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Voacanga grandifolia (Miq.) Rolfe protects against alcohol-induced liver toxicity in rats

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ABSTRACT

Objective: To evaluate the ethanol extract of *Voacanga grandifolia* for hepatoprotective and antioxidant potential against ethanol-induced liver toxicity in rats.

Methods: Sprague-Dawley rats were administered ethanol (7 g/kg) and then treated with 100 and 200 mg/kg of *Voacanga grandifolia* extract. The phytochemical constituents and antioxidant potential of *Voacanga grandifolia* extract were evaluated by GC-MS and *in vitro* antioxidant assays. Biochemical indicators for liver damage and pro-apoptotic and antiapoptotic gene expression were determined using biochemical kits, ELISA, and qRT-PCR, respectively. Additionally, histopathological study of the liver was performed.

Results: GC-MS identified propanoic acid, meso-erythritol, *D*-pinitol, myo-inositol, and hexadecanoic acid in *Voacanga grandifolia* extract. *Voacanga grandifolia* extract (100 and 200 mg/kg) increased the concentration of enzymatic antioxidants while diminishing the levels of inflammatory cytokines and biochemical indicators. qRT-PCR assay showed that *Voacanga grandifolia* extracts upregulated antiapoptotic gene expression while downregulating pro-apoptotic gene expression. Furthermore, the plant extract improved the hepatic architecture of ethanol-intoxicated rats.

Conclusions: *Voacanga grandifolia* extract demonstrates hepatoprotective activity against alcohol-induced liver injury in rats and could be a potential hepatoprotective agent.

KEYWORDS: Antioxidant; Ethanol-induced toxicity; Hepatoprotective; Hepatic markers; *Voacanga grandifolia*

1. Introduction

Alcohol is the most common psychotropic or pleasurable stimulant after coffee. Long-term alcohol intake increases the risk of major health problems that include injuries, irritability, cancer, and liver problems. The World Health Organization states that drinking alcohol may be more detrimental to hypertension and hypercholesterolemia than smoking cigarettes. The liver is the organ that is responsible for metabolizing alcohol that has been consumed[1]. Alcohol causes oxidative stress in liver cells, resulting in acetaldehyde generation, cell membrane and mitochondrial

Significance

Alcohol intake is detrimental to the health of the liver. Furthermore, the adverse side effects of existing synthetic hepatoprotective medications, as well as the increasing risk factors for hepatotoxicity, necessitate the development of new drugs of natural origin. The present findings demonstrate that the *Voacanga grandifolia* plant has hepatoprotective effects against alcohol-induced liver toxicity and suggest that it may be utilized as a possible option for the treatment of hepatic toxicity.

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impairment, hypoxia, immunologic dysfunction, cytokine generation, CYP2E1 induction, and iron mobilization. Fatty liver, steatosis, alcoholic hepatitis, and liver cirrhosis are the stages of alcoholic liver disease. The development of alcoholic liver disease may be triggered by reactive intermediates generated during the reduction of molecular oxygen. Reactive oxygen species (ROS) are thought to be a cause of chronic liver illness, and previous research suggests that ethanol or its metabolic products may act as pro-oxidants or induce a decline in antioxidants in the body as well as a surge in ROS[2]. ROS are potent oxidizing radicals that severely damage DNA, proteins, and lipids. An imbalance between free radicals and antioxidants causes oxidative stress, which threatens human life by increasing the risk of developing cancer, diabetes, heart disease, and brain abnormalities[3]. In order to survive and maintain a healthy lifestyle, strengthening defense mechanisms is a constant necessity. Antioxidants are regarded as a good approach to minimizing oxidative stress because they restrict the development of ROS, stop the chain reaction, scavenge free radicals, and chelate transition metals that enhance the formation of free radicals[4]. To alleviate oxidative stress, a number of synthetic antioxidants are commercially available; however, they all have adverse side effects[5,6]. Because of their synthetic nature and composite structure, plant-derived or herbal compounds may function as antioxidants[7]. Polyphenols, a class of phytochemicals with a phenolic hydroxyl structure that has attracted the interest of researchers due to their potent antioxidant and hepatoprotective properties, are abundant in plants[8].

Many plants of the *Voacanga* genus (Apocynaceae) have been examined for biological activities and bioactive constituents. A few species in the genus *Voacanga* have been employed as traditional medicines for a long time. The *Voacanga africana* Staph fruit bark and leaf extracts were utilized to treat infant convulsions, diarrhea, generalized edema, leprosy, and madness in West Africa[9]. In subsequent studies on rats with pylorus ligation, the aqueous extract improved stomach mucosal damage, but did not affect gastric juice acidity[10]. The plant *Voacanga grandifolia* (*V. grandifolia*) has been underutilized for medicinal uses. However, novel phytocompounds, primarily the indole alkaloids, have been found in *V. grandifolia*. Desacetylvindoline was used to isolate voacinol, a stereochemically symmetric alkaloid related to bisindole alkaloid, from *V. grandifolia* leaves[11]. Researchers discovered five new indole alkaloids called voacalgines A-E[12]. The leaves of *V. grandifolia* contained two new bisindole alkaloids, 12′-*O*-demethyl-vobtusine-5-lactam, and isovobtusine-*N*-oxide, as well as two previously identified bisindole alkaloids[13]. Non-alkaloid compounds found in *V. grandifolia* extract included lupeol acetate, -sitosterol, and alkaloid rhazine[14]. As part of our ongoing study on medicinal plants, the extract of *V. grandifolia* leaves growing at the botanical garden of National Botanical Research Institute was studied for its hepatoprotective potential in the present study.

2. Materials and methods

2.1. Chemicals and kits

Sigma Aldrich supplied ethylenediaminetetraacetic acid and the Enhanced Avian HS RT-PCR kit (USA). Transasia Bio-Medicals Ltd. supplied a biochemical reagent kit (India). Ethanol and an ELISA kit were procured from Sigma Aldrich and Elabscience. All other analytical-grade chemicals and reagents were procured from Merck and Himedia Pvt. Ltd. (India).

2.2. Extract preparation and phytochemical characterization

V. grandifolia leaves were obtained in May 2019 in Lucknow, Uttar Pradesh, India. The collected sample was identified by Dr. A.K.S. Rawat, a scientist at the National Botanical Research Institute in Lucknow. The herbarium (NBRI/G/976/2019) was deposited in the institute for future reference. The leaves were ground (to a weight of 500 g) and extracted in 1.5 L of ethanol for up to 7 d. A rotary evaporator was used to concentrate the extract, and a lyophilizer was used to freeze-dry it. The dried extract was kept for hepatoprotective investigations. Total phenolic content was estimated as mg of gallic acid equivalent (GAE)/g of *V. grandifolia* extract[15]. Total flavonoid content was calculated and expressed as mg of quercetin equivalent (QE)/g of *V. grandifolia* extract[16]. GC-MS was used to examine the phytoconstituents of *V. grandifolia* leaf extract. *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide was used to derivatize the sample (*V. grandifolia* leaf extract). GC-MS equipment, which consisted of a gas chromatograph (Thermo Trace GC Ultra) and a mass spectrometer, was employed to examine the derivatized extract sample (Thermo Fisher DSQ II). A mass-selective electron impact mode detector with a 70-eV ionization energy, 2.0 mA ionization current, and a mass range of 50-800 *m/z* was utilized to capture the data. The software analyzes the *m/z* ratio values of each metabolite fragment seen in mass spectra using GC-MS spectral library databases like WILLY and NIST. A percentage of the peak area was used to estimate the relative content of the identified metabolites.

2.3. In vitro antioxidant activity

The antioxidant activity of *V. grandifolia* leaf extract was assessed using the DPPH radical[15]. The reducing potential of *V. grandifolia* leaf extract was calculated using linear regression and a calibration curve at 515 nm. The results of *V. grandifolia* leaf extract were compared to those of ascorbic acid, a standard antioxidant. The equation was used to examine the reduction of DPPH radicals.

$$DPPH^{\cdot} \text{ radical inhibition} = (\text{Control}–\text{Sample})/\text{Control}\times 100$$

The ferric-reducing power was measured and expressed as mg ascorbic acid equivalents per gram of *V. grandifolia* leaf extract using the ferric-reducing power assay[16].

2.4. Experimental animal

Sprague Dawley rats were purchased from the CSIR-Central Drug Research Institute in India, and they were kept in a controlled environment [(22 ± 1)℃ temperature; 45%-55% relative humidity] with a 12 h light/12 h dark cycle, as well as had free access to food pellets and water. Acute toxicity testing was carried out in accordance with OECD Guideline 423. Administration of *V. grandifolia* leaf extract at 300 mg/kg b.w. to rats was demonstrated to be safe, with no aberrant behavior or death. Twenty rats were separated into four groups of five animals each (n=5). Except group Ⅰ, all groups of the animals were given ethanol (7 g/kg) orally for 28 d. Groups Ⅲ and Ⅳ were administered *V. grandifolia* leaf extract (100 and 200 mg/kg, *p.o.*) once daiy. Group Ⅱ animals were given 0.5% sodium carboxyl methyl cellulose[17]. The animals were fasted overnight and sacrificed by cervical dislocation after two weeks of treatment. Blood was drawn and serum was separated for biochemical marker analysis by centrifugation at 1 000 ×g for 15 min. The vital organs were excised and cleaned in phosphate buffered saline before being preserved in formalin. For histological examination, the liver tissue was sectioned into 3-5 μm thick slices and stained by hematoxylin and eosin according to standard laboratory methods. Finally, the stained sections were analyzed for cell injury or modifications in the morphology of specific tissues under the microscope (Leica DM6 B Germany)[18]. The remaining tissue was stored at −80℃ for enzymatic antioxidants and molecular analysis.

2.5. Determination of biochemical parameters

Biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), and total bilirubin were determined using biochemical kits (Transasia Biomedicals Ltd) from obtained blood serum using an auto chemistry analyzer (Csense 100).

2.6. Determination of antioxidant enzymes and oxidative stress marker

A total of 400 mg of hepatic tissue was homogenized in phosphate buffer (10 mM, pH 7.4) with KCl (1.15%) and ethylenediaminetetraacetic acid (1.15%, pH 7.4), followed by centrifugation at 1 000 ×g for 15 min to obtain liver tissue homogenate.

Superoxide dismutase (SOD) (EC 1.15.1.1) was measured according to the method of Aebi[19], and the activity of catalase (EC1.11.1.6) was measured as a mole of H_2O_2 consumed per mg of protein. The activity of glutathione peroxidase (GPX) was evaluated using the method described by Rotruck *et al*[20].

The activity of glutathione S-transferase (GST; EC 2.5.1.13) was also measured[21]. Moreover, MDA level was determined to evaluate lipid peroxidation by quantifying thiobarbituric acid reactive chemicals[22]. Since ethanol-induced free radicals and oxidative stress can decline glutathione (GSH) levels, GSH content was also determined in this study[23].

2.7. Determination of interleukins and TNF-α in hepatic tissue

Interleukins and TNF-α were determined using ELISA kits (Elabscience Biotech Co. ltd. Wuhan, Hubei, China) based on principle of standard sandwich ELISA technology.

2.8. Quantitative real-time PCR (qRT-PCR) analysis

The RNA was isolated using the TRizol reagent. At 260/280 nm, the NanoDrop device was used to assess the purity and quantity of RNA. The Enhanced Avian HS RT-PCR kit was used to synthesize cDNA from extracted total RNA (Sigma Aldrich, USA). To determine total transcript levels, this cDNA was utilized as a template for qRT-PCR in a StepOne real-time PCR machine with SYBR Green PCR Master Mix (Applied Biosystems, USA). $2^{-\Delta\Delta Ct}$ method was used to evaluate the gene expressions[24]. The primer sequences are listed in Table 1.

2.9. Statistical analysis

All results were expressed as mean±SD of pentaplicate and analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test using the IBM SPSS package (v20). P<0.05 was considered significantly different.

2.10. Ethical statement

Male Sprague Dawley rats were used in this study according to the regulations of the Institutional Animal Care Committee (CPCSEA) India (Approval no. 1732/GO/Re/s/13/CPCSEA).

Table 1. The sequences of primers used in the study.

Gene	Sequences of primers (5'-3')
Bcl-2	F 5'GTGGATGACTGAGTACCTGAAC 3'
	R 5' CAGCCAGGAGAAATCAAACAG 3'
p53	F 5'GCCATCTACAAGCAGTCACAG 3'
	R 5'TCATCCAAATACTCCACACGC 3'
Bax	F 5'AGTAACATGGAGCTGCAGAG 3'
	R 5' AGTAGAAAAGGGCGACAACC 3'
Caspase-3	F 5'ACTGGACTGTGGCATTGAG 3'
	R 5'GAGCCATCCTTTGAATTTCGC 3'
Caspase-9	F 5' AGTTCCCGGGTGCTGTCTAT 3'
	R 5' GCCATGGTCTTTCTGCTCAC 3'
GAPDH	F 5' GCATGGCCTTCCGTGTTCC 3'
	R 5' GGGTGGTCCAGGGTTTCTTACTC 3'

F: forward; R: reverse.

3. Results

3.1. GC–MS analysis of *V. grandifolia* extract

The phytochemical profile of *V. grandifolia* extract was investigated by GC-MS. Table 2 shows a list of the identified compounds, together with their chemical formula, retention time, and peak area (%). The major detected compounds are propanoic acid (77.5%), meso-erythritol (36.2%), 2,3,4 trihydroxybutyric acid (35.6%), *D*-pinitol (44.9%), myo-inositol (40.4%), hexadecanoic acid (91.8%) and octadecanoic acid (43.3%) (Table 2, Supplementary Figure 1).

3.2. Total phenolic and flavonoid content of *V. grandifolia* extract

The extraction yield of *V. grandifolia* leaf extract was 20.71% *w/w*. The total phenolic content of *V. grandifolia* leaf extract was (36.00±2.13) mg GAE/g *V. grandifolia* extract, while the total flavonoid content was (21.00±1.21) mg QE/g *V. grandifolia* extract.

3.3. In vitro antioxidant activity of *V. grandifolia* extract

With increasing concentration, the effect of *V. grandifolia* leaf extract on DPPH radical scavenging increased. The scavenging activities of *V. grandifolia* leaf extract on DPPH radical were 22%, 35%, 47%, 66%, and 85% at 25, 50, 75, 100, and 125 μg/mL, respectively with an IC_{50} value of 56 μg/mL. The reducing power of *V. grandifolia* leaf extract demonstrated the antioxidant capacity to reduce Fe^{3+} to Fe^{2+}. The reducing power of *V. grandifolia* leaf extract was (31.21±1.43) mg ascorbic acid equivalents/g *V. grandifolia* leaf extract.

3.4. Effect of *V. grandifolia* leaf extract on biochemical parameters

Ethanol significantly increased the levels of ALT, AST, ALP, GGT, and TBL as compared to the normal control group. Treatment with *V. grandifolia* leaf extracts at both doses markedly reduced these parameters (Table 3). *V. grandifolia* leaf extract at 200 mg/kg showed a more significant effect on normalizing the levels of ALT, AST, ALP, GGT, and TBL compared with 100 mg/kg *V. grandifolia* extract.

3.5. Effect of *V. grandifolia* leaf extract on antioxidant activity and lipid peroxidation

Ethanol administration prominently declined antioxidant activities including SOD, GST, GSH, GPX, and catalase, and concomitantly increased MDA level. *V. grandifolia* leaf extract (100 mg/kg and 200 mg/kg) markedly enhanced the activities of SOD, GST, GSH, GPX, and catalase and decreased MDA level (Table 4).

Table 2. Phytochemical constituents of *Voacanga grandifolia* ethanol extract detected by GC-MS.

Compound	Retention time (min)	Molecular weight	Molecular formula	Peak area (%)
Propanoic acid	15.38	74.08	$CH_3CH_2CO_2H$	77.5
meso-Erythritol	18.19	122.12	$C_4H_{10}O_4$	36.2
2,3,4-Trihydroxybutyric acid	20.00	136.10	$C_4H_8O_5$	35.6
D-Pinitol	28.44	194.18	$C_7H_{14}O_6$	44.9
Myo-inositol	29.54	180.15	$C_6H_{12}O_6$	40.4
Hexadecanoic acid, trimethylsilyl ester	32.27	256.43	$C_{16}H_{32}O_2$	91.8
Octadecanoic acid, trimethylsilyl ester	35.78	284.48	$C_{18}H_{36}O_2$	43.3

Table 3. Effect of *Voacanga grandifolia* leaf extract on the levels of biochemical markers in rats with ethanol-induced liver toxicity.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	TBL (mg/dL)
Normal control	46.20 ± 4.45^a	74.30 ± 3.96^a	69.70 ± 4.57^a	43.50 ± 3.52^a	0.921 ± 0.103^a
Ethanol	218.90 ± 12.64^d	269.70 ± 13.91^d	253.10 ± 14.34^d	170.90 ± 13.84^d	5.534 ± 0.587^d
VGE (100 mg/kg)	126.50 ± 11.45^c	167.70 ± 11.13^c	169.70 ± 9.45^c	101.10 ± 8.35^c	2.887 ± 0.368^c
VGE (200 mg/kg)	53.10 ± 6.34^b	89.90 ± 8.15^b	79.40 ± 7.36^b	51.60 ± 3.89^b	1.215 ± 0.294^b

All values are expressed as mean ± SD of pentaplicate and analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Values with different letters show a significant difference at $P<0.05$. VGE: *Voacanga grandifolia* leaf extract. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: γ-glutamyl transferase; TBL: total bilirubin.

Table 4. Effect of *Voacanga grandifolia* leaf extract on antioxidant enzyme activity and lipid peroxidation.

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GPX (U/mg protein)	GST (U/mg protein)	GSH (U/mg protein)	MDA (nmol/mg protein)
Normal control	186.33 ± 6.45^a	58.65 ± 3.77^a	28.76 ± 1.41^a	14.65 ± 0.92^a	178.63 ± 6.53^a	23.63 ± 1.97^a
Ethanol	49.56 ± 5.68^d	18.47 ± 2.19^d	7.78 ± 0.87^d	3.12 ± 0.32^d	29.53 ± 2.43^d	83.63 ± 2.92^d
VGE (100 mg/kg)	106.18 ± 7.91^c	29.43 ± 2.89^c	16.41 ± 1.21^c	9.62 ± 0.82^c	96.77 ± 5.72^c	51.73 ± 2.27^c
VGE (200 mg/kg)	164.78 ± 9.21^b	49.35 ± 3.93^b	24.96 ± 1.56^b	12.92 ± 1.15^b	161.45 ± 6.48^b	28.65 ± 1.55^b

All values are expressed as mean ± SD of pentaplicate and analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Values with different letters show a significant difference at $P<0.05$. SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; GST: glutathione S-transferase; GSH: reduced glutathione; MDA: malondialdehyde.

Table 5. Effect of *Voacanga grandifolia* leaf extract on the level of IL-6, IL-1β and TNF-α in ethanol-induced rats (ng/mL).

Groups	IL-6	IL-1β	TNF-α
Normal control	7.575 ± 0.850[a]	4.345 ± 0.410[a]	3.151 ± 0.320[a]
Ethanol	29.254 ± 1.890[d]	18.363 ± 1.270[d]	9.994 ± 0.790[d]
VGE (100 mg/kg)	18.543 ± 1.460[c]	12.875 ± 1.180[c]	6.723 ± 0.520[c]
VGE (200 mg/kg)	10.968 ± 0.750[b]	8.478 ± 0.850[b]	3.957 ± 0.290[b]

All values are expressed as mean ± SD of pentaplicate and analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Values with different letters show a significant difference at $P<0.05$. IL-6: interleukin 6, IL-1β: interleukin-1β; TNF-α: tumor necrosis factor alpha.

3.6. Effect of *V. grandifolia* leaf extract on interleukins and TNF-α

The increased levels of IL-6, IL-1β, and TNF-α were found in ethanol-intoxicated rats as compared to the normal control rats. Treatment with *V. grandifolia* leaf extract lowered ethanol-induced IL-6, IL-1β, and TNF-α, 200 mg/kg of which showed a better effect (Table 5).

3.7. Effect of *V. grandifolia* leaf extract on the expression of antiapoptotic and pro-apoptotic genes

Ethanol administration significantly upregulated the expressions of *p53*, *Bax*, *caspase-3*, and *caspase-9* while reducing the expression of *Bcl-2*. *V. grandifolia* leaf extract significantly reversed the ethanol-induced changes in these gene expressions. Additionally, *V. grandifolia* leaf extract at 200 mg/kg was more effective than 100 mg/kg in improving impaired gene expressions (Figure 1).

3.8. Histopathological results

The liver sections of the normal control rats showed normal cell morphology with visible cellular edges, nucleus and nucleolus, and well-preserved granulated cytoplasm. The ethanol-intoxicated rats showed disorganized hepatic architecture, an increase in the number of inflammatory cells, as well as cellular disintegration with

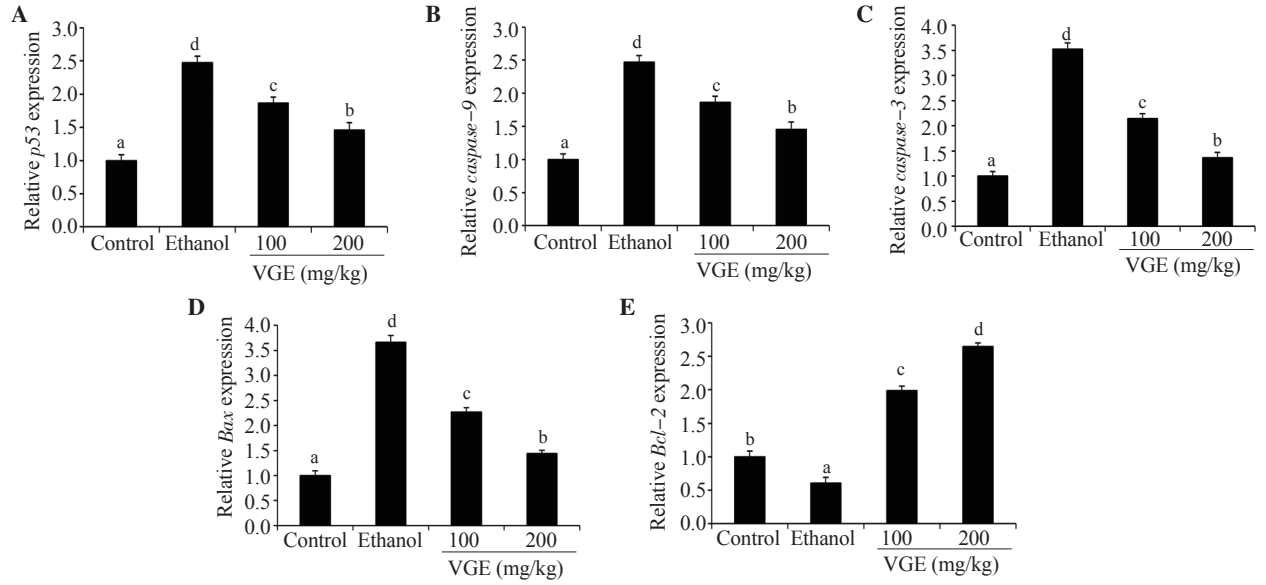

Figure 1. Effect of *Voacanga grandifolia* extract on the expression of antiapoptotic and pro-apoptotic genes. The gene expressions of (A) *p53*, (B) *caspase-9*, (C) *caspase-3*, (D) *Bax*, and (E) *Bcl-2* were determined by qRT-PCR. All values are expressed as mean ± SD of pentaplicate and analyzed by Duncan's multiple range test. Bars with different letters show a significant difference at $P<0.05$.

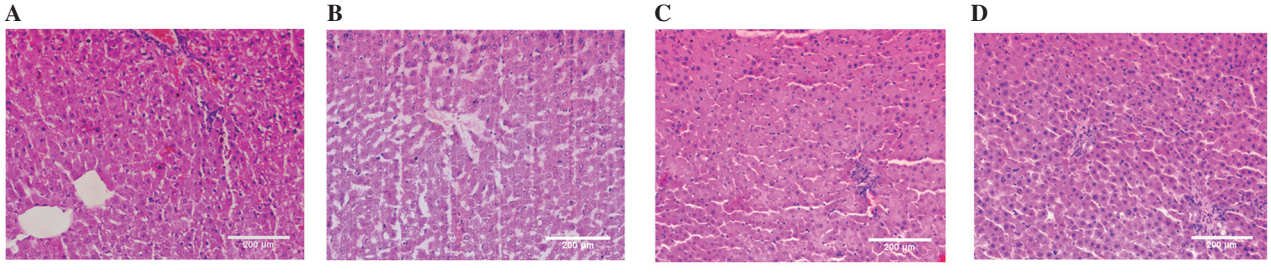

Figure 2. Effect of *Voacanga grandifolia* leaf extract on hepatic architecture of ethanol-intoxicated rats (Magnification: 40×). (A) H &E stained liver section of the normal control rats shows normal hepatic cells with well-preserved granulated cytoplasm, normal nucleus and nucleolus. (B) The ethanol-intoxicated rats show impaired hepatic architecture with increased inflammatory cells and centrilobular necrosis. (C) The rats treated with 100 mg/kg *Voacanga grandifolia* leaf extract show a normal architecture, with some hepatocytes showing isokaryosis and minimal inflammatory cell infiltration around the portal triads. (D) The rats treated with 200 mg/kg *Voacanga grandifolia* leaf extract show prominently improved hepatic architecture compared with 100 mg/kg *Voacanga grandifolia*.

centrilobular necrosis. In contrast, rats treated with *V. grandifolia* leaf extract demonstrated improved hepatocyte architecture and reduced inflammatory cells and necrosis with clear nucleus and nucleolus (Figure 2).

4. Discussion

Alcohol is both a food and a drug. Alcohol is rich in energy, and in many societies alcoholic beverages are considered part of the basic food supply. Alcohol is also consumed for its mood-altering effects and is thereby a psychoactive drug. Under the both circumstances, a larger intake of alcohol causes serious adverse effects[25]. Ethanol is a peculiar substance with a quick and effective action that is absorbed from the stomach and intestine and then rapidly diffuses into the blood circulation, where it is dispersed throughout the body. This is because ethanol is soluble in both water and lipids. The majority of ethanol is metabolized in the liver. Ethanol consumption disturbs the delicate balance of protein homeostasis in the liver, causing intracellular protein accumulation due to a disruption of hepatic protein catabolism. Evidence indicates that ethanol or its metabolism impairs trafficking events in the liver, including the process of macroautophagy, which is the engulfment and degradation of cytoplasmic constituents by the lysosomal system[26]. Alcohol consumption induces hepatic necrosis by increasing the level of serum markers[27]. Elevated ALT, AST, ALP, GGT, and TBL levels are well-known markers of liver injury[28]. When ethanol is transformed into acetaldehyde and then to acetate by the microsomal oxidizing mechanism *via* cytochrome P450, it increases the formation of ROS inside the biological system[27]. Steatosis is the most extreme change that occurs in the liver as a result of alcohol usage. Lipid peroxidation occurs as a result of a redox state imbalance. In organisms, GSH is an essential antioxidant molecule that can mitigate the effects produced by ROS. MDA is a marker of oxidative stress and is formed because of lipid peroxidation. Ethanol-induced free radicals and oxidative stress enhance MDA levels while decreasing GSH levels[29]. Excessive free radicals may activate Kupffer cells, influencing the inflammatory response in the liver *via* TNF-α and other pro-inflammatory cytokines[30]. Metallic nanomaterials that have been developed in the past few decades have been utilized to minimize cell damage due to their improved sensitivity, cellular antioxidant capacity, lowered cytotoxic activity, and customized delivery. Defining the synthesis, physical and chemical properties, and mechanism of action of a nano-antioxidant composite is essential for achieving optimum enzymatic and biological activity. Extensive toxicity investigations for non-biodegradable and insoluble nanoparticles are also important before any additional biological uses can be performed. In comparison to natural extracts, these nanoparticles often have several adverse effects in addition to their positive ones[31]. Therefore, finding new therapeutic agents of natural origin with fewer side effects is necessary.

V. grandifolia leaf extract was investigated for its hepatoprotective benefits in the present study. The findings show that *V. grandifolia* leaf extract had potential hepatoprotective activity, which may be attributable to its antioxidant capacities or phytocompounds such as phenolics and flavonoids that reduce oxidative stress and its associated consequences, including liver damage and inflammation induced by ethanol.

Pro-inflammatory factors and cytokines (such as TNF-α, IL-1β, and IL-6) were found enhanced in ethanol-induced groups, which may be due to NF-κB activation, TNF-α, and other pro-inflammatory cytokines[30]. Treatment with *V. grandifolia* leaf extract lowered ethanol-induced IL-6, IL-1β, and TNF-α, by enhancing antioxidant capability against alcohol-induced oxidative stress. Histopathological results aslo further confirm the hepatoprotective properties of *V. grandifolia* leaf extract. In terms of efficacy, *V. grandifolia* leaf extract at 200 mg/kg shows a better effect than 100 mg/kg.

To assess apoptosis, the expression levels of Bax, Bcl-2, caspases-3, caspase-9, and p53 have been determined. The activation of the mitochondrial apoptotic pathway has been linked to the Bcl-2 protein family[32]. A pro-apoptotic protein called Bax enters the mitochondria and interacts with Bcl-2. An increase in the Bax/Bcl2 ratio causes mitochondrial dysfunction and cytochrome C leakage. Caspases are proteolytic enzymes that cause cell injury by cleaving proteins in the nucleus and cytoplasm. When mitochondrial cytochrome c enters the cytoplasm, it activates caspase 9, promoting caspase-3 activation *via* cell membrane death receptors[33]. Researchers have found increased mRNA levels of the Bax/Bcl2 ratio, as well as caspase-3 and -9 levels, in the livers of ethanol-induced rats by real-time PCR assay, demonstrating that ethanol may decrease Bcl-2 while augmenting Bax, which is consistent with previous findings[34]. However, it is essential to conduct a detailed toxicological and safety evaluation of *V. grandifolia* extract and its bioactive constituent, which will be included in our future study. The GC-MS analysis revealed pharmacologically active phytocompounds in *V. grandifolia* extract. *D*-Pinitol and hexadecanoic acid detected in the extract effectively protected against carbon tetrachloride-induced hepatotoxicity in rats and reduced xenobiotic metabolic activation *via* their antioxidant properties[35]. Meso-erythritol inhibited hepatocarcinogenesis by suppressing neuropilin-1[36]. Thus, in ethanol-intoxicated animals, these pharmacologically active phytocompounds may play a key role in restoring altered biochemical indicators.

In conclusion, GC-MS analysis revealed the presence of different bioactive compounds in *V. grandifolia* extract. This study also showed that alcohol administration caused alterations in liver functions by inducing oxidative stress and inflammation in the liver. *V. grandifolia* leaf extract was found to inhibit liver toxicity by

improving the level of enzymatic antioxidants and decreasing the levels of MDA, inflammatory cytokines, and biochemical indicators of liver damage. Additionally, pro-apoptotic gene expression was decreased while antiapoptotic gene expression increased. Histological study showed that *V. grandifolia* leaf extract improved hepatic architecture of ethanol-intoxicated rats. *V. grandifolia* possesses hepatoprotective capabilities as a result of all of the above-mentioned significant advantages. Therefore, future studies will attempt to isolate the most effective bioactive phytocompounds from the bioactive fraction and explore their mechanisms of action as a hepatoprotective agent.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

LCP and SA designed the experiments. LCP carried out the experimental work. LCP contributed in original draft preparation. AG and LCP reviewed and edited the manuscript. All authors read and approved the manuscript.

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