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Original Article

Assessing the hepatoprotective efficacy of *Vitis gracilis* Wall. against doxorubicin-induced hepatic injury in rats

[Evaluación de la eficacia hepatoprotectora de *Vitis gracilis* Wall. contra la lesión hepática inducida por doxorrubicina en ratas]

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Abstract

Context: Doxorubicin is an anticancer drug that can adversely affect the liver. Therefore, seeking medical attention to manage pain and minimize side effects is crucial.

Aims: To evaluate the hepatoprotective potential of *Vitis gracilis* Wall. nanoherbs against doxorubicin-induced hepatotoxicity.

Methods: The study included five treatment groups: T0 (negative control), T+ (positive control with doxorubicin alone), TC (doxorubicin + vitamin C at 0.2 mg/kg body weight/day), T125 (doxorubicin + *V. gracilis* at 125 mg/kg body weight/day), and T150 (doxorubicin + *V. gracilis* at 150 mg/kg body weight/day). Doxorubicin, administered intraperitoneally at 0.0019 mg/kg body weight once a week for three weeks, induced hepatotoxicity in the T+ group.

Results: Serum transaminases were significantly reduced in the T125 and T150 groups, indicative of the hepatoprotective effects of *V. gracilis*. Immunohistochemical analysis demonstrated the modulation of key markers, with decreased levels of pro-inflammatory tumor necrosis factor-α and increased levels of anti-inflammatory interleukin 10 in the T125 and T150 groups. The activities of oxidative stress markers, including superoxide dismutase and caspase 3, were favorably influenced by *V. gracilis* nanoherb treatment.

Conclusions: These findings suggest that *V. gracilis* nanoherbs may effectively attenuate doxorubicin-induced hepatotoxicity, evidenced by biochemical and immunohistochemical changes. This study provides a foundation for further exploration of *V. gracilis* nanoherbs as a potential adjunctive therapy in preventing chemotherapy-associated liver damage.

Keywords: CASP3; IL-10; serum transaminases; SOD; TNF-α.

Resumen

Contexto: La doxorrubicina es un fármaco anticancerígeno que puede afectar negativamente al hígado. Por lo tanto, es fundamental buscar atención médica para controlar el dolor y minimizar los efectos secundarios.

Objetivos: Evaluar el potencial hepatoprotector de *Vitis gracilis* Wall. nanohierbas contra la hepatotoxicidad inducida por doxorrubicina.

Métodos: El estudio incluyó cinco grupos de tratamiento: T0 (control negativo), T+ (control positivo con doxorrubicina sola), TC (doxorrubicina + vitamina C a 0,2 mg/kg de peso corporal/día), T125 (doxorrubicina + *V. gracilis* a 125 mg/kg de peso corporal/día) y T150 (doxorrubicina + *V. gracilis* a 150 mg/kg de peso corporal/día). La doxorrubicina, administrada por vía intraperitoneal a 0,0019 mg/kg de peso corporal una vez por semana durante tres semanas, indujo hepatotoxicidad en el grupo T+.

Resultados: Las transaminasas séricas se redujeron significativamente en los grupos T125 y T150, lo que indica los efectos hepatoprotectores de *V. gracilis*. El análisis inmunohistoquímico demostró la modulación de marcadores clave, con niveles reducidos de factor de necrosis tumoral α proinflamatorio y niveles elevados de interleucina 10 antiinflamatoria en los grupos T125 y T150. Las actividades de los marcadores de estrés oxidativo, incluidas la superóxido dismutasa y la caspasa 3, se vieron influenciadas favorablemente por el tratamiento con nanoherbas de *V. gracilis*.

Conclusiones: Estos hallazgos sugieren que las nanohierbas de *V. gracilis* pueden atenuar eficazmente la hepatotoxicidad inducida por doxorrubicina, evidenciada por cambios bioquímicos e inmunohistoquímicos. Este estudio proporciona una base para una mayor exploración de las nanohierbas de *V. gracilis* como posible terapia complementaria para prevenir el daño hepático asociado a la quimioterapia.

Palabras Clave: CASP3; IL-10; SOD; TNF-α; transaminasas séricas.

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INTRODUCTION

Liver diseases (Ballotin et al., 2021) encompass a spectrum of disorders and pose a formidable challenge to global health. Drug-induced hepatotoxicity, particularly associated with chemotherapeutic agents, remains a significant concern, limiting the therapeutic potential of these agents. Doxorubicin, an anthracycline widely used in cancer treatment (Li et al., 2020), is known for its potent antineoplastic effects but frequently has adverse side effects, including hepatotoxicity. This study examines the hepatoprotective potential of *Vitis gracilis* Wall. (Ilyas et al., 2023; Midoen et al., 2023; Wasnis et al., 2022) against doxorubicin-induced liver damage, focusing on biochemical markers and immunohistochemical (IHC) changes.

Doxorubicin-induced hepatotoxicity (Ballotin et al., 2021) is a multifaceted phenomenon characterized by elevated liver enzymes, oxidative stress, and inflammatory responses. The intricate balance between doxorubicin's efficacy and potential harm to normal tissues, especially the liver (Gajendiran et al., 2018; Sabri et al., 2018; Silitonga et al., 2014; Situmorang et al., 2019; Situmorang and Ilyas, 2018), necessitates the exploration of complementary interventions to mitigate its hepatotoxicity. While an effective anticancer agent, doxorubicin has cytotoxic effects on cancer cells and healthy tissues, including the liver. Its hepatotoxic effects can manifest as increased serum transaminases, such as serum glutamic oxaloacetic transaminase [SGOT; also called aspartate aminotransferase (AST)] and serum glutamic pyruvic transaminase [SGPT; also called alanine aminotransferase (ALT)], reflecting liver damage.

V. gracilis, a plant rich in bioactive compounds, has been traditionally used for its medicinal properties. Its antioxidant, anti-inflammatory, and hepatoprotective potential make it an intriguing candidate for mitigating drug-induced liver damage. Nanoherbs derived from *V. gracilis* (VGN) represent a novel approach to harnessing its medicinal properties (Ilyas et al., 2023). The unique characteristics of nanoherbs, including enhanced bioavailability and targeted delivery (Wasnis et al., 2022), make them promising candidates for hepatoprotective interventions.

Beyond conventional biochemical markers, IHC (Ilyas et al., 2023; Santoso et al., 2023) provides insights into the molecular mechanisms underlying hepatotoxicity. Key markers such as tumor necrosis factor-alpha (TNF-α) (Erekat, 2022; Hamaidia and Soltani, 2019), interleukin 10 (IL-10) (Nagata and Nishiyama, 2021), superoxide dismutases (SOD) (Midoen et al., 2023) and caspase 3 (CASP3) (Ilyas, 2014; Ilyas et al., 2019a; Stoessel and Majewska, 2021) play pivotal roles in inflammation (Allison et al., 2023), oxidative stress (Allameh et al., 2023; Johra et al., 2023), and apoptosis, offering a comprehensive understanding of the cellular responses.

The primary objective of this study was to assess the hepatoprotective potential of *V. gracilis* Wall. against doxorubicin-induced liver damage. Its specific aims were to evaluate changes in serum transaminases (SGOT, SGPT) (Ilyas et al., 2019a; 2019b) and explore changes in IHC markers TNF-α (Chang et al., 2013; Hammer et al., 2019; Kustarini et al., 2012; Khairani and Sumarmin, 2018; Nagata and Nishiyama, 2021; El-Gindy et al., 2023), IL-10 (Hammer et al., 2019; Nagata and Nishiyama, 2021), SOD (Alshanwani et al., 2022; Iruoghene et al., 2024; Taepongsorat and Phadungkit, 2018), and CASP3 (Alshanwani et al., 2022; Iruoghene et al., 2024).

Hepatoprotection refers to the ability of a substance to protect the liver from damage or injury, which is critical given the liver's vital role in metabolism, detoxification, and overall physiological homeostasis. Doxorubicin, a potent chemotherapeutic agent used in cancer treatment, is known to induce hepatotoxicity as one of its adverse effects, making it an ideal model for studying potential hepatoprotective agents (Alshanwani et al., 2022).

V. gracilis, a grapevine species native to specific regions, has unique properties that make it a promising candidate for hepatoprotection research. It may contain bioactive compounds such as polyphenols, flavonoids, and other phytochemicals that have shown antioxidant, anti-inflammatory, and hepatoprotective properties in various preclinical and clinical studies. By investigating the hepatoprotective efficacy of *V. gracilis*, this study seeks to contribute to identifying natural remedies for hepatic injuries, potentially offering safer and more accessible treatment options for liver-related disorders (Fathy et al., 2023).

Studying the ethnomedical uses of plants, including *V. gracilis*, provides valuable insights into their therapeutic potential and pharmacological activities. By bridging ethnomedical knowledge with scientific research, this study aims to validate and elucidate the hepatoprotective properties attributed to *V. gracilis* by traditional healers and communities. Understanding the relationship between the ethnomedical uses and demonstrated activity of *V. gracilis* against doxorubicin-induced hepatic injury can enrich our understanding of its medicinal properties and guide the development of evidence-based therapies rooted in traditional medicine practices (Arman et al., 2022).

Overall, encapsulating the plant extract from *V. gracilis* in nanoherbs represents a promising strategy

to overcome the limitations of conventional dosage forms and unlock the full therapeutic potential of herbal remedies for hepatoprotection. This innovative approach holds great promise for improving treatment outcomes and addressing unmet clinical needs in liver health management. This study aimed to assess the hepatoprotective efficacy of *V. gracilis* against doxorubicin-induced hepatic injury in rats. This study holds significant clinical relevance by potentially unraveling a natural remedy for doxorubicin-induced hepatotoxicity. If successful, using *V. gracilis*, particularly in nanoherb form, could enhance the safety profile of doxorubicin-based chemotherapy, enabling more effective cancer treatment.

MATERIAL AND METHODS

Reagents

The reagents used were SGOT (AIM SGOT/AST 5 reagent kit; Indonesia), SGPT (ONECARE Reagen SGPT ALT [OCR-GPT100]), TNF-α (IHC monoclonal antibody [MBS438099], ELISA kit [MBS175904]; USA), IL-10 (monoclonal antibody [MBS225651], IHC/ELISA kit; USA), SOD (rat superoxide dismutase ELISA kit [MBS036924]; USA), CASP3 (caspase-3 polyclonal antibody [E-AB-63510], rat CASP3 (caspase 3) ELISA kit [E-EL-R0160]; USA).

Plant material

The herbarium specimens of *V. gracilis* were collected in the Tangkahan area, which includes selected villages (Sei Serdang and Namo Sialang) in Batang Serangan district, North Sumatra Province, Indonesia (coordinates: X = 395928.28 and Y = 408308.48). The foliage of *V. gracilis* was harvested in Kalong Cave, a limestone cavity with a thermal spring nearby located between Namo Sialang and Sei Serdang. The Batang Sarangan River follows the Kalong Cave. This cave's structure is composed of fine clay and sharp-edged rocks with a light shade of brown. The Plant Systematics Laboratory Herbarium team at the Universitas Sumatera Utara (USU) identified the plant (voucher number 201/MEDA/2023) as *V. gracilis*.

Nanoherb preparation

The extract's particle size was adjusted using high energy milling (HEM; Emax [4292201006-AL2-106857201]; Germany) with a ball milling mass ratio of 1:20 and a milling time of three, six, or nine hours (Ilyas et al., 2023). Before milling, the *V. gracilis* samples and alumina milling balls were weighed and transferred into the milling vial at the appropriate ratio. The HEM machine ran for 10 minutes, followed by a one-hour break until the specified accumulated time was reached. *V. gracilis* was procured, authenticated, and used to prepare nanoherbs (VGN). The plant material was processed following standard procedures to obtain a concentrated and bioactive form suitable for experimental use (Irianti et al., 2020).

Experimental animals

This study used 25 male Wistar rats (200–250 g, 10–15 weeks old) obtained from the Biology Laboratory at USU. The rats were acclimated in animal housing at the Animal Physiology Laboratory of the Biology Study Program at USU for two weeks. The housing was pre-cleaned by radiation and was maintained with a 12/12-hour light/dark cycle and 35–60% humidity. The rats had free access to water, corn, and pellets. The rats were placed in 40 × 30 cm plastic containers for the experiments. This study was approved by the Health Research Ethics Committee of USU FMIPA Medan (approval number 0908/KEPH-FMIPA/2023).

Group allocation and treatment protocol

The rats were randomly assigned to five experimental groups (n = 5/group): T0 (negative control) = no treatment; T+ (positive control) = treated with doxorubicin at 0.0019 mg/kg body weight (BW) intraperitoneally (i.p.) once a week for three weeks; TC (treatment control) = treated with doxorubicin (0.0019 mg/kg BW, i.p., 1×/week) and vitamin C (0.2 mg/kg BW/day) for three weeks; T125 = treated with doxorubicin (0.0019 mg/kg BW, i.p., 1×/week) and VGN (125 mg/kg BW/day) for three weeks; T150 = treated with doxorubicin (0.0019 mg/kg BW, i.p., 1×/week) and VGN (150 mg/kg BW/day) for three weeks.

Blood sample collection

The rats were euthanized at the end of the experimental period via injection of a lethal dose of euthanasia solution into a muscle, such as the thigh muscle; this method may take slightly longer to take effect than intravenous injection. Then, blood samples were collected by cardiac puncture and allowed to clot before the serum was separated by centrifugation for subsequent biochemical analyses (Ajami et al., 2020; Raghavan et al., 2023; Shen et al., 2023; Stamataki et al., 2020).

Biochemical analyses

The serum levels of hepatic biomarkers, including SGOT and SGPT, were quantified using standard enzymatic assays (Yulizal et al., 2020). These markers served as indicators of hepatocellular damage.

IHC analyses

Liver tissues were collected, fixed in formalin, and processed for IHC analyses. The levels of the following key markers were assessed: TNF-α and IL-10 as inflammatory response indicators, SOD as an antioxidant indicator, and CASP3 as an apoptosis indicator (Erekat, 2017; Satria et al., 2019).

Statistical analysis

The data were analyzed using the SPSS software. The data are expressed as the mean ± standard deviation and were compared between groups using analysis of variance tests followed by post-hoc Duncan's tests. Significance was set at $p<0.05$ (Yashkin et al., 2023).

RESULTS

Effect on hepatic enzymes

SGOT and SGPT levels were significantly higher in the doxorubicin-treated T+ (positive control) group than in the negative control (T0) and treatment control (TC) groups. Notably, SGOT and SGPT levels were significantly lower in the VGN-treated T125 and T150 groups than in the T+ group, suggesting that both VGN concentrations have hepatoprotective effects against doxorubicin-induced liver damage (Fig. 1).

Inflammatory cytokines

TNF-α levels were significantly higher in the T+ group than in the T0 group, indicating doxorubicin-induced inflammation. However, TNF-α levels were significantly lower in the TC, T125, and T150 groups than in the T+ group (Fig. 2A). Moreover, IL-10 levels were significantly higher in the TC, T125, and T150 groups than in the T+ group, suggesting a potential anti-inflammatory response (Fig. 2B). TNF-alpha and IL-10 expression of rats administered with *Vitis gracilis* nanoherb can be seen in Fig. 3.

Antioxidant enzyme activity

SOD activity was significantly lower in the T+ group than in the T0 group, indicating oxidative stress due to doxorubicin. In contrast, SOD activity was significantly higher in the TC, T125, and T150 groups, suggesting enhanced antioxidant defense against doxorubicin-induced oxidative damage (Fig. 4).

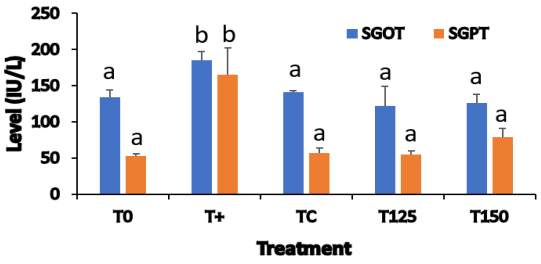

Figure 1. SGOT and SGPT levels of rats administered with *Vitis gracilis* nanoherb.

Data are expressed as mean ± SD (n = 5). Treatments not sharing the same letters in the same row and color significantly differ by ANOVA followed by Duncan's test ($^{a,b}p<0.05$). T0: negative control; T+: DXR; TC: DXR + vitamin C at 0.2 mg/kg body weight/day; T125: DXR + nanoherbal dose 125 mg/kg body weight and T150: DXR + herbal dose 150 mg/kg body weight; and DXR: doxorubicin.

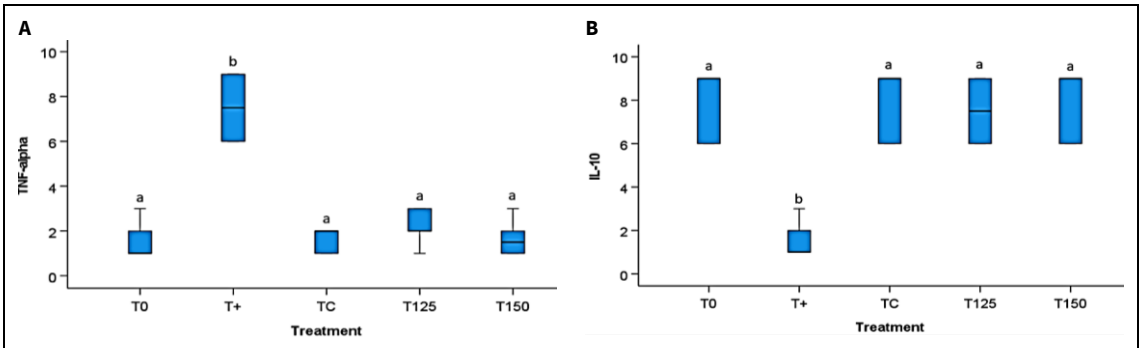

Figure 2. TNF-alpha and IL-10 levels of rats administered with *Vitis gracilis* nanoherb.

Data are expressed as median and min-max (n = 5). Treatments not sharing the same letters in the same row and color significantly differ by the Kruskal-Wallis test followed by Mann-Whitney's test ($^{a,b}p<0.05$). T0: negative control; T+ DXR, TC: DXR + vitamin C at 0.2 mg/kg body weight/day; T125: DXR + nanoherbal dose 125 mg/kg body weight; T150: DXR + herbal dose 150 mg/kg body weight; and DXR: doxorubicin.

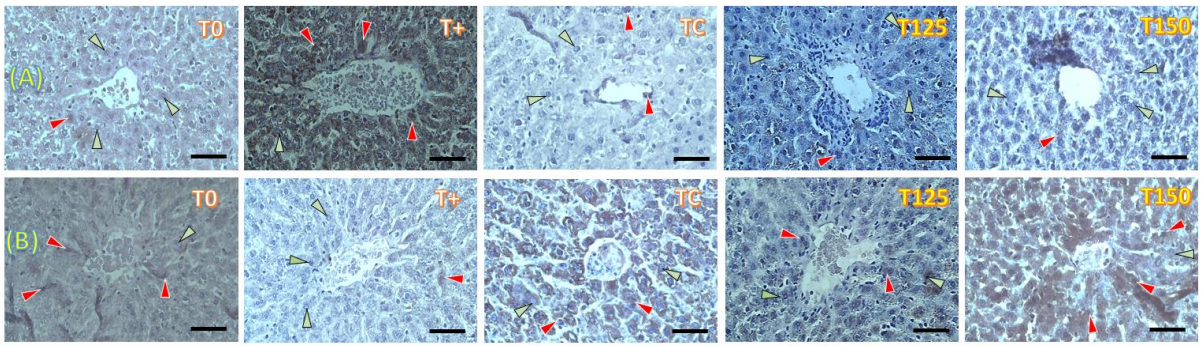

Figure 3. TNF-alpha and IL-10 expression of rats administered with *Vitis gracilis* nanoherb.

T0: negative control; T+: DXR; TC: DXR + vitamin C at 0.2 mg/kg body weight/day; T125: DXR + nanoherbal dose 125 mg/kg body weight; T150: DXR + herbal dose 150 mg/kg body weight; and DXR: doxorubicin. **(A)** Red arrows: positive TNF-alpha expression, green arrow: negative TNF-alpha **(B)** Red arrows: positive IL-10 expression, green arrow: negative IL-10 expression. Magnification 400× and scale 200 µm.

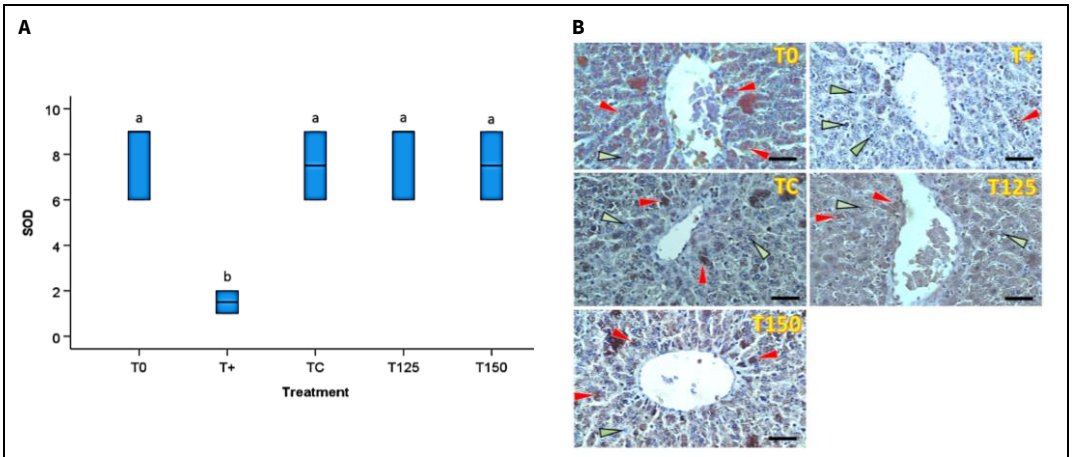

Figure 4. (A) SOD levels and **(B)** SOD expression of rats administered with *Vitis gracilis* nanoherb.

In **(A)**, data are expressed as median and min-max (n = 5). Treatments not sharing the same letters significantly differ by the Kruskal-Wallis test followed by Mann-Whitney's test ($^{a,b}p<0.05$). In **(B)**, red arrows: positive SOD expression, green arrows: negative SOD expression. T0: negative control; T+: DXR, TC: DXR + vitamin C at 0.2 mg/kg body weight/day; T125: DXR + nanoherbal dose 125 mg/kg body weight; T150: DXR + herbal dose 150 mg/kg body weight; and DXR: doxorubicin. Magnification 400× and scale 200 µm.

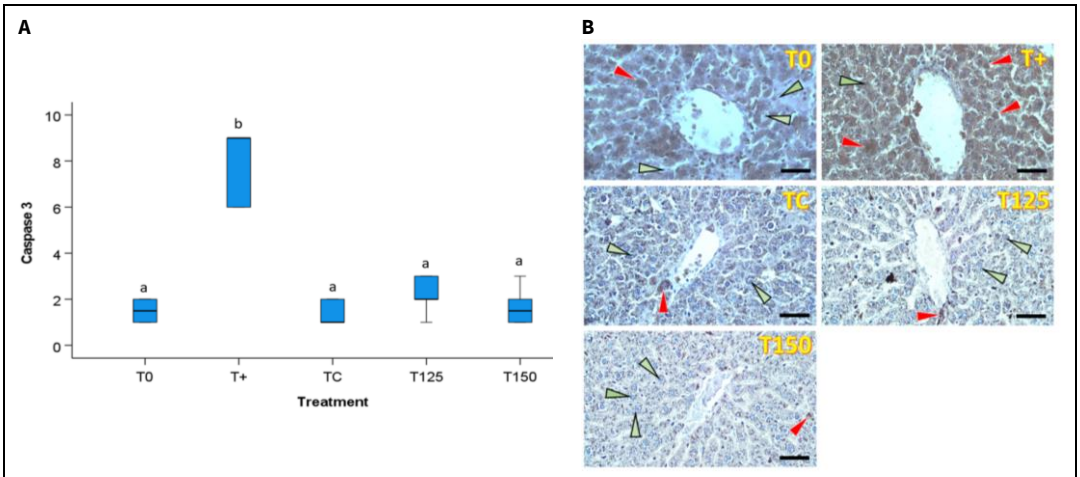

Figure 5. (A) Caspase-3 levels and **(B)** caspase-3 expression of rats administered with *Vitis gracilis* nanoherbs.

In **(A)**, data are expressed as median and min-max (n = 5). Treatments not sharing the same letters significantly differ by the Kruskal-Wallis test followed by Mann-Whitney's test ($^{a,b}p<0.05$). In **(B)**, red arrows: positive caspase-3 expression, green arrows: negative caspase-3 expression. T0: negative control; T+: DXR; TC: DXR + vitamin C at 0.2 mg/kg body weight/day; T125: DXR + nanoherbal dose 125 mg/kg body weight; T150: DXR + herbal dose 150 mg/kg body weight; and DXR: doxorubicin. Magnification 400× and scale 200 µm.

DISCUSSION

VGN affected hepatic enzymes. The elevated SGOT and SGPT levels in the T+ group are consistent with doxorubicin-induced hepatotoxicity. However, their significant reductions in the T125 (125 mg/kg BW) and T150 (150 mg/kg BW) suggest VGN protects against doxorubicin-induced liver damage. Doxorubicin can trigger increases in liver enzymes such as SGOT and SGPT through live cell damage. It is an effective chemotherapeutic agent, but it can also cause liver toxicity. The increases in SGOT and SGPT after doxorubicin administration may be due to oxidative damage, mitochondrial damage, inflammation, and oxidative stress.

Doxorubicin can cause the formation of free radicals and reactive oxygen species, known as oxidative stress, which can damage liver cells, called oxidative damage (Kong et al., 2022). VGN contains antioxidants such as xanthone, kinoprene, and diphyllin (Ilyas et al., 2023), which attenuate the effects of oxidants on liver cells. Doxorubicin can also damage mitochondria in liver cells, which are organelles vital for cellular energy production (Gajendiran et al., 2018). Mitochondrial damage can release liver enzymes, including SGOT and SGPT, into the blood circulation (Mohammad et al., 2024). Additionally, doxorubicin can induce an inflammatory response in the liver, which can cause liver cells to release more enzymes into the bloodstream, such as SGPT and SGOT (Alshanwani et al., 2022). Moreover, doxorubicin can interact with and damage DNA at the molecular level (Linders et al., 2024). Furthermore, it can activate cellular responses that affect liver function (AlAsmari et al., 2021).

Notably, TNF-α is a pro-inflammatory cytokine associated with liver damage (Shati et al., 2024). Our results suggest that VGN may have an anti-inflammatory effect, possibly by the decrease in TNF-α levels observed in the T125 and T150 groups. The increase in IL-10, an anti-inflammatory cytokine, further supports this potential. Some VGN components exhibit potent anti-inflammatory activity, such as 1-monolinolenoyl-rac-glycerol (glycerolipid). They can decrease pro-inflammatory TNF-α and increase anti-inflammatory IL-10 (Chen et al., 2024).

The decreased SOD activity in the T+ group suggests doxorubicin-induced oxidative stress. However, the significant increase in SOD activity in the TC, T125, and T150 groups indicates the potential of VGN to enhance antioxidant defenses, mitigating oxidative damage. This effect is likely due to kinoprene (a fatty acid) and 1-monolinolenoyl-rac-glycerol (glycerolipid) in VGN, which function as antioxidants (Ilyas et al., 2023) and increased SOD levels in rat livers (Sheweita et al., 2023).

CASP3 activation in the T+ group indicates doxorubicin-induced apoptosis. However, the decrease in CASP3 activity in the TC, T125, and T150 groups suggests that VPN may have an anti-apoptotic effect, supporting its hepatoprotective role. The 1-monolinolenoyl-rac-glycerol (glycerolipid) found in VGN functions as an anti-inflammatory agent, thus serving as a hepatoprotectant (Ilyas et al., 2023).

The observed hepatoprotective effects of *V. gracilis* components may be attributed to their anti-inflammatory (Fathy et al., 2023), antioxidant (Januszewski et al., 2024), and anti-apoptotic properties (Xiaobo et al., 2023). These components may scavenge free radicals, modulate inflammatory pathways, and inhibit apoptotic processes, collectively contributing to liver protection (Senavirathna et al., 2024). Our findings suggest the potential therapeutic use of VGN in mitigating doxorubicin-induced hepatotoxicity. Further research is needed to identify the specific bioactive compounds responsible for the observed effects and to optimize dosage regimens for clinical use.

CONCLUSION

Administering *V. gracilis* as a nanoherb demonstrated promising hepatoprotective potential against doxorubicin-induced liver damage. The observed effects on hepatic enzymes, inflammatory markers, antioxidant defenses, and apoptosis highlight the multifaceted protective mechanisms of *V. gracilis*. Our study provides a foundation for future investigations aiming to harness the therapeutic potential of *V. gracilis* in clinical settings.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Midoen YH	Ilyas S	Santoso P
Concepts or ideas	x	x	
Design	x	x	
Definition of intellectual content		x	x
Literature search			x
Experimental studies			x
Data acquisition	x	x	
Data analysis	x	x	
Statistical analysis	x		
Manuscript preparation	x		x
Manuscript editing	x		
Manuscript review	x	x	x

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