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Effect of *Vernonia Cinerea* Water Extract on the Protection of Liver Tissue in Chronic Nicotine-Treated Rats

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Abstract

Nicotine, a highly toxic alkaloid found in tobacco, is known for its addictive properties and systemic side effects, including carcinogenic potential and multi-organ damage. Recent evidence indicates that nicotine can significantly impair liver function and regeneration by promoting fibrogenesis and oxidative stress. This study aimed to investigate the protective effects of *Vernonia cinerea* (VC) against nicotine-induced liver damage in Wistar rats, focusing on inflammation and pro-fibrosis markers. Thirty male Wistar rats were divided into three groups: a control group, a nicotine group (1 mg/kg/day), and a nicotine plus VC group (1 mg/kg/day nicotine and 100 mg/kg/day VC). Liver tissues were examined using Hematoxylin and Eosin staining, and Masson's trichrome staining to evaluate histological changes. Kupffer cell counts were determined using a Panoramic digital slide scanner. Statistical analyses were conducted to compare the groups. Nicotine exposure led to significant liver damage, characterized by increased inflammation, collagen deposition, and disruption of liver architecture. VC supplementation ameliorated these effects, reducing inflammation and fibrosis markers. Kupffer cell counts were lower in the nicotine plus VC group compared to the nicotine group alone. Conclusion: VC demonstrates a protective role in mitigating nicotine-induced liver damage, highlighting its potential as a therapeutic agent for preventing liver fibrosis and maintaining liver health in nicotine-exposed individuals.

Keywords**:** *Vernonia cinerea less.; chronic nicotine; kupffer cell; liver inflammation; liver fibrosis*

1. Introduction

Nicotine is a major chemical compound found in tobacco and tobacco products. It is well known to have serious systemic side effects in addition to being highly addictive (Teerapong et al., 2019). Nicotine is a highly toxic alkaloid soluble in both polar and nonpolar solvents (log K_{ow} = 1.17), and its low molecular weight (162.2 g/mol) facilitates rapid absorption into the body through the skin, oral mucosa, intestines, and lungs, reaching the brain directly (Hukkanen et al., 2005). Scientific studies have consistently demonstrated its carcinogenic potential and its contribution to the pathogenesis of various diseases. It affects the heart and blood vessels, reproductive system, lungs, kidneys, gastrointestinal organs, and more. Several studies have shown that nicotine promotes neo-angiogenesis, cell division, and cell proliferation, affecting neurons and non-neurons through specific pathways downstream of nicotinic acetylcholine receptors (nAChRs) (Sansone

et al., 2023). Combined with its highly acute toxic effect and low lethal dose of about 0.5–1 mg/kg LD_{50}, or 30–60 mg for an adult human, nicotine poses a significant health risk (Mayer, 2014).

Recent evidence increasingly confirms that nicotine can lead to significant liver damage and disrupt essential liver functions. Despite the liver's remarkable capacity for regeneration, chronic nicotine exposure impairs this ability. Nicotine interferes with the signaling pathways that regulate hepatocyte proliferation and tissue repair, impairing the liver's regenerative capacity and leading to progressive liver dysfunction. Nicotine is metabolized primarily by liver enzymes such as cytochrome P450 A6 (CYP2A6), UDP-glucuronosyltransferase (UGT), and flavin-containing monooxygenase (FMO) (Moesan et al., 2024; Benowitz, 2010). This metabolism generates various reactive metabolites that can induce oxidative stress and cellular damage. Nicotine exerts its biological effects throughout the body by binding to nAChRs located on the plasma membrane of cells (Picciotto et al., 2000).

One key mechanism through which nicotine damages the liver tissue is through promoting the development of collagen fibers, or fibrogenesis, via nAChRs expressed on Ito (hepatic stellate or perisinusoidal) cells (Soeda et al., 2012). Hepatoblasts, a population of epithelial cells lining the three-dimensional network of bile ducts known as the biliary tree, are also affected. Jensen et al. (2013) reported that chronic nicotine exposure contributes to biliary fibrosis by activating hepatoblast proliferation and the expression of profibrotic genes in rats.

Liver fibrosis, characterized by the excessive deposition of extracellular matrix (ECM) proteins including collagen, occurs in most chronic liver diseases (Bataller, & Brenner, 2005; Kierszenbaum, & Tres, 2019). Research indicates that exposure to toxic substances stimulates the proliferation of Kupffer cells, which produce both anti-inflammatory and pro-inflammatory cytokines. Pro-inflammatory cytokines, in particular, can cause liver tissue damage, endothelium degeneration, accumulation of collagen type I within the perisinusoidal space, and extensive hepatocyte necrosis (Kierszenbaum, & Tres, 2019; Liu et al., 2017, Liu et al., 2020). Furthermore, liver fibrosis is associated with changes in liver sinusoidal endothelial cells (LSECs), including capillarization and the loss of fenestrae (Hwang et al., 2023). In addition to its potential role in initiating injury, nicotine increases serum levels of liver enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), and nitric oxide secretion in animals. Elevated levels of ALP, ALT, and AST are markers of liver injury, inflammation, or disease (Bissel et al., 2001). Consequently, several cell types, including hepatocytes, Kupffer cells, endothelial cells of blood sinusoids, hepatoblasts (cholangiocytes), and Ito cells, play essential roles in maintaining liver balance and are implicated in the pathological processes of liver disorders.

The treatment of nicotine-related liver diseases involves a multifaceted approach aimed at mitigating liver damage, promoting liver regeneration, and addressing nicotine dependence. Evidence from pharmacological interventions such as N-acetylcysteine (NAC) and silymarin (milk thistle extract) has shown the potential to reduce oxidative stress and inflammation in the liver, thereby aiding in the treatment of liver fibrosis (Demiroren et al., 2018; Emadi et al., 2022). Additionally, the use of anti-fibrotic agents like angiotensin II receptor blockers (ARBs) and peroxisome proliferator-activated receptor (PPAR) agonists has been explored to reverse liver fibrosis and improve liver function (Kim et al., 2017).

Some herbal treatments provide promising alternative approaches for managing nicotine-related liver diseases by leveraging the natural hepatoprotective properties of certain plants. For example, milk thistle (Silybum marianum) exhibits antioxidant, anti-inflammatory, and anti-fibrotic effects, aiding in liver regeneration and reducing inflammation (Loguercio, & Festi, 2011). Curcumin, the active ingredient in turmeric (Curcuma longa), protects liver cells from oxidative damage and inhibits hepatic stellate cell activation, thereby preventing fibrosis (Singh, & Sharma, 2011). Dandelion (Taraxacum officinale) root improves bile flow, aids detoxification, and exhibits hepatoprotective effects by reducing oxidative stress and inflammation (Borém et al., 2018; Pfingstgraf et al., 2021). Licorice root (Glycyrrhiza glabra) contains glycyrrhizin, which reduces liver enzyme levels and inhibits fibrosis by suppressing hepatic stellate cell proliferation (Van Rossum et al., 1998). Green tea (Camellia sinensis) catechins, particularly epigallocatechin gallate (EGCG), have strong antioxidant properties that reduce oxidative stress and inflammation in the liver, preventing liver damage and fibrosis (Neuman et al., 2015; Musial et al., 2020). Schisandra (Schisandra chinensis) enhances the liver detoxification processes and protects against liver damage by promoting

antioxidant enzyme activity and reducing inflammation (Panossian, & Wikman, 2008).

Vernonia cinerea (L.) Less, also known as Cyanthillium cinereum, is proposed as a promising treatment for nicotine-related liver diseases due to its long-standing use in traditional medicine throughout Southeast Asia (Monton et al., 2023). This perennial plant, belonging to the Asteraceae family, is commonly found throughout Thailand, known as Ya Dok Khao or Ya Moh Noi (Srithanee et. al., 2022). Traditionally, *Vernonia cinerea* has been used to treat a range of ailments, including fever, cough, eye infections, urinary bladder disorders, diarrhea, stomach pain, gastrointestinal disorders, and malaria when it is mixed with quinine (Pratheeshkumar, & Kuttan, 2011).

Scientific studies have demonstrated the diverse pharmacological activities of *Vernonia cinerea* (VC), including nephroprotective (Amuthan et al., 2021), antimicrobial, antioxidant (Sonibare et al., 2016), hepatoprotective against carbon tetrachloride-induced damage (Leelaprakash et al., 2011), and anti-inflammatory effects (Mazumder et al., 2003). These therapeutic benefits are attributed to its rich phytochemical profile, which includes sterols, flavonoids, triterpenoids, sesquiterpenes, and tannins. Trang et. al, (2024) highlighted the role of these phytochemicals in treating inflammatory diseases and preventing oxidative stress. Moreover, Al-Khayri et al., (2022) emphasized the potential of flavonoids in *Vernonia cinerea* to reduce the activity of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1).

Despite the extensive traditional and scientific reports on Vernonia cinerea's medicinal properties, its use in protecting against liver damage specifically caused by chronic nicotine exposure remains underexplored. Therefore, this study aims to investigate the morphological changes in parenchymal and non-parenchymal cells in liver tissue subjected to prolonged nicotine exposure and the potential hepatoprotective effects of *Vernonia cinerea*.

Given the broad spectrum of Vernonia cinerea's medicinal applications, this research seeks to provide evidence supporting its use as an alternative treatment for nicotine-related liver diseases. By exploring its systemic protective effects, this study aims to establish *Vernonia cinerea* as a viable option for mitigating liver damage induced by chronic nicotine exposure.

2. Objectives

The objective of this study is to investigate the effects of aqueous extracts from the Ya Dok Khao herb (*Vernonia cinerea*) in protecting liver tissue from inflammation and fibrosis in rats chronically exposed to nicotine.

3. Materials and methods

In this work, we used Hematoxylin and Eosin staining to examine the histological morphologies in liver tissue and investigated the pathological changes of liver tissue by the special stain of Masson. Next, we analyzed the histological appearances representing the characteristics of how *Vernonia cinerea* protected against inflammation and slowed pro-fibrosis in hepatic tissue in nicotine-exposed rats.

3.1 VC Collection

The whole plant was gathered from the Ongkharak Campus of Srinakharinwirot University (Thailand). *Vernonia cinerea* aqueous extract was prepared at the Laboratory Center of the Faculty of Medicine, Srinakharinwirot University, Bangkok (Thailand).

3.2 Plant Extraction

The whole VC plant was cleaned with running tap water, sun-dried, and then incubated in a hot air oven. The dried plants were powdered for the preparation of extracts. The crude extract was prepared by shaking 500 g of the powdered sample in 2.0 L of distilled water, followed by an ultrasonic bath at 40°C for 2 hours. The solvent was filtered through Whatman-grade filter paper. The filtrate was divided into flasks and stored in a deep freezer (–20°C), then further stored in a freezer (–80°C) and finally dried by a freeze-dryer. The crude extract yield was calculated using the formula: (weight of extract/weight of VC) x 100.

3.3 Animals

Thirty male Wistar rats (6 weeks old; 180 – 200 g) were obtained from the National Laboratory Animal Office, Mahidol University Salaya Campus. The animals were maintained on a balanced diet with free access to water and food for 3 months under controlled conditions of light/dark cycles (12-hour light/12-hour dark), room temperature (25 ± 2°C), and relative humidity (40 – 75%). The management and care of animals were administered at the Laboratory Center of the Faculty of Medicine, Srinakharinwirot

University, Bangkok. The 30 animals were divided into 3 groups based on the treatment duration:

The control group received 0.5 mL of Normal saline solution (NSS) 0.9%/day.

The nicotine group (N) received 1 mg/kg of nicotine/day.

The N+V group received 1 mg/kg of nicotine/day and 100 mg/kg/day of *Vernonia cinerea* aqueous extract orally.

All administrations were performed intra-peritoneally (i.p.) with concentrations measured as mg/kg. The weight of the rats was measured daily during the experimental period.

3.4 Tissue Preparation and Staining Method

The animal models were terminated by an anesthetic overdose of sodium pentobarbital (PB). Visceral organs were exposed, and the liver was perfused through the aorta. The liver was then fixed with 4% formaldehyde and Bouin's solution (Gibco, Billings, MT, USA), dehydrated, embedded in paraffin, cut into 5 μm thick slices, and mounted on glass slides. Liver tissues were collected to investigate pathological morphology using H&E techniques according to the Sigma Aldrich protocol. Liver fibrosis was assessed by staining with Masson's trichrome (Sigma Aldrich, St. Louis, MO, USA) according to the Sigma Aldrich protocol.

3.5 Kupffer Cell Counts

Histological slides were examined using the Panoramic digital slide scanner (3DHISTECH Co). Whole slide imaging (WSI) was used to evaluate histopathological features by translating tissue morphology from glass slides into a digital form similar to standard microscopy. All glass slides were scanned using a high-resolution camera at ×400 magnification. For semi-quantifying the Kupffer cell population, nucleated Kupffer profiles were counted under a light microscope. Twenty fields with an area of 0.1 mm² were delineated by the viewfinder of the photomicroscope, and ten sections were viewed per rat.

3.6 Statistical Analysis

The results were presented as the mean ± standard error of the mean (SEM). Kupffer cell density was analyzed by comparing the control and experimental groups. Data from two groups were compared using an unpaired t-test. Data from three groups were analyzed using one-way ANOVA with Tukey's multiple comparison test. Significance was based on P values < 0.05 using the GraphPad Prism 5.1 application.

4. Results and Discussion

4.1 Pathological Changes

Figure 1 presents photomicrographs of Hematoxylin & Eosin (H&E)-stained sections of liver tissue. The images compare liver tissues from control rats (Figures 1A and 1D), nicotine-exposed rats (Figures 1B and 1E), and nicotine-exposed rats treated with *Vernonia cinerea* (VC) extract (Figures 1C and 1F).

4.1.1 Control Group (Figure 1A and 1D)

In the control group (Figure 1A), the liver tissue exhibits the normal size and structure of a hepatic acinus with functional metabolic zones between the central vein (cv) and the portal triad. The normal hepatic acinus of Rappaport is uniform and divided into three zones: zone 1 (periportal zone), zone 2 (middle zone), and zone 3 (pericentral zone). The hepatocytes in the control group are large, polygonal cells with cytoplasmic acidophilic (pink) granules, round nuclei, and prominent nucleoli. The hepatic sinusoids are normal, connecting the three zones and radiating from the central vein to the portal triad. The central vein is lined by intact endothelium, and the hepatic cords are well-arranged with sinusoids appearing between the rows of hepatic cords (H&E x 100) (Figure 1D).

4.1.2 Nicotine-Exposed Rats (Figures 1B and 1E)

In the nicotine-exposed rats (Figure 1B), significant liver damage was observed. The structure of the hepatic lobules was disrupted, with less clearly defined zones. The central vein (cv) showed damage, indicated by black arrows, and there was an increased presence of Kupffer cells (asterisks), suggesting an inflammatory response. The hepatic sinusoids (S) were dilated and irregular, indicating liver stress and potential fibrosis (H&E x100) (Figure 1E).

It should be mentioned here that Kupffer cells, the liver's resident macrophages, play a crucial role in defending and clearing the blood of pathogens and foreign particles. The migration of Kupffer cells to lesion sites contributes to the inflammatory progression mainly through the increased production of inflammatory mediators (Latha et al., 2010). These mediators, including cytokines, chemokines, and proteolytic enzymes, enhance cytotoxicity and chemotaxis, thereby exacerbating tissue damage (Jongrungruangchok et al., 2019; Slevin et al., 2020).

Moreover, previous studies have shown that inflammatory cell infiltration, including Kupffer cells, along with degenerative changes in hepatocytes, blood vessel congestion, and sinusoid dilation, represent the main histological features of liver tissue inflammation.

4.1.3 Nicotine-Exposed Rats Treated with Vernonia cinerea Extract

In the nicotine-exposed rats treated with *Vernonia cinerea* extract (Figures 1C and 1F), the liver tissue showed significant improvement compared to the untreated nicotine group. The hepatic acinus structure was more defined, resembling the control group. The central vein (cv) exhibits reduced damage, and the hepatic sinusoids (S) appear more regular and less dilated. The number of Kupffer cells (asterisks) decreased, indicating a reduction in inflammation. The improved tissue architecture, including the better-defined zones and reduced signs of inflammation and fibrosis, suggests that *Vernonia cinerea* extract has hepatoprotective properties (H&E x 100) (Figure 1F).

The pathological changes of liver tissues indicate that the control group of male rats exhibited normal hepatic acini with distinct functional metabolic zones (zone 1, zone 2, and zone 3), characterized by healthy hepatocytes, normal hepatic sinusoids, and central veins. In contrast, chronic nicotine exposure significantly disrupted this normal structure, resulting in notable damage and inflammation. Specifically, nicotine-induced liver tissue inflammation was evidenced by the disturbance of hepatic lobules and marked injury in the central vein, including mild endothelial degeneration. The periportal and pericentral zones of the hepatic acinus displayed moderate sinusoid dilation, and a substantial presence of Kupffer cells was observed in the pericentral area (Figures 1B and 1E). However, treatment with *Vernonia cinerea* extracts ameliorated these adverse effects, demonstrating its potential as a therapeutic agent for nicotine-induced liver damage.

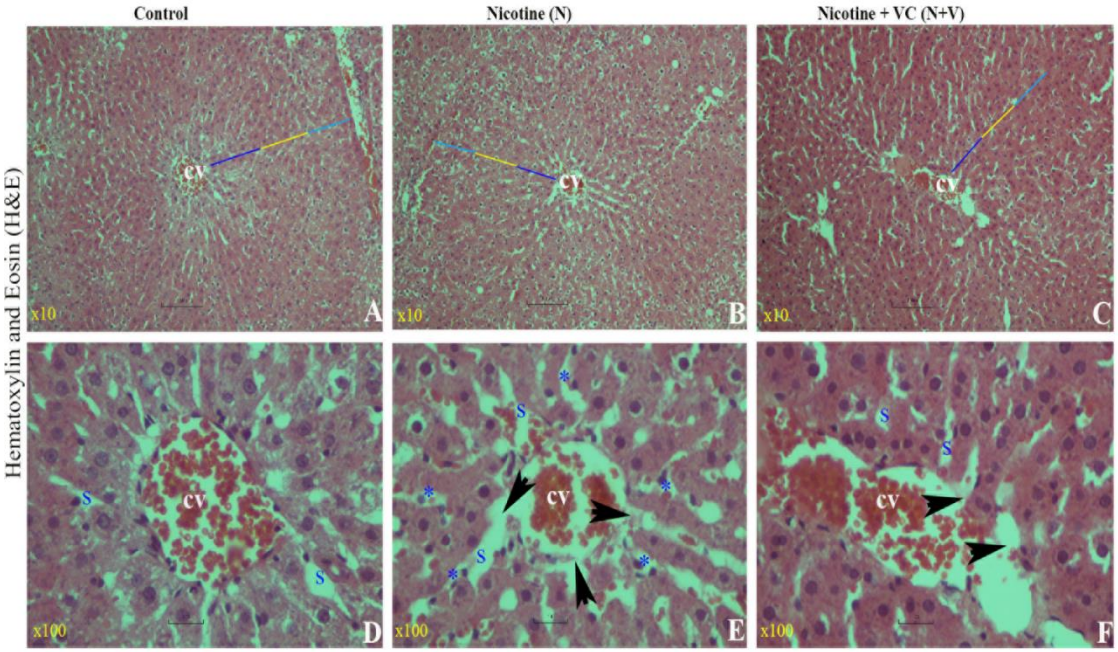

Figure 1 Photomicrographs of Hematoxylin & Eosin (H&E)-stained liver sections. Top row (x10): (A) Control, (B) Nicotine-exposed, (C) Nicotine-exposed treated with *Vernonia cinerea* extract. Bottom row (x100): (D) Control, (E) Nicotine-exposed, (F) Nicotine-exposed treated with *Vernonia cinerea* extract. cv = central vein, s = hepatic sinusoid, asterisk (*) = Kupffer cells, 1 = periportal zone, 2 = middle zone, 3 = pericentral zone, and black arrows indicate damaged endothelium of the central vein.

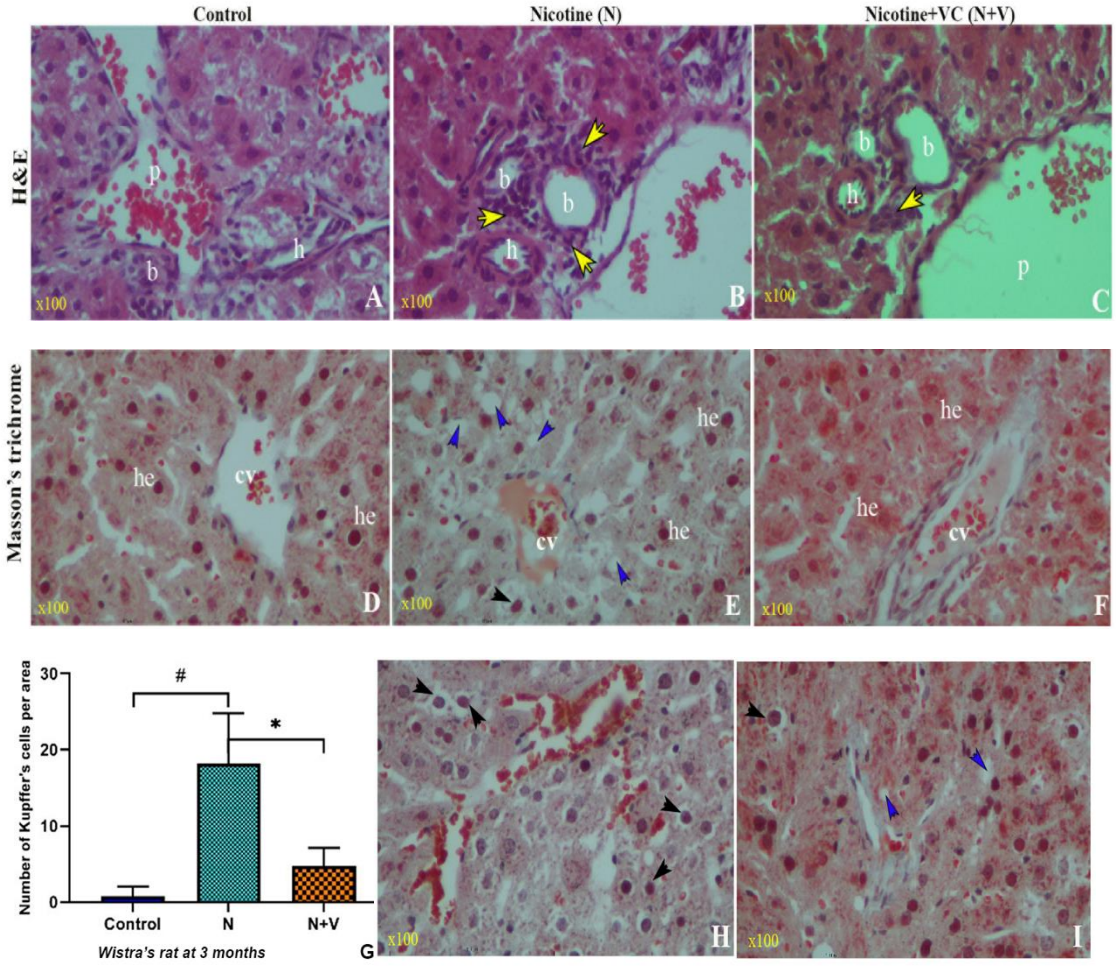

Figure 2 Photomicrographs of liver tissue stained with Hematoxylin & Eosin (H&E) and Masson's trichrome under high magnification (x100). Top row (H&E staining): (A) Control group with normal hepatic architecture, (B) Nicotine-exposed (N) group with pro-inflammatory cells (yellow arrows) around the portal triad, and (C) Nicotine-exposed treated with *Vernonia cinerea* (N+V) group showing reduced inflammation. Middle row (Masson's trichrome staining): (D) Control group with normal hepatocytes (he) and central vein (cv), (E) Nicotine-exposed group with cytoplasmic vacuoles (blue arrows) and central vein injury, and (F) Nicotine-exposed treated with *Vernonia cinerea* group showing reduced vacuolation and degeneration. Bottom row: (G) Graph showing a significant reduction in Kupffer cells in the N+V group compared to the N group (*P<0.01, #P<0.01). (H) Nicotine-exposed group with increased Kupffer cells (black arrows), and (I) Nicotine-exposed treated with *Vernonia cinerea* group with fewer Kupffer cells

cv = central vein, s = hepatic sinusoid, * = Kupffer cells, 1 = periportal zone, 2 = middle zone, 3 = pericentral zone, yellow arrows = pro-inflammatory cells, blue arrows = cytoplasmic vacuoles, black arrows = hepatocyte degeneration.

4.2 *Vernonia cinerea* with Anti-Inflammation

Figure 2 shows liver tissue stained with Hematoxylin & Eosin (H&E) (Figures 2A-2C) and Masson's trichrome (Figures 2D-2F, 2H, and 2I) under high magnification (x100) in Control (Figure 2A, 2D), Nicotine-exposed (N) group (Figure 2B, 2E, and 2H), and Nicotine-exposed treated with *Vernonia cinerea* extract (N+V) group (Figures 2C, 2F, and 2I).

Control Group (Figures 2A and 2D): The liver tissue of control rats displayed normal hepatic architecture with well-defined hepatocytes (he), central veins (cv), and normal branches of the bile duct (b), hepatic artery (h), and portal vein (p).

Nicotine-Exposed Group (Figures 2B, 2E, and 2H): In the nicotine-exposed group, there was a marked aggregation of pro-inflammatory cells around

the portal triad (yellow arrows in Figure 2B). Moderate fatty changes and vacuolation (blue arrows) around the portal areas, portal triad, and central veins were observed (Figures 2E and 2H). Hepatocytes exhibited mild degenerative nuclei, markedly vacuolated cytoplasm, and cell necrotic debris, indicating significant liver damage compared to the control group.

Kupffer cells, which are liver-fixed macrophages, increased in number, contributing to inflammatory progression through the production of inflammatory mediators such as cytokines and chemokines (Figures 2B and 2E). The involvement of these cells in the inflammatory response is supported by previous studies (Slevin et al., 2020), highlighting their role in liver disease initiation.

Nicotine-Exposed + *Vernonia cinerea* Extract Group (Figures 2C, 2F, and 2I): Treatment with *Vernonia cinerea* extract showed notable protective effects on liver tissue. The periportal zone of the hepatic acinus exhibited fewer pro-inflammatory cells and Kupffer cell infiltrates compared to the nicotine-exposed group (Figure 2C). Slight vacuolation and single-cell nuclei degeneration were observed (Figures 2F and 2I), indicating reduced damage and inflammation. The *Vernonia cinerea* extract appears to block or delay the progression of collagen fiber development, which is essential for wound repair and reducing fibrogenesis.

Quantitative Analysis (Figure 2G): The graph illustrates the intensity of Kupffer cells in zone 1 and zone 2 of the hepatic acinus at 3 months, with the experimental results expressed as mean ± standard error of the mean (SEM). There was a significant reduction in Kupffer cell count in the N+V group compared to the N group (*P<0.01, #P<0.01).

Overall, these results demonstrate that *Vernonia cinerea* extract has a protective effect against nicotine-induced liver damage, reducing inflammation and fibrogenesis. This is consistent with previous studies reporting the hepatoprotective, anti-inflammatory, and antioxidant properties of *Vernonia cinerea* (Leelaprakash et al., 2011; Leelavathi et al., 2023).

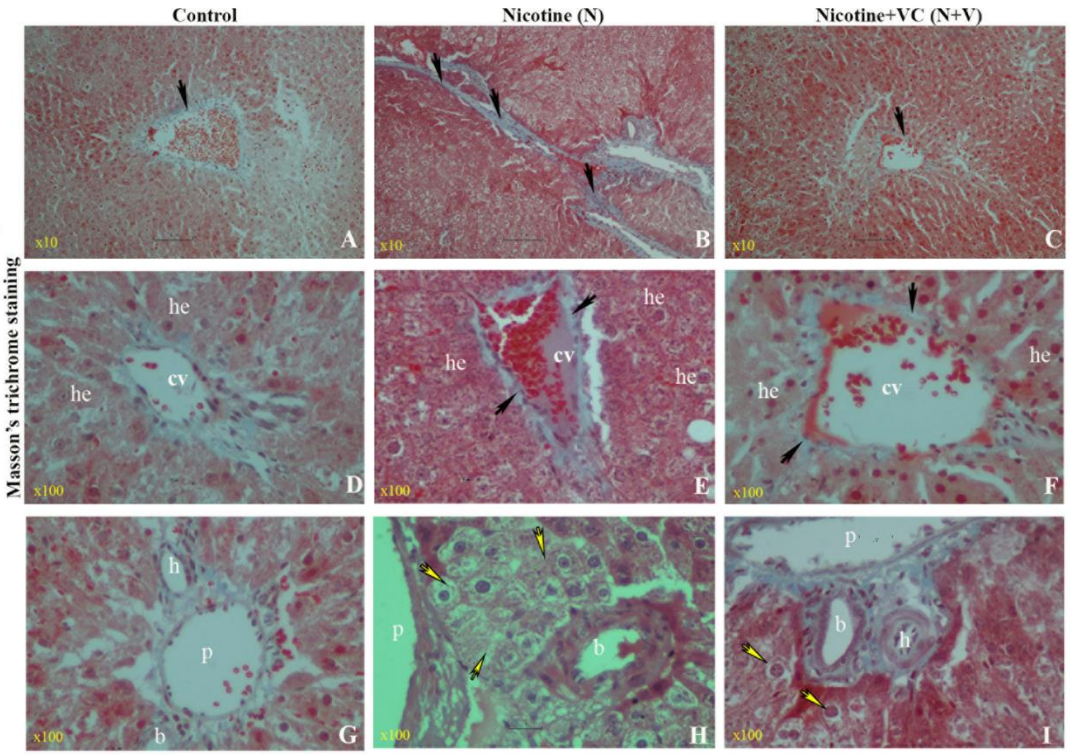

Figure 3 Photomicrographs of Masson's trichrome-stained liver sections. (A, D, G) Control rats, (B, E, H) Nicotine-exposed rats (N), (C, F, I) Nicotine-exposed rats treated with *Vernonia cinerea* extract (N+V). (A-F) Tissue sections highlighting the central vein (cv), collagen bundles (black arrows, x10), and hepatocytes (he). (G-I) Tissue sections highlighting the portal triad, including the portal vein (p), common bile duct (b), hepatic artery (h), and hepatocyte hypertrophy (yellow arrows)

4.3 *Vernonia cinerea* with Anti-profibrotic Activity

In the control group (Figures 3A, 3D, and 3G), liver tissue shows normal architecture with no significant collagen deposition. Hepatocytes (he) appear healthy, and the central vein (cv) and portal triad structures, including the portal vein (p), common bile duct (b), and hepatic artery (h), are well-organized with no signs of hypertrophy or fibrosis.

In the nicotine-exposed group (Figures 3B, 3E, and 3H), there is significant deposition of thick collagen fibers, indicated by black arrows, particularly around the central vein and portal triad. Hepatocyte hypertrophy and increased collagen deposition suggest active fibrogenesis and liver damage due to nicotine exposure.

Conversely, in the nicotine-exposed rats treated with *Vernonia cinerea* extract (Figures 3C, 3F, and 3I), there is a notable reduction in collagen fiber accumulation. The collagen fibers are less prominent around the central vein and portal triad, indicating a protective effect of the *Vernonia cinerea* extract against fibrogenesis. Hepatocyte hypertrophy is also reduced, as shown by the fewer yellow arrows indicating hypertrophic cells.

These findings demonstrate that *Vernonia cinerea* extract effectively mitigates collagen deposition and hepatocyte hypertrophy, suggesting its potential role in protecting against nicotine-induced liver fibrosis. The reduction in collagen fibers in the N+V group supports the hypothesis that *Vernonia cinerea* extract has anti-fibrotic and hepatoprotective properties, likely by blocking or delaying the progression of collagen fiber development during wound repair. Further studies are needed to confirm these effects and explore the underlying mechanisms.

5. Conclusion

The pathological examination of liver tissues revealed that the control group of male rats exhibited normal hepatic architecture with healthy hepatocytes, distinct metabolic zones, and well-structured hepatic sinusoids, and central veins. Chronic nicotine exposure significantly disrupted this normal hepatic structure, leading to notable damage and inflammation, characterized by disturbed hepatic lobules, central vein injury, and sinusoid dilation, particularly in the periportal and pericentral zones. These adverse effects were accompanied by an increased presence of Kupffer cells, indicating an inflammatory response.

The treatment with *Vernonia cinerea* extract showed a marked protective effect against nicotine-induced liver damage. The *Vernonia cinerea* extract mitigated inflammation and fibrogenesis, demonstrating its hepatoprotective, anti-inflammatory, and antioxidant properties. Specifically, *Vernonia cinerea* extract significantly reduced collagen deposition and hepatocyte hypertrophy, highlighting its potential in preventing nicotine-induced liver fibrosis. The observed reduction in collagen fibers supports the hypothesis that *Vernonia cinerea* extract possesses anti-fibrotic properties, likely by inhibiting or delaying the progression of collagen fiber development during the wound repair process.

The results of this work consistently demonstrate that *Vernonia cinerea* extract has a protective effect against nicotine-induced liver damage across various parameters. These findings align with previous studies, further reinforcing the consistency and reliability of these outcomes. Moreover, the anti-inflammatory and anti-fibrogenic properties of *Vernonia cinerea* suggest its potential therapeutic application for chronic lung diseases induced by nicotine exposure.

In summary, *Vernonia cinerea* extract shows significant potential as a therapeutic agent for nicotine-induced liver damage and possibly chronic lung conditions. Further studies are warranted to confirm these protective effects, elucidate the underlying mechanisms, and explore its efficacy in treating other chronic diseases.

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