. Proveniente dos rizomas, a Cúrcuma é um membro perene da família Zingiberácea da mesma família do gengibre e apresenta em sua constituição três curcuminóides: a curcumina (diferuloimetano, 77%), desmetoxicucumina (17%) e bisdemetozicurcumina (3%), além de óleos voláteis (turmerona, atlantone e zingibereno), proteínas, açúcares e resinas (SHARMA et al., 2004; SETHI et al., 2015).

A habilidade desta substância em regular os níveis de estresse oxidativo tem sido amplamente investigada, revelando-se um componente fundamental na mediação de seus efeitos antitumorais. Segundo Valko *et al.* (2007), o estresse oxidativo resulta da produção exacerbada de espécies reativas de oxigênio (ROS), subprodutos tóxicos de processos metabólicos e imunológicos que, quando em excesso, resultam em danos biomoleculares significativos. Tais danos incluem mutações no DNA, peroxidação lipídica e oxidação de proteínas, eventos que propiciam o início e a progressão do câncer. Dentre as principais formas de ROS envolvidas estão os peróxidos lipídicos, ânions superóxido, radicais hidroxila, oxigênio singlete e peróxido de hidrogênio. Estas espécies reativas comprometem a integridade celular e ativam vias oncogênicas, incluindo a via inflamatória mediada por NF-κB, fator de transcrição central na perpetuação da inflamação e na promoção tumoral (KARIN, 2006).

A inflamação crônica, por sua vez, corresponde a um fator de risco relevante para neoplasias, especialmente em sítios como o cólon e o pâncreas. Ela favorece um microambiente propício à malignização por meio da estimulação da proliferação celular, da evasão da apoptose e do aumento da angiogênese. Elementos moleculares como os fatores de transcrição NF-κB e STAT3, as enzimas pró-inflamatórias COX-2 e MMP-9, bem como citocinas como IL-1, IL-6, IL-8 e TNF-α, são implicados na patogênese de tumores associados à inflamação (GRIVENNIKOV; KARIN, 2010; DIDONATO; MERCURIO; KARIN, 2012).

Particularmente relevante é a via IL-6/STAT3, cuja ativação sustentada tem sido associada ao desenvolvimento e à progressão de diversos cânceres, incluindo os de fígado, estômago e colorretal. Segundo Yu, Pardoll e Jove (2009), o aumento da sinalização via IL-6/ STAT3 correlaciona-se com pior prognóstico e resistência terapêutica, tornando-se um alvo estratégico em oncologia translacional. Estudos apontam que a curcumina exerce efeito

inibitório sobre essa via, reduzindo a expressão de IL-6R e bloqueando a fosforilação de STAT3, o que pode reverter a resistência tumoral e sensibilizar as células cancerosas aos tratamentos citotóxicos. Além disso, há evidências de que a curcumina interfere negativamente na via de sinalização de NF-κB em células B, potencializando seus efeitos antimetastáticos (KUNNUMAKKARA et al.,2009; CAO et al., 2021).

Dessa forma, a curcumina se consolida como uma candidata terapêutica promissora na quimioterapia, graças ao seu amplo espectro de atividades biológicas. Seu perfil anti-inflamatório, antioxidante e antineoplásico contribui não apenas para a potencialização da resposta terapêutica, mas também para a mitigação dos efeitos adversos relacionados aos quimioterápicos, reforçando seu valor na medicina oncológica.

1.2.4. Biomoléculas no tratamento oncológico

A integridade do DNA segregado tem relação direta com a atividade dos microtúbulos, os quais consistem em polímeros celulares extremamente dinâmicos que orquestram importante função durante a mitose. Desta forma, correspondem a um significativo alvo na terapêutica moderna do câncer. O antineoplásico Docetaxel faz parte do rol de drogas antitumorais da classe dos taxanos e suas características naturais tem o objetivo de estabilizar os microtúbulos. Seu mecanismo de ação difere do de outros antimicrotúbulos, como alcalóides Vinca, que impedem a polimerização da tubulina. A função dos taxanos consiste em bloquear a progressão do ciclo celular por meio de comprometimento centrossomal, indução de fuso anormal e supressão da dinâmica dos microtúbulos do fuso. Ele promove a agregação da tubulina em microtúbulos estáveis e impede a sua dissociação, levando a uma significativa redução de tubulina livre. A ligação do docetaxel aos microtúbulos não altera o número de protofilamentos. Foi comprovado que ele bloqueia a rede microtubular nas células, fundamental para as funções celulares vitais, como a mitose e interfase. É imprescindível que seja realizado acompanhamento rigoroso dos pacientes e monitorização dos efeitos adversos deste quimioterápico, como neutropenia, diarréia, neuropatia, reações de hipersensibilidade, reações cutâneas, retenção hídrica, toxicidade pulmonar e cardíaca e afecções oculares (TAVARES, 2019).

Como forma de aumentar o poder do tratamento, associações entre diferentes quimioterápicos são realizados, no entanto os efeitos colaterais nesses casos acabam aumentando também. (SCHULZ et al., 2003; STEELMAN et al., 2008). Docetaxel é uma droga que pertence a classe dos taxanos, cuja propriedade farmacológica é a de comprometer a estabilização da dinâmica dos microtúbulos e, consequentemente prejudicar a formação do fuso mitótico e, posteriormente, o ciclo celular. Algumas das indicações do uso do desta medicação são implicadas no tratamento dos seguintes tumores: mama, pulmão, estômago e junção esofagogástrica, próstata e cabeça e pescoço, sendo que a dosagem pode variar entre 50 a 75mg/m^2. A farmacocinética do docetaxel sofre interferência direta da variabilidade genética dos pacientes, podendo ficar entre 30 e 45%, o que faz dele uma droga que deve ser monitorada de perto devido a riscos de intoxicação (ABAL et al., 2003; NIEUWEBOER et al., 2015).

As reações adversas são extremamente comuns em decorrência do uso de quimioterápicos. Em se tratando de tratamento com taxanos, os mais frequentemente observados são: neutropenia, alopécia, mialgias, mucosites, neuropatias, assim como retenção hídrica e complicações cutâneas (PAZDUR et al., 1993).

1.2.5. *C. longa* no tratamento contra o câncer

O estudo proposto é baseado no princípio de interpretar os efeitos do extrato da C. longa em pacientes oncológicos que estejam em tratamento quimioterápico com Docetaxel, com a finalidade de identificar possíveis propriedades que possam agregar benefícios aos mesmos, bem como minimizar a toxicidade da terapêutica padrão.

Análises recentes demonstraram que a C. longa possui importante atividade na manutenção da homeostase, angiogênese, assim como na reabilitação de doenças dos olhos, como por exemplo a catarata, glaucoma, uveíte e retinopatia diabética (RADOMSKA-LES; NIEWSK et al., 2019). Em um trabalho randomizado duplo-cego com 46 pacientes com diabetes tipo-2 que apresentavam albuminúria ≥300 mg/24 e taxa de filtração glomerular ≥30 mL/min/1.73 m2 os autores observaram que houve melhora no quadro clínico-laboratorial dos pacientes após dois meses de utilização da C. longa (AZAM ET AL., 2019). Atualmente, diversos ensaios clínicos estão em andamento associando a cúrcuma, o câncer e doenças

relacionadas, ao passo que, estudos clínicos de fase I já demonstraram sua segurança mesmo em altas doses (12g/dia) (SKRZYPCZAK-JANKUN et al., 2003).

Cabe ressaltar que recentemente foi publicado um estudo com a finalidade de avaliar a atividade de *C. longa* em células de tumores de mama e fibroblastos humanos sadios cultivados in vitro. Neste trabalho os autores observaram que os fibroblastos são mais sensíveis às doses da planta que a células tumorais, ou seja, doses elevadas da planta podem trazer efeitos deletérios (tóxicos) superiores aos benéficos. (CIANFRUGLI et al., 2019)

A metabolização plasmática da Cúrcuma pode se dar através de vias de redução e de conjugação tanto em roedores quanto em humanos. Após sua administração por via oral, é submetida a conjugação, dando origem a dois compostos, glucuronido curcumina e sulfatos, e uma vez administrada via intraperitoneal ou intravenosa, é reduzida em hexahidrocurcumina, tetrahidrocurcumina e dihidrocurcumina. O tempo de meia-vida dos metabólitos originados é muito curto e tem permeabilidade celular baixa. Após a sua suplementação, o pico sérico é atingido entre uma e duas horas, e posteriormente, sofre rápida queda. Em virtude disso, as modificações nos suplementos são investigadas para aumentar a concentração da substância que atinge a circulação.Sua combinação com piperidina do extrato de pimenta preta (inibidor da glucuronidação) é conhecida por aumentar a biodisponibilidade em vinte vezes quando se usa 20 mg de piperidina ao lado de 2.000mg de Cúrcuma. (SHOBA et al., 1998; ALLAPPAT, et al., 2010; DI PIERRO et al., 2013)

A utilização da Cúrcuma como suplemento já foi aprovada em vários países, como Japão, Turquia, Índia, Estados Unidos, Nepal, África do Sul, Coreia, Tailândia e China. Diversas formulações foram elaboradas como emulsões, cápsulas, liofilizado, comprimidos, lipossomas e nanopartículas; estes, no intuito de expandir sua biodisponibilidade e aprimorar sua função farmacológica (CHIBA et al., 2012)

Conforme Nieuweboer et al. (2015), a quimioterapia tradicional baseia-se na utilização de drogas que tem por finalidade fazer a destruição celular, através da interferência do ciclo delas. Essa interferência pode se dar pela ação direta da duplicação do DNA ou em eventos posteriores, como por exemplo na segregação cromossômica. Ressalta-se que o ataque celular não é seletivo às células tumorais, somente, de forma que, infelizmente, células sadias acabam sendo destruídas também e, como consequência disso, inúmeros efeitos colaterais indesejados acabam aparecendo ao longo do tratamento.

Há vários anos estuda-se os principais mecanismos de ação e funções da Cúrcuma. Pesquisas mostraram que tal composto possui propriedades anti-oxidantes, anti-inflamatórias, anti-proliferativas e anti-angiogênicas contra vários tipos de células cancerígenas e também demonstra atividades antimicrobianas (YU; HUANG, 2012). Atualmente, diversos ensaios clínicos estão em andamento relacionando-a, com o câncer e doenças associadas, ao passo que, estudos clínicos de fase I já demonstraram sua segurança mesmo em altas doses (12g/ dia) (SKRZYPCZAK-JANKUN et al., 2003).

Devido a sua fraca solubilidade em água e meia-vida curta, há dificuldade no avanço clínico desta substância natural promissora, ofertando baixa biodisponibilidade no plasma e nos tecidos (CHANG et al., 2012) De fato, após a utilização de Cúrcuma livre por via oral (até 12g/dia), somente concentrações nanomolares ou metabólitos correspondentes foram encontradas no soro do paciente (KARIN *et al.*, 2997) Um ensaio clínico de fase II recentemente mostrou que mesmo baixas concentrações desta substância conseguiram alcançar um impacto biológico em NF-κB, COX-2 e phosphoSTAT-3 em células mononucleares de sangue periférico de pacientes tratados do que nas observadas in vitro com 5-50 μM de Cúrcuma. A alta hidrofobicidade e baixa biodisponibilidade dela devem ser superadas em breve em futuras aplicações clínicas por administração endovenosa. (SKRZYPCZAK-JANKUN et al., 2003).

Foi demonstrado que a Cúrcuma inibe a atividade da lipoxigenase e especificamente a expressão da ciclooxigenase-2. Inibe também o início da carcinogênese bloqueando a atividade enzimática do citocromo P-450 e aumentando os níveis de glutationa-S-transferase. Desta forma, sua ação elimina as fases de progressão da carcinogênese. Seu efeito antitumoral foi atribuído parcialmente ao aprisionamento de células neoplásicas nas fases S, G2 / M do ciclo celular e indução da apoptose. A Cúrcuma inibe o crescimento de reparo de DNA incompatível defectivo (DNA-MMRd) de células do câncer de colon. Desta forma, seu efeito como agente quimioterapêutico pode ser considerado seguro, não tóxico e de fácil utilização para o tratamento de tumores que apresentam fenótipo DNA-MMRd e instabilidade de microsatélites. (SKRZYPCZAK-JANKUN et al., 2003)

1.2.6. *C. longa* como adjuvante no tratamento contra o câncer

A curcumina, composto bioativo da *Curcuma longa*, tem despertado crescente interesse como agente adjuvante na Oncologia, sobretudo pela sua potencial ação protetora contra efeitos adversos induzidos por quimioterapia e radioterapia. Chen et al. (2024) realizaram uma meta-análise combinada com farmacologia de rede, acoplamento molecular e simulações de dinâmica molecular, com o objetivo de avaliar a eficácia da curcumina na prevenção da mucosite oral induzida por tratamento oncológico. O trabalho incluiu 12 estudos clínicos envolvendo 565 pacientes, demonstrando que a administração de curcumina reduziu significativamente a incidência de mucosite grave (graus 3–4), além de atenuar a gravidade média da toxicidade oral nos pacientes tratados. A farmacologia de rede identificou 11 genes-alvo centrais, como *TNF*, *MAPK3* e *SRC*, associados à inflamação e apoptose. A curcumina apresentou alta afinidade e estabilidade de ligação com esses alvos, sugerindo um mecanismo molecular robusto de ação anti-inflamatória e citoprotetora.

Gutsche et al. (2025) também realizaram uma revisão sistemática abrangente sobre o uso da curcumina como tratamento complementar em Oncologia. O estudo incluiu 34 ensaios clínicos randomizados, envolvendo diferentes tipos de câncer, como mama, cabeça e pescoço, colorretal e próstata. A curcumina foi avaliada quanto à sua eficácia na redução de sintomas relacionados ao tratamento antineoplásico, como mucosite oral, fadiga, perda de peso e marcadores inflamatórios sistêmicos. A maioria dos estudos relatou benefícios clínicos com o uso da curcumina como adjuvante, incluindo melhora da qualidade de vida, modulação de citocinas inflamatórias (como TNF-α e IL-6), e redução de toxicidades induzidas por quimio e radioterapia. No entanto, os autores alertam para a heterogeneidade metodológica dos estudos, diferenças nas formulações de curcumina, vias de administração e desfechos avaliados, o que dificulta a consolidação de recomendações clínicas definitivas. A conclusão ressalta o potencial terapêutico da curcumina, mas destaca a necessidade de ensaios clínicos maiores, mais bem desenhados e padronizados.

Um outro estudo clínico conduzido por Hindu et al. (2020) avaliou os efeitos da suplementação de curcumina em pacientes com câncer de mama submetidos à quimioterapia com paclitaxel, com ênfase na toxicidade do tratamento e nos desfechos clínicos associados. O estudo consistiu em uma série de casos clínicos, na qual os pacientes receberam curcumina

por 21 dias concomitantemente ao ciclo de paclitaxel. Os resultados foram expressivos, indicando que a curcumina promoveu melhora significativa na qualidade de vida dos pacientes ($p = 0,004$), com impacto positivo sobre diversos sintomas relacionados ao tratamento oncológico. Houve redução estatisticamente significativa na intensidade de fadiga ($p = 0,000$), náuseas e vômitos ($p = 0,026$), dor ($p = 0,000$), insônia ($p = 0,000$), perda de apetite ($p = 0,000$), constipação ($p = 0,000$) e diarreia ($p = 0,033$). Além disso, observou-se melhora em parâmetros hematológicos relevantes, com elevação significativa nos níveis de hemoglobina ($p = 0,001$), leucócitos totais ($p = 0,001$) e neutrófilos ($p = 0,03$), sugerindo um efeito protetor da curcumina sobre a hematopoese.

Esses achados reforçam a hipótese de que a curcumina pode atuar como agente adjuvante eficaz na mitigação da toxicidade associada à quimioterapia, especialmente em esquemas baseados em taxanos, como o paclitaxel. A capacidade da curcumina de modular vias inflamatórias, oxidativas e apoptóticas contribui para seus efeitos benéficos, sem comprometer a eficácia antitumoral dos quimioterápicos. Assim, a integração da curcumina como suplemento durante o tratamento do câncer se apresenta como uma estratégia promissora para melhorar os desfechos clínicos e a tolerabilidade terapêutica.

Recentemente nosso grupo realizou um estudo semelhante ao proposto agora, no entanto as análises laboratoriais foram realizadas 30 dais após a exposição ao docetaxel (padrão de tratamento). Neste trabalho pôde-se observar que a C. longa promoveu melhorias na função renal e hepática (TAVARES, 2019).

Diante do exposto, este projeto tem como objetivo contribuir para o avanço do conhecimento científico no campo do tratamento oncológico, buscando identificar e desenvolver estratégias inovadoras que aprimorem a eficácia e a segurança das abordagens terapêuticas. Considerando a crescente incidência de neoplasias e seu impacto significativo na saúde pública, torna-se imprescindível incentivar estudos que promovam avanços científicos substanciais. Esses esforços são essenciais para gerar evidências robustas que possam ser traduzidas em benefícios clínicos concretos, melhorando os desfechos para os pacientes e contribuindo para o desenvolvimento sustentável da oncologia moderna.

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1.4 Objetivo

Avaliar, de forma sistemática e comparativa, os efeitos protetores de diferentes concentrações do extrato de *Curcuma longa* sobre a toxicidade induzida pelo quimioterápico docetaxel em ratos Wistar sadios, analisando parâmetros hematológicos, bioquímicos e morfológicos em órgãos-alvo (fígado, rins, intestino, pulmões, coração e baço), ao longo de distintos períodos de exposição. O estudo visa elucidar potenciais mecanismos citoprotetores e cronodependentes da *Curcuma longa*, com ênfase em sua ação antioxidante, anti-inflamatória e imunomoduladora.

CAPÍTULO 2

TÍTULO COMPLETO DO ARTIGO CIENTÍFICO 1:

IMPACT OF *CURCUMA LONGA* ON HEMATOPOIESIS AND SPLENIC MASS IN AN ANIMAL MODEL UNDERGOING DOCETAXEL CHEMOTHERAPY

Artigo editado de acordo com as normas de publicação da Revista Drug and Chemical Toxicology – Print ISSN: 0148-0545 Online ISSN: 1525-6014

RESUMO

Introdução: A toxicidade hematológica induzida por quimioterapia compromete significativamente a continuidade do tratamento e a qualidade de vida dos pacientes. Embora eficaz, o docetaxel está associado à supressão cumulativa da medula óssea e a alterações esplênicas. A *Curcuma longa*, um fitoterápico com propriedades antioxidantes e anti-inflamatórias, pode oferecer benefícios citoprotetores. **Objetivo:** Avaliar os efeitos hematológicos e esplênicos da *Curcuma longa* em ratos Wistar submetidos à quimioterapia com docetaxel. **Métodos:** Ratos machos da linhagem Wistar foram distribuídos em cinco subgrupos e tratados por 7, 14 ou 21 dias com placebo, docetaxel isolado ou docetaxel combinado com *Curcuma longa* nas doses de 25, 50 ou 500 mg/kg/dia. Foram avaliados parâmetros hematológicos e o peso relativo do baço. **Resultados:** O docetaxel induziu reduções progressivas na contagem de eritrócitos (RBC), hemoglobina (Hb), hematócrito (Ht), leucócitos (WBC) e plaquetas (PLT), especialmente no 21º dia. A coadministração com *Curcuma longa* atenuou esses efeitos de forma dose- e tempo-dependente, com destaque para as doses intermediárias (25–50 mg/kg), que apresentaram os efeitos hematoprotetores mais consistentes. O peso relativo do baço apresentou aumentos significativos no grupo de 500 mg/kg, sugerindo hematopoiese extramedular ou ativação imune. **Conclusão:** A *Curcuma longa* pode exercer efeito protetor contra a toxicidade hematológica induzida por docetaxel e modular a morfologia esplênica. Esses achados sustentam futuras investigações translacionais sobre o uso da *Curcuma longa* como adjuvante na oncologia clínica.

Palavras-chave: *Curcuma longa*; Quimioterapia; Parâmetros hematológicos; Citoproteção; Hematopoiese esplênica

ABSTRACT

Background: Chemotherapy-induced hematological toxicity significantly compromises treatment continuity and patient quality of life. Docetaxel, while effective, is associated with cumulative bone marrow suppression and splenic alterations. *Curcuma longa*, a phytochemical with antioxidant and anti-inflammatory properties, may offer cytoprotective benefits. **Objective:** To evaluate the hematological and splenic effects of *Curcuma longa* in Wistar rats undergoing docetaxel chemotherapy. **Methods:** Male Wistar rats were assigned to 5 subgroups and treated for 7, 14, or 21 days with placebo, docetaxel alone, or docetaxel combined with *Curcuma longa* at 25, 50, or 500 mg/kg/day. Hematological parameters and relative spleen weight were assessed. **Results:** Docetaxel induced progressive reductions in red blood cell (RBC) count, hemoglobin (Hb), hematocrit (Ht), white blood cells (WBC), and platelets (PLT), particularly by day 21. *Curcuma longa* co-treatment attenuated these effects in a dose- and time-dependent manner, with intermediate doses (25–50 mg/kg) demonstrating the most consistent hematoprotective effects. Relative spleen weight showed significant increases in the 500 mg/kg group, suggestive of extramedullary hematopoiesis or immune activation. **Conclusion:** *Curcuma longa* may protect against docetaxel-induced hematological toxicity and modulate splenic morphology. These findings support further translational research exploring *Curcuma longa* as an adjuvant in oncologic care.

Keywords: *Curcuma longa*; Chemotherapy; Hematological parameters; Cytoprotection; Spleen-associated hematopoiesis

IMPACT OF *CURCUMA LONGA* ON HEMATOPOIESIS AND SPLENIC MASS IN AN ANIMAL MODEL UNDERGOING DOCETAXEL CHEMOTHERAPY

Introduction

Cancer remains a leading global health burden, with over 20 million new cases and 9.7 million cancer-related deaths reported in 2022, according to the World Health Organization (WHO).[1] Despite advances in precision oncology, cytotoxic chemotherapy remains a mainstay in the treatment of solid tumors. However, chemotherapy-induced adverse effects, particularly hematological toxicity, significantly impact patient outcomes, often leading to dose reductions, treatment delays, and increased morbidity.[2]

Docetaxel, a widely used taxane-based chemotherapeutic agent, is associated with high rates of myelosuppression and splenic alterations, which compromise hematopoietic function.[3-4] Neutropenia, anemia, and thrombocytopenia are among the most common complications, frequently resulting from direct toxicity to bone marrow and hematopoietic organs.[3-5] These effects are particularly concerning given the cumulative nature of docetaxel toxicity and its narrow therapeutic index.[4-5]

In recent years, natural compounds with anti-inflammatory and antioxidant properties have gained attention for their potential to mitigate chemotherapy-induced toxicity. *Curcuma longa L*. (turmeric) and its principal bioactive constituent, curcumin, have demonstrated cytoprotective effects in various experimental models[8-9]. Preclinical studies have shown that curcumin may protect against chemotherapy-related organ damage by modulating oxidative stress, inflammation, and apoptosis.[8-12] Furthermore, combination strategies involving phytochemicals and cytotoxic agents have shown promise in enhancing antitumor efficacy while minimizing systemic toxicity.[13]

Nevertheless, the potential of *Curcuma longa* to attenuate docetaxel-induced hematological toxicity and splenic morphological alterations remains poorly understood in in vivo models. Therefore, the present study aimed to investigate the effects of *Curcuma longa* on hematopoiesis and splenic structure in a Wistar rat model undergoing docetaxel chemotherapy. This research seeks to explore whether phytotherapeutic intervention can preserve hematopoietic function and reduce structural damage in key immune organs during cytotoxic treatment.

Materials and Methods

This controlled case experimental study involved 105 male Wistar rats (n = 7 per subgroup; 8 weeks of age), obtained from the Animal Facility of Colombo, Paraná, Brazil. The animals were randomly allocated into three primary treatment groups based on duration: Group A (7 days), Group B (14 days), and Group C (21 days). Each primary group was subdivided into five treatment arms as follows:

Group A – 7-day treatment

Subgroups G1A to G5A received treatments as follows:

- G1A: oral placebo (water), daily for 7 days (control group).
- G2A: single intraperitoneal dose of docetaxel on Day 1.
- G3A: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 25 mg/kg/day.
- G4A: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 50 mg/kg/day.
- G5A: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 500 mg/kg/day.

Group B – 14-day treatment

Subgroups G1B to G5B received treatments as follows:

- G1B: oral placebo (water), daily for 14 days (control group).
- G2B: single intraperitoneal dose of docetaxel on Day 1.
- G3B: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 25 mg/kg/day.
- G4B: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 50 mg/kg/day.
- G5B: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 500 mg/kg/day.

Group C – 21-day treatment

Subgroups G1C to G5C received treatments as follows:

- G1C: oral placebo (water), daily for 21 days (control group).
- G2C: single intraperitoneal dose of docetaxel on Day 1.

- G3C: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 25 mg/kg/day.
- G4C: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 50 mg/kg/day.
- G5C: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 500 mg/kg/day.

The number of animals per subgroup (n = 7) was defined based on previous experimental studies employing similar designs, which demonstrated that this sample size is sufficient to detect significant differences in hematological, biochemical, and histopathological parameters. The selection also complied with international ethical guidelines for animal experimentation, adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement), thereby ensuring the minimum number of animals required to obtain statistically reliable results.

The dose of docetaxel was extrapolated for rats using allometric scaling from a human reference (70 kg), based on the methodology proposed by Pachaly (2006)[17]. Calculations were based on specific metabolic rate (SMR) values:

- SMRref (human): $70 \times 70^{-0.25} = 24$
- SMRrat (300 g): $70 \times 0.3^{-0.25} = 95$

To reach the target dose in rats, the human equivalent dose (HED) was adjusted to 0.63 mg/kg. Thus:

- Rat dose = $0.63/24 \times 95 = 2.5$ mg/kg

Therefore, a final intraperitoneal dose of 2.5 mg/kg docetaxel was established, in a volume up to 1 mL/kg.

Example for a 250 g rat:

2.5 mg – 1000 g

X mg – 250 g

X = 0.625 mg

The *Curcuma longa* extract (95% curcuminoids) was obtained from Farmácia Farmavida (Umuarama, Paraná, Brazil). It was administered by gavage in doses of 25, 50, or 500 mg/kg/day, diluted in 1 mL/kg of water (Fig. A).

Fig. A: *Curcuma longa*

Fonte: https://www.easytogrowbulbs.com/products/ginger-curcuma-longa-turmeric . Acessado em: 25 de maio de 2025

Animals were housed in polypropylene cages in a temperature-controlled room (22 ± 2°C) with a 12-h light/dark cycle, and had ad libitum access to standard chow and water throughout the experimental period (Fig. B). After administration, they were placed on a flat surface and observed continuously for 1 hour, then hourly for the first 6 hours, and subsequently once daily or 7, 14, or 21 days, according to the respective group, with the aim of analyzing their behavior.. At the end of each experimental period, animals were fasted for 12 hours (with access to water), anesthetized using isoflurane, and euthanized via decapitation in accordance with the euthanasia guidelines of the Brazilian CONCEA (2015)[18].

Fig. B: Animals

Collected samples included whole blood, obtained via retro-orbital plexus puncture, which was analyzed for complete blood count (CBC) parameters (Fig. C). The spleen was harvested and weighed for the calculation of relative organ weight, which was determined by the ratio of organ weight (g) to final body weight (g) of the respective animal and expressed as a percentage, according to the following formula: Relative Weight (%) = (Organ Weight / Final Body Weight) × 100.

Fig. C: Laboratory

Carcasses were stored at −20°C in labeled plastic bags until collected by a certified biological waste disposal company. Samples were homogenized and stored at 4°C until analysis.

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of University Paranaense under protocol number 40130, and conducted in compliance with the guidelines of the Brazilian CONCEA (2015).

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism version 10.4.2.

Results

A time-dependent evaluation of hematological parameters revealed distinct trends associated with docetaxel-induced toxicity and the potential mitigating effect of *Curcuma longa* supplementation. The experimental model allowed comparative analysis at 7, 14, and 21 days of treatment across five subgroups: control, docetaxel-only, and docetaxel combined with *Curcuma longa* at 25 mg/kg, 50 mg/kg, and 500 mg/kg.

Red Blood Cell Count (RBC) and Hemoglobin (Hb) levels showed slight fluctuations across all timepoints, with a consistent pattern of decline in the docetaxel-only groups relative to controls. This decline was more pronounced at 14 and 21 days, suggesting cumulative bone marrow suppression. Interestingly, co-administration of *Curcuma longa*, particularly at 50 mg/kg, maintained more stable erythrocytic indices over time, indicating a possible hematoprotective effect (Fig. 1-2).

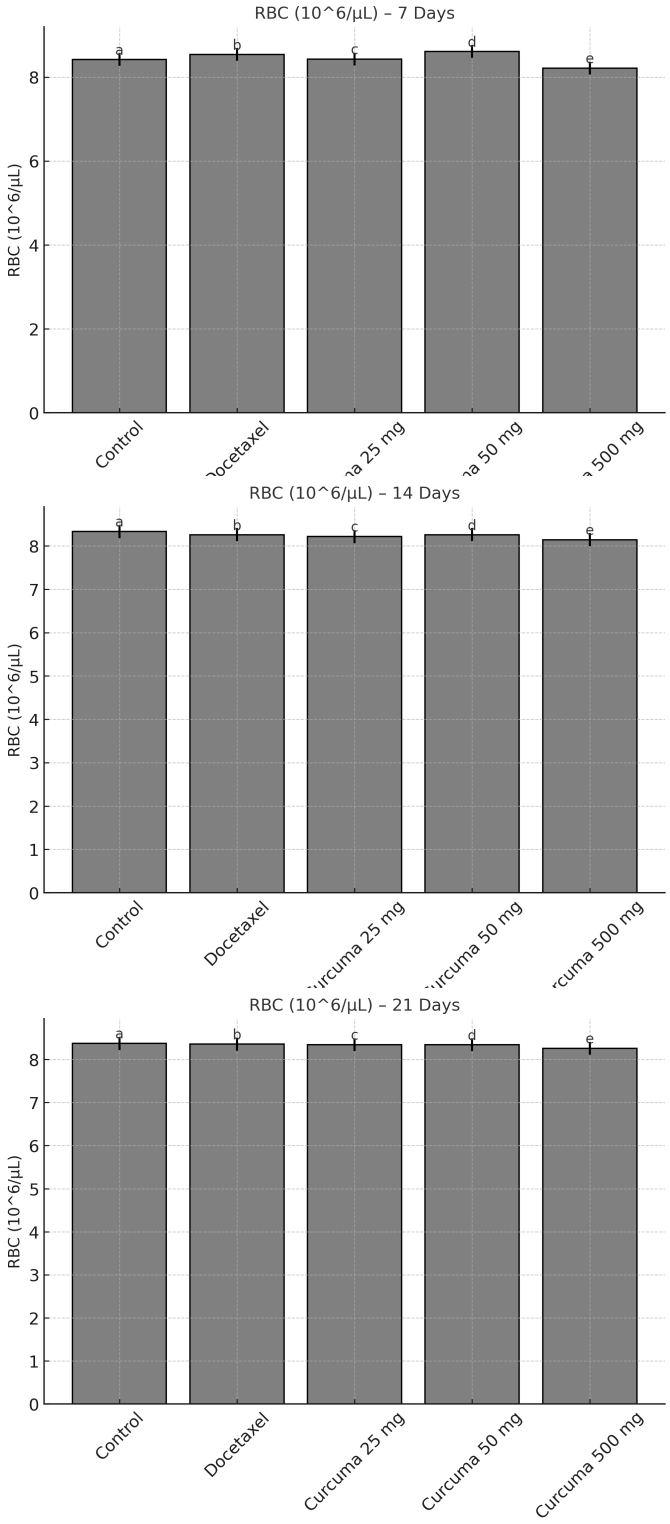


Fig. 1.: RBC ($10^6/\mu$L). A) Mean red blood cell count in experimental groups after 7 days of treatment. Bars represent mean ± SD (n = 7). Different letters indicate statistically significant differences between groups (ANOVA followed by Tukey's post hoc test, $p < 0.05$). **B)** Mean red blood cell count in experimental groups after 14 days of treatment. Bars represent mean ± SD (n = 7). Different superscript letters indicate statistically significant differences between groups (ANOVA followed by Tukey's post hoc test, $p < 0.05$). **C)** Red blood cell counts in all treatment groups after 21 days. Data are expressed as mean ± SD (n = 7). Superscript letters indicate significant differences between groups (ANOVA followed by Tukey's post hoc test, $p < 0.05$).

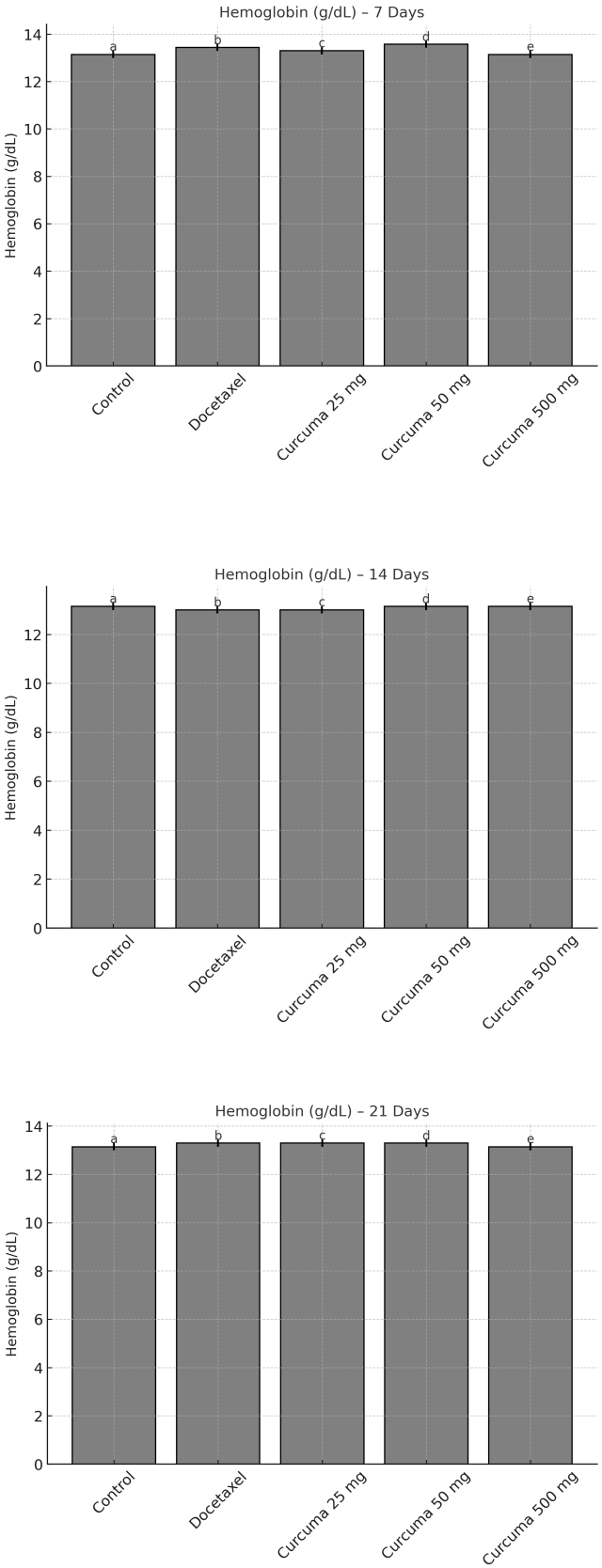


Fig. 2. Hemoglobin (g/dL). A) Mean hemoglobin concentration across treatment groups after 7 days. Bars represent mean ± SD (n = 7). Distinct letters denote significant differences ($p < 0.05$). **B)** Average hemoglobin concentration following 14 days of treatment. Data are expressed as mean ± SD. Groups labeled with different letters are significantly different ($p < 0.05$). **C)** Mean hemoglobin concentration in experimental groups after 21 days of treatment. Error bars represent SD. Statistically different groups are labeled with distinct letters ($p < 0.05$).

Hematocrit (Ht) followed a similar temporal trajectory, with the lowest values observed in the 21-day docetaxel group. The protective trend of *Curcuma longa* was evident again, particularly in the 7- and 14-day regimens, though slightly attenuated at 21 days (Fig. 3).

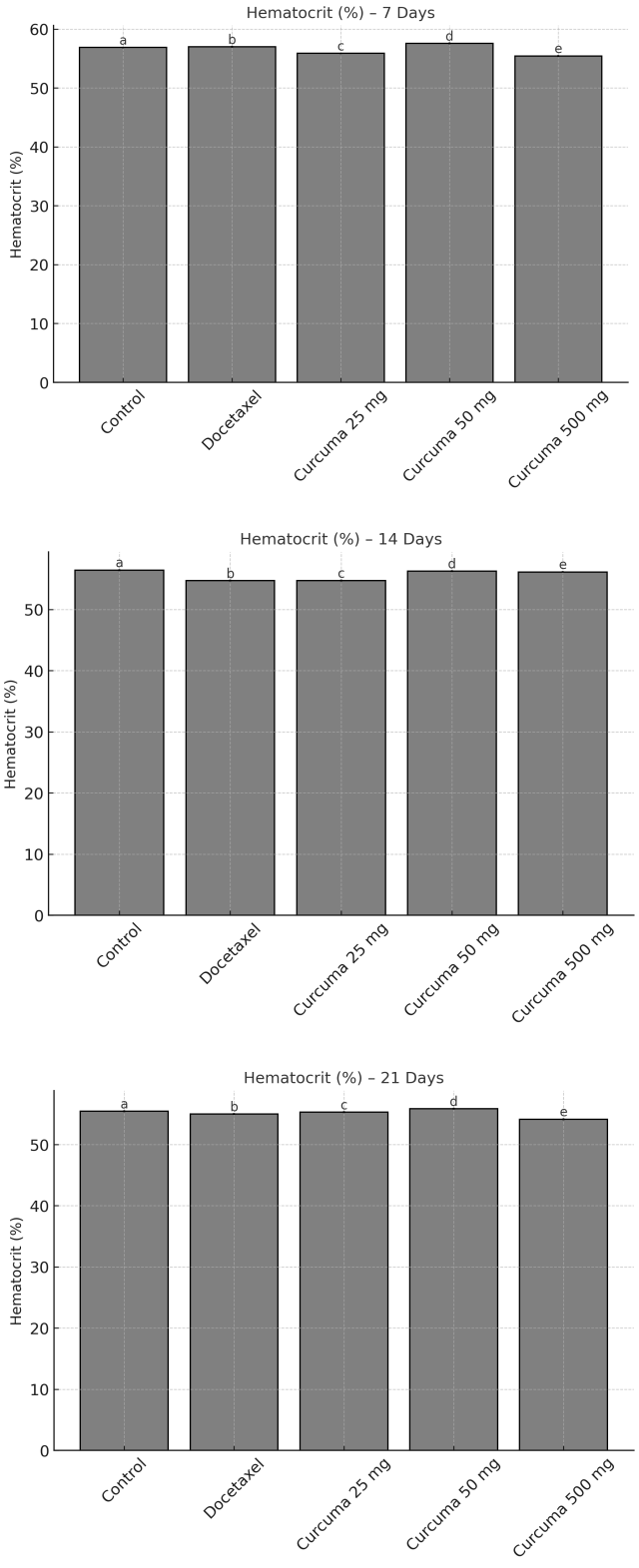
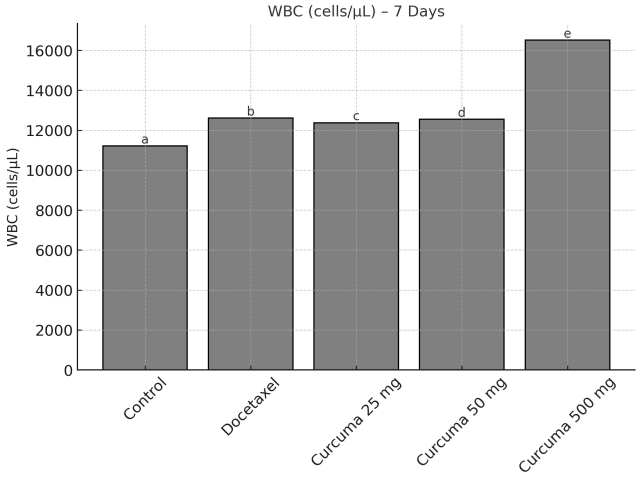
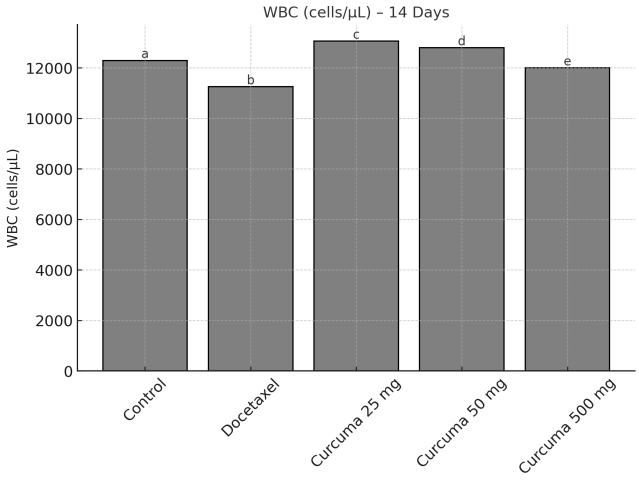


Fig. 3. Hematocrit (%). A) Percentage of hematocrit in each group on day 7. Error bars denote SD. Different letters indicate significant differences ($p < 0.05$). **B)** Hematocrit values across experimental conditions after 14 days. Bars show mean ± SD. Letters denote statistical differences ($p < 0.05$). **C)** Hematocrit percentage following 21-day treatment. Mean ± SD is shown. Groups with different letters are significantly different ($p < 0.05$).

White Blood Cell (WBC) counts revealed a progressive elevation in the groups treated with docetaxel plus *Curcuma longa*, especially at 14 and 21 days, suggesting immunomodulatory support. The docetaxel-only group consistently showed mild leukopenia across timepoints, consistent with expected chemotherapy-induced myelosuppression (Fig. 4).





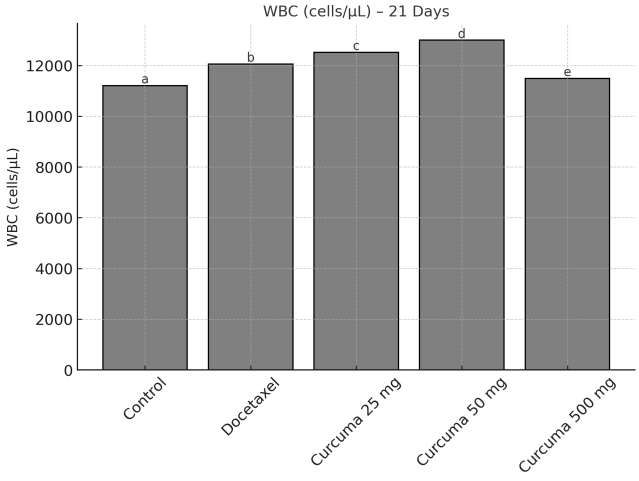


Fig. 4. WBC (cells/μL). A) White blood cell counts among experimental groups after 7 days. Bars represent mean ± SD. Different superscript letters indicate statistical significance ($p < 0.05$). **B)** White blood cell counts in all groups after 14 days. Values are presented as mean ± SD. Statistically significant differences indicated by different letters ($p < 0.05$). **C)** White blood cell counts in each group after 21 days. Bars represent mean ± SD. Superscript letters denote significant differences ($p < 0.05$).

Platelet counts (PLT) declined significantly in the docetaxel-only groups over time, particularly by day 21. Co-treatment with *Curcuma longa* mitigated this reduction, with the 25 and 50 mg/kg doses showing the most favorable trends, reinforcing its potential role in preserving megakaryocytic function (Fig. 5).

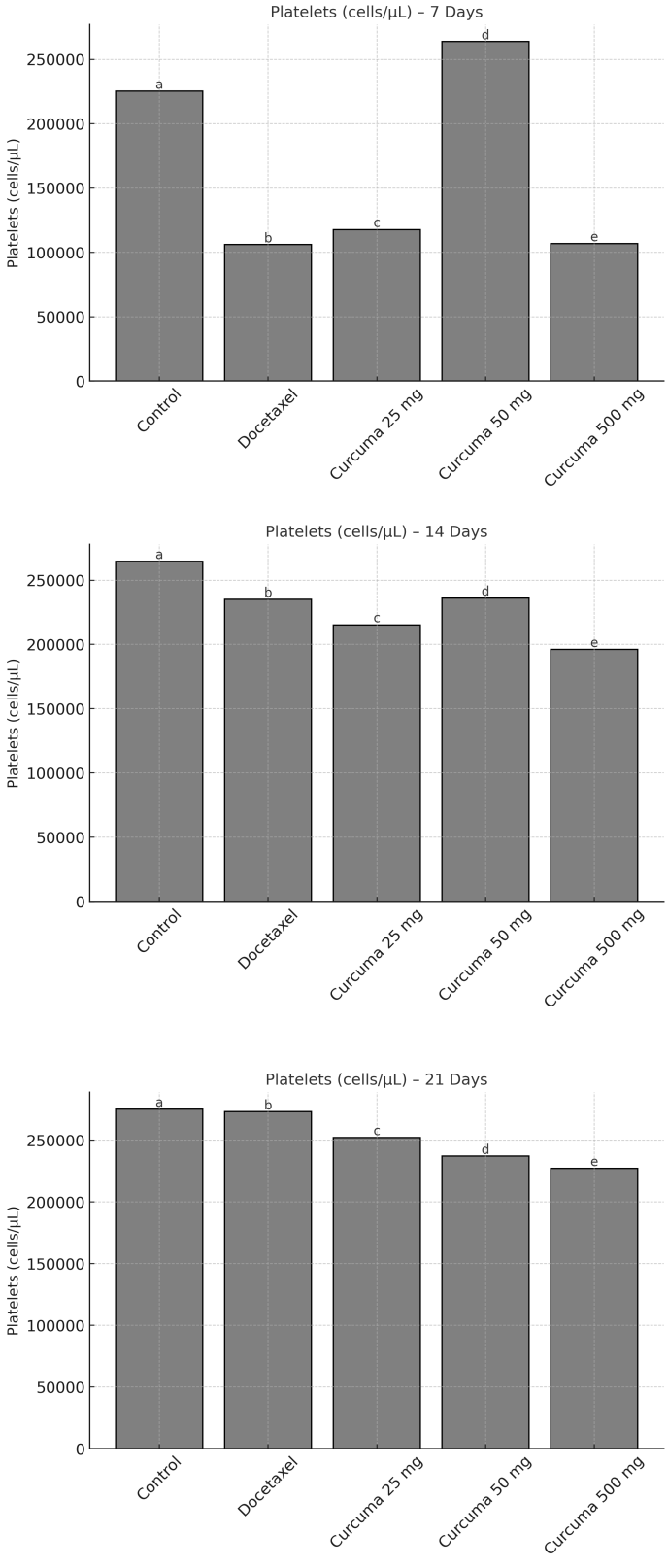


Fig. 5. Platelets (cells/μL). A) Mean platelet counts following 7-day treatment. Results are shown as mean ± SD. Distinct letters denote significant group differences ($p < 0.05$). **B)** Platelet counts observed in animals after 14 days of treatment. Mean ± SD shown. Superscript letters indicate $p < 0.05$. **C)** Platelet counts after 21 days of treatment. Data are presented as mean ± SD. Different letters indicate statistically significant differences ($p < 0.05$).

Among the red cell indices, MCV, MCH, and MCHC remained within a relatively narrow range across all groups and timepoints, although a gradual increase in MCH was noted in *Curcuma longa*-treated animals by day 21, potentially reflecting improved red cell hemoglobinization (Fig. 6-8).

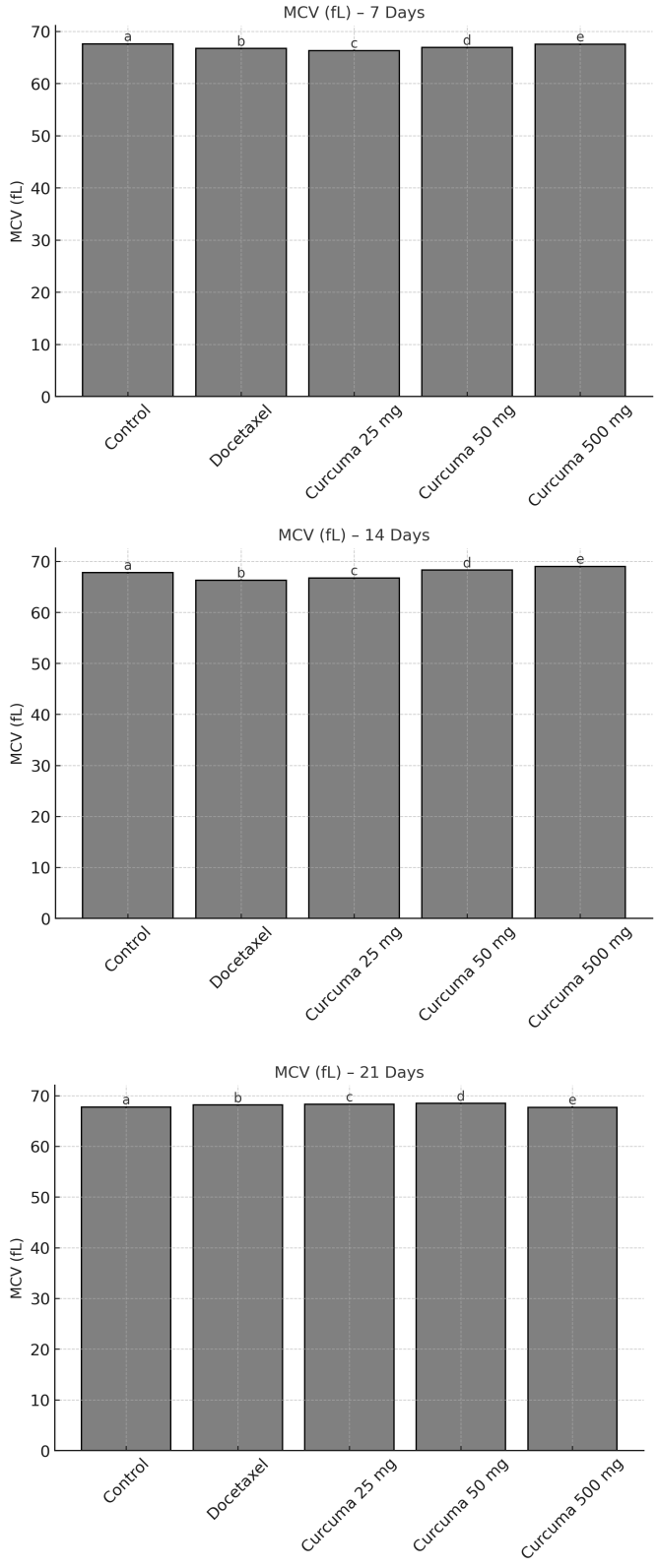
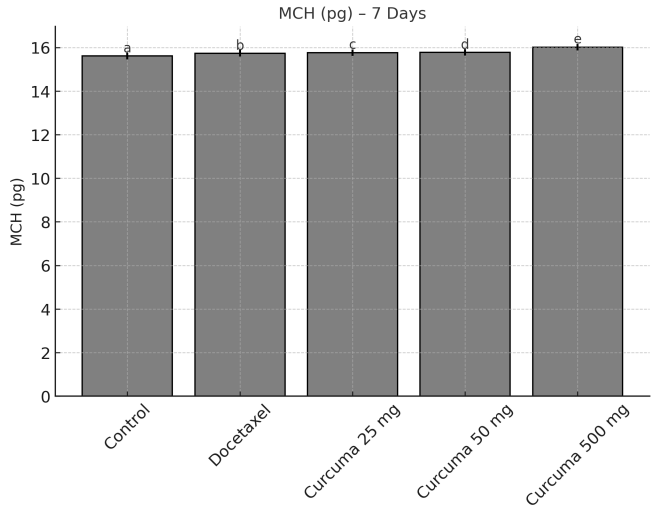
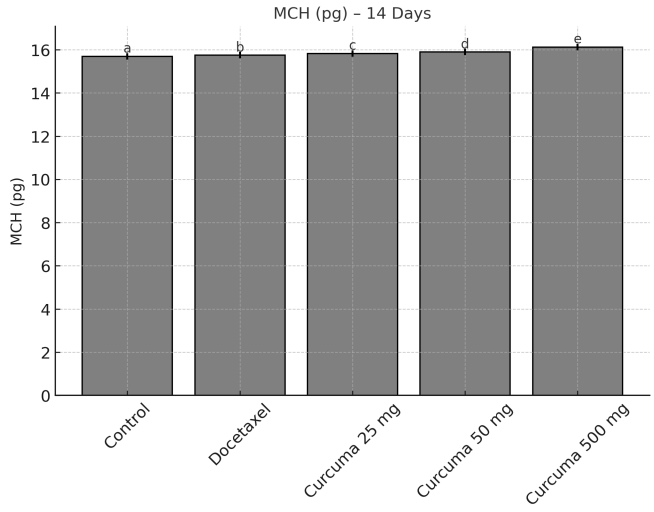


Fig. 6. MCV (fL). A) Mean corpuscular volume values in treated groups after 7 days. Bars show mean **±** SD. Letters above bars reflect statistically significant differences ($p < 0.05$). **B)** Mean corpuscular volume in the five groups after 14 days. Bars represent mean **±** SD. Significant group differences are marked by distinct letters ($p < 0.05$). **C)** Mean corpuscular volume across treatment groups after 21 days. Bars show mean **±** SD; letters denote significance ($p < 0.05$).





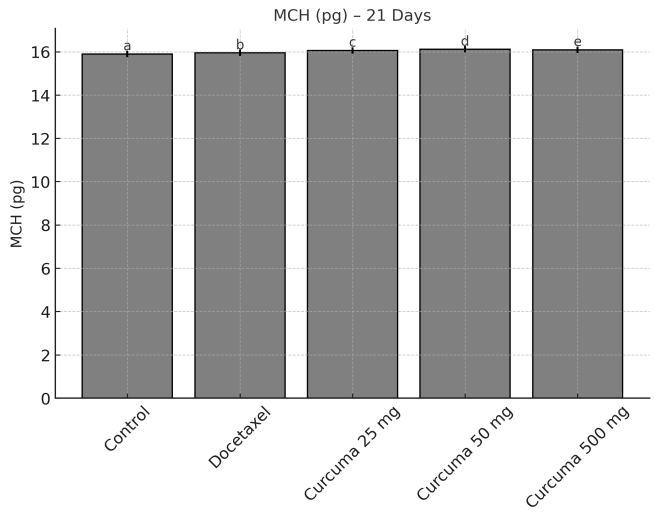


Fig. 7. MCH (pg). A) Mean corpuscular hemoglobin values in treated groups after 7 days. Mean **±** SD shown. Statistical differences indicated by letter superscripts ($p < 0.05$). **B)** Mean corpuscular hemoglobin values in treated groups after 14 days. Error bars show SD. Statistical comparisons are shown via letter annotations ($p < 0.05$). **C)** Mean corpuscular hemoglobin in animals treated for 21 days. Statistically distinct groups are indicated with superscript letters ($p < 0.05$).

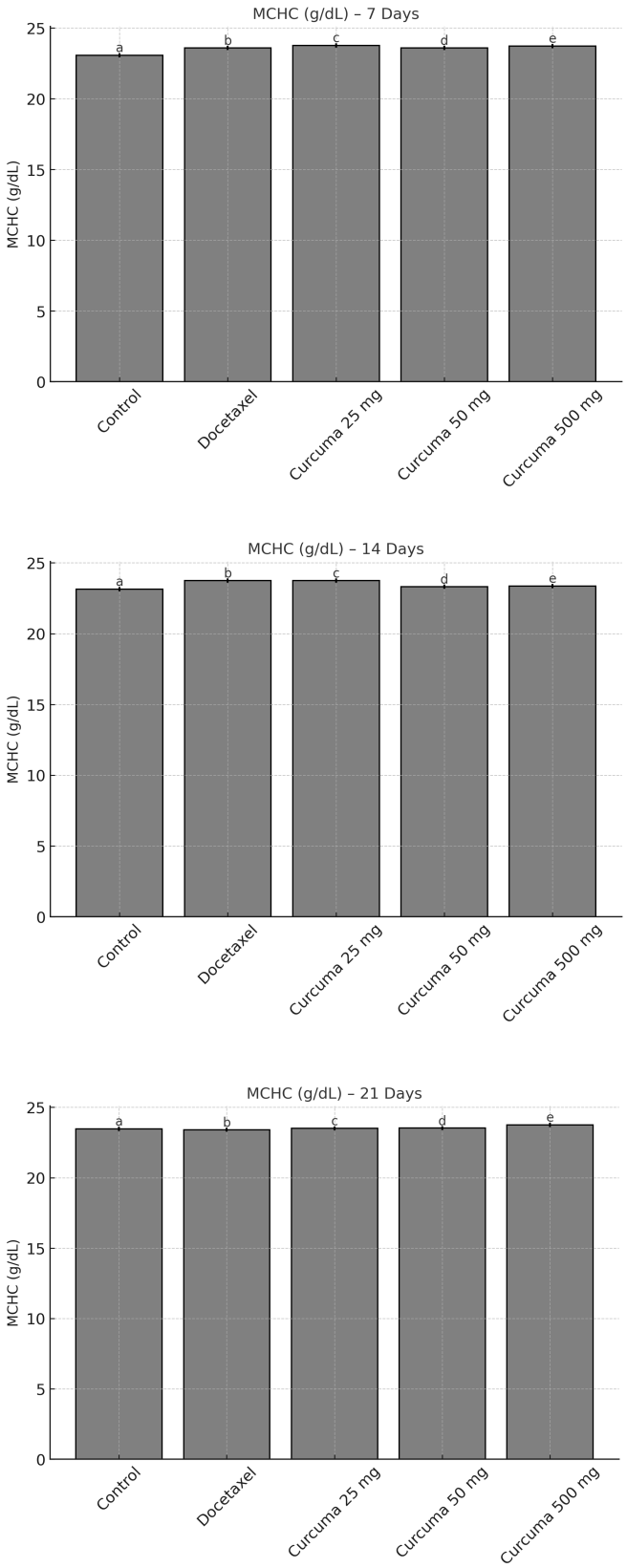


Fig. 8. MCHC (g/dL). A) Mean corpuscular hemoglobin concentration values in treated groups after 7 days. Distinct letters indicate significant differences ($p < 0.05$). **B)** Mean corpuscular hemoglobin concentration in treated groups after 14 days. Differences with statistical significance ($p < 0.05$) are marked by distinct letters. **C)** Mean corpuscular hemoglobin concentration values after 21 days treatment. Bars indicate mean **±** SD. Statistically significant differences indicated by different letters ($p < 0.05$).

RDW (%) increased slightly with time in all groups but remained relatively stable within the Curcuma-supplemented groups, suggesting reduced anisocytosis and better erythrocyte morphology preservation (Fig, 9).

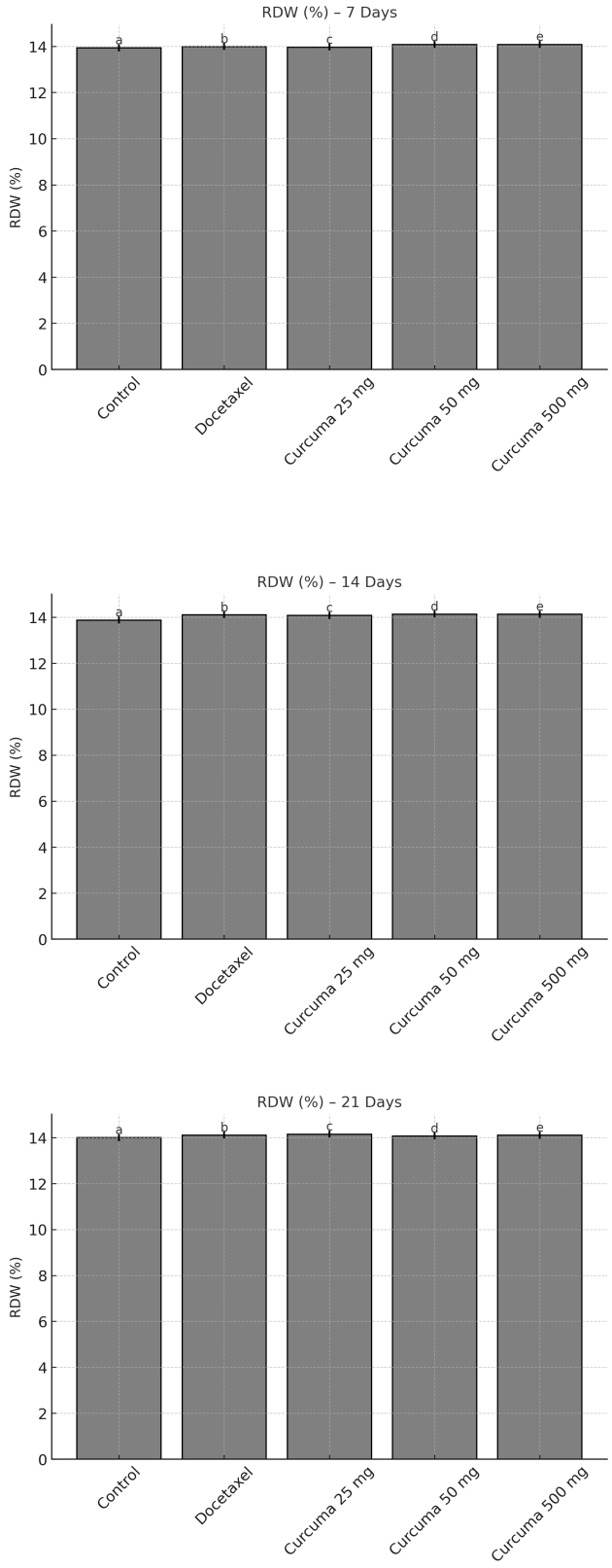


Fig. 9. RDW (%). A) Red cell distribution width results after 7 days. Data are mean ± SD. Statistical significance represented by letter annotations ($p < 0.05$). **B)** Red cell distribution width in rats following 14-day treatment. Data are mean ± SD. Letters indicate statistically significant differences ($p < 0.05$). **C)** Red cell distribution width in each group following 21-day treatment. Mean ± SD shown. Letters above bars indicate statistically different groups ($p < 0.05$).

The differential leukocyte count revealed stability in neutrophil and lymphocyte proportions across all timepoints, with modest variation. Notably, monocyte percentages tended to rise progressively in the groups treated with *Curcuma longa*, possibly indicating enhanced innate immune surveillance (Fig 10-12).

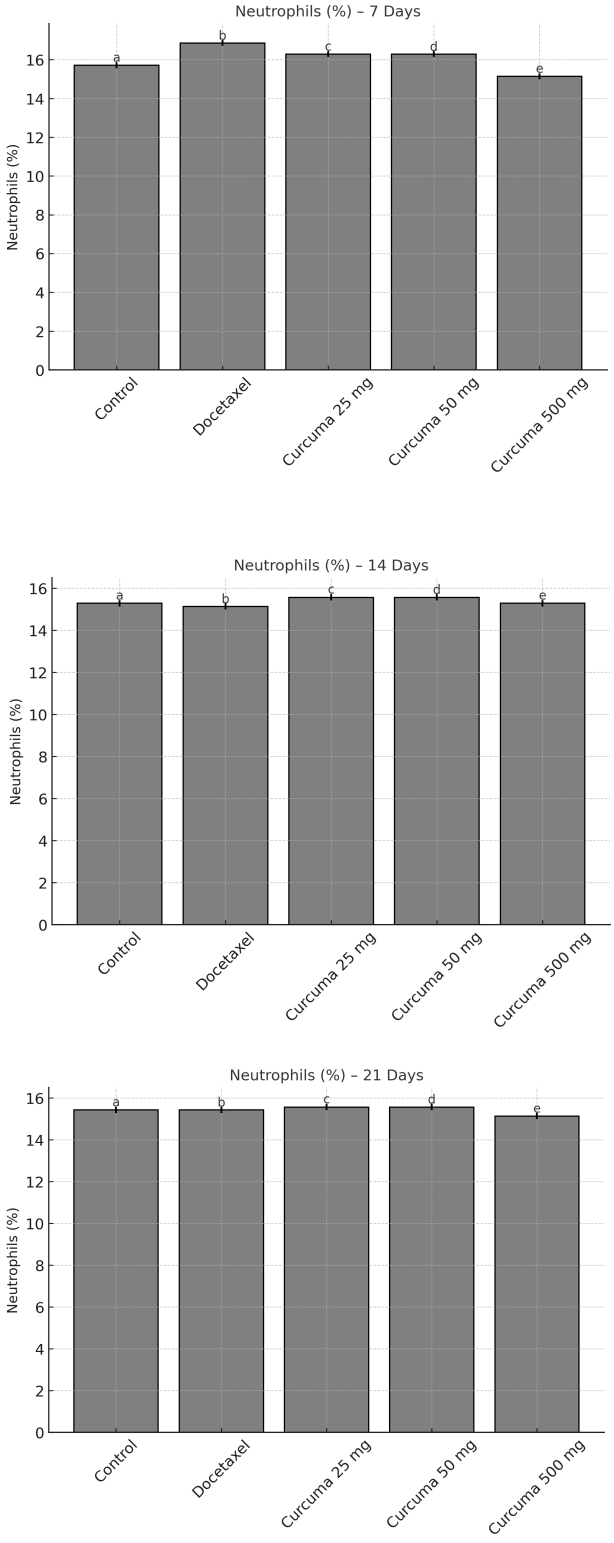
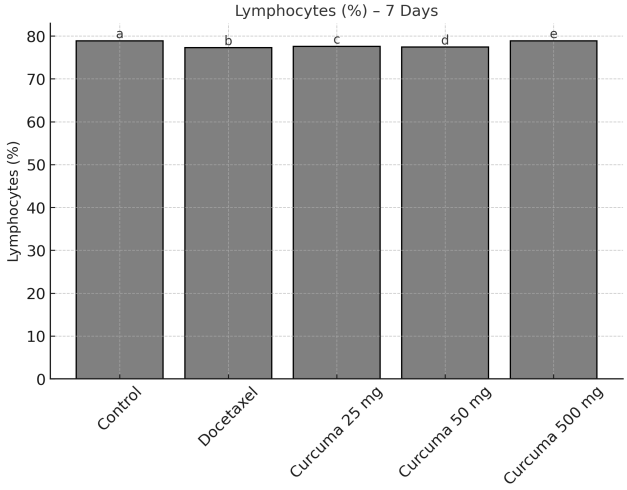
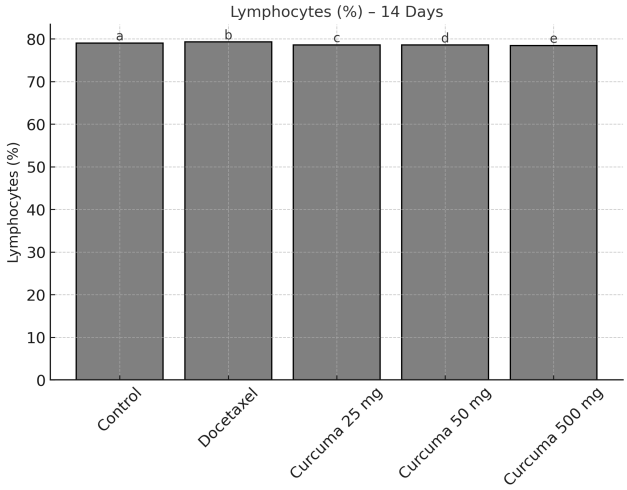


Fig. 10. Neutrophils (%). A) Relative percentage of segmented neutrophils after 7-day treatment. Distinct letters indicate $p < 0.05$ by ANOVA and Tukey post hoc analysis. **B)** Neutrophil percentage in peripheral blood of rats after 14 days. Superscript letters denote statistical differences ($p < 0.05$). **C)** Neutrophil percentage following 21 days of treatment. Values are mean ± SD. Letters represent statistically significant differences ($p < 0.05$).





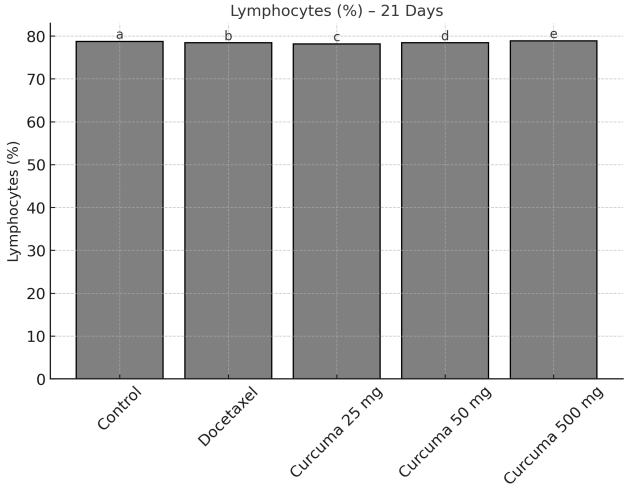
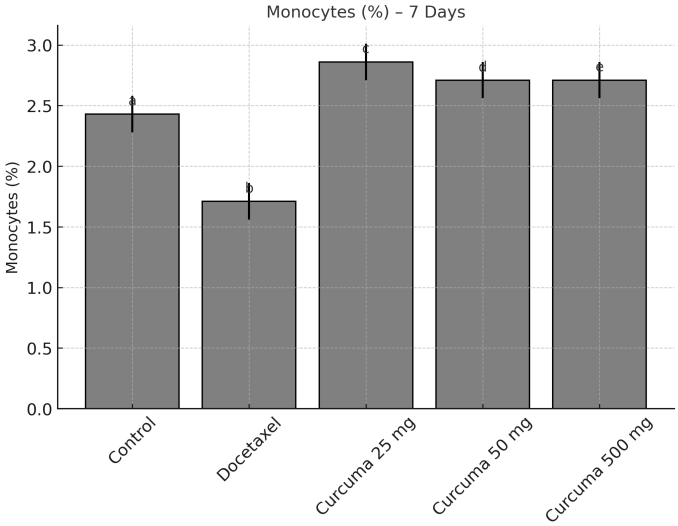
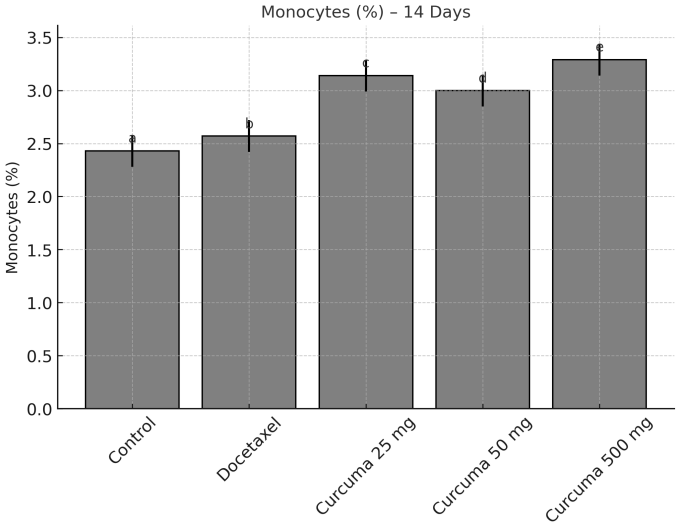


Fig. 11. Lymphocytes (%). A) Lymphocyte percentage distribution on day 7. Bars represent mean ± SD. Groups with distinct letters are significantly different ($p < 0.05$). **B)** Percentage of lymphocytes following 14-day treatment. Bars denote mean ± SD. Superscript letters show statistical significance ($p < 0.05$). **C)** Lymphocyte percentages across groups after 21 days. Different letters reflect statistical differences ($p < 0.05$).





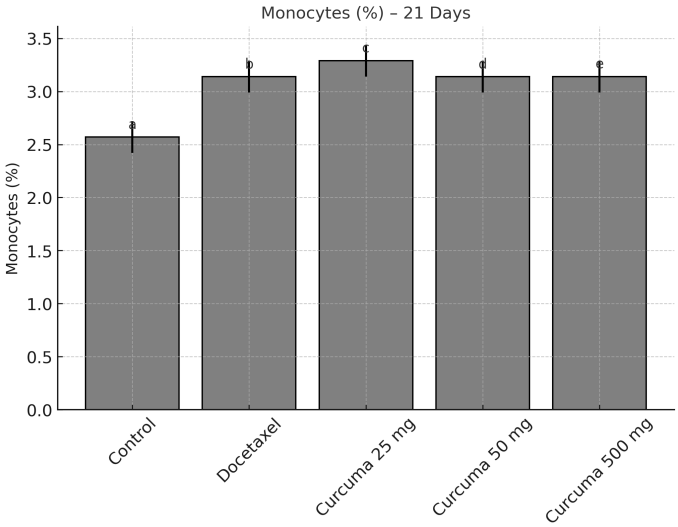
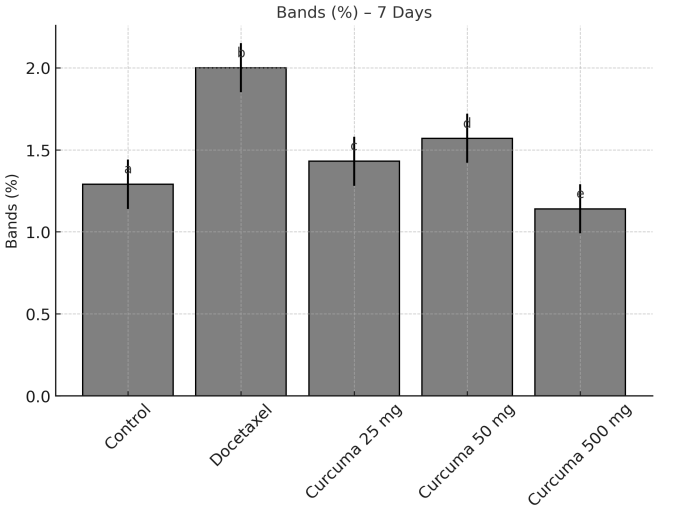
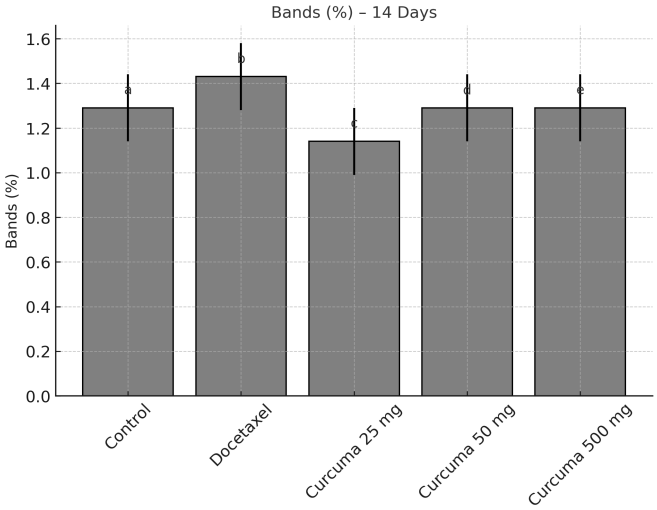


Fig. 12. Monocytes (%). A) Monocyte percentages after 7-days treatment. Bars represent mean ± SD; statistical differences are represented by letter annotations ($p < 0.05$). **B)** Monocyte percentages following 14-day treatment. Statistically distinct groups are marked with different letters (ANOVA and Tukey test, $p < 0.05$). **C)** Monocyte distribution following 21-day treatment. Bars show mean ± SD. Groups with different superscript letters are significantly different ($p < 0.05$).

Other white cell populations—including bands, eosinophils, and basophils — showed minimal variation across the three timepoints, with no significant time-dependent trends (Fig. 13-16).





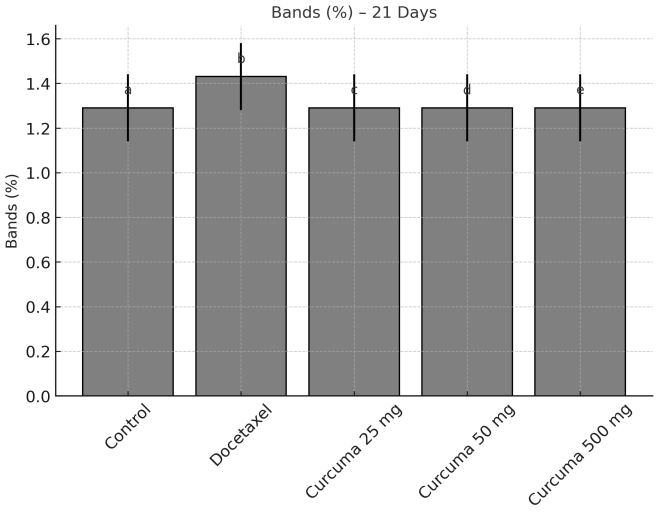
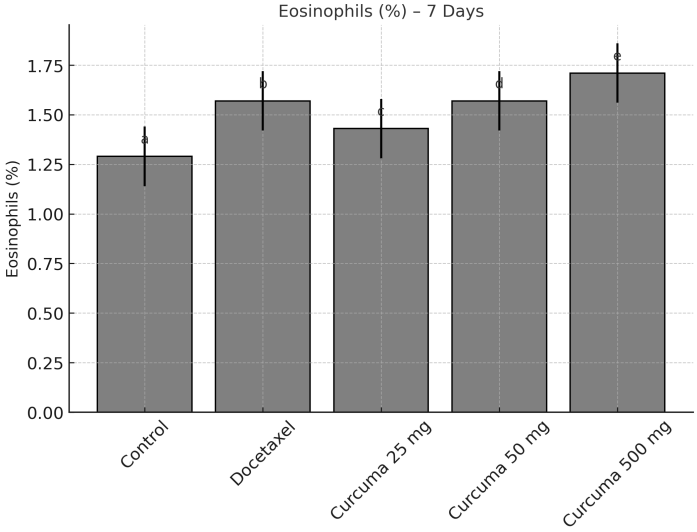
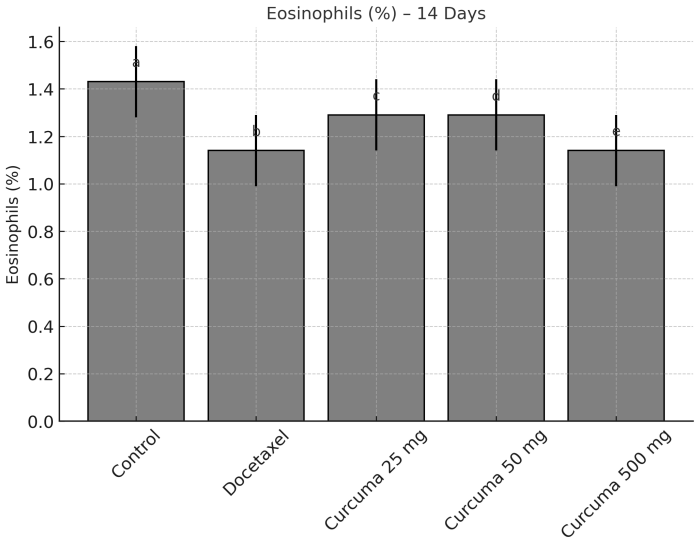


Fig. 13. Bands (%). A) Proportion of band neutrophils after 7-days treatment. Mean ± SD, with significant differences denoted by different letters ($p < 0.05$). **B)** Percentage of band neutrophils across groups after 14 days. Bars represent mean ± SD. Distinct letters correspond to $p < 0.05$. **C)** Percentage of band neutrophils per group after 21 days. Data expressed as mean ± SD. Different letters indicate significant differences ($p < 0.05$).





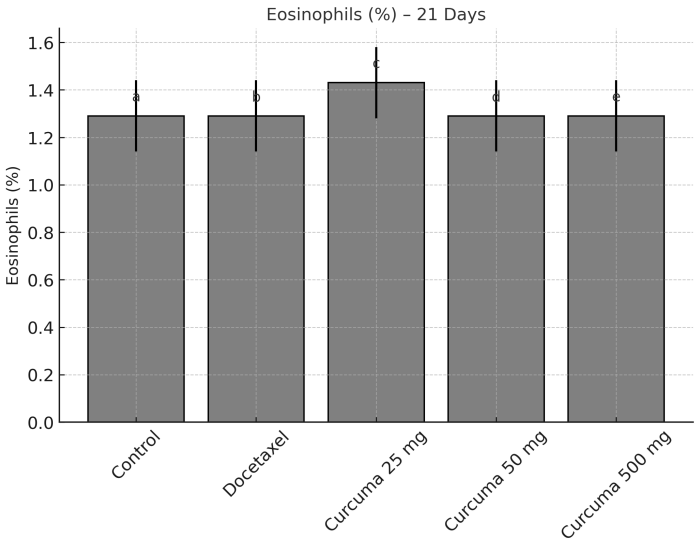
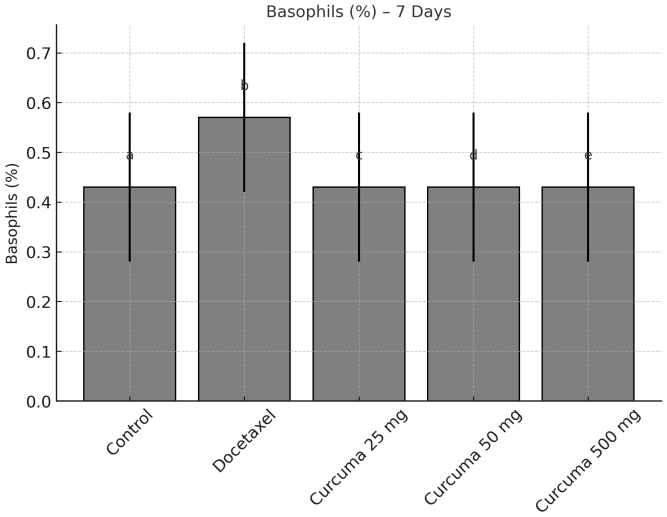
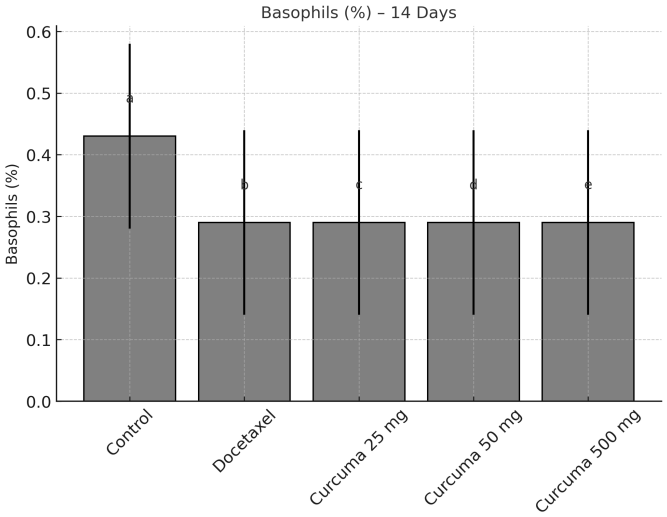


Fig. 14. Eosinophils (%). A) Percentage of eosinophils after 7-days treatment. Bars represent mean ± SD. Letters above bars denote statistical significance ($p < 0.05$). **B)** Eosinophil distribution following treatment for 14 days. Mean ± SD shown; distinct letters represent significant group differences ($p < 0.05$). **C)** Mean eosinophil percentage in peripheral blood of treated rats after 21 days. Statistically distinct groups are labeled with different letters ($p < 0.05$).





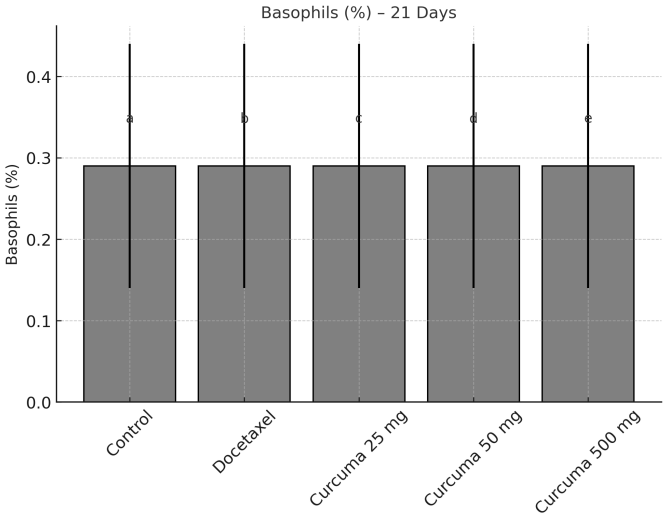


Fig. 15. Basophils (%). A) Basophil proportion on day 7. Different letters signify statistically significant differences between groups ($p < 0.05$). **B)** Proportion of basophils in experimental groups after 14 days. Significant differences indicated by different letters ($p < 0.05$). **C)** Basophil proportions observed after 21 days. Bars show mean ± SD; letters denote significant differences ($p < 0.05$).

After 7 days of treatment, the relative spleen weight remained comparable across most groups, except for a mild but statistically significant increase in the 500 mg/kg *Curcuma longa* group ($p < 0.05$ vs. Curcuma 50 mg/kg and docetaxel) (Fig 16). In the 14-day protocol, administration of docetaxel and *Curcuma longa* 25 mg/kg led to a moderate reduction in spleen weight, with the latter showing significantly lower values compared to control ($p < 0.05$). By day 21, a marked splenomegaly was observed in the group treated with *Curcuma longa* at 500 mg/kg, which exhibited a more than 9-fold increase in relative spleen weight compared to the control group ($p < 0.001$), indicating a dose- and time-dependent splenic response.

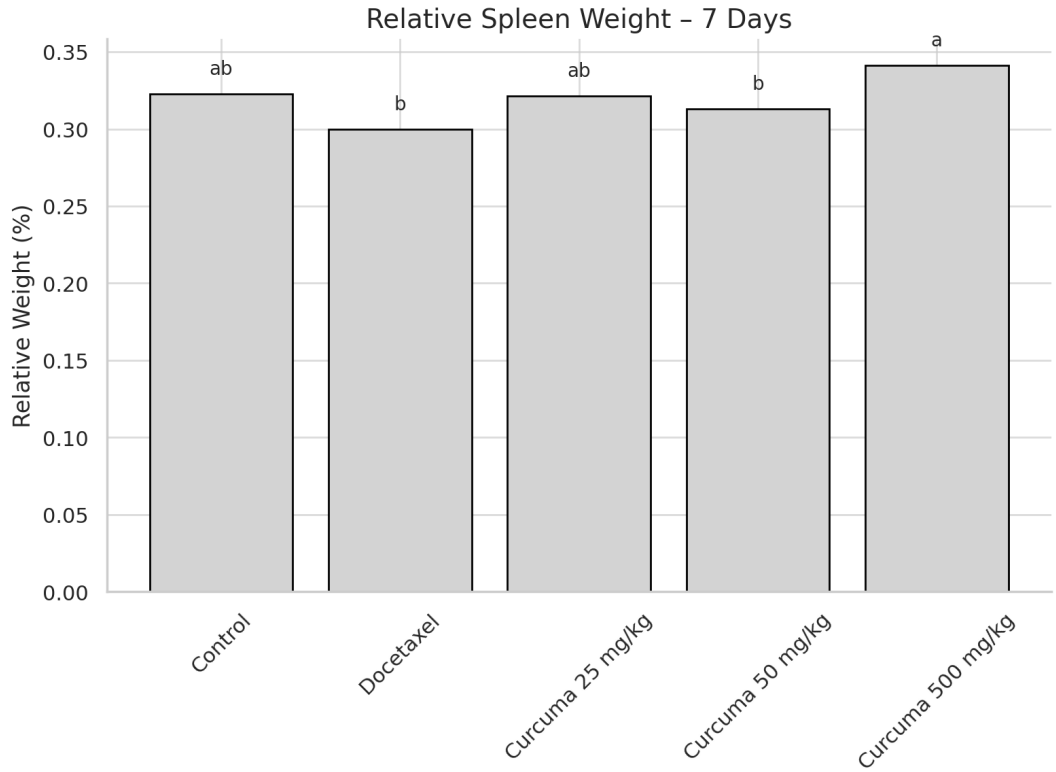
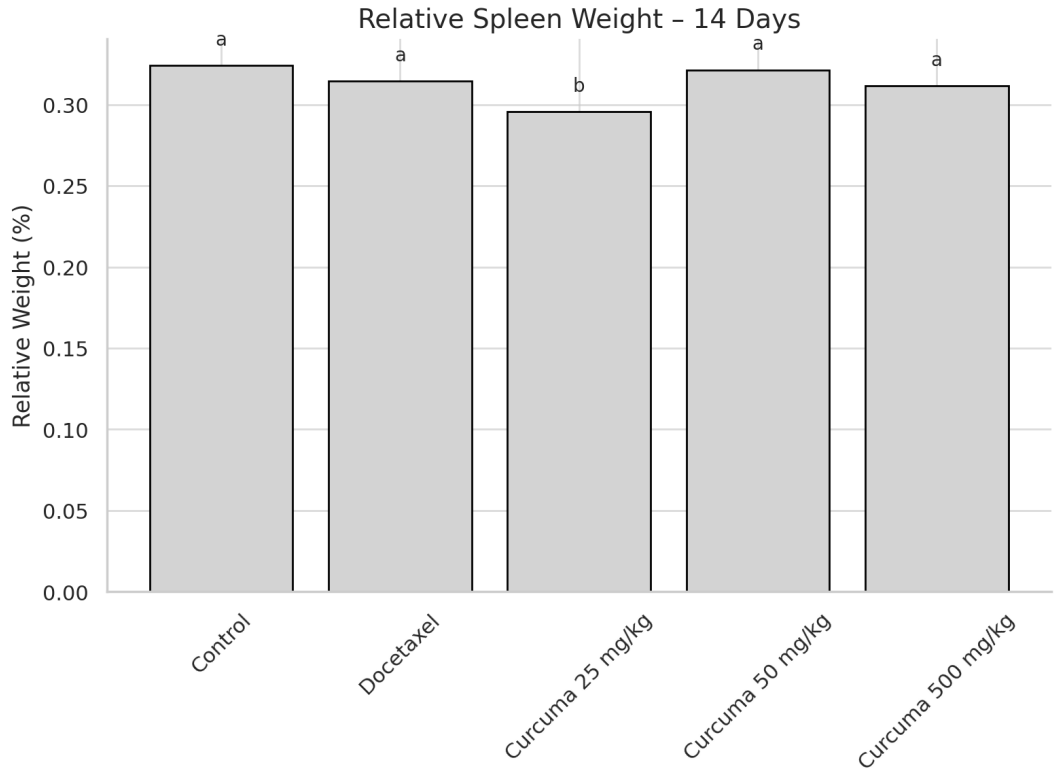


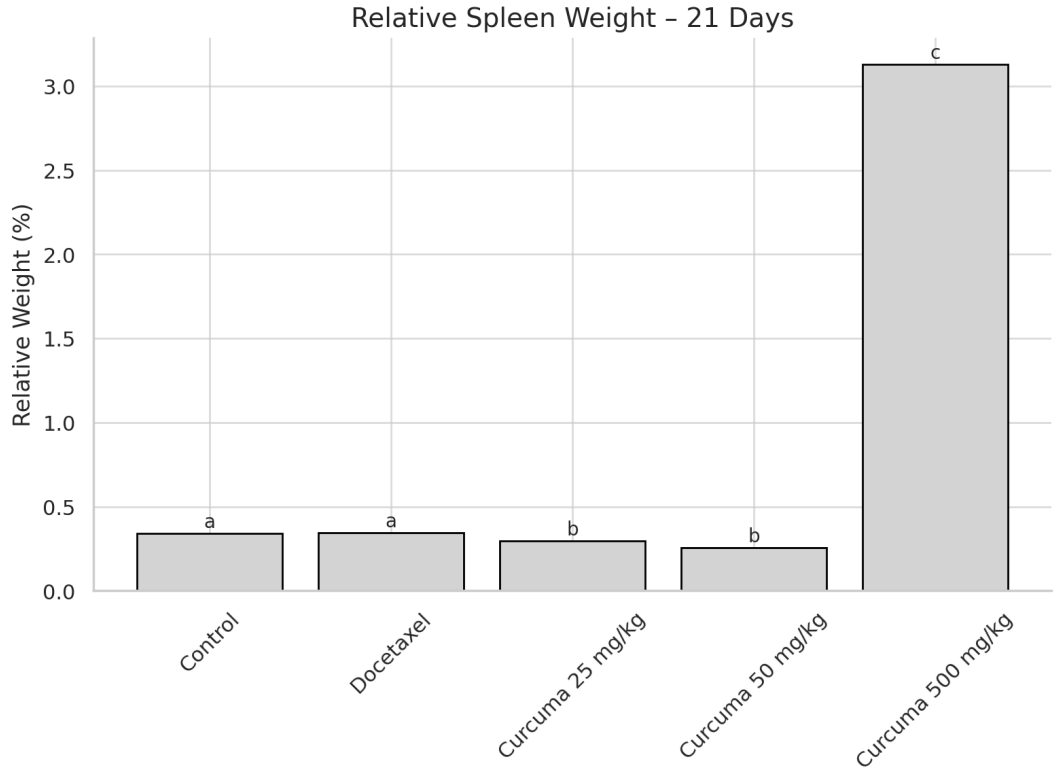
Fig. 16 Relative spleen weight. A) Relative spleen weight in Wistar rats after 7 days of treatment. Bar heights show mean spleen weight (%). The administration of *Curcuma longa* at

500 mg/kg significantly increased spleen weight compared to docetaxel and 50 mg/kg groups ($p < 0.05$, Tukey's test).



Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

B) Relative spleen weight in Wistar rats after 14 days of treatment. The Curcuma 25 mg/kg group showed significantly lower spleen weight than control and other groups ($p < 0.05$).



Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

C) Relative spleen weight in Wistar rats after 21 days of treatment. Curcuma 500 mg/kg produced an extreme increase in spleen weight, significantly different from all other groups ($p < 0.05$).

These results suggest a distinct modulation of splenic mass by high-dose *Curcuma longa,* potentially reflecting its impact on hematopoietic or immunomodulatory mechanisms within the spleen, particularly when administered in prolonged exposure. The findings collectively support the hypothesis that *Curcuma longa*, when used as an adjuvant to docetaxel, may provide a time-dependent protective effect on hematopoietic parameters. While the toxic effects of docetaxel became more apparent with treatment duration—particularly regarding RBC, PLT, and WBC profiles—co-administration with *Curcuma longa* consistently mitigated these changes, especially in the early and mid phases (7 and 14 days). By day 21, the protective effects remained present but were slightly attenuated, suggesting a possible ceiling to curcumin's efficacy under prolonged cytotoxic stress. Importantly, no

deaths, treatment discontinuations, or any signs of clinical toxicity related to *Curcuma longa* were observed in any of the experimental groups.

Discussion

Hematological Toxicity: A Limiting Factor in Oncology

Despite advances in precision oncology, including targeted therapies and immunotherapies, cytotoxic chemotherapy remains the cornerstone of treatment for most solid tumors. However, chemotherapy-associated adverse events (CAAEs) continue to pose a substantial clinical and economic burden, contributing to significant morbidity and mortality, and negatively impacting patients' physical, emotional, and social well-being. Hematological toxicities such as neutropenia, anemia, and thrombocytopenia are among the most frequent complications, often leading to treatment delays, dose reductions, or discontinuation—factors that may compromise therapeutic efficacy and increase the risk of disease recurrence[1,2,3].

Hematological abnormalities are frequently observed in cancer patients, both prior to and following therapeutic interventions (Fig. 1)[3]. Pre-treatment hematological alterations are largely attributed to bone marrow suppression secondary to neoplastic infiltration, which compromises hematopoietic function[3-4]. Numerous studies have reported a high incidence of baseline anemia, neutropenia, and thrombocytopenia in oncology populations. Post-treatment hematological derangements, in contrast, may arise not only from the continued impact of the malignancy but also from the cytotoxicity associated with chemotherapy and radiotherapy regimens[3,4,5].

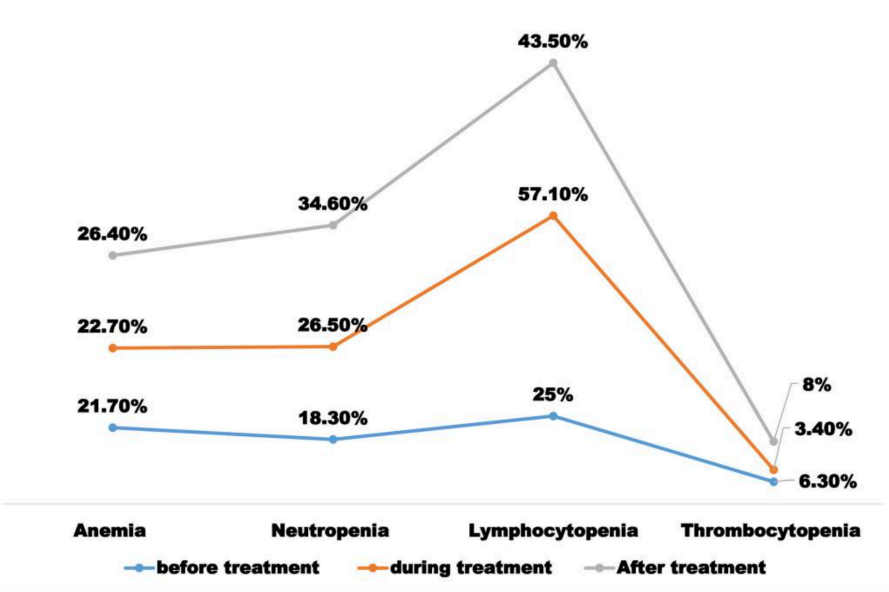


Figure 1. Hematological abnormalities observed throughout the treatment cycle in breast cancer patients treated at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia.
Adapted and translated from Aynalem M et al.[3]

Among these abnormalities, anemia is the most prevalent and is associated with multiple underlying mechanisms, including chronic tumor-related bleeding, direct bone marrow infiltration, tumor-induced malnutrition, altered iron metabolism, renal impairment, and general suppression of hematopoiesis. Leukopenia—especially neutropenia—is primarily linked to the myelosuppressive nature of antineoplastic agents[3,4,5]. Thrombocytopenia, on the other hand, is often attributed to tumor-mediated activation of the coagulation cascade, resulting in increased platelet consumption. Conversely, thrombocytosis has been identified as a negative prognostic marker, correlating with reduced progression-free survival and worse overall outcomes in various malignancies[3,7].

In light of these challenges, there is a growing demand for integrative therapeutic strategies capable of attenuating chemotherapy-induced toxicity without diminishing its antitumor potential. Within this context, phytochemicals such as *Curcuma longa* have emerged as promising adjuvants, owing to their anti-inflammatory, antioxidant, and cytoprotective properties demonstrated in preclinical models[8].

The Therapeutic Promise of *Curcuma longa*

Growing preclinical and clinical evidence has demonstrated that curcumin and its structural analogs possess a broad spectrum of pharmacological properties, including potent antioxidant, anti-inflammatory, and antineoplastic activities. Among these, its anticancer potential is particularly noteworthy. Curcumin has been shown to induce apoptosis and exert antiproliferative effects across a wide range of tumor cell lines, such as those derived from prostate, breast, colorectal, pancreatic, and renal cancers. Notably, doses up to 12 g/day administered over 3 months have been reported as safe in humans[8-9].

Mechanistically, curcumin modulates several oncogenic and tumor-suppressor pathways. It inhibits telomerase reverse transcriptase activity and downregulates anti-apoptotic proteins such as Bcl-2[8,9,10]. At the molecular level, curcumin interacts with key regulators of angiogenesis, metastasis, and cell survival, and interferes with dysregulated signaling cascades including PI3K/Akt and NF-κB. It also modulates crucial cellular processes through its effects on p53, MAPKs, PTEN, and microRNAs. NF-κB, a transcription factor implicated in both inflammation and tumorigenesis, is a major molecular target of curcumin. Under pathological stimuli—such as cytokines, carcinogens, free radicals, or ionizing radiation—NF-κB is upregulated via TNF-α-mediated activation of the IκB kinase (IKK) complex. Curcumin effectively inhibits this process by suppressing IKK activity, thereby preventing NF-κB nuclear translocation and downstream gene expression[8,11,12].

In both in vitro and in vivo models, curcumin has demonstrated cytotoxicity against pancreatic cancer cells through the suppression of oxidative stress and angiogenesis, and through the induction of programmed cell death. Collectively, these data reinforce curcumin's role as a promising adjuvant compound in oncology, capable of targeting multiple hallmarks of cancer with a favorable safety profile[8].

Recent preclinical data suggest that combination regimens integrating natural compounds and cytotoxic agents may enhance antineoplastic efficacy without exacerbating systemic toxicity[13]. Docetaxel, a microtubule-stabilizing agent approved at doses of 30 to 75 mg for the treatment of metastatic castration-resistant prostate cancer (mCRPC), remains a cornerstone of standard therapy[14]. Nonetheless, prolonged exposure to docetaxel has been associated with significant cumulative toxicity, often limiting treatment adherence and compromising patient outcomes[15].

Synergism Between Curcumin and Docetaxel

In this context, Banerjee et al.[16] demonstrated that the co-administration of curcumin (20 μM) and docetaxel (10 nM) significantly enhanced cytotoxicity in prostate cancer cell lines (DU145 and PC-3), compared to monotherapy with either agent. The combined treatment potentiated apoptosis and markedly suppressed cell proliferation through the downregulation of key oncogenic pathways, including COX-2, phospho-Akt, PI3K, NF-κB, p53, and receptor tyrosine kinases (RTKs). These findings highlight the mechanistic synergy between curcumin and docetaxel, suggesting that phytochemical-based adjuvants may augment therapeutic efficacy while potentially attenuating chemotherapy-induced toxicity and overcoming resistance mechanisms in prostate cancer.

Given the substantial burden of hematological toxicity associated with cytotoxic chemotherapy, particularly with agents such as docetaxel, this study aimed to evaluate the extent of hematological alterations induced by docetaxel and the potential mitigating role of *Curcuma longa* as an adjuvant therapeutic agent. The proposed hypothesis is that *Curcuma longa*, owing to its known anti-inflammatory and antioxidant properties, may contribute to the preservation of hematopoietic integrity and attenuation of myelosuppressive effects when used in combination with standard chemotherapy. This investigation seeks to contribute to the growing body of evidence supporting the integration of phytotherapeutic agents into oncologic care as a complementary strategy to improve treatment tolerability and protect physiological homeostasis.

Hematological Preservation in Docetaxel-Treated Animals

In light of these challenges, the present study investigated the potential adjuvant role of *Curcuma longa* in modulating hematologic toxicity induced by docetaxel. The results demonstrated a time- and dose-dependent protective effect of *Curcuma longa* on key hematological parameters, including red blood cell count, hemoglobin concentration, hematocrit, white blood cell count, and platelet levels. These findings are in agreement with preclinical evidence indicating that curcumin—*Curcuma longa*'s principal bioactive

component—possesses anti-inflammatory and antioxidant properties capable of mitigating chemotherapy-induced damage[19].

Notably, the attenuation of leukopenia and thrombocytopenia, particularly at intermediate doses (25–50 mg/kg), suggests a protective effect on both myeloid and megakaryocytic lineages. This may be mediated by the suppression of oxidative stress and inflammatory cascades, as curcumin is known to inhibit NF-κB and modulate PI3K/Akt signaling—key pathways implicated in cytotoxic drug resistance and hematopoietic regulation[20-22].

Splenic Response and Immunomodulation

The most striking morphological finding was the dose- and time-dependent alteration in relative spleen weight. At day 7, splenic hypertrophy was modest, becoming more pronounced at day 21, particularly in the group receiving 500 mg/kg of *Curcuma longa*. Splenomegaly in the context of chemotherapeutic exposure may reflect extramedullary hematopoiesis, increased reticuloendothelial activity, or compensatory immune cell proliferation[23]. High-dose curcumin has been shown to upregulate hematopoietic cytokines and increase splenic macrophage activity in experimental models[19], which may explain the observed findings.

The splenic response observed in this study, characterized by dose- and time-dependent variations in spleen weight, supports the hypothesis that *Curcuma longa* may influence extramedullary hematopoiesis or immune cell trafficking, consistent with prior studies showing curcumin's immunomodulatory potential[23-24]. Particularly, the pronounced splenomegaly observed at 21 days with the 500 mg/kg dose raises important questions regarding the balance between therapeutic benefit and immunological overstimulation in prolonged exposures.

However, the excessive splenic enlargement at 21 days in the high-dose group (>9-fold increase in relative weight) warrants caution. While it may indicate a robust immunohematologic response, it also raises concerns about potential overstimulation of splenic architecture, which could predispose to fibrosis or functional hypersplenism with prolonged exposure. These effects underline the importance of optimizing the therapeutic window of *Curcuma longa* to balance benefit and risk.

The spleen, as a central organ involved in hematopoietic regulation and immune surveillance, plays a critical role in the systemic response to cytotoxic insult. In the present study, the relative spleen weight exhibited a striking time- and dose-dependent increase, particularly pronounced in the group receiving *Curcuma longa* at 500 mg/kg for 21 days. The pronounced splenomegaly observed in the high-dose group after 21 days may represent a compensatory extramedullary hematopoiesis response or an accumulation of immune effector cells, such as macrophages, stimulated by sustained curcumin exposure.

Extramedullary hematopoiesis is a well-documented phenomenon in murine models subjected to hematopoietic stress, infection, or inflammation, wherein the spleen reactivates its embryonic hematopoietic function in response to bone marrow suppression or systemic immunological demand[31]. Given the cytotoxic nature of docetaxel and the associated marrow suppression observed in this model, it is plausible that splenic hypertrophy reflects a physiological attempt to restore hematopoietic balance.

Furthermore, curcumin's immunomodulatory effects may contribute to this splenic response by enhancing the recruitment and activation of monocytes/macrophages and stimulating cytokines such as IL-6, TNF-α, and GM-CSF—mediators known to drive splenic expansion and myeloid cell proliferation[22,23,25]. Indeed, high-dose curcumin has been associated with increased splenic macrophage activity and elevated hematopoietic cytokine expression in previous experimental models[19,24].

However, the magnitude of splenic enlargement observed in the 500 mg/kg group (>9-fold relative weight increase compared to control) raises questions regarding the balance between adaptive and potentially maladaptive responses. Chronic splenic stimulation may predispose to architectural distortion, fibrosis, or even functional hypersplenism—a condition associated with cytopenias and immune dysregulation[32–34]. Thus, while moderate splenic activation may reflect beneficial immune or hematopoietic support, exaggerated responses warrant further scrutiny regarding the long-term safety of high-dose *Curcuma longa* administration.

Future investigations incorporating spleen histomorphology, immunophenotyping, and cytokine profiling would provide critical insight into the underlying mechanisms of splenic remodeling and its systemic implications. Together, these results reinforce the notion that *Curcuma longa*, when used in conjunction with docetaxel, may preserve hematological

homeostasis and limit cytotoxic damage in a time-sensitive and dose-dependent manner. Future studies employing bone marrow histopathology and flow cytometry could further elucidate the mechanisms underlying these effects.

Red Cell Indices and Erythropoietic Efficiency

Red cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), remained within physiological ranges but displayed modest elevation in MCH in *Curcuma longa*-treated animals over time. These changes may reflect enhanced hemoglobin synthesis or improved erythrocyte morphology, although further ultrastructural analysis would be required to confirm this.

Red Cell Distribution Width (RDW) is a quantitative index of erythrocyte size heterogeneity and is commonly elevated in conditions associated with ineffective erythropoiesis, increased red cell turnover, or oxidative stress. In this study, RDW levels showed a mild, progressive elevation over time in animals exposed to docetaxel alone, particularly at the 21-day mark, suggesting a disruption in erythroid maturation or red cell membrane integrity likely mediated by oxidative injury to hematopoietic progenitors.

Notably, co-treatment with *Curcuma longa*—especially at 25 and 50 mg/kg doses—was associated with stabilization of RDW values across timepoints, with values remaining within a narrow physiological range. This observation may reflect improved erythrocyte morphology preservation and more synchronized erythropoiesis under adjunctive phytotherapy. Curcumin, the principal bioactive component of *Curcuma longa*, is well documented to possess potent antioxidant and anti-inflammatory activities, including the scavenging of reactive oxygen species (ROS), inhibition of lipid peroxidation, and suppression of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α[20-22]. These properties may reduce oxidative stress and inflammation in the bone marrow microenvironment, thereby supporting the survival and proper maturation of erythroid progenitor cells.

Furthermore, experimental data suggest that curcumin enhances the activity of the Nrf2–ARE (antioxidant response element) pathway, which plays a central role in cellular defense against oxidative damage and is essential for the maintenance of hematopoietic stem

cell function[20]. By promoting redox homeostasis and reducing inflammation-induced erythroid dysregulation, curcumin may indirectly contribute to the normalization of RDW. Therefore, the observed stabilization of RDW in the intermediate dose groups may serve as a surrogate marker for preserved erythropoietic efficiency and cellular integrity, reinforcing the hematoprotective potential of *Curcuma longa* under cytotoxic stress.

These hematologic findings provide additional insight into the temporal dynamics of bone marrow suppression and potential hematologic recovery under adjunctive phytotherapy. Docetaxel, a microtubule-stabilizing chemotherapeutic agent, is well-documented for inducing dose-limiting myelosuppression, particularly neutropenia and thrombocytopenia, through inhibition of mitotic spindle assembly and consequent apoptosis in proliferating hematopoietic progenitor cells[19]. In this experimental model, a progressive decline in red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (Ht) was observed in animals exposed solely to docetaxel, with the most significant alterations noted on day 21, confirming the cumulative cytotoxic effect of the agent.

The co-treatment with *Curcuma longa*—especially at doses of 25 and 50 mg/kg—attenuated the extent of RBC, Hb, and Ht reduction over time. This suggests a potential erythropoietic protection, likely attributable to the antioxidative action of curcumin, which has been shown to upregulate nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of antioxidant defense mechanisms and hematopoietic integrity[20-23]. Curcumin has also been shown to modulate hepcidin and iron metabolism, factors critical to erythropoiesis under inflammatory and cytotoxic stress[21].

Preservation of Myelopoiesis and Immune Competence Under Chemotherapy Stress

The leukocyte response is equally relevant. Docetaxel-induced leukopenia, predominantly neutropenia, poses substantial clinical challenges and increases the risk of infection. In this study, leukocyte counts in the docetaxel-only group declined over time, as expected. In contrast, animals receiving *Curcuma longa* in combination therapy showed relative preservation—and in some cases enhancement—of white blood cell (WBC) levels, particularly neutrophils and monocytes, across the 14- and 21-day groups. This immunomodulatory effect aligns with existing literature showing that curcumin can enhance

myelopoiesis and innate immune function via modulation of IL-6, TNF-α, and colony-stimulating factors[22,25].

The observed trends in neutrophil and lymphocyte counts across the experimental groups provide important insights into the potential immunological modulation conferred by *Curcuma longa*. In contrast to the progressive leukopenia commonly associated with docetaxel monotherapy, animals receiving adjunctive *Curcuma longa*—particularly at intermediate doses—displayed relative preservation or mild elevation in total leukocyte count, including neutrophils and lymphocytes, particularly at the 14- and 21-day timepoints.

These findings suggest that *Curcuma longa* may exert a mild immunomodulatory effect, potentially preserving myelopoiesis and promoting a more balanced immune cell profile under cytotoxic stress. This hypothesis is supported by preclinical evidence showing that curcumin modulates the expression of key hematopoietic and immune-related cytokines, such as IL-6, TNF-α, and GM-CSF, which regulate myeloid and lymphoid lineage differentiation[22,25,26]. Additionally, curcumin has been shown to enhance the functional capacity of immune effector cells, including macrophages, NK cells, and cytotoxic T lymphocytes, in various models of inflammation and malignancy[26].

From a translational perspective, these immune trends underscore the relevance of assessing inflammatory biomarkers such as the neutrophil-to-lymphocyte ratio (NLR) in future studies. The NLR has emerged as a robust prognostic indicator in several solid tumors, reflecting the balance between pro-tumor inflammation (neutrophils) and antitumor immune surveillance (lymphocytes). An elevated NLR has been associated with poor outcomes and reduced treatment response in patients receiving chemotherapy or immunotherapy, particularly in lung, colorectal, and breast cancers[27,28].

The relatively stable or slightly elevated white blood cell counts observed in animals co-treated with *Curcuma longa* suggest preservation of myelopoiesis or a mild immunomodulatory effect, especially in the 14- and 21-day groups. This effect may be particularly valuable in an adjuvant treatment setting, where maintaining immune competence is essential to reduce infection risk and support antitumor surveillance.

Curcumin, the principal bioactive compound of *Curcuma longa*, has been shown to modulate the production of cytokines such as IL-6 and TNF-α, enhance leukocyte recovery,

and mitigate oxidative stress within the tumor microenvironment, all of which may contribute to improved immunologic resilience during chemotherapy[29,30].

In future studies, evaluation of neutrophil-to-lymphocyte ratio (NLR) could offer additional insights. NLR is a well-established prognostic marker in several malignancies, with higher ratios correlating with systemic inflammation and poorer clinical outcomes[27,28]. In this context, monitoring NLR in response to *Curcuma longa* supplementation may help elucidate the compound's immun and inform its clinical applicability in adjuvant oncology settings.

Thrombopoiesis Preservation and Platelet Recovery

Furthermore, the platelet count (PLT) followed a consistent suppressive pattern with docetaxel monotherapy, while co-treatment with *Curcuma longa*—notably at the 25 and 50 mg/kg doses—ameliorated this effect. This may be due to curcumin's reported ability to protect megakaryocyte progenitors from apoptosis and improve thrombopoiesis via antioxidative and anti-apoptotic pathways[26].

Thrombocytopenia is a common and often dose-limiting adverse effect of cytotoxic chemotherapy, which may lead to bleeding events, need for platelet transfusions, treatment delays, and overall compromise in oncologic outcomes. Therefore, the observed attenuation of PLT decline in the *Curcuma longa*-treated groups holds significant clinical relevance.

Curcumin has been shown to protect megakaryocytic progenitor cells from oxidative stress-induced apoptosis, likely via activation of antioxidant response elements and suppression of inflammatory mediators such as NF-κB and ROS-related pathways[19,22,26]. Additionally, curcumin may enhance thrombopoietin signaling and support platelet production through the modulation of cytokine networks involved in megakaryopoiesis.

The preservation of PLT counts in this experimental model may indicate a direct or indirect stimulatory effect of *Curcuma longa* on megakaryocyte differentiation and maturation. This hypothesis warrants further investigation. Future studies involving bone marrow histopathological analysis and immunohistochemical staining for megakaryocytic markers (e.g., CD41, CD42b) would be valuable in elucidating the specific mechanisms by which *Curcuma longa* modulates thrombopoiesis.

Ultimately, maintaining platelet levels within a functional range can reduce the risk of hemorrhagic complications and minimize interruptions in the treatment regimen—an essential goal in the clinical management of cancer patients receiving chemotherapy.

Comparison with Other Cytotoxic Agents

The hematopoietic alterations observed in this docetaxel-based experimental model are in line with findings reported for other commonly used cytotoxic agents, such as cisplatin and cyclophosphamide. Both agents are well-documented for inducing dose-dependent bone marrow suppression, leading to reductions in peripheral blood cell counts, including erythrocytes, leukocytes, and platelets[35–37]. Cisplatin, a platinum-based compound, exerts its cytotoxicity through DNA cross-linking and has been associated with significant anemia and leukopenia in preclinical models, particularly through suppression of erythroid progenitors and oxidative damage to bone marrow stromal cells[35]. Similarly, cyclophosphamide, an alkylating agent, causes profound and sustained suppression of hematopoiesis via DNA alkylation, with marked effects on lymphocyte and megakaryocyte lineages[36].

Notably, several studies investigating phytotherapeutic adjuvants—such as *Curcuma longa*, *Panax ginseng*, and *Camellia sinensis*—have reported hematoprotective effects across different chemotherapy models, highlighting their potential to mitigate oxidative stress and inflammation-mediated marrow suppression[37–39]. The temporal patterns of cytopenias and recovery observed in the present docetaxel model bear resemblance to cisplatin and cyclophosphamide studies, reinforcing the construct validity of the experimental framework and suggesting that the protective trends induced by *Curcuma longa* may hold relevance across diverse cytotoxic contexts.

These parallels strengthen the translational relevance of the findings and provide a compelling rationale for broader investigation of *Curcuma longa* as an adjunctive agent in chemotherapy protocols involving various myelotoxic regimens.

Translational Implications and Clinical Perspectives

The hematopoietic modulation demonstrated by *Curcuma longa* in this preclinical model carries significant implications for translational oncology. One of the major barriers to optimal chemotherapy delivery in clinical practice is treatment-limiting hematological

toxicity, which often necessitates dose reductions, cycle delays, or even early discontinuation. These modifications compromise dose intensity—a key determinant of therapeutic efficacy in many oncologic protocols—and have been associated with inferior outcomes across various solid tumors[2,3,40].

The present findings suggest that *Curcuma longa*, through its hematoprotective and immunomodulatory actions, may help preserve bone marrow function during cytotoxic chemotherapy, thereby maintaining treatment continuity and full-dose intensity. This is particularly relevant for agents such as docetaxel, whose clinical benefit is closely linked to adherence to scheduled dosing regimens[14,15,40]. The ability of *Curcuma longa* to attenuate erythrocyte, leukocyte, and platelet suppression—especially during the early and intermediate treatment phases—could translate into reduced need for supportive interventions such as growth factors, transfusions, or hospitalization.

Furthermore, the observed dose- and time-dependent effects highlight the importance of identifying an optimal therapeutic window that maximizes hematologic support while minimizing the risk of immune overstimulation or organomegaly. These results provide a robust rationale for future clinical trials investigating *Curcuma longa* as an adjuvant agent in cancer patients undergoing chemotherapy. Such studies could assess endpoints including hematologic toxicity profiles, chemotherapy dose intensity, infection rates, and quality of life metrics, offering a holistic view of its clinical utility.

Given its favorable safety profile, low cost, and accessibility, *Curcuma longa* represents a promising phytotherapeutic candidate for integration into multimodal oncologic care strategies aimed at improving tolerability and enhancing treatment outcomes.

Overall, these results provide strong support for the hematoprotective and immunomodulatory effects of *Curcuma longa* in the context of docetaxel chemotherapy, particularly during the early and intermediate phases of treatment. The combination of favorable erythropoietic, leukopoietic, and thrombopoietic outcomes, coupled with dynamic splenic responses, underscores the potential of *Curcuma longa* as an effective adjunct in mitigating hematologic toxicity. Nonetheless, long-term safety, optimal dosing, and mechanistic insights into splenic activation require further investigation through histopathological and molecular analyses.

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Declaration of interest statement

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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TÍTULO COMPLETO DO ARTIGO CIENTÍFICO 2 - EXPERIMENTAL EVALUATION OF *CURCUMA LONGA* IN DOCETAXEL-INDUCED SYSTEMIC TOXICITY: FUNCTIONAL HEPATORENAL AND TARGET ORGAN ANALYSIS

Artigo editado de acordo com as normas de publicação da Revista Drug and Chemical Toxicology – Print ISSN: 0148-0545 Online ISSN: 1525-6014

RESUMO

Introdução: O docetaxel é um quimioterápico amplamente utilizado no tratamento de tumores sólidos, porém sua aplicação clínica é frequentemente limitada por toxicidades sistêmicas, especialmente hepáticas e renais. A *Curcuma longa*, planta com propriedades antioxidantes e anti-inflamatórias, pode exercer efeitos organoprotetores. **Objetivo**: Avaliar os efeitos protetores da *Curcuma longa* administrada por via oral frente à toxicidade sistêmica induzida por docetaxel em ratos Wistar. **Métodos**: Ratos machos Wistar foram distribuídos em 30 subgrupos (n=7) e tratados por 7, 14 ou 21 dias com placebo, docetaxel (2,5 mg/kg, i.p.) ou docetaxel associado à *Curcuma longa* (25, 50 ou 500 mg/kg/dia, oral). Foram avaliados biomarcadores séricos de função hepática (AST, ALT, bilirrubinas) e renal (ureia, creatinina, eletrólitos), além do peso relativo de fígado, rins, coração, pulmões e intestino delgado. **Resultados**: O docetaxel induziu alterações significativas nos parâmetros bioquímicos e nos pesos relativos dos órgãos. A coadministração com *Curcuma longa* atenuou essas alterações de forma dose- e tempo-dependente. A dose de 50 mg/kg apresentou o perfil protetor mais consistente. Altas doses foram associadas a esplenomegalia e hipertrofia intestinal. **Conclusão**: A *Curcuma longa* demonstrou potencial citoprotetor frente à toxicidade induzida por docetaxel, possivelmente por meio da modulação das vias Nrf2, NF-κB e PI3K/ Akt. Esses achados reforçam seu valor como agente adjuvante em oncologia.

ABSTRACT

Background: Docetaxel is a widely used chemotherapeutic agent effective against solid tumors, but its clinical use is limited by systemic toxicities, especially hepatotoxicity and nephrotoxicity. *Curcuma longa*, a phytochemical with antioxidant and anti-inflammatory properties, may offer organoprotective effects. **Objective:** To evaluate the protective effects of orally administered *Curcuma longa* against docetaxel-induced systemic toxicity in Wistar rats. **Methods:** Male Wistar rats were assigned to 5 subgroups (n=7/group) and treated for 7, 14, or 21 days with placebo, docetaxel (2.5 mg/kg, i.p.), or docetaxel combined with *Curcuma longa* (25, 50, or 500 mg/kg/day, oral). Serum biomarkers of hepatic (AST, ALT, bilirubins) and renal (urea, creatinine, electrolytes) function were measured, alongside relative weights of liver, kidneys, heart, lungs, and small intestine. **Results:** Docetaxel induced significant elevations in hepatic and renal biomarkers and altered organ weights. *Curcuma longa* co-treatment attenuated these effects in a dose- and time-dependent manner. The 50 mg/kg dose consistently provided optimal protection. High-dose treatment was associated with marked splenic and intestinal hypertrophy. **Conclusion:** *Curcuma longa* demonstrates cytoprotective potential against docetaxel-induced toxicity, likely via Nrf2, NF-κB, and PI3K/Akt modulation. These findings support further translational research on *Curcuma longa* as an adjuvant in oncologic therapy.

Keywords: Chemotherapy-related toxicity; Phytotherapy; Hepatorenal function; Experimental toxicology; Organ-specific effects.

EXPERIMENTAL EVALUATION OF *CURCUMA LONGA* IN DOCETAXEL-INDUCED SYSTEMIC TOXICITY: FUNCTIONAL HEPATORENAL AND TARGET ORGAN ANALYSIS

Introduction

Docetaxel is a widely used antineoplastic agent belonging to the taxane class, with proven efficacy in the treatment of various solid tumors, including breast, prostate, and non-small cell lung cancers.[1] However, its clinical use is frequently limited by dose-dependent systemic toxicities, especially involving hepatic and renal function, and by off-target organ damage such as splenic and intestinal alterations. These toxic effects are often attributed to mechanisms including oxidative stress, inflammation, and impaired cellular metabolism.[6]

In recent years, there has been growing scientific interest in the use of natural compounds with antioxidant and cytoprotective potential to reduce the adverse effects of chemotherapeutic agents. *Curcuma longa L.* (turmeric), a plant traditionally used in Ayurvedic and Chinese medicine, is recognized for its bioactive polyphenolic compounds, particularly curcumin.[2-5] These substances have been shown to exhibit anti-inflammatory, hepatoprotective, nephroprotective, and immunomodulatory activities. Curcumin's pharmacological profile also includes modulation of cellular signaling pathways, inhibition of pro-inflammatory cytokines, and attenuation of oxidative damage in various experimental models.[3-7]

Despite encouraging evidence, the protective role of *Curcuma longa* in the context of docetaxel-induced systemic toxicity remains insufficiently explored, particularly regarding functional and structural outcomes in critical organs. Additionally, dose–response relationships and organ-specific effects have not been consistently addressed in preclinical models.

The present study aimed to evaluate the potential protective effects of *Curcuma longa* extract, administered orally, on docetaxel-induced systemic toxicity in Wistar rats. The investigation focused on biochemical parameters of hepatorenal function, modulation of inflammatory cytokines, and physiopathological alterations in key target organs, including the liver, kidneys, spleen, and small intestine. This research seeks to contribute to the

understanding of phytotherapeutic strategies for mitigating chemotherapy-associated organ toxicity.

Materials and Methods

This controlled case experimental study involved 105 male Wistar rats (n = 7 per subgroup; 8 weeks of age), obtained from the Animal Facility of Colombo, Paraná, Brazil. The animals were randomly allocated into three primary treatment groups based on duration: Group A (7 days), Group B (14 days), and Group C (21 days). Each primary group was subdivided into five treatment arms as follows:

Group A – 7-day treatment

Subgroups G1A to G5A received treatments as follows:

- G1A: oral placebo (water), daily for 7 days (control group).
- G2A: single intraperitoneal dose of docetaxel on Day 1.
- G3A: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 25 mg/kg/day.
- G4A: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 50 mg/kg/day.
- G5A: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 500 mg/kg/day.

Group B – 14-day treatment

Subgroups G1B to G5B received treatments as follows:

- G1B: oral placebo (water), daily for 14 days (control group).
- G2B: single intraperitoneal dose of docetaxel on Day 1.
- G3B: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 25 mg/kg/day.
- G4B: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 50 mg/kg/day.
- G5B: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 500 mg/kg/day.

Group C – 21-day treatment

Subgroups G1C to G5C received treatments as follows:

- G1C: oral placebo (water), daily for 21 days (control group).
- G2C: single intraperitoneal dose of docetaxel on Day 1.
- G3C: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 25 mg/kg/day.
- G4C: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 50 mg/kg/day.
- G5C: single intraperitoneal dose of docetaxel on Day 1 + *Curcuma longa* orally at 500 mg/kg/day.

The number of animals per subgroup (n = 7) was defined based on previous experimental studies employing similar designs, which demonstrated that this sample size is sufficient to detect significant differences in hematological, biochemical, and histopathological parameters. The selection also complied with international ethical guidelines for animal experimentation, adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement), thereby ensuring the minimum number of animals required to obtain statistically reliable results

The dose of docetaxel was extrapolated for rats using allometric scaling from a human reference (70 kg), based on the methodology proposed by Pachaly (2006)[43]. Calculations were based on specific metabolic rate (SMR) values:

- SMRref (human): $70 \times 70^{-0.25} = 24$
- SMRrat (300 g): $70 \times 0.3^{-0.25} = 95$

To reach the target dose in rats, the human equivalent dose (HED) was adjusted to 0.63 mg/kg. Thus:

- Rat dose = $0.63/24 \times 95 = 2.5$ mg/kg

Therefore, a final intraperitoneal dose of 2.5 mg/kg docetaxel was established, in a volume up to 1 mL/kg.

Example for a 250 g rat:

2.5 mg – 1000 g

X mg – 250 g

X = 0.625 mg

The *Curcuma longa* extract (95% curcuminoids) was obtained from Farmácia Farmavida (Umuarama, Paraná, Brazil). It was administered by gavage in doses of 25, 50, or 500 mg/kg/day, diluted in 1 mL/kg of water (Fig. A).

Fig. A: *Curcuma longa*

Fonte: https://www.easytogrowbulbs.com/products/ginger-curcuma-longa-turmeric . Acessado em: 25 de maio de 2025

Animals were housed in polypropylene cages in a temperature-controlled room (22 ± 2°C) with a 12-h light/dark cycle, and had ad libitum access to standard chow and water throughout the experimental period (Fig. B). After administration, they were placed on a flat surface and observed continuously for 1 hour, then hourly for the first 6 hours, and subsequently once daily for 7, 14, or 21 days, according to the respective group, with the aim of analyzing their behavior. At the end of each experimental period, animals were fasted for 12 hours (with access to water), anesthetized using isoflurane, and euthanized via decapitation in accordance with the euthanasia guidelines of the Brazilian CONCEA (2015)[18].

Fig. B: Animals

Additional biological samples included blood for the assessment of liver function markers—namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin—and renal function parameters, including urea, creatinine, sodium, and potassium levels. Solid organs—including the liver, kidneys, small intestine, and heart—were surgically excised, cleaned of adherent tissue, and weighed with precision. Relative organ weight was subsequently calculated for each specimen as the ratio of the organ mass (g) to the final body weight (g) of the corresponding animal, and expressed as a percentage, according to the formula: Relative Weight (%) = (Organ Weight / Final Body Weight) × 100.

Fig. C: Laboratory

Carcasses were stored at −20°C in labeled plastic bags until collected by a certified biological waste disposal company. Samples were homogenized and stored at 4°C until analysis.

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of University Paranaense under protocol number 40130, and conducted in compliance with the guidelines of the Brazilian CONCEA (2015).

Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism version 10.4.2.

Results

Biochemical profiling revealed dynamic alterations in hepatic and renal markers across treatment groups and durations. In the total bilirubin assay, docetaxel-treated animals exhibited a significant increase ($p < 0.05$) in serum levels compared to controls at all time points, with the peak occurring on day 14. Notably, co-treatment with *Curcuma longa* led to a dose-dependent attenuation of bilirubin elevation. The highest dose (500 mg/kg) resulted in total bilirubin values statistically comparable to the control group by day 21, suggesting hepatoprotective potential (Fig 1).

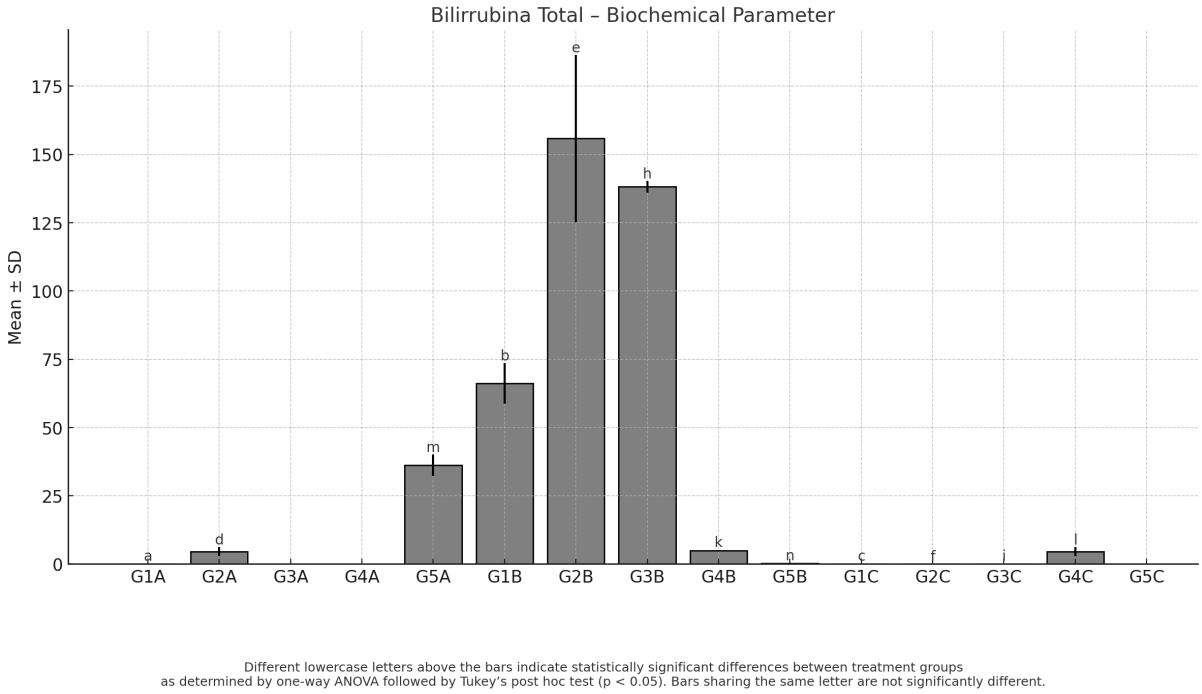


Fig 1.: Total Bilirubin levels (mg/dL)

Mean ± standard deviation of total bilirubin concentrations in Wistar rats treated with docetaxel and/or Curcuma longa for 7, 14, and 21 days. Different lowercase letters indicate statistically significant differences between groups (ANOVA followed by Tukey's post hoc test, $p < 0.05$).

Similar trends were observed in direct and indirect bilirubin. Docetaxel monotherapy significantly elevated both fractions, particularly direct bilirubin at day 14. Administration of *Curcuma longa* at 50 and 500 mg/kg significantly reduced these levels by day 21 ($p < 0.05$), demonstrating potential in mitigating cholestatic effects (Fig 2-3).

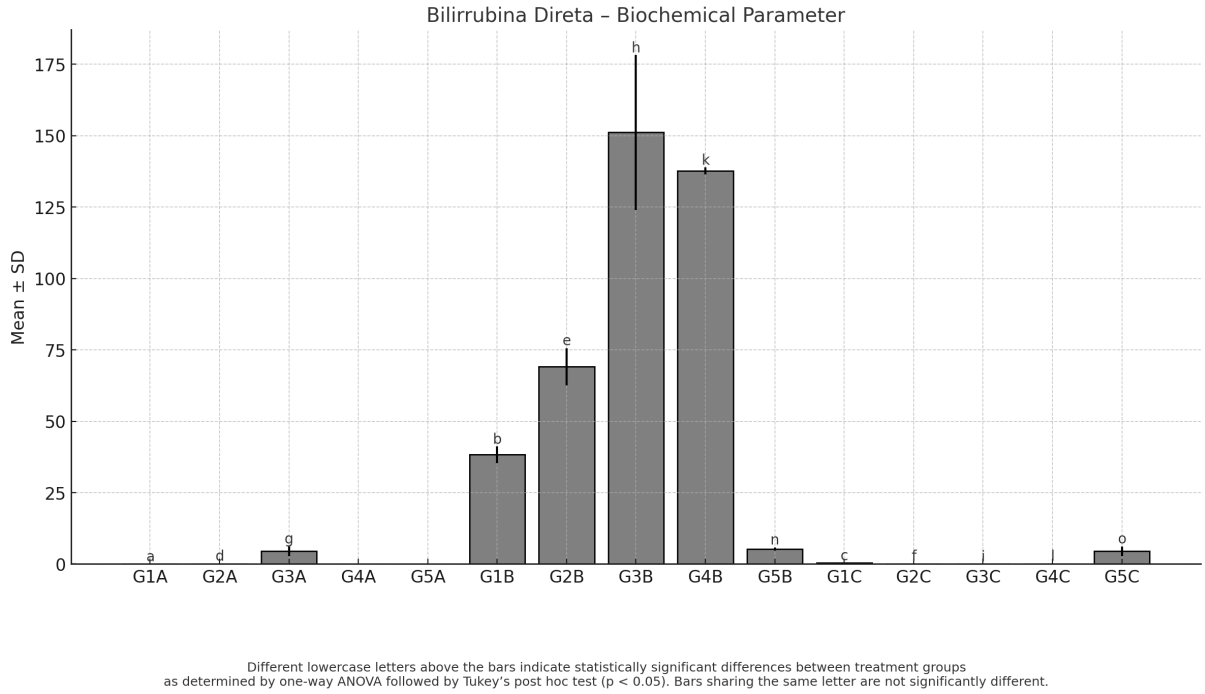


Fig. 2.: Direct Bilirubin levels (mg/dL)

Assessment of direct bilirubin concentrations across treatment groups and time points. Values are expressed as mean ± SD. Groups not sharing the same letter are significantly different ($p < 0.05$).

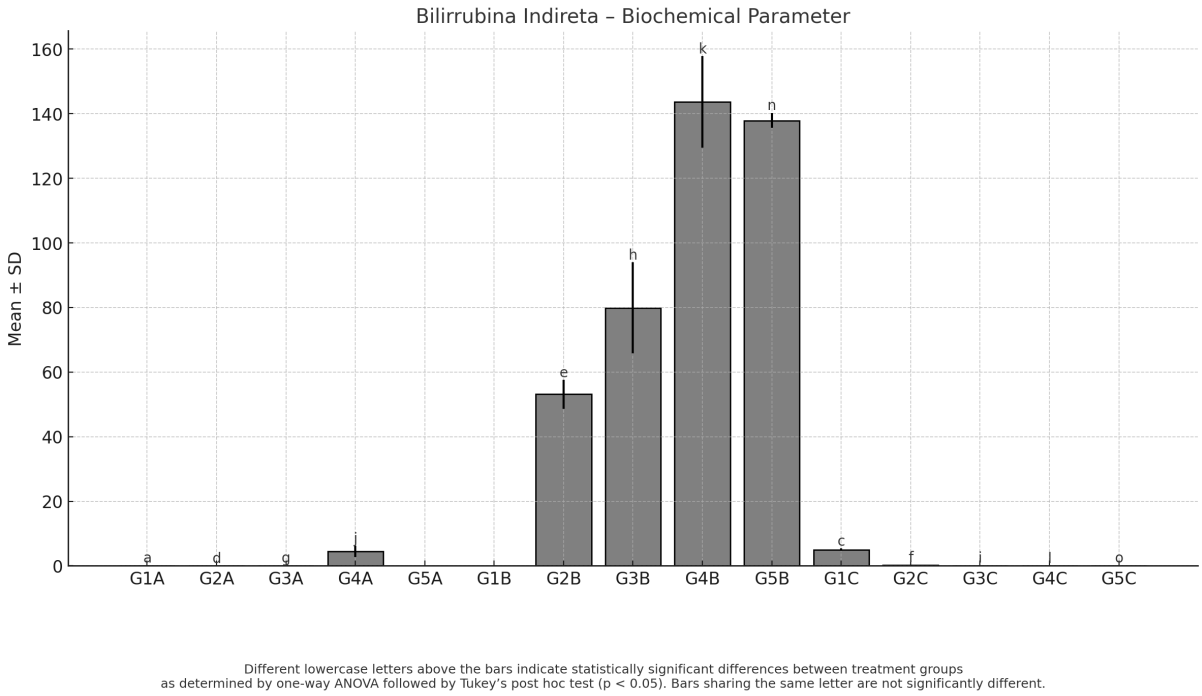


Fig. 3.: Indirect Bilirubin levels (mg/dL)

Mean ± SD of indirect bilirubin concentrations in animals exposed to different combinations of docetaxel and Curcuma longa. Statistically significant differences between groups are indicated by distinct lowercase letters.

Serum creatinine levels, indicative of renal function, were modestly but significantly elevated in the docetaxel-only group at all time points compared to controls ($p < 0.05$). A protective effect was observed in rats co-treated with *Curcuma longa,* particularly at the 500 mg/kg dose, where creatinine values returned to baseline by day 21 (Fig. 4).

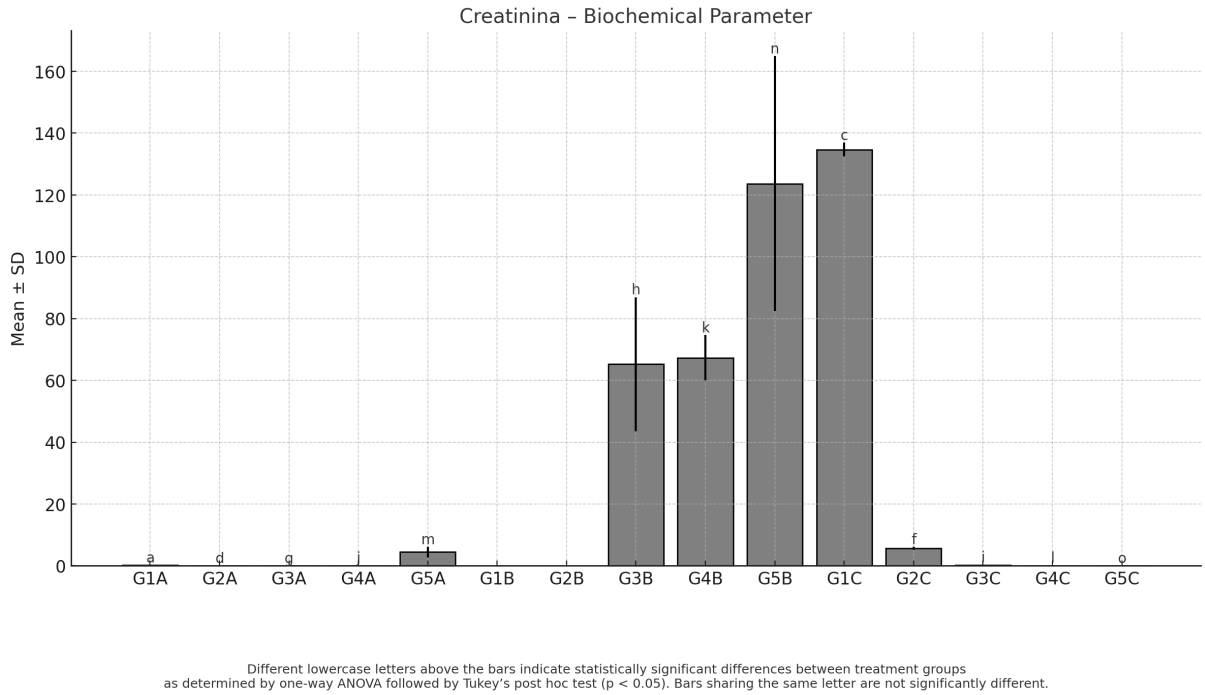


Fig. 4.: Creatinine levels (mg/dL)

Creatinine concentrations across experimental groups. Data shown as mean ± SD. Letters above bars denote statistically significant differences ($p < 0.05$).

In the urea assay, a similar pattern emerged: docetaxel monotherapy led to sustained increases in serum urea across all durations. Co-administration of *Curcuma longa* significantly decreased urea levels in a dose- and time-dependent manner. By day 21, rats receiving the highest dose of *Curcuma longa* displayed urea concentrations indistinguishable from controls (Fig. 5).

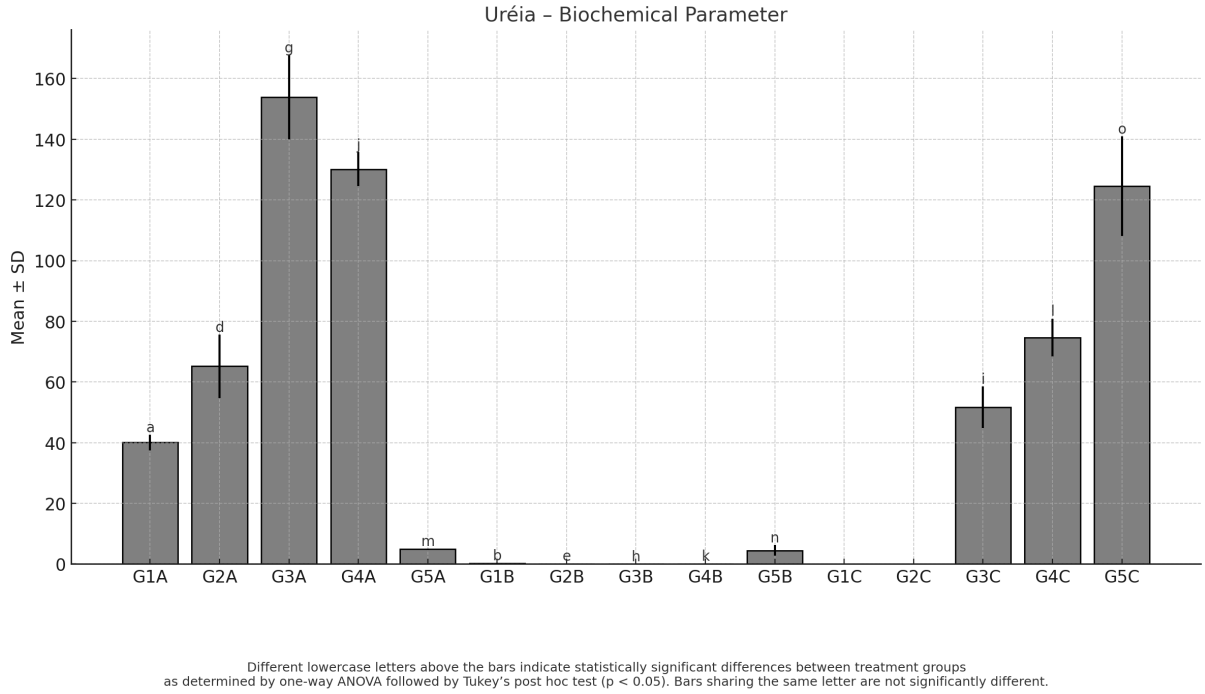


Fig. 5.: Urea levels (mg/dL)

Urea levels in treated Wistar rats across 7, 14, and 21-day protocols. Mean ± SD presented for each subgroup. Bars with different letters are significantly different (ANOVA + Tukey).

Electrolyte homeostasis was also evaluated. Serum potassium levels were significantly reduced in the docetaxel group at day 14 ($p < 0.05$), a disturbance that was partially restored with *Curcuma longa* treatment. Sodium levels, in contrast, remained relatively stable across groups, with mild variations that did not reach statistical significance except in the low-dose *Curcuma longa* group at day 7 ($p < 0.05$) (Fig. 6-7).

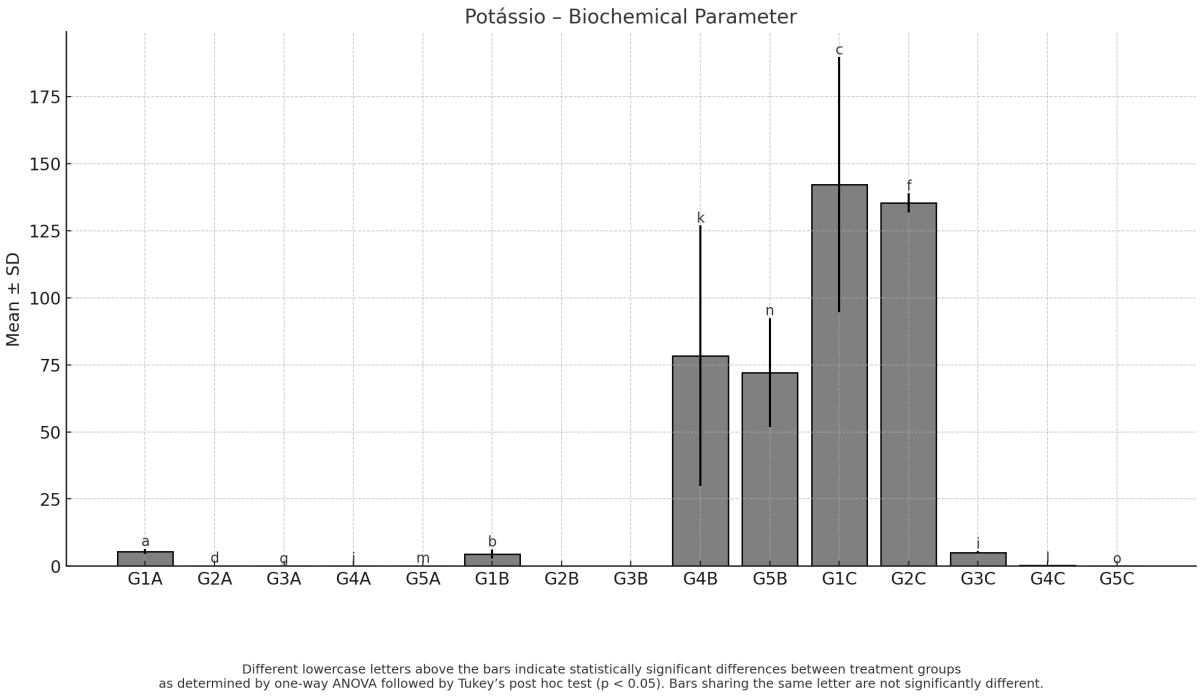


Fig. 6.: Potassium levels (mEq/L)

Serum potassium concentrations analyzed across all treatment durations. Values are reported as mean ± SD. Lowercase letters indicate statistically distinct groups ($p < 0.05$).

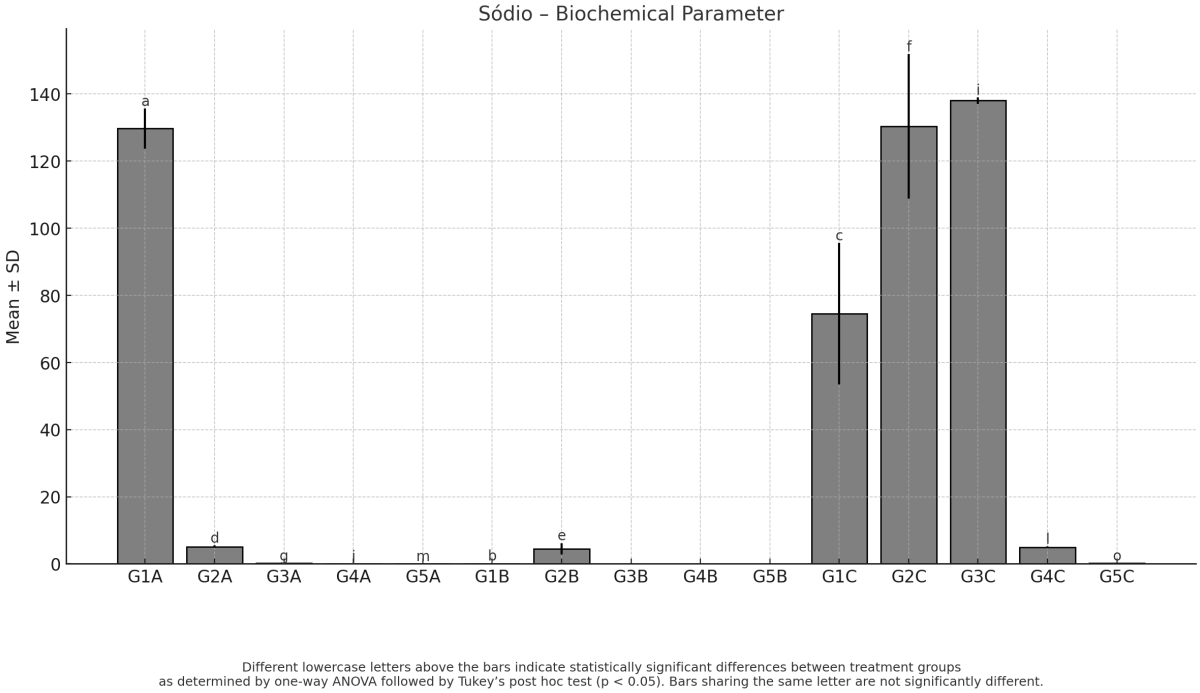


Fig. 7.: Sodium levels (mEq/L)

Mean ± SD of serum sodium levels in experimental animals. Statistical significance between groups indicated by distinct lowercase letters based on Tukey's post hoc analysis.

Regarding hepatic enzymes, AST (TGO) and ALT (TGP) levels were markedly elevated following docetaxel exposure, particularly at day 14. This hepatocellular injury was significantly ameliorated by *Curcuma longa* in a dose-dependent fashion. At the highest dose and longest duration, AST and ALT levels were reduced to near-control values ($p < 0.05$), highlighting the anti-inflammatory and hepatoprotective effects of curcuminoids (Fig. 8-9).

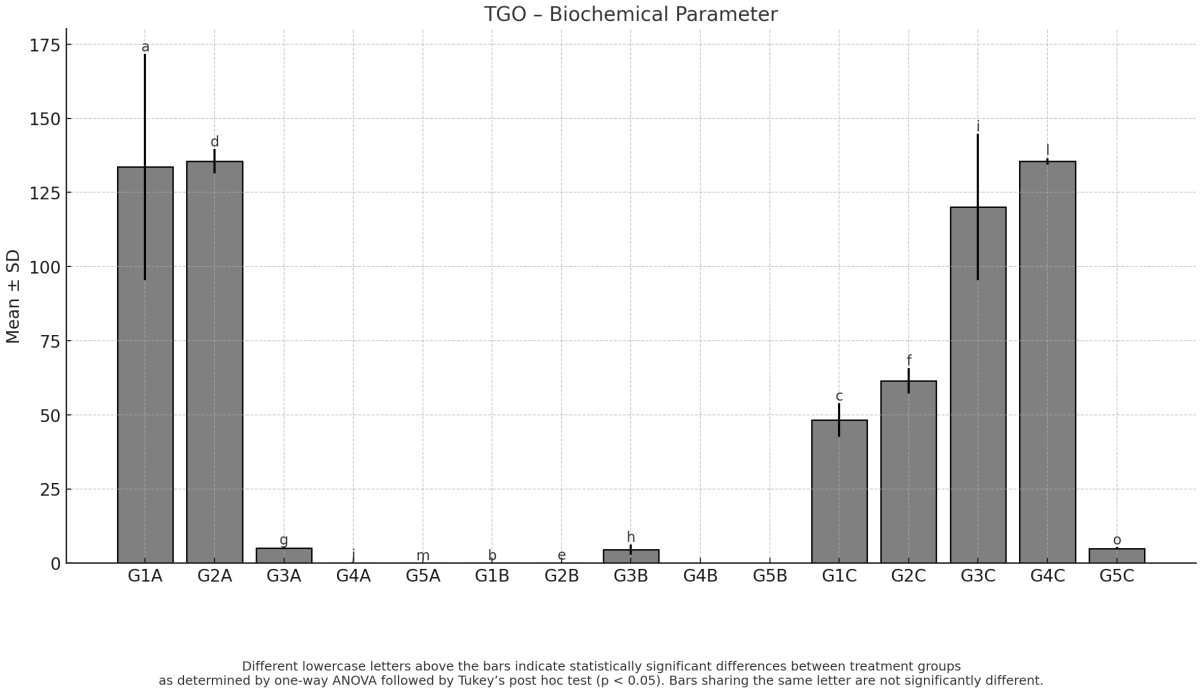


Fig. 8.: Aspartate Aminotransferase (AST/TGO, U/L)

Plasma AST activity in response to docetaxel and/or Curcuma longa. Bars represent mean ± SD. Significant differences ($p < 0.05$) indicated by distinct lowercase letters.

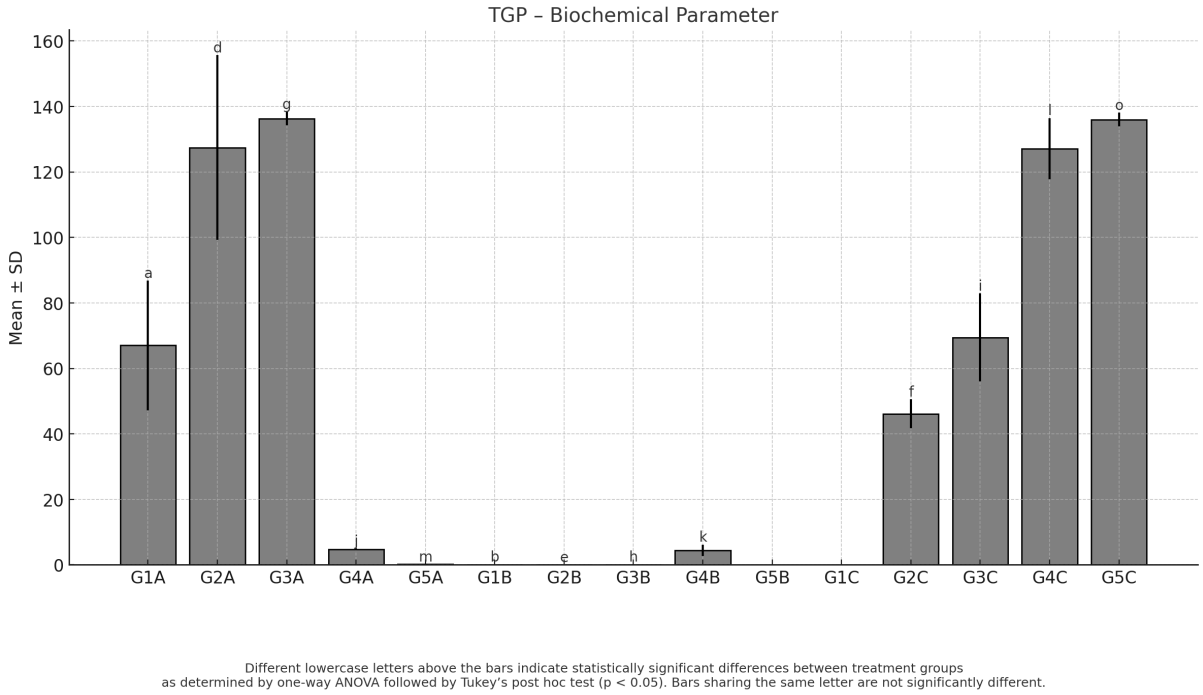


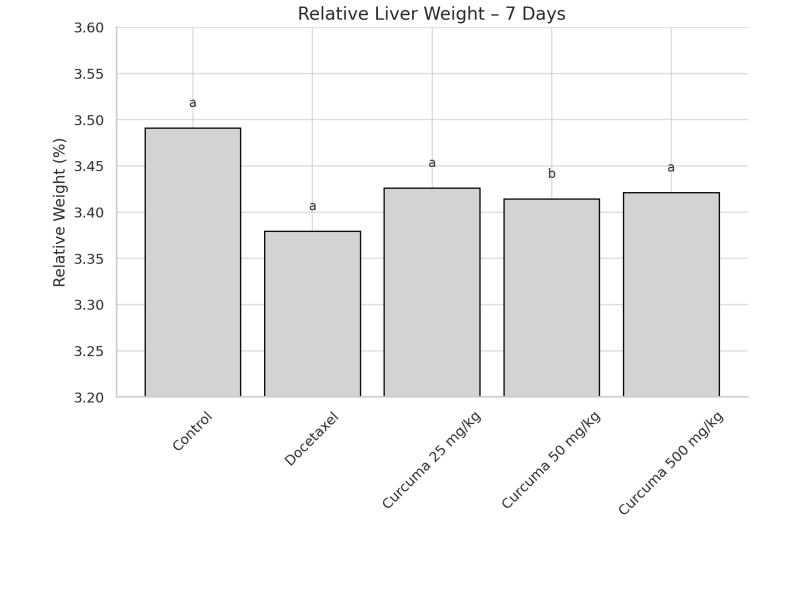
Fig. 9.: Alanine Aminotransferase (ALT/TGP, U/L)

ALT activity among experimental groups exposed to chemotherapy and phytotherapy. Results are shown as mean ± SD. Statistical differences marked by different letters above the bars.

Collectively, these findings reinforce the systemic toxicity induced by docetaxel and underscore the therapeutic promise of *Curcuma longa* as a biochemical stabilizer capable of preserving hepatic and renal integrity under chemotherapeutic stress.

Docetaxel administration consistently altered the relative weight of several organs compared to control, indicative of systemic toxicity. Notably, hepatic hypertrophy was observed from day 7 and persisted through day 21, with significant increases in liver weight seen particularly on day 14 and 21 ($p < 0.05$ vs. control). Co-treatment with *Curcuma longa*,

especially at higher doses (50 and 500 mg/kg), appeared to mitigate this effect, restoring hepatic weight closer to baseline (Fig. 10).



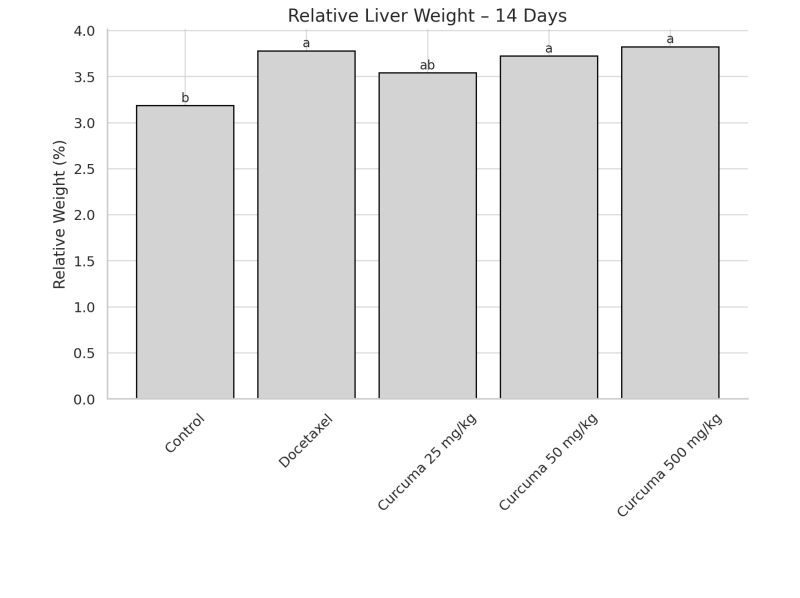
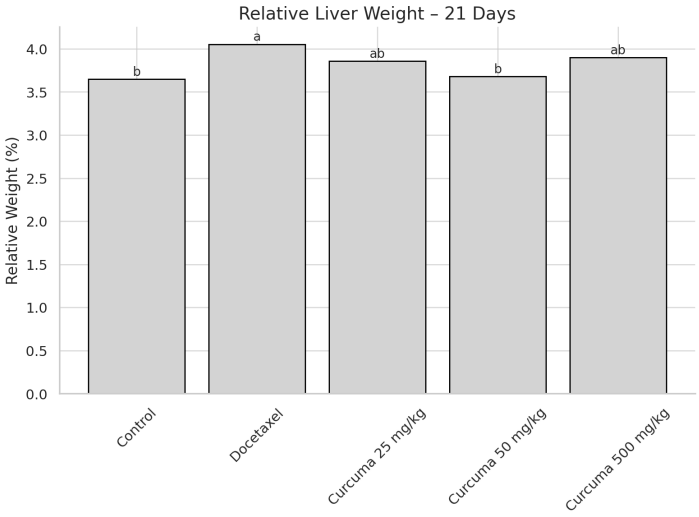


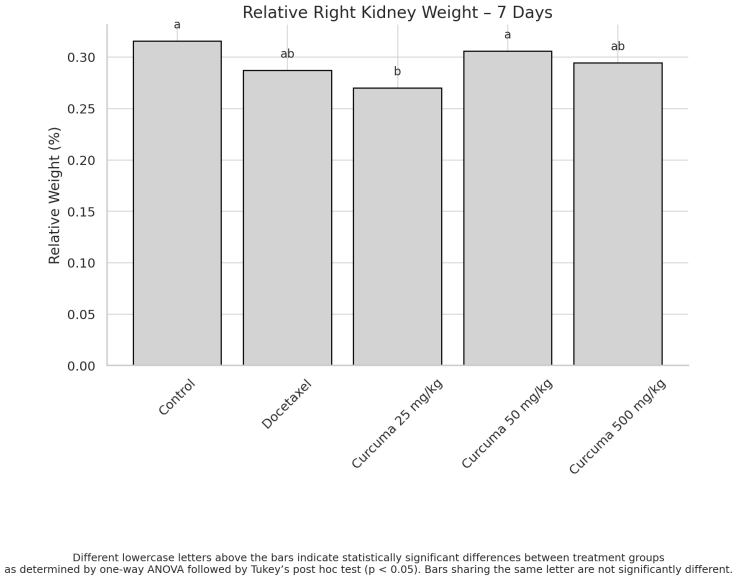
Fig. 10.: Relative liver weight. A) Relative liver weight in Wistar rats after 7 days of treatment. Bar heights represent the mean relative liver weight (%) for each group: Control, Docetaxel, and C*urcuma longa* at 25, 50, and 500 mg/kg. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Different lowercase letters above the bars indicate statistically significant differences between treatment groups. Bars sharing the same letter are not significantly different. **B)** Relative liver weight in Wistar rats after 14 days of treatment. Bar heights represent the mean



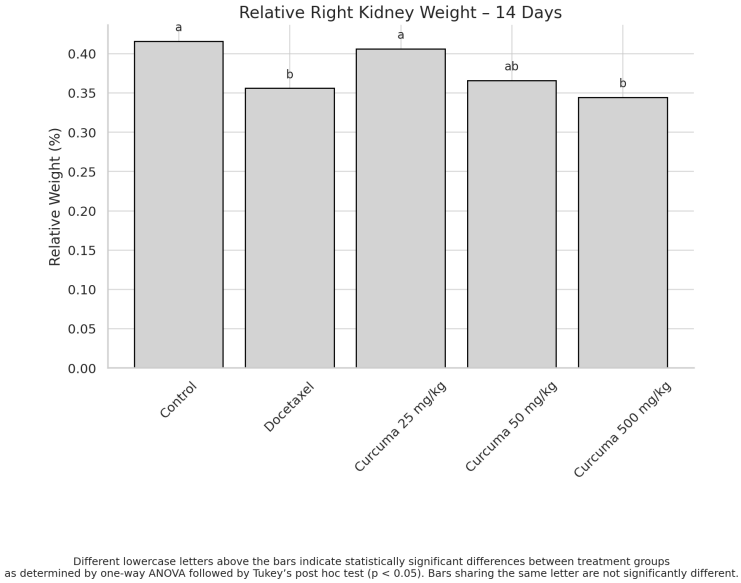
Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

relative liver weight (%) for each group: Control, Docetaxel, and C*urcuma longa* at 25, 50, and 500 mg/kg. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Different lowercase letters above the bars indicate statistically significant differences between treatment groups. **C)** Relative liver weight in Wistar rats after 21 days of treatment. Docetaxel significantly increased liver weight compared to control, while co-administration with *Curcuma longa* (50 and 500 mg/kg) mitigated this effect. Different lowercase letters indicate significant differences ($p < 0.05$, ANOVA/Tukey).

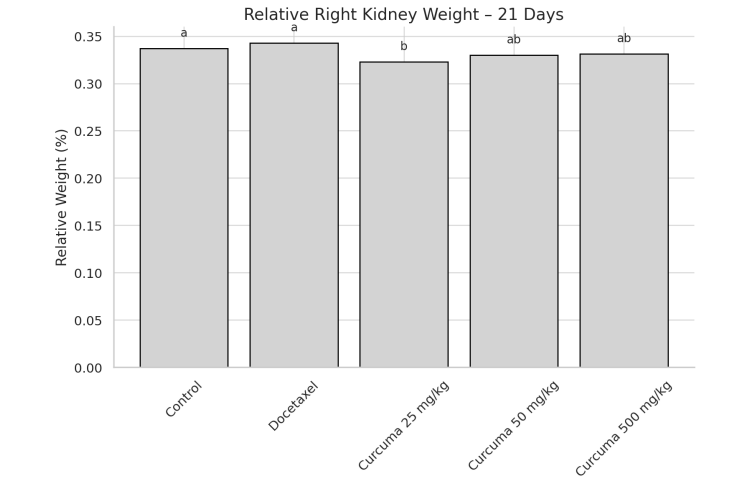
In the renal compartments, docetaxel reduced both right and left kidney weights by day 14, a finding partially reversed by Curcuma 25 mg/kg. By day 21, kidney weights remained lower in the Curcuma 25 mg/kg group compared to docetaxel, though the differences did not reach the same magnitude as in earlier phases (Fig, 11-12).



Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

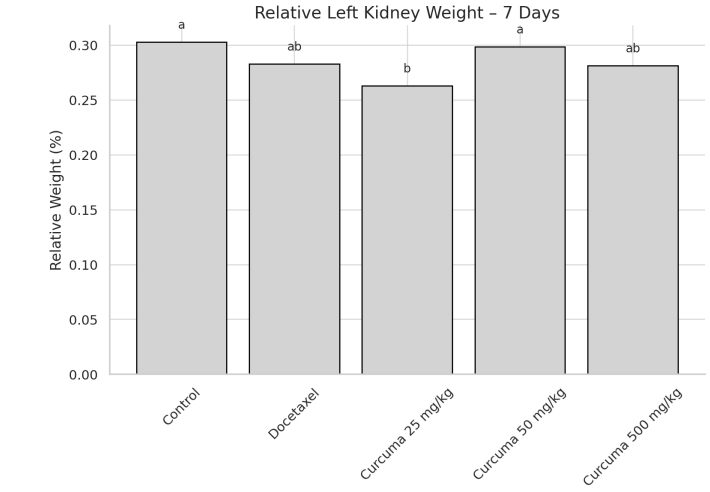


Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

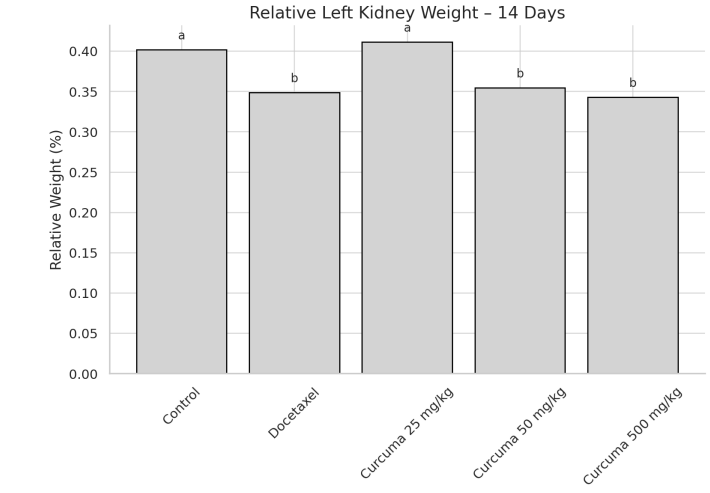


Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

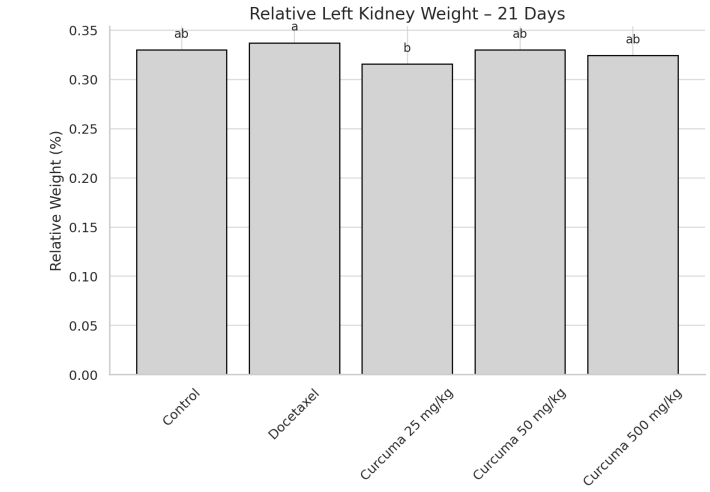
Fig. 11. Relative right kidney weight. **A)** Relative right kidney weight in Wistar rats after 7 days of treatment. Bar heights represent the mean relative right kidney weight (%). Statistical differences were determined by one-way ANOVA and Tukey's test. Groups not sharing the same letter differ significantly ($p < 0.05$). **B)** Relative right kidney weight in Wistar rats after 14 days of treatment. Curcuma 25 mg/kg showed comparable values to control. Docetaxel and Curcuma 500 mg/kg significantly reduced right kidney weight ($p < 0.05$). **C) R**elative right kidney weight in Wistar rats after 21 days of treatment. Curcuma 25 mg/kg significantly reduced kidney weight compared to control and docetaxel groups ($p < 0.05$).



Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.



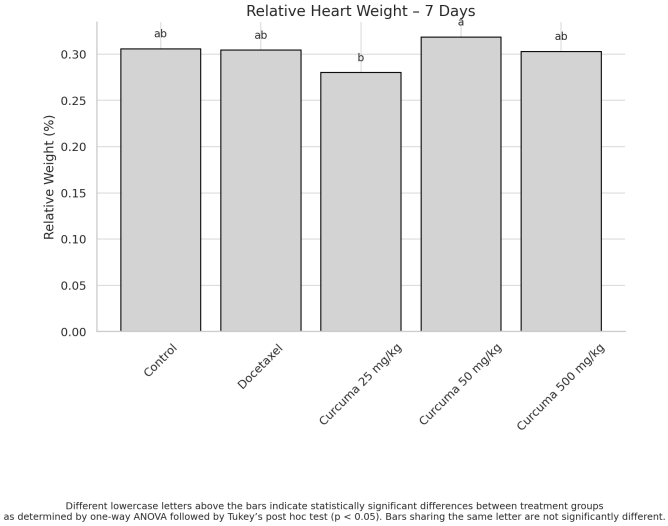
Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

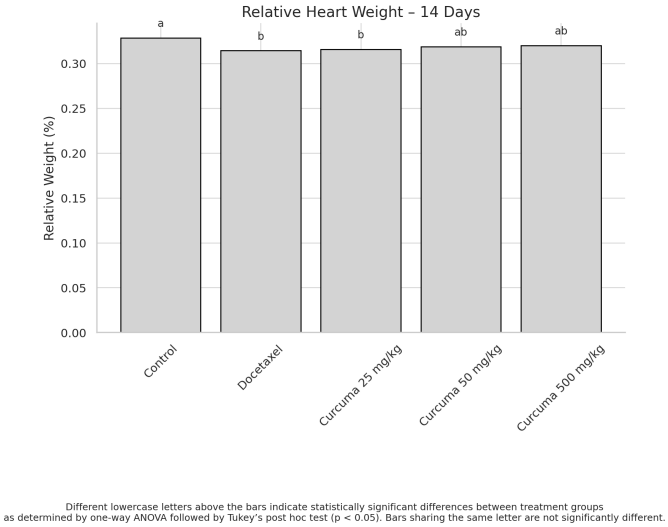


Different lowercase letters above the bars indicate statistically significant differences between treatment groups as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different.

Fig. 12.: Relative left kidney weight. A) Relative left kidney weight in Wistar rats after 7 days of treatment. Same statistical methodology and notation as described above. **B)** Relative left kidney weight in Wistar rats after 14 days of treatment. Curcuma 25 mg/kg preserved kidney weight compared to docetaxel and high-dose groups ($p < 0.05$). **C)** Relative left kidney weight in Wistar rats after 21 days of treatment. The Curcuma 25 mg/kg group exhibited significantly lower weight compared to control and docetaxel groups.

Cardiac mass was also impacted, with docetaxel decreasing relative heart weight significantly on day 14 ($p < 0.05$ vs. control), whereas Curcuma groups maintained near-normal values throughout all treatment durations (Fig. 13).





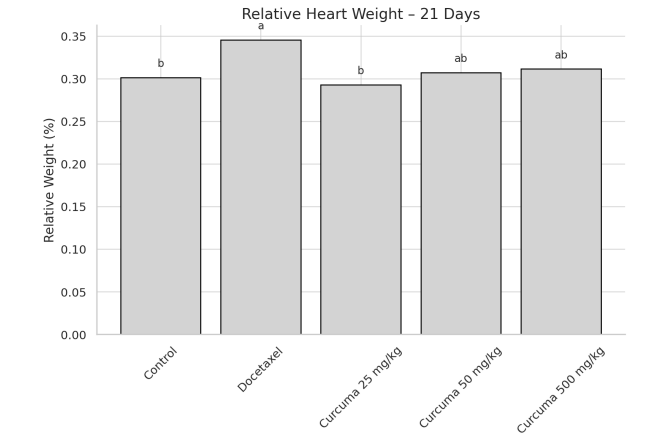
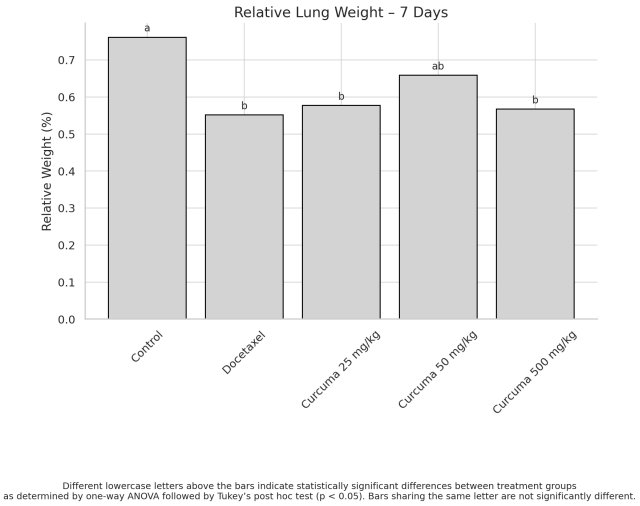
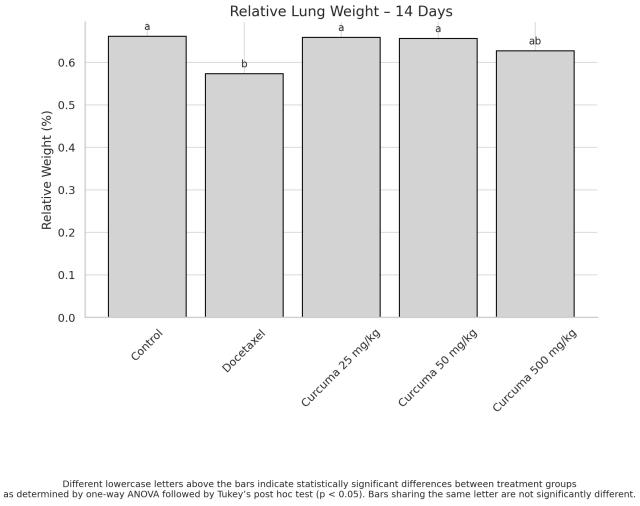


Fig. 13.: Relative heart weight. A) Relative heart weight in Wistar rats after 7 days of treatment. Heart weight was statistically higher in the Curcuma 50 mg/kg group compared to Curcuma 25 mg/kg. Other comparisons did not reach significance. **B)** Relative heart weight in Wistar rats after 14 days of treatment. All Curcuma groups showed heart weights comparable to control, while docetaxel significantly reduced heart mass ($p < 0.05$). **C)** Relative heart weight in Wistar rats after 21 days of treatment. Docetaxel significantly increased heart weight. Control and Curcuma groups remained within a comparable range.

For the lungs, docetaxel caused a marked increase in relative weight, particularly on day 21 ($p < 0.01$), likely reflecting pulmonary toxicity or edema. *Curcuma longa*, across all doses, demonstrated a trend toward normalization, albeit without complete reversal of the docetaxel-induced increase (Fig. 14).





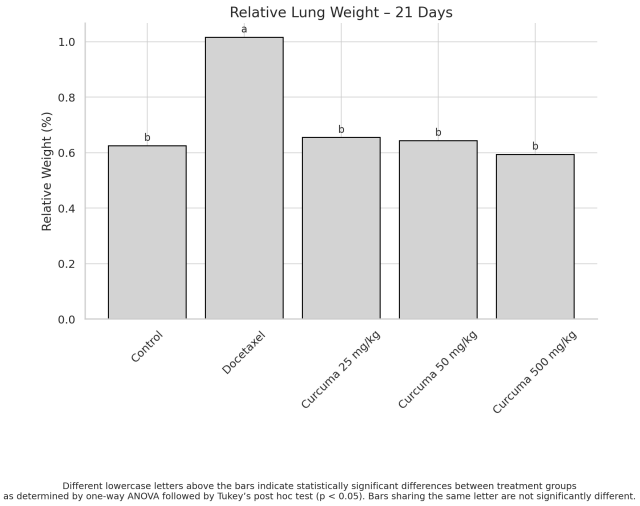


Fig. 14.: A) Relative lung weight in Wistar rats after 7 days of treatment. Docetaxel significantly reduced lung weight compared to control. *Curcuma longa* showed partial recovery, but did not fully restore lung weight to baseline ($p < 0.05$).

B) Relative lung weight in Wistar rats after 14 days of treatment. Docetaxel significantly reduced lung weight compared to control. Curcuma groups partially restored the values ($p < 0.05$).

C) Relative lung weight in Wistar rats after 21 days of treatment. Docetaxel markedly increased lung weight. Curcuma-treated groups showed lower values ($p < 0.05$).

Finally, the intestinal weight revealed a unique pattern. While early alterations were minimal, a dramatic increase in intestinal mass was noted on day 14 in the group treated with Curcuma longa 50 mg/kg ($p < 0.01$ vs. all groups), which persisted in part at 21 days. Although a standardized method for sample sectioning was not employed, the findings may indicate a proliferative or trophic effect of *Curcuma longa* on intestinal tissues, particularly at intermediate doses. Importantly, no deaths, treatment discontinuations, or any signs of clinical toxicity related to *Curcuma longa* were observed in any of the experimental groups.

Discussion

Docetaxel, a widely used chemotherapeutic agent, is known for its potent antineoplastic activity against a range of solid tumors. However, its clinical utility is frequently hampered by dose-dependent systemic toxicities, notably hepatotoxicity and nephrotoxicity, which are major limiting factors in treatment adherence and overall patient outcomes[1]. In the present study, we demonstrated that docetaxel induced significant biochemical and morphophysiological disturbances in liver and kidney function, consistent with previous preclinical and clinical findings[6].

Importantly, our data show that *Curcuma longa,* particularly at intermediate and high doses, exerted a protective effect on key biochemical markers of hepatic (AST, ALT, total bilirubin) and renal (urea, creatinine) function. The time- and dose-dependent attenuation of these markers aligns with earlier evidence of curcumin's hepatoprotective properties, which are mediated through its ability to modulate oxidative stress pathways, inhibit lipid peroxidation, and suppress pro-inflammatory cytokines such as TNF-α and IL-6[2-5]. The reversal of elevated AST and ALT by *Curcuma longa* at 50 and 500 mg/kg corroborates its effect on stabilizing hepatocellular membranes and restoring hepatic enzymatic activity[3].

Elevations in total and direct bilirubin levels following docetaxel administration suggest an impairment in hepatobiliary excretion and potential cholestatic damage. The mitigation of bilirubin accumulation by curcumin may involve its influence on bile acid homeostasis and transporter regulation, as suggested in murine models of liver injury[4]. Curcumin has been shown to downregulate pro-apoptotic proteins and upregulate cytoprotective enzymes such as heme oxygenase-1 (HO-1), enhancing hepatocyte resilience against chemotoxic insults[7].

Hepatic Toxicity and *Curcuma longa*'s Hepatoprotective Effects

Docetaxel administration was associated with a marked elevation of serum liver enzymes—AST and ALT—as well as total and fractionated bilirubin levels, indicating significant hepatocellular injury and cholestatic dysfunction. These effects were most pronounced at day 14, suggesting a cumulative hepatic burden during the subacute phase of exposure. Similar hepatotoxic profiles have been reported with taxane-based regimens, which can induce mitochondrial dysfunction, endoplasmic reticulum stress, and pro-inflammatory cytokine release in hepatocytes[8].

The observed normalization of liver enzyme levels and bilirubin concentrations following co-treatment with *Curcuma longa*, particularly at the 50 and 500 mg/kg doses, underscores its potential hepatoprotective action. These effects are consistent with previous reports demonstrating that curcumin, the principal bioactive constituent of *Curcuma longa*, exerts robust hepatoprotective properties through multiple pathways. Mechanistically, curcumin has been shown to attenuate hepatic oxidative stress by scavenging reactive oxygen species (ROS) and enhancing endogenous antioxidant defenses via activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway[9–10]. Nrf2 translocation into the nucleus leads to upregulation of heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), and glutamate-cysteine ligase, which collectively restore redox balance and protect hepatocytes from apoptosis and necrosis[11].

Furthermore, curcumin downregulates nuclear factor-kappa B (NF-κB), a transcription factor implicated in inflammation-mediated liver damage. By inhibiting IκB kinase (IKK), curcumin prevents NF-κB nuclear translocation and transcription of pro-inflammatory mediators such as TNF-α, IL-1β, and IL-6[12]. These anti-inflammatory effects reduce leukocyte infiltration and cytokine-induced hepatocellular apoptosis, mitigating liver injury under toxic insults.

Of note, curcumin also interferes with hepatic cytochrome P450 (CYP) enzymes, particularly CYP3A4 and CYP2E1, which are involved in the bioactivation of toxic intermediates during chemotherapeutic metabolism[13]. This enzymatic modulation may partially explain the reduction in docetaxel-induced hepatotoxicity in animals receiving *Curcuma longa* supplementation.

The dose-dependent nature of the hepatoprotective effect observed in this study aligns with data from other experimental models of drug-induced liver injury, including those induced by acetaminophen, isoniazid, and cisplatin[14]. In these studies, curcumin administration significantly attenuated transaminase elevation, lipid peroxidation, and histopathologic damage.

In the present model, the 500 mg/kg dose appeared most effective in restoring biochemical markers to near-baseline values by day 21. However, the 50 mg/kg dose also produced meaningful reductions in hepatotoxic markers, suggesting that intermediate dosing may offer an optimal balance between efficacy and safety.

Taken together, these findings reinforce the hepatoprotective profile of *Curcuma longa* and its potential utility as an adjuvant agent in chemotherapy protocols. Future investigations incorporating liver histopathology and molecular profiling will be essential to confirm the protective mechanisms and identify biomarkers predictive of response.

Renal Function Markers and Nephroprotection

Renal function parameters also showed marked alterations under docetaxel exposure. Serum creatinine and urea levels were elevated, consistent with glomerular and tubular dysfunction observed in taxane-induced nephrotoxicity[6]. Co-treatment with *Curcuma longa* significantly normalized these parameters by day 21, particularly at 500 mg/kg, indicating nephroprotective activity. This effect is likely mediated by curcumin's antioxidative modulation of Nrf2–ARE signaling, reduction of renal inflammation, and maintenance of mitochondrial function, as described in models of cisplatin- and gentamicin-induced renal injury[3,4].

Renal dysfunction is a well-established complication of taxane-based chemotherapy, with docetaxel exhibiting nephrotoxic potential primarily through mechanisms involving oxidative stress, mitochondrial dysfunction, and inflammatory injury to the renal tubular epithelium[15]. In the present study, the docetaxel-only groups demonstrated significant and sustained elevations in serum creatinine and urea levels at all evaluated timepoints (7, 14, and 21 days), indicative of impaired glomerular filtration and possible subclinical nephron damage. These biochemical alterations are in agreement with previously described patterns of taxane-induced nephrotoxicity in preclinical models[16].

Of particular note, co-administration of *Curcuma longa* extract produced a dose-dependent nephroprotective effect, with the 500 mg/kg group demonstrating near-complete normalization of creatinine and urea values by day 21. This finding suggests that *Curcuma longa* may enhance renal clearance and mitigate chemotherapy-induced renal injury over time.

The nephroprotective mechanisms of curcumin have been extensively documented in various models of renal injury. One of its principal actions involves attenuation of oxidative stress via activation of the Nrf2–ARE (antioxidant response element) pathway, which upregulates endogenous antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase[17–18]. By reducing reactive oxygen species (ROS) accumulation within renal tubular cells, curcumin preserves mitochondrial function and prevents lipid peroxidation —a key mediator of nephrotoxicity[19].

Additionally, curcumin has been shown to downregulate the expression of pro-apoptotic mediators (e.g., Bax, caspase-3) while enhancing anti-apoptotic pathways (e.g., Bcl-2), thereby preserving tubular epithelial cell integrity[20]. These anti-apoptotic effects have been correlated with improved histopathological scores in cisplatin-induced nephrotoxicity models, suggesting translational relevance across chemotherapeutic classes[21].

Moreover, curcumin's ability to modulate inflammatory cytokines—such as TNF-α, IL-1β, and IL-6—contributes to reduced leukocyte infiltration and attenuation of glomerular inflammation[22]. Experimental studies in models of ischemia-reperfusion and diabetic nephropathy have shown that curcumin ameliorates renal injury by decreasing macrophage accumulation and improving microvascular perfusion[23].

In this study, the 500 mg/kg dose was the most effective in reversing biochemical markers of renal dysfunction, suggesting a threshold effect in achieving nephroprotection. However, the 50 mg/kg dose also showed partial efficacy, particularly at later timepoints, which may offer a clinically safer and equally effective window for translation.

Comparative studies with other nephrotoxic agents, such as cisplatin, reinforce the findings observed here. Al-Moundhri et al. demonstrated that post-treatment with curcumin significantly reduced cisplatin-induced elevations in serum creatinine and urea, preserved glomerular structure, and reduced tubular necrosis in rats[24]. Similarly, Jeong et al. reported

that curcumin protected against cyclophosphamide-induced renal injury by restoring oxidative balance and preventing apoptosis in renal tissues[25].

Taken together, the data support the conclusion that *Curcuma longa*—particularly at higher doses—exerts a strong nephroprotective effect in the context of docetaxel-induced toxicity. These effects appear to be mediated by a combination of antioxidant, anti-inflammatory, and anti-apoptotic actions, with potential relevance for mitigating renal adverse effects in clinical oncology.

Electrolyte Balance and Systemic Homeostasis

Electrolyte disturbances, especially hypokalemia observed on day 14, are consistent with chemotherapy-related tubular injury. Although mild and transient, the partial reversal by *Curcuma longa* reflects a stabilizing effect on renal tubular integrity, which may preserve ionic transport systems. Interestingly, sodium levels remained stable across most groups, indicating a selective tubular impact.

Chemotherapy-induced disturbances in electrolyte homeostasis are clinically relevant manifestations of systemic toxicity, often reflecting underlying renal tubular dysfunction, gastrointestinal losses, or neurohormonal dysregulation. In the present study, docetaxel monotherapy induced a significant reduction in serum potassium levels at day 14 ($p < 0.05$ vs. control), consistent with hypokalemia. Although sodium levels remained relatively stable across treatment groups, mild hyponatremia was noted in the *Curcuma longa* 25 mg/kg group at day 7, but without sustained alterations thereafter.

Hypokalemia in the context of taxane-based chemotherapy may result from several mechanisms: increased urinary potassium excretion due to impaired tubular reabsorption, gastrointestinal potassium loss secondary to mucosal toxicity and diarrhea, or intracellular potassium shifts mediated by altered insulin or catecholamine activity[26–27]. In our model, the mid-phase (day 14) was particularly susceptible to hypokalemia, likely due to the cumulative nephrotoxic and enterotoxic effects of docetaxel. Notably, co-administration of Curcuma longa, especially at 50 and 500 mg/kg, attenuated this potassium loss and stabilized serum levels by day 21, indicating a potential role in restoring ionic equilibrium.

Curcumin’s contribution to electrolyte homeostasis may be linked to its protective effects on renal tubular integrity and its ability to suppress oxidative stress and

proinflammatory cytokine production within the nephron microenvironment[28]. Oxidative damage to renal tubular cells, particularly in the thick ascending limb of Henle's loop and distal tubules, is a known contributor to impaired sodium and potassium handling during systemic toxic insults[29]. By mitigating mitochondrial dysfunction and reducing ROS accumulation, curcumin preserves tubular transporter function and promotes ion reabsorption efficiency.

Additionally, curcumin has been shown to modulate key regulators of ion balance, including Na^+/K^+-ATPase and aquaporin channels, in experimental nephrotoxicity models[30]. It also exhibits indirect effects via its anti-inflammatory action, suppressing TNF-α and IL-1β, which are known to impair tubular handling of electrolytes and induce tubular apoptosis[28–29].

Clinically, maintaining electrolyte balance is paramount during chemotherapy to prevent arrhythmias, neuromuscular dysfunction, and acute kidney injury, particularly in regimens involving nephrotoxic agents such as cisplatin, cyclophosphamide, and taxanes[31]. The capacity of *Curcuma longa* to preserve potassium levels and avoid significant sodium fluctuations may enhance patient safety and reduce the need for corrective interventions such as electrolyte repletion or hospitalization.

These findings reinforce the relevance of phytotherapeutic adjuvants in systemic toxicity management, not only for organ-specific protection but also for preserving essential physiological parameters such as electrolyte balance.

Relative Organ Weight Changes: Functional Interpretation

Changes in relative organ weights are sensitive biomarkers of systemic toxicity and physiological stress induced by chemotherapeutic agents. In the present experimental model, docetaxel treatment led to distinct alterations in the relative mass of multiple organs, indicating multisystem involvement. Co-administration of *Curcuma longa* modulated several of these effects, demonstrating potential protective and regenerative actions in a dose- and time-dependent manner.

The organ weight analysis revealed systemic effects of docetaxel on several tissues. Liver hypertrophy, likely secondary to inflammation, congestion, and hepatocyte injury, was reversed with *Curcuma longa*, supporting its role in limiting hepatomegaly and tissue remodeling[4]. Conversely, renal hypotrophy, observed at day 14, may represent structural

atrophy due to cytotoxic damage. *Curcuma longa* demonstrated only partial recovery, particularly at low doses, suggesting a more complex dose-response relationship requiring further exploration.

Cardiac and pulmonary weights were also affected. The reduction in heart weight under docetaxel treatment could reflect cardiomyocyte atrophy or loss, as observed in preclinical cardiotoxicity models with taxanes[6]. *Curcuma longa* preserved cardiac mass, aligning with previous reports of its cardioprotective effects via antioxidant and antiapoptotic mechanisms[2]. Lung weight increases, likely indicative of pulmonary edema or inflammation, were attenuated by *Curcuma longa* but not fully normalized, suggesting a partial protective effect.

An unexpected finding was the increase in intestinal weight, notably in the group treated with 50 mg/kg *Curcuma longa* on day 14. This may reflect mucosal regeneration or enhanced epithelial turnover stimulated by curcumin, which has been previously associated with intestinal trophism and anti-ulcerogenic properties[5]. Further histopathological analysis would be necessary to clarify whether this finding denotes a beneficial or adverse response.

Liver

Hepatomegaly observed in docetaxel-treated groups, particularly at days 14 and 21, may reflect acute inflammatory response and/or hepatocellular regeneration following cytotoxic insult. Previous studies report hepatic hypertrophy linked to leukocyte infiltration, hepatocyte swelling, and proliferative activity in response to oxidative stress caused by chemotherapeutic agents[32].

In groups co-treated with *Curcuma longa*—notably at 50 and 500 mg/kg doses—this hypertrophy was significantly attenuated. This suggests that curcuminoids reduce hepatic oxidative damage and inflammation, while promoting redox homeostasis and controlled hepatocyte renewal without inducing reactive hyperplasia[33,34].

Kidneys

The observed reduction in kidney weight in docetaxel-only groups, especially at day 14, may be indicative of renal hypoperfusion, tubular necrosis, or apoptotic degeneration, which are well-documented outcomes of chemotherapy-induced nephrotoxicity[35]. In rats

receiving *Curcuma longa*—particularly at 25 mg/kg—partial preservation of kidney mass was noted, supporting a renoprotective effect. This may be mediated through activation of endogenous antioxidant defenses (e.g., Nrf2–ARE pathway) and inhibition of tubulointerstitial fibrosis[36].

Heart

Docetaxel-treated animals showed a significant reduction in cardiac mass at day 14, which may be attributed to mitochondrial oxidative stress and cardiomyocyte atrophy—a recognized complication of taxane therapy[37]. Notably, *Curcuma longa*-treated groups maintained near-normal heart weight throughout, suggesting cardioprotective properties likely driven by anti-apoptotic and mitochondrial stabilizing effects of curcumin[38].

Lungs

An increase in lung weight, particularly in the docetaxel-only group at day 21, suggests pulmonary inflammation or edema. Taxane-induced pulmonary toxicity has been associated with interstitial inflammation, capillary leakage, and oxidative damage[39]. While *Curcuma longa* did not fully reverse these changes, it demonstrated a trend toward normalization of lung mass, supporting its potential anti-inflammatory effects at the alveolar–capillary interface[40].

Small Intestine

The most pronounced change in intestinal weight was observed in the group treated with *Curcuma longa* 50 mg/kg at day 14, showing a statistically significant increase. This may reflect a trophic effect of curcumin on intestinal mucosa, possibly through stimulation of crypt cell proliferation and enhancement of epithelial integrity. Curcumin has been shown to modulate intestinal growth factors such as EGF and TGF-β, supporting epithelial regeneration and mucosal barrier function[41,42]. Although this finding could represent a favorable adaptation, further histological investigation is warranted to rule out dysplastic or hyperplastic changes under prolonged exposure.

Dose- and Time-Dependent Protective Profile of *Curcuma longa*

The present findings demonstrate a clear dose- and time-dependent profile for *Curcuma longa*'s protective effects against docetaxel-induced systemic toxicity. Among the three tested doses (25, 50, and 500 mg/kg/day), the intermediate dose of 50 mg/kg emerged as the most consistently effective in mitigating functional alterations in hepatic and renal biomarkers, preserving organ weights, and supporting systemic homeostasis—particularly at the later timepoint (day 21).

This temporal dynamic aligns with the known pharmacodynamics of curcumin, which requires sustained exposure to accumulate intracellularly and exert long-lasting anti-inflammatory and antioxidative effects through pathways such as NF-κB inhibition, Nrf2 activation, and suppression of cytokine cascades (e.g., IL-6, TNF-α)[3,7,33,36].

Interestingly, while the highest dose (500 mg/kg) was effective in reversing elevations in bilirubin, urea, and creatinine, it also led to pronounced splenic and intestinal hypertrophy by day 21. These findings suggest a dual-edged response: enhanced detoxification and cytoprotection on one hand, and potential overstimulation of immune or regenerative pathways on the other. High-dose curcumin has previously been associated with immune cell recruitment, macrophage proliferation, and splenic architectural remodeling, which—if sustained—may predispose to organomegaly, fibrosis, or functional dysregulation[19,24,34].

This raises the possibility of a therapeutic efficacy plateau beyond which further dose escalation may not confer additional benefit and could instead trigger maladaptive responses. Such a biphasic pattern underscores the importance of optimizing dosing regimens to maximize therapeutic gain while avoiding overactivation of compensatory physiological mechanisms.

Moreover, the timing of protective effects is relevant. At earlier stages (days 7 and 14), modest biochemical improvements were observed, but the full extent of protection—particularly normalization of AST, ALT, creatinine, and bilirubin levels—was only evident by day 21. This lag suggests a cumulative protective action, possibly through gene expression modulation and restoration of metabolic homeostasis, consistent with previous in vivo models of phytochemical co-treatment during chemotherapy[2,4,41].

In summary, these results support the hypothesis that *Curcuma longa* exhibits a time-sensitive and dose-limited protective profile, with 50 mg/kg representing a potentially optimal

therapeutic window in this preclinical setting. Future studies should further explore the long-term safety of high-dose administration and seek to determine the molecular thresholds beyond which curcumin's beneficial effects may diminish or reverse.

Mechanistic Insights: Curcumin and Molecular Pathways

The protective effects of *Curcuma longa* observed in this study—particularly the normalization of hepatic enzymes, improvement in renal function, stabilization of electrolyte balance, and modulation of organ weights—are consistent with curcumin's multifaceted interaction with several intracellular signaling pathways. Among these, the Nrf2, NF-κB, MAPK, and PI3K/Akt pathways stand out as key molecular mediators of the cytoprotective and anti-inflammatory actions of curcumin.

The Nrf2 (nuclear factor erythroid 2-related factor 2) pathway plays a pivotal role in the cellular defense against oxidative stress. Upon activation, Nrf2 translocates to the nucleus and upregulates the transcription of antioxidant response elements (ARE), including heme oxygenase-1 (HO-1), NAD(P)H quinone dehydrogenase 1 (NQO1), and glutathione S-transferases[7,38,39]. In the current model, the time- and dose-dependent reductions in AST, ALT, bilirubins, creatinine, and urea suggest enhanced antioxidant capacity and restoration of redox homeostasis, likely mediated by Nrf2-driven transcriptional activity. This hypothesis is reinforced by the delayed yet robust protective effects observed on day 21, consistent with the kinetics of Nrf2-dependent gene expression.

Conversely, curcumin has been widely recognized as a potent inhibitor of NF-κB, a transcription factor central to the propagation of inflammatory responses. Under chemotherapeutic stress—such as that induced by docetaxel—NF-κB is activated by cytokines like TNF-α and IL-1β, leading to increased expression of pro-inflammatory genes, including COX-2, iNOS, and various interleukins[3,33,40]. Curcumin attenuates this cascade by inhibiting IκB kinase (IKK), thereby preventing NF-κB nuclear translocation and downstream inflammatory amplification[3,5]. The reductions in hepatic and renal injury markers in the co-treated groups support this anti-inflammatory mechanism.

The MAPK (mitogen-activated protein kinase) signaling pathway—including ERK, JNK, and p38 kinases—has also been implicated in drug-induced organ injury via regulation of apoptosis, inflammation, and cell proliferation. Curcumin modulates MAPK signaling in a

context-dependent manner: it can inhibit p38 and JNK in inflammatory settings while promoting ERK activation in regenerative and anti-apoptotic contexts[41]. These dual effects may account for the preserved organ weights and functional parameters seen in liver and kidney tissues, particularly at intermediate doses.

Moreover, the PI3K/Akt pathway, which is critically involved in cell survival, metabolism, and autophagy, is another target of curcumin modulation. In chemotherapy models, inhibition of PI3K/Akt has been associated with enhanced apoptosis and tissue damage. Curcumin has demonstrated the ability to restore PI3K/Akt signaling balance, enhancing cellular resilience to cytotoxic insults while preventing pathological proliferation[3,7,42]. In this context, the partial recovery of intestinal and splenic mass, alongside biochemical normalization, suggests Akt-mediated cell survival and regenerative cues.

At the cytokine and protein expression level, curcumin has been shown to downregulate TNF-α, IL-6, and IL-1β, while simultaneously upregulating anti-apoptotic proteins such as Bcl-2 and suppressing pro-apoptotic markers like Bax and caspase-3[3,6,43]. These effects are likely instrumental in the mitigation of hepatocyte and tubular epithelial cell death observed in our study, particularly under high-dose *Curcuma longa* supplementation.

Taken together, these molecular insights provide a plausible mechanistic framework for the systemic cytoprotection observed in our experimental model. By orchestrating a multi-target regulatory network—spanning oxidative stress defense, inflammatory suppression, and apoptosis modulation—curcumin exerts comprehensive organoprotective effects that align with the observed attenuation of docetaxel-induced hepatotoxicity, nephrotoxicity, and systemic imbalance.

Translational Relevance and Future Directions

The findings of this study offer meaningful translational potential for improving the management of chemotherapy-associated toxicity in oncology patients. Docetaxel, while highly effective against various solid tumors, is often limited by its systemic side effects—particularly hepatotoxicity and nephrotoxicity—that compromise therapeutic intensity, continuity, and patient quality of life[1,6]. The demonstration that *Curcuma longa* extract, particularly at intermediate and high doses, attenuates these toxic effects without overt

adverse outcomes supports its candidacy as an adjuvant cytoprotective agent during chemotherapeutic regimens.

From a clinical perspective, *Curcuma longa* could serve to preserve hepatic and renal function, reduce the incidence of treatment delays or dose reductions, and thereby maintain the planned chemotherapy dose-intensity—a well-established determinant of clinical efficacy in oncologic protocols[40]. This is especially relevant in settings where cumulative toxicity often dictates therapeutic limitations, such as in breast, prostate, and lung cancers treated with taxanes.

The results also lay the foundation for the design of future clinical trials, ideally randomized, placebo-controlled, and dose-escalated, to evaluate the safety and efficacy of *Curcuma longa* as an adjunctive intervention. Primary endpoints could include biochemical markers of liver and kidney function (AST, ALT, bilirubin, creatinine, urea), while secondary endpoints may assess patient-reported outcomes, quality of life, and treatment adherence. Additionally, the identification and validation of predictive biomarkers—such as cytokine profiles (e.g., IL-6, TNF-α), oxidative stress markers (e.g., malondialdehyde, glutathione), and apoptosis-related proteins (e.g., Bcl-2, Bax, caspase-3)—could optimize patient selection and therapeutic monitoring.

To further elucidate the mechanisms underlying the observed organoprotection, histopathological and immunohistochemical studies are warranted in future investigations. Such analyses should assess structural integrity, inflammatory infiltration, fibrosis, and vascular remodeling in hepatic, renal, and intestinal tissues. The integration of molecular pathway analysis, including the evaluation of Nrf2, NF-κB, MAPKs, and PI3K/Akt signaling cascades, will be instrumental in clarifying the intracellular targets of *Curcuma longa* in the context of chemotherapeutic injury.

Finally, emerging biotechnological tools—such as transcriptomic profiling, proteomics, and metabolomic screening—can provide a systems-level understanding of *Curcuma longa*'s pleiotropic actions and help to identify synergistic interactions when combined with cytotoxic agents. Such insights may ultimately guide the personalization of supportive care in oncology, aligning phytotherapeutic strategies with individual toxicity profiles and treatment regimens.

In conclusion, this study provides preclinical evidence supporting the adjuvant use of *Curcuma longa* in reducing docetaxel-induced systemic toxicity, paving the way for translational applications in oncologic care. Ongoing and future investigations will be critical to validate these findings, optimize dosing strategies, and ensure the safe integration of phytochemicals into standard chemotherapy protocols.

Overall, our findings support the hypothesis that *Curcuma longa* mitigates docetaxel-induced systemic toxicity through multifactorial mechanisms, including antioxidative defense, anti-inflammatory modulation, cellular protection, and restoration of physiological function in hepatic and renal systems. The dose- and time-dependent patterns observed highlight the importance of identifying optimal therapeutic windows for phytotherapeutic intervention.

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Declaration of interest statement

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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3. CONCLUSÃO

1. O presente estudo fornece evidências pré-clínicas robustas de que a *Curcuma longa* exerce efeitos hematoprotetores dependentes do tempo e da dose em um modelo experimental de mielossupressão induzida por docetaxel em ratos. A administração adjuvante de *Curcuma longa*, especialmente em doses intermediárias (25–50 mg/kg), foi associada à preservação dos parâmetros eritroides, leucocitários e megacariocíticos, atenuando as citopenias características observadas com o uso isolado de docetaxel. Além disso, a estabilização observada na largura de distribuição dos eritrócitos (RDW) e a modulação da morfologia esplênica sugerem que a *Curcuma longa* pode exercer efeitos regulatórios mais amplos sobre a homeostase hematopoiética, possivelmente por meio de mecanismos antioxidantes, anti-inflamatórios e imunomoduladores.

A esplenomegalia pronunciada observada em doses elevadas e exposições prolongadas levanta considerações relevantes quanto aos limites de segurança e ao risco de hiperestimulação imunológica. Esses achados ressaltam a importância de definir a janela terapêutica ideal para intervenções fitoterápicas no contexto da quimioterapia.

Em conjunto, os resultados deste estudo apoiam o potencial da *Curcuma longa* como adjuvante de baixa toxicidade, capaz de aumentar a resiliência hematológica durante o tratamento citotóxico. As implicações translacionais desta pesquisa justificam investigações adicionais, incluindo estudos mecanísticos, análises histopatológicas de medula óssea e ensaios clínicos em cenários oncológicos, com o objetivo final de melhorar a tolerabilidade do tratamento e preservar a intensidade posológica na terapia do câncer;

2. O presente estudo demonstrou, de forma abrangente, que a *Curcuma longa* exerce efeitos citoprotetores significativos frente à toxicidade sistêmica induzida por docetaxel em modelo experimental com ratos Wistar. A administração adjuvante de *Curcuma longa*, especialmente nas doses de 50 e 500 mg/kg/dia, foi capaz de atenuar marcadamente as alterações bioquímicas relacionadas à função hepática (AST, ALT, bilirrubinas) e renal (ureia, creatinina), além de estabilizar o balanço eletrolítico e preservar a integridade funcional de órgãos-alvo, como fígado, rins, pulmões, coração e intestino.

Os efeitos protetores observados revelaram-se dependentes do tempo e da dose, com perfil mais consistente de proteção observado ao final do protocolo (21 dias), sugerindo ação

cumulativa por mecanismos moleculares complexos. Entre eles, destacam-se a ativação da via antioxidante Nrf2, a inibição da cascata inflamatória mediada por NF-κB, e a modulação de vias de sobrevivência celular como MAPK e PI3K/Akt.

A esplenomegalia e hipertrofia intestinal associadas ao uso prolongado de altas doses levantam hipóteses sobre possível hiperestimulação imunológica e requerem análise histológica adicional. No entanto, os achados reforçam o valor translacional da *Curcuma longa* como adjuvante fitoterápico seguro e promissor na oncologia, com potencial para ampliar a tolerabilidade da quimioterapia, reduzir efeitos adversos e preservar a funcionalidade orgânica durante o tratamento antineoplásico.

4 ANEXOS

ANEXO 1 - Normas da Revista Drug and Chemical Toxicology

Research Article

- Should be written with the following elements in the following order: title page; abstract; keywords; main text introduction, materials and methods, results, discussion; acknowledgments; declaration of interest statement; references; appendices (as appropriate); table(s) with caption(s) (on individual pages); figures; figure captions (as a list).
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ANEXO 2 - Certificado de aprovação do Comitê de Ética em Pesquisa envolvendo experimentação animal (CEPEA)

UNIVERSIDADE PARANAENSE - UNIPAR
Reconhecida pela Portaria - MEC Nº 1580, DE 09/11/93 - D.O.U. 10/11/93
Mantenedora: Universidade Paranaense - UNIPAR LTDA
Coordenadoria de Pós-Graduação - COPG

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO EXPERIMENTAÇÃO ANIMAL (CEPEEA)

CERTIFICADO

Certificamos que o projeto intitulado "ATIVIDADE PROTETORA E IMUNOMODULADORA DA CÚRCUMA LONGA EM ANIMAIS SADIOS EXPOSTOS A DOSES TERAPÊUTICAS DE DOCETAXEL", protocolo 40130/2023, sob a responsabilidade de LEONARDO GARCIA VELASQUEZ, - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata , subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº. 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), tendo sido aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Universidade Paranaense - UNIPAR em reunião realizada em 16/02/2023.

We hereby certify that the project "ATIVIDADE PROTETORA E IMUNOMODULADORA DA CÚRCUMA LONGA EM ANIMAIS SADIOS EXPOSTOS A DOSES TERAPÊUTICAS DE DOCETAXEL", protocol n.40130/2023, under the responsibility of LEONARDO GARCIA VELASQUEZ – involving production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (with the exception of Man), for scientific or teaching purposes – complies with Law n. 11,794, published on October 8, 2008, by Decree n. 6,899 of July 15, 2009, and with norms published by the Brazilian Council for the Control of Animal Experiments (CONCEA), and approved by the COMMITTEE FOR ETHICS IN THE USE OF ANIMALS (CEUA) of UNIPAR - Universidade Paranaense at the meeting held on 02/16/2023.

UMUARAMA - PR, 28/08/2023.

Salviano Tramontin Belettini
Presidente CEPEEA/UNIPAR

Registro Nº:40130

UNIVERSIDADE PARANAENSE - UNIPAR
Reconhecida pela Portaria - MEC Nº 1580, DE 09/11/93 - D.O.U. 10/11/93
Mantenedora: Universidade Paranaense - UNIPAR LTDA
Coordenadoria de Pós-Graduação - COPG

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO EXPERIMENTAÇÃO ANIMAL (CEPEEA)

Vigência do Projeto / Duration of the Project	Fevereiro a Dezembro de 2023
Espécie/Linhagem / Species/Pedigree	Rato heterogênico / Wistar
Nº de animais / N. of animals	105
Peso/Idade / Weight/Age	300 g / 8 semanas
Sexo / Sex	Não Definido / Undefined
Origem / Origin	

ANEXO 3 - Certificado de Análise da *Curcuma Longa*

SOVITA
ATIVOS PARA A VIDA

QUALIDADE ÚNICA FDA & BPDF ANVISA GRUPO FAGRON

CERTIFICADO DE ANÁLISE

Insumo:	CUREIT -Ext. Curcuma Longa Biodisponivel		
Lote Interno:	24D11-B013-207973	**Lote Fabricante:**	2402071001
Data de Fabricação:	07-02-2024	**Data de Validade:**	06-02-2026
Origem:	India	**Procedência:**	India
Data de Análise:	17-05-2024	**Ordem de Fracionamento:**	207973
Condições de Armazenamento:	Temperatura até 25°C		

DCB:	-		**DCI:**	-
CAS:	-		**Peso Molecular:**	-
Fórmula Molecular:	-			
	-			
	-			
Observações:	**Parte Utilizada:**	Rizoma	**Nome Científico:**	Curcuma longa

Testes	Especificações	Resultados	Unidade	Referências
Descrição *	Grânulos, amarelo a marrom.	Conforme		Fabricante
Umidade *	< 6,00 (Em 100 mg)	4,44	%	Fabricante
Cinzas totais *	< 2,00	0,62	%	Fabricante
Chumbo	< 3,0000	0,3422	ppm	Fabricante
Mercúrio	< 0,1000	< 0,0500	ppm	Fabricante
Arsênio	< 1,0000	0,0827	ppm	Fabricante
Cádmio	< 1,0000	< 0,0500	ppm	Fabricante
Densidade aparente *	0,5000 a 0,8000 (Com compactação)	0,6446	g/mL	Fabricante
Densidade aparente *	0,4500 a 0,7500 (Sem compactação)	0,7252	g/mL	Fabricante
Solvente residual (CG) *	Etanol < 5000	Não detectado	ppm	Fabricante
Curcuminóides totais (HPLC) *	45,00 - 50,00	49,96	%	Fabricante
Testes microbiológicos				
Contagem total de bactérias *	< 1000	< 10	UFC/g	Fabricante
Fungos e leveduras *	< 100	< 10	UFC/g	Fabricante
Coliformes	< 3	Conforme	MPN/g	Fabricante
Escherichia coli *	< 10	Ausente	UFC/g	Fabricante
Salmonella *	Ausente	Ausente		Fabricante

* Resultados obtidos em análises realizadas no Laboratório de Controle de Qualidade SM EMPREENDIMENTOS FARMACÊUTICOS LTDA. E os demais foram transcritos conforme certificado de análise do fabricante.

Conclusão:

Aprovado (X)

Reprovado ( )

5 APENDICE

1. Parâmetros bioquímicos

1.1 Análise geral

Tabela 1.1. ANOVA dos parâmetros bioquímicos (Macro) ao nível de 5% de significância

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Bilirrubina.Total	0.01	0.00	3.84	0.0247	*
Bilirrubina.Direta	0.00	0.00	3.62	0.0303	*
Bilirrubina.Indireta	0.01	0.01	6.68	0.0019	*
Creatinina	1.05	0.53	46.88	<0.001	*
Potássio	0.12	0.06	0.23	0.7943	
Sódio	253.26	126.63	10.16	<0.001	*
TGO	5343.42	2671.71	3.12	0.0486	*
TGP	691.06	345.53	2.05	0.1342	
Uréia	7309.18	3654.59	14.89	<0.001	*

Tabela 1.2. Teste de Tukey para comparação múltipla dos parâmetros bioquímicos

Variável	A 7 DIAS	B 14 DIAS	C 21 DIAS
Bilirrubina.Direta	0.04±0.01 a	0.03±0.02 b	0.04±0.01 ab
Bilirrubina.Indireta	0.09±0.02 b	0.12±0.04 a	0.11±0.04 a
Bilirrubina.Total	0.13±0.02 b	0.15±0.03 a	0.14±0.03 ab
Creatinina	0.18±0.04 b	0.41±0.17 a	0.23±0.05 b
Potássio	5.13±0.59 a	5.05±0.45 a	5.07±0.48 a
Sódio	133.71±5.15 b	137.26±2.27 a	136.69±2.4 a
TGO	133.45±31 ab	142.62±35.42 a	125.15±18.89 b
TGP	67.89±13.68 a	72.93±15.23 a	67.16±9.33 a
Uréia	39.52±4.18 b	59.31±26.29 a	44.34±5.86 b

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

1.1 Análise por período

Tabela 1.3. ANOVA dos parâmetros bioquímicos em animais tratados por 7 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Bilirrubina.Total	0.00	0.00	0.21	0.9313	
Bilirrubina.Direta	0.00	0.00	1.42	0.2516	
Bilirrubina.Indireta	0.00	0.00	0.53	0.7145	
Creatinina	0.00	0.00	0.66	0.6271	
Potássio	2.72	0.68	2.24	0.0885	
Sódio	198.29	49.57	2.12	0.1034	
TGO	7037.37	1759.34	2.06	0.1113	
TGP	313.85	78.46	0.39	0.8147	
Uréia	83.24	20.81	1.22	0.3228	

Tabela 1.4. Teste de Tukey para comparação múltipla dos parâmetros bioquímicos em 7 dias

Variável	AG25	AG50	AG500	AGC	AGQ
Bilirrubina.Direta	0.04±0.01 a	0.04±0.01 a	0.04±0.01 a	0.05±0.01 a	0.04±0.01 a
Bilirrubina.Indireta	0.09±0.02 a	0.09±0.02 a	0.09±0.03 a	0.08±0.03 a	0.09±0.02 a
Bilirrubina.Total	0.13±0.02 a	0.13±0.02 a	0.14±0.02 a	0.13±0.02 a	0.13±0.01 a
Creatinina	0.2±0 a	0.17±0.05 a	0.19±0.04 a	0.17±0.05 a	0.19±0.04 a
Potássio	5.14±0.36 a	4.72±0.34 a	4.99±0.42 a	5.57±0.97 a	5.22±0.39 a
Sódio	136.71±2.29 a	131.43±6.48 a	135±3.37 a	130.43±6.32 a	135±4.32 a
TGO	156.86±15.98 a	117.4±20 a	120.44±30 a	138.99±40.72 a	133.56±32.55 a
TGP	73.3±6.29 a	67.43±17.96 a	64.46±8.67 a	66.03±19.95 a	68.21±13.15 a
Uréia	40.85±2.43 a	37.32±4.93 a	37.99±3.05 a	40.79±3.2 a	40.67±5.96 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

Tabela 1.5. ANOVA dos parâmetros bioquímicos em animais tratados por 14 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Bilirrubina.Total	0.01	0.00	1.48	0.2344	
Bilirrubina.Direta	0.00	0.00	0.99	0.4294	
Bilirrubina.Indireta	0.01	0.00	1.84	0.1463	
Creatinina	0.21	0.05	1.92	0.1327	
Potássio	1.20	0.30	1.54	0.2152	
Sódio	40.69	10.17	2.28	0.0842	
TGO	4056.16	1014.04	0.79	0.5421	
TGP	478.15	119.54	0.48	0.7472	
Uréia	4917.00	1229.25	1.99	0.1221	

Tabela 1.6. Teste de Tukey para comparação múltipla dos parâmetros bioquímicos em 14 dias

Variável	BG25	BG50	BG500	BGC	BGQ
Bilirrubina.Direta	0.04±0.02 a	0.02±0.02 a	0.03±0.02 a	0.03±0.03 a	0.03±0.02 a
Bilirrubina.Indireta	0.11±0.04 a	0.14±0.03 a	0.13±0.04 a	0.09±0.03 a	0.12±0.03 a
Bilirrubina.Total	0.15±0.04 a	0.16±0.02 a	0.16±0.03 a	0.13±0.04 a	0.15±0.02 a
Creatinina	0.51±0.29 a	0.37±0.05 a	0.36±0.05 a	0.33±0.08 a	0.5±0.2 a
Potássio	5±0.21 a	5.18±0.61 a	4.97±0.39 a	5.31±0.48 a	4.77±0.42 a
Sódio	137.43±1.4 a	138±2.38 a	135.14±2.54 a	138±1.53 a	137.71±2.43 a
TGO	138.34±19.83 a	127.59±42.57 a	137.2±49.33 a	154.81±30.74 a	155.16±29.15 a
TGP	68.07±7.59 a	74.97±21.63 a	73.67±21.21 a	69.61±6.64 a	78.31±14.67 a
Uréia	80.74±48.99 a	49.04±3.12 a	48.68±5.74 a	53.97±5 a	62.66±22.74 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

Tabela 1.7. ANOVA dos parâmetros bioquímicos em animais tratados por 21 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Bilirrubina.Total	0.00	0.00	0.93	0.4623	
Bilirrubina.Direta	0.00	0.00	1.35	0.2743	
Bilirrubina.Indireta	0.01	0.00	1.55	0.2137	
Creatinina	0.01	0.00	1.02	0.4115	
Potássio	2.41	0.60	3.43	0.0201	*
Sódio	21.26	5.31	0.91	0.4681	
TGO	384.88	96.22	0.25	0.9100	
TGP	752.62	188.15	2.56	0.0590	
Uréia	419.91	104.98	4.26	0.0078	*

Tabela 1.8. Teste de Tukey para comparação múltipla dos parâmetros bioquímicos em 21 dias

Variável	CG25	CG50	CG500	CGC	CGQ
Bilirrubina.Direta	0.03±0.01 a	0.04±0.01 a	0.04±0 a	0.03±0.02 a	0.04±0.01 a
Bilirrubina.Indireta	0.13±0.03 a	0.09±0.01 a	0.1±0.01 a	0.12±0.06 a	0.1±0.03 a
Bilirrubina.Total	0.16±0.03 a	0.13±0.02 a	0.14±0.02 a	0.15±0.06 a	0.14±0.02 a
Creatinina	0.23±0.05 a	0.21±0.04 a	0.21±0.04 a	0.23±0.05 a	0.26±0.05 a
Potássio	4.95±0.29 ab	5.03±0.46 ab	4.8±0.23 b	5.57±0.55 a	5.02±0.47 ab
Sódio	136.14±2.04 a	136.14±2.12 a	136.86±1.46 a	136.14±4.14 a	138.14±1.07 a
TGO	124.39±11.8 a	123.3±16.76 a	127.93±21.49 a	129.73±21.59 a	120.43±24.69 a
TGP	75.53±6.69 a	64.5±8.9 ab	65.57±5.73 ab	61.99±4.51 b	68.21±13.81 ab
Uréia	43.75±3.55 ab	40.66±4.42 b	40.72±1.81 b	46.05±4.43 ab	49.99±8.21 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

2. Peso corporal dos órgãos

2.1 Análise geral

Tabela 2.1. ANOVA para peso corporal e órgãos (Macro) ao nível de 5% de significância.

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Peso.Corporal	13390.70	6695.35	3.16	0.0465	*
Fígado	60.93	30.46	6.86	0.0016	*
Rim.Direito	0.77	0.39	11.87	<0.001	*
Rim.Esquerdo	0.89	0.45	12.43	<0.001	*
Baço	69.27	34.64	1.01	0.3673	
Coração	0.07	0.03	1.16	0.3177	
Pulmão	2.88	1.44	2.70	0.072	
Intestino	5.27	2.64	1.30	0.2771	

Tabela 2.2. Teste de Tukey para comparação múltipla de peso corporal e órgãos

Variável	A 7 DIAS	B 14 DIAS	C 21 DIAS
Baço	1.11±0.21 a	1.02±0.24 a	2.79±10.13 a
Coração	1.05±0.12 a	1.03±0.14 a	1.09±0.23 a
Fígado	11.91±1.49 b	11.65±2.58 b	13.38±2.11 a
Intestino	1.7±0.55 a	1.19±1.73 a	1.27±1.67 a
Peso.Corporal	348.91±40.84 a	325.43±46.63 a	349.83±50.07 a
Pulmão	2.14±0.59 a	2.05±0.3 a	2.44±1.08 a
Rim.Direito	1.02±0.12 b	1.22±0.24 a	1.16±0.17 a
Rim.Esquerdo	0.99±0.12 b	1.21±0.25 a	1.14±0.17 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

2.2 Análise por período

Tabela 2.3. ANOVA para peso corporal e órgãos em animais tratados por 7 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Peso.Corporal	2945.31	736.33	0.41	0.7994	
Fígado	3.22	0.80	0.33	0.8527	
Rim.Direito	0.03	0.01	0.55	0.6973	
Rim.Esquerdo	0.02	0.00	0.30	0.8761	
Baço	0.10	0.02	0.54	0.7046	
Coração	0.02	0.00	0.23	0.9202	
Pulmão	1.40	0.35	1.01	0.4164	
Intestino	2.39	0.60	2.27	0.0847	

Tabela 2.4. Teste de Tukey para comparação múltipla de peso corporal e órgãos em 7 dias

Variável	AG25	AG50	AG500	AGC	AGQ
Baço	1.16±0.22 a	1.05±0.17 a	1.18±0.23 a	1.11±0.27 a	1.06±0.15 a
Coração	1.01±0.15 a	1.06±0.12 a	1.05±0.09 a	1.05±0.18 a	1.07±0.08 a
Fígado	12.33±1.87 a	11.4±0.92 a	11.88±1.78 a	11.87±1.4 a	12.06±1.61 a
Intestino	1.64±0.37 a	2.19±0.57 a	1.71±0.67 a	1.5±0.58 a	1.46±0.27 a
Peso.Corporal	360±33.1 a	334.29±26.89 a	349.43±38.5 a	344.14±62.84 a	356.71±41.36 a
Pulmão	2.07±0.32 a	2.19±0.42 a	2±0.41 a	2.51±1.11 a	1.96±0.22 a
Rim.Direito	0.97±0.1 a	1.02±0.14 a	1.02±0.1 a	1.06±0.13 a	1.02±0.12 a
Rim.Esquerdo	0.95±0.11 a	0.99±0.15 a	0.98±0.12 a	1.01±0.11 a	1±0.11 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

Tabela 2.5. ANOVA para peso corporal e órgãos em animais tratados por 14 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Peso.Corporal	12118.29	3029.57	1.47	0.2357	
Fígado	47.87	11.97	2.01	0.1181	
Rim.Direito	0.50	0.13	2.65	0.0523	
Rim.Esquerdo	0.56	0.14	2.53	0.0613	
Baço	0.14	0.04	0.61	0.6567	
Coração	0.08	0.02	0.98	0.4321	
Pulmão	0.45	0.11	1.27	0.3042	
Intestino	13.24	3.31	1.12	0.3650	

Tabela 2.6. Teste de Tukey para comparação múltipla de peso corporal e órgãos em 14 dias

Variável	BG25	BG50	BG500	BGC	BGQ
Baço	0.99±0.17 a	1.02±0.11 a	0.93±0.16 a	1.02±0.22 a	1.13±0.42 a
Coração	1.07±0.16 a	1.01±0.2 a	0.96±0.15 a	1.03±0.05 a	1.09±0.11 a
Fígado	11.99±2.09 a	11.83±1.35 a	11.45±1.48 a	9.67±3.96 a	13.3±2.38 a
Intestino	0.93±0.23 a	2.42±3.82 a	0.77±0.1 a	0.94±0.28 a	0.91±0.18 a
Peso.Corporal	338.71±54.44 a	318.71±32.79 a	300.14±38.53 a	316.14±41.83 a	353.43±55.01 a
Pulmão	2.22±0.39 a	2.08±0.12 a	1.87±0.2 a	2.07±0.34 a	2.02±0.34 a
Rim.Direito	1.37±0.4 a	1.16±0.09 ab	1.03±0.1 b	1.3±0.11 ab	1.26±0.22 ab
Rim.Esquerdo	1.39±0.42 a	1.13±0.11 ab	1.02±0.09 b	1.26±0.13 ab	1.23±0.25 ab

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

Tabela 2.7. ANOVA para peso corporal e órgãos em animais tratados por 21 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Peso.Corporal	30750.11	7687.53	4.23	0.0078	*
Fígado	51.12	12.78	3.82	0.0126	*
Rim.Direito	0.28	0.07	3.23	0.0256	*
Rim.Esquerdo	0.27	0.07	2.82	0.0425	*
Baço	395.37	98.84	0.96	0.4446	
Coração	0.33	0.08	1.78	0.1583	
Pulmão	9.66	2.41	2.43	0.0696	
Intestino	10.40	2.60	0.92	0.4628	

Tabela 2.8. Teste de Tukey para comparação múltipla de peso corporal e órgãos em 21 dias

Variável	CG25	CG50	CG500	CGC	CGQ
Baço	1.17±0.21 a	0.96±0.15 a	9.51±22.71 a	1.12±0.16 a	1.18±0.13 a
Coração	1.15±0.12 a	1.15±0.23 a	0.96±0.08 a	0.99±0.15 a	1.2±0.37 a
Fígado	15.15±1.98 a	13.7±2 ab	12.05±1.25 b	11.99±1.69 b	14.01±2.09 ab
Intestino	2.33±3.73 a	0.95±0.19 a	0.97±0.11 a	0.86±0.18 a	1.24±0.28 a
Peso.Corporal	392.29±42.28 a	372.71±49.19 ab	309.86±35.19 b	328.57±31.87 ab	345.71±51.2 ab
Pulmão	2.55±0.45 a	2.41±0.54 a	1.83±0.28 a	2.05±0.29 a	3.36±2.08 a
Rim.Direito	1.26±0.09 a	1.23±0.2 ab	1.01±0.06 b	1.1±0.12 ab	1.19±0.21 ab
Rim.Esquerdo	1.23±0.12 a	1.23±0.21 a	1±0.06 a	1.08±0.13 a	1.16±0.2 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

3. Parâmetros Hematológicos

3.1 Análise geral

Tabela 3.1. ANOVA para parâmetros hematológicos (Macro) ao nível de 5% de significância

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Hemáceas	9.92	4.96	6.60	0.0020	*
Hemoglobina	22.36	11.18	5.70	0.0045	*
Hematócrito	128.15	64.08	1.45	0.2401	
VCM	79.13	39.57	2.41	0.0948	
HCM	7.26	3.63	5.59	0.0050	*
CHCM	10.42	5.21	2.00	0.1408	
RDW	17.59	8.79	6.11	0.0031	*
Leucócitos	2.32×10^8	1.16×10^8	6.16	0.0030	*
Neutrófilos	456.51	228.26	3.10	0.0492	*
Linfócitos	513.44	256.72	2.95	0.0567	
Monócitos	0.61	0.30	0.11	0.8939	
Plaquetas	2.51×10^{10}	1.26×10^{10}	0.96	0.3865	
VPM	0.80	0.40	1.87	0.1593	

Tabela 3.2. Teste de Tukey para comparação múltipla dos parâmetros hematológicos

Variável	A 7 DIAS	B 14 DIAS	C 21 DIAS
CHCM	22.82±2.33 a	23.43±0.56 a	22.71±1.47 a
HCM	15.71±0.68 a	15.06±0.94 b	15.38±0.78 ab
Hematócrito	56.56±3.6 a	59.11±2.67 a	57.03±10.67 a
Hemoglobina	13.32±0.94 ab	13.71±0.67 a	12.59±2.14 b
Hemáceas	8.45±0.58 ab	8.82±0.48 a	8.06±1.3 b
Leucócitos	13068.07±4453.45 ab	15214.29±5244.39 a	11570.59±2972.25 b
Linfócitos	65.74±7.31 a	60.29±11.17 b	63.21±9.03 ab
Monócitos	4.03±1.82 a	4±1.7 a	3.85±1.4 a
Neutrófilos	30±6.16 b	35.14±10.86 a	32.74±7.96 ab
Plaquetas	160959.12±192933.5 a	134289.14±34126.59 a	123678.53±35142.58 a
RDW	14.88±1.34 b	15.63±1 a	15.85±1.23 a
VCM	66.29±3.71 a	66.6±3.11 a	68.29±5.1 a
VPM	6.38±0.42 a	6.59±0.53 a	6.5±0.42 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

3.2 Análise por período

Tabela 3.3. ANOVA para parâmetros hematológicos em animais tratados por 7 dias

Variável	**Sum sq**	**Mean sq**	**Statistic**	**p value**	**Sig**
Hemáceas	0.79	0.20	0.55	0.70	
Hemoglobina	1.25	0.31	0.32	0.86	
Hematócrito	24.12	6.03	0.43	0.78	
VCM	79.65	19.91	1.55	0.21	
HCM	1.34	0.34	0.71	0.59	
CHCM	66.39	16.60	4.28	0.01	*
RDW	4.20	1.05	0.55	0.70	
Leucócitos	1.14×10^8	0.28×10^8	1.52	0.22	
Neutrófilos	172.88	43.22	1.16	0.35	
Linfócitos	261.86	65.46	1.26	0.31	
Monócitos	6.14	1.53	0.43	0.78	
Plaquetas	14.74×10^{10}	3.68×10^{10}	0.99	0.43	
VPM	1.57	0.39	2.66	0.05	

Tabela 3.4. Teste de Tukey para comparação múltipla dos parâmetros hematológicos em 7 dias

Variável	AG25	AG50	AG500	AGC	AGQ
CHCM	23.43±0.79 a	19.83±4.58 b	23.57±0.53 a	23.14±0.38 a	23.71±0.49 a
HCM	15.43±0.98 a	16±0.63 a	15.86±0.38 a	15.57±0.79 a	15.71±0.49 a
Hematócr	55.86±2.12 a	57.83±3.06 a	55.43±6.63 a	56.86±2.85 a	57±1.73 a
Hemoglo	13.29±0.76 a	13.67±0.82 a	13.14±1.68 a	13.14±0.69 a	13.43±0.53 a
Hemácea	8.43±0.47 a	8.68±0.35 a	8.21±1.04 a	8.42±0.51 a	8.54±0.26 a
Leucócit	12371.43±1953.39	12552.42±7812.95 a	16514.29±5108.63	11214.29±2193.5 a	12614.29±2133.4
Linfócito	64.86±8.36 a	71.67±2.88 a	64.71±7.32 a	63.71±7.11 a	64.57±8.34 a
Monócito	4±1.83 a	3.17±0.75 a	4.29±2.14 a	4.43±2.64 a	4.14±1.35 a
Neutrófil	30.86±6.72 a	25.17±2.4 a	30.86±6.2 a	31.57±6.35 a	30.86±7.17 a
Plaquetas	117628.57±65491.	263883.33±331829.	106742.86±46057.9	225244.29±282297.	106000±48048.1
RDW	14.71±1.6 a	14.67±2.25 a	15.57±1.27 a	14.71±0.49 a	14.71±0.76 a
VCM	66.57±3.55 a	63.17±6.55 a	67.57±2.07 a	67.43±2.07 a	66.29±2.29 a
VPM	6.11±0.23 a	6.38±0.28 a	6.69±0.54 a	6.53±0.47 a	6.19±0.28 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

Tabela 3.5. ANOVA para parâmetros hematológicos em animais tratados por 14 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Hemáceas	0.86	0.21	0.92	0.4648	
Hemoglobina	0.86	0.21	0.45	0.7715	
Hematócrito	22.11	5.53	0.76	0.5621	
VCM	50.97	12.74	1.38	0.2651	
HCM	14.46	3.61	7.03	<0.001	*
CHCM	2.00	0.50	1.75	0.1652	
RDW	16.17	4.04	6.74	<0.001	*
Leucócitos	3.45×10^8	0.86×10^8	4.39	0.0065	*
Neutrófilos	1301.43	325.36	3.61	0.0162	*
Linfócitos	927.43	231.86	2.10	0.1055	
Monócitos	12.29	3.07	1.07	0.3863	
Plaquetas	0.41×10^{10}	0.10×10^{10}	0.87	0.4908	
VPM	1.33	0.33	1.20	0.3297	

Tabela 3.6. Teste de Tukey para comparação múltipla dos parâmetros hematológicos em 14 dias

Variável	BG25	BG50	BG500	BGC	BGQ
CHCM	23.29±0.49 a	23.71±0.49 a	23.71±0.49 a	23.29±0.76 a	23.14±0.38 a
HCM	13.86±0.69 b	15.71±0.76 a	15.29±0.49 a	15±0.58 a	15.43±0.98 a
Hematócr	59.29±1.38 a	58±2.16 a	58.71±2.87 a	59.14±3.34 a	60.43±3.26 a
Hemoglo	13.71±0.49 a	13.43±0.53 a	13.86±0.69 a	13.71±0.76 a	13.86±0.9 a
Hemácea	8.76±0.28 a	8.54±0.52 a	8.99±0.32 a	8.87±0.5 a	8.93±0.68 a
Leucócit	12714.29±1573.14	21042.86±7104.8	14871.43±4525.38	12214.29±2664.23	15228.57±4214.15
Linfócito	70.14±4.18 a	57.86±16.51 a	58.71±9.34 a	55.14±9.96 a	59.57±8.7 a
Monócito	4.57±2.44 a	4.57±2.3 a	4.14±1.46 a	3.71±0.95 a	3±0 a
Neutrófil	23.29±0.49 b	37.43±14.64 ab	37±8.76 ab	40.86±9.55 a	37.14±8.28 ab
Plaquetas	141342.86±13792.8	145900±14369.5	125188.57±28663.3	141285.71±64085.4	117728.57±24191.9
RDW	15.71±0.76 ab	16.29±0.76 a	15±0.58 b	16.43±0.53 a	14.71±1.11 b
VCM	67.57±2.99 a	67.29±3.2 a	64.43±1.27 a	66.14±2.85 a	67.57±4.16 a
VPM	6.39±0.72 a	6.94±0.28 a	6.47±0.35 a	6.51±0.57 a	6.66±0.58 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

Tabela 3.7. ANOVA para parâmetros hematológicos em animais tratados por 21 dias

Variável	Sum sq	Mean sq	Statistic	p value	Sig
Hemáceas	4.23	1.06	0.59	0.6714	
Hemoglobina	13.17	3.29	0.69	0.6043	
Hematócrito	622.68	155.67	1.44	0.2459	
VCM	540.42	135.10	12.30	<0.001	*
HCM	6.98	1.75	3.88	0.0121	*
CHCM	59.92	14.98	38.98	<0.001	*
RDW	18.48	4.62	4.21	0.0082	*
Leucócitos	0.08×10^8	0.02×10^8	0.19	0.9408	
Neutrófilos	346.14	86.54	1.44	0.247	
Linfócitos	458.23	114.56	1.49	0.2313	
Monócitos	10.07	2.52	1.35	0.2762	
Plaquetas	0.62×10^{10}	0.16×10^{10}	1.30	0.2917	
VPM	0.72	0.18	1.02	0.4113	

Tabela 3.8. Teste de Tukey para comparação múltipla dos parâmetros hematológicos em 21 dias

Variável	CG25	CG50	CG500	CGC	CGQ
CHCM	21.43±0.79 b	20.86±0.38 b	23.86±0.38 a	23.57±0.79 a	24±0.63 a
HCM	15.86±0.69 a	15±1 ab	15.71±0.49 ab	14.71±0.49 b	15.67±0.52 ab
Hematóc	64.14±2.85 a	58±18.2 a	54.57±4.69 a	51.43±10.83 a	57±7.24 a
Hemoglo	13.64±0.52 a	12.21±3.87 a	12.86±1.35 a	11.89±2.27 a	12.32±0.98 a
Hemácea	8.67±0.27 a	7.97±2.35 a	8.14±0.71 a	7.81±1.52 a	7.63±0.54 a
Leucócit	11414.29±3151.42	11185.71±3943.53	12471.43±2752.4 a	11471.43±3270.43	11266.67±2073.32
Linfócito	65.14±3.18 a	67.29±4.75 a	64.86±14.62 a	56.86±5.73 a	61.67±10.54 a
Monócit	3.57±0.53 a	3.29±0.76 a	3.86±1.95 a	4.86±1.95 a	3.67±0.82 a
Neutrófil	31.29±3.2 a	29.14±4.41 a	31±12.54 a	38.14±4.67 a	34.33±9.93 a
Plaquetas	117628.57±29484.	144242.86±54778.	133471.43±31323.	108038.57±28034.	113566.67±11992.
RDW	16.71±0.76 a	16.71±0.76 a	15±1.15 b	15.29±1.5 ab	15.5±0.84 ab
VCM	73.71±3.77 a	72.14±3.67 a	64.86±4.45 b	64±1.63 b	66.5±1.87 b
VPM	6.56±0.36 a	6.23±0.36 a	6.64±0.17 a	6.49±0.53 a	6.58±0.58 a

Nota: valores expressos em media ± o desvio padrão da media. Diferenças de médias foram avaliadas pelo teste de Anova, seguida do teste post hoc de Tukey em que, médias seguidas da mesma letra em linha não diferem significativamente a 5% de significância.

4. Correlação entre Peso dos Órgãos e Parâmetros Bioquímicos

Tabela 4. Correlação entre variáveis (coeficiente de Pearson (ρ), p-valor e significância)

Grupo	Variável X	Variável Y	Correlação (ρ)	Valor-p	Sig.
7 DIAS	Média dos Rins	Sódio	0.07	0.70	
7 DIAS	Média dos Rins	Potássio	-0.08	0.66	
7 DIAS	Média dos Rins	Uréia	0.02	0.91	
7 DIAS	Fígado	Bilirrubina Total	0.15	0.39	
7 DIAS	Fígado	Bilirrubina Direta	-0.24	0.16	
7 DIAS	Fígado	Bilirrubina Indireta	0.23	0.18	
7 DIAS	Fígado	TGO	-0.23	0.19	
7 DIAS	Fígado	TGP	-0.19	0.29	
14 DIAS	Média dos Rins	Sódio	0.21	0.23	
14 DIAS	Média dos Rins	Potássio	-0.21	0.23	
14 DIAS	Média dos Rins	Uréia	0.80	<0.01	*
14 DIAS	Fígado	Bilirrubina Total	0.47	<0.01	*
14 DIAS	Fígado	Bilirrubina Direta	0.10	0.55	
14 DIAS	Fígado	Bilirrubina Indireta	0.36	0.03	*
14 DIAS	Fígado	TGO	-0.04	0.83	
14 DIAS	Fígado	TGP	0.18	0.31	
21 DIAS	Média dos Rins	Sódio	0.22	0.21	
21 DIAS	Média dos Rins	Potássio	0.29	0.10	
21 DIAS	Média dos Rins	Uréia	0.23	0.18	
21 DIAS	Fígado	Bilirrubina Total	0.05	0.77	
21 DIAS	Fígado	Bilirrubina Direta	0.08	0.67	
21 DIAS	Fígado	Bilirrubina Indireta	0.02	0.92	
21 DIAS	Fígado	TGO	-0.1	0.58	
21 DIAS	Fígado	TGP	0.3	0.08	

As correlações foram avaliadas pelo coeficiente de Pearson ao nível de significância de 5% ($\alpha < 0{,}05$).