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Biochemical Studies on the Protective Role of Moringa oleifera Leaves Against Sodium Nitrite-Induced Hepatotoxicity in Male Albino Rats

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Abstract:
Moringa oleifera leaves (MOL), widely recognized for their nutritional and medicinal properties, are rich in natural antioxidants, proteins, vitamins, and essential minerals. The present study was conducted to evaluate the hepatoprotective and therapeutic potential of MOL extract against sodium nitrite ($NaNO_2$)-induced hepatotoxicity in adult male rats. Thirty-five rats were randomly allocated into five groups (n = 7): the control group, $NaNO_2$ group (75 mg/kg body weight), MOL group (400 mg/kg body weight), the therapeutic group ($NaNO_2$ for 21 days, followed by MOL), and the protective group (MOL for 21 days, followed by $NaNO_2$). Moreover, Gas chromatography-mass spectroscopy (GC-MS), Serum biomarkers of liver functions, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were measured. GC-MS exhibited the existence of varied bioactive compounds, while the main chemical constituents were 9-Octadecenami de (16.44%), Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-m ethyl (8.46%), 1,2,4-Metheno-1H-cycl obuta[cd]pentalene-3, 5-diol, octahydro (2.94%), 4H-1-benzopyran -4-one, 2-(3,4-dimethoxyp henyl)-3,5-dihydr oxy-7-methoxy (2.44%), $NaNO_2$ administration significantly elevated ALT, AST, ALP, and GGT ($P < 0.001$) compared with the control group, indicating severe liver damage. Conversely, MOL supplementation either before or after $NaNO_2$ exposure significantly ameliorated these alterations and improved hepatic histological architecture. These findings demonstrate that MOL exerts both protective and therapeutic effects against $NaNO_2$-induced liver injury, supporting its potential as a natural hepatoprotective agent.

Keywords: Sodium nitrite; Hepatotoxicity; Liver enzymes, *Moringa oleifera* leaves

1. Introduction

Nitrites occur in various chemical forms and are widely present in natural water sources [1]. They are extensively used in the food industry as preservatives, color stabilizers, and flavor enhancers, particularly in processed meats. Among nitrites, sodium nitrite ($NaNO_2$) is the only inorganic compound with approved therapeutic use, primarily as an antidote for cyanide poisoning [2]. However, despite its clinical applications, sodium nitrite is considered an environmental toxicant with significant health risks, especially when consumed in high amounts. In acidic gastric conditions, nitrites can react with amines to form nitrosamines, which are known carcinogens [3]. Moreover, sodium nitrite induces hypoxia by converting hemoglobin into methemoglobin (MHb), a form incapable of effectively transporting oxygen, thereby leading to impaired tissue oxygenation. This transformation involves the oxidation of the heme iron from its ferrous (Fe^{2+}) to ferric (Fe^{3+}) state, disrupting oxygen delivery and resulting in systemic effects such as metabolic acidosis, cyanosis, and potentially death. Toxic effects of sodium nitrite have been documented in both animal and human studies, including oxidative stress, disruption of apoptosis, angiogenesis, erythropoiesis, and cell proliferation [4]. Although its use in food is internationally regulated due to its dose-dependent toxicity, $NaNO_2$ remains easily accessible, including online, which has contributed to its misuse in deliberate self-poisoning cases, particularly among youth. Its affordability, water solubility, and biological reactivity further increase public health concerns, highlighting the need for stricter regulation in trade and public awareness regarding its risks [5]. $NaNO_2$ can undergo endogenous conversion into nitrosamines, which are highly reactive and carcinogenic compounds. Furthermore, $NaNO_2$ contributes to the generation of RNS and reactive oxygen species (ROS), leading to oxidative stress, tissue injury, and inflammation [3, 6].

Among the organs affected by $NaNO_2$ toxicity, the liver is particularly vulnerable due to its central role in metabolism and detoxification [7]. Hepatocellular damage induced by nitrite is often characterized by elevated liver enzymes such as ALT, AST, and ALP [8]. The liver is the largest internal organ in the human body, located in the upper right quadrant of the abdomen beneath the diaphragm. It plays a central role in metabolism, detoxification, and homeostasis [9]. One of its primary functions is the detoxification of harmful substances,

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including drugs, alcohol, and metabolic waste, by converting them into less toxic compounds that can be excreted in bile or urine [10]. *Moringa oleifera* (MO), belonging to the genus Moringa and family Moringaceae, is commonly known by several names, including *Moringa*, Drumstick tree, and Horseradish tree. It is widely cultivated in various regions worldwide [11]. It is the most well-known and widely used species among the 14 recognized species within the Moringa genus. [12]. MO is used globally for both medicinal purposes and food source, with numerous studies confirming its health benefits, including its medicinal properties and nutritional value [13]. Its global fame is largely due to its diverse medicinal uses, which have earned it the title of the "miracle tree" [14]. The MOL are highly abundant in phytonutrients such as alkaloids, flavonoids, and phenolics, which are often referred to as nutrient protectors. These compounds have shown a diverse array of biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects [15]. Additionally, several studies have been conducted on its detoxification and antioxidant properties [16]. Although *Moringa oleifera* has been demonstrated to possess antioxidant, anti-inflammatory, and hepatoprotective effects in various models (e.g., against paracetamol, lead, and nickel toxicity) [17-19], there remains a significant gap in studies specifically targeting sodium nitrite ($NaNO_2$)-induced hepatic injury, particularly comparing pre-treatment (protective) versus post-treatment (therapeutic) effects. Recent studies have examined *Moringa oleifera* leaf powder in rats exposed to sodium nitrate and shown amelioration of oxidative stress and enzyme alterations [20]. Still, the corresponding investigations for sodium nitrite are scarce. Therefore, this study aims to fill that gap by evaluating and comparing the protective and therapeutic potential of the aqueous leaf extract of *Moringa oleifera* in a $NaNO_2$-induced liver damage model in adult male albino rats.

2. Materials and methods

2.1 Plant material

Leaves of *Moringa oleifera* Lam. were collected from the Faculty of Agriculture, Sohag University (Egypt) in December 2023. The raw materials were thoroughly washed with tap water to remove environmental contaminants, such as dust, sand, and waste from birds and insects. The plant materials were then dried in the open air, away from direct sunlight

2.2 Chemicals

$NaNO_2$ was obtained from Biochem Company, 6th October City, Giza, Egypt. Biochemical analysis kits for ALT, AST, ALP, and albumin were purchased from the Egyptian Company for Biotechnology (Spectrum Diagnostics, Cairo, Egypt).

2.3. Sample preparation and extraction

The dried plant material was ground using a mechanical grinder. The fine powder of MOL was extracted using the maceration method with 100% distilled water. The extract was then filtered through glass wool and Whatman filter paper No. 1. Subsequently, the extract was concentrated at Assiut University by freeze-dryer (**VirTis**, model 6KBTES-55, Albany, NY, USA) to obtain the crude extract.

2.4 Gas chromatography–mass spectrometry (GC-MS) analysis

The tentative characterization of bioactive substances in MOL using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 35 °C and then increased by 3°C /min to 200°C, held for 3 min. increased to the final temperature 280°C by 3°C /min and hold for 10 min. The injector and MS transfer line temperatures were kept at 250, 260°C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min, and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral databases

2.5. Animal experimental model

All experimental procedures were conducted according to the guidelines for the care and use of laboratory animals established by Sohag University. Ethical approval for the study was obtained from the Institutional Ethical Committee, Faculty of Science, Sohag University (Approval No.: CSRE-8-25). A total of 35 adult male albino rats (200 ± 10 g) were obtained from the Animal House, Faculty of Science, Sohag University (Sohag, Egypt). Before the start of the experiment, the animals were acclimatized for one week under standard laboratory conditions, including a 12-hour light/dark cycle (lights on in the morning), a temperature of 25 ± 1°C, and free access to food and water. The rats were housed in five separate cages, with seven animals per cage for each experimental group, as follows:

Group 1 (Control group): Received distilled water for 21 days and served as a negative control.

Group 2 (Toxin group): Received a single daily intraperitoneal (IP) injection of sodium nitrite (75 mg/kg body weight) for 21 consecutive days [21].

Group 3 (MOL group): Received a single daily IP injection of *Moringa oleifera* leaf extract (400 mg/kg body weight) for 21 consecutive days [22].

Group 4 (Protective group): Received a single daily IP injection of MOL extract (400 mg/kg body weight) for 21 days, followed by $NaNO_2$ (75 mg/kg body weight, IP) once daily for the next 21 days.

Group 5 (Therapeutic group): Received $NaNO_2$ (75 mg/kg body weight, IP) once daily for 21 days, followed by MOL extract (400 mg/kg body weight, IP) once daily for the next 21 days.

2.6. Blood sampling

Blood samples from all groups were collected from the heart into plain tubes and centrifuged at 4000 rpm for 10 min to separate the serum, then divided into several aliquots and stored at -20°C until analysis was performed.

2.7. Biochemical studies

Serum samples obtained from each experimental group were analyzed to assess liver function biomarkers. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were measured using commercially available diagnostic kits (Spectrum Diagnostics, Cairo, Egypt) according to the manufacturer's instructions. The principle of each assay was based on standard colorimetric methods. ALT and AST activities were determined by monitoring the formation of pyruvate and oxaloacetate, respectively, which react with 2,4-dinitrophenylhydrazine to form a colored hydrazone complex measurable at 546 nm. ALP activity was determined using p-nitrophenyl phosphate as the substrate, which is hydrolyzed to p-nitrophenol under alkaline conditions and read at 405 nm. GGT activity was measured using γ-glutamyl-p-nitroanilide as the substrate, and the liberated p-nitroaniline was quantified spectrophotometrically at 405 nm. All biochemical parameters were expressed as units per liter (U/L), and the data were statistically analyzed as described in the Statistical Analysis section.

2.8. Histological Study

After blood collection, the animals were sacrificed by cervical dislocation, and within one minute, the livers were carefully removed, cleaned of surrounding fat, and washed three times with sterile saline. The liver tissues were then fixed in 10% neutral buffered formalin for 24 hours to prevent tissue dehydration and preserve histological structure. Fixed liver tissues were dehydrated, embedded in paraffin, and sectioned at a thickness of 4–5 µm using a microtome. The sections were stained with hematoxylin for 20 minutes, followed by differentiation in 1% hydrochloric acid in alcohol for a few seconds, and then counterstained with eosin for a few seconds. Finally, the stained sections were mounted using a neutral resin and examined under a light microscope for histological evaluation.

2.9. Statistical analysis

SPSS Statistics 25 was utilized for data analysis. The findings are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way variance (ANOVA), followed by Tukey's multiple comparison test to analyze the difference between groups, with a significance threshold of $p < 0.05$. Significant results ($p < 0.05$) are indicated by *, and highly significant results ($p < 0.001$) by ***. Non-significant results are defined as $p > 0.05$ as NS.

3 Results

3.1. GC-MS analysis:

The bioactive substances of the MOL extract were tentatively identified using GC-MS. The results of this analysis were summarized in (Table), the total ion chromatogram (TIC) was presented in Fig 1. The data exhibited various phytochemical constituents, however, the major compounds were 9,12,15-Octadecatrien oic acid, 2-(acetyloxy)-1-[(acety loxy)methyl]ethyl ester, (Z,Z,Z)- (1) 1,3,5-triazine-2,4-diamine, 6-chloro-n-ethy l (2), 3-Oxo-20-methyl11-à-hydroxycon anine-1,4-diene (3), 2-Aminoethaneth iol hydrogen sulfate (ester) (4), Oleic Acid (5), Estra-1,3,5(10)-trien-17 á-ol (6) 9-Octadecenoic acid (Z)--(7), Phenol, 2,2'-methylenebis[6-(1 ,1-dimethylethyl)-4-m ethyl (8), 1,3,5-Triazine-2,4- diamine, 6-chloro-n-ethy l (9) 13,16-Octadecadi ynoic acid, methyl ester, (10), 7-Methyl-Z-tetradecen1-ol acetate (11), 2-Acetyl-3-(2-cin namido)ethyl-7-m ethoxyindole (12), 9-Octadecenoic acid (Z)- (13), Ethyl iso-allocholateEthyl iso-allocholate (14), 1-Heptatriacotanol (15), 4H-1-benzopyran -4-one, 2-(3,4-dimethoxyp henyl)-3,5-dihydr oxy-7-methoxy (16)

Table 1: Estimation of total phenolic and flavonoid contents of MOL extract

S/ No	Name of Compound	Mol. formula	Mol. Wt(g)	RT (min)	% TIC
1	9,12,15-Octadecatrien oic acid, 2-(acetyloxy)-1-[(acety loxy)methyl]ethyl ester, (Z,Z,Z)-	C25H40O6	436	10 .25	1.97
2	1H-Indol-5-ol, 3-(2-aminoethyl)	C10H12N2O	176	11 .64	1.27
3	1,2,4-Metheno-1H-cycl obuta[cd]pentalene-3, 5-diol, octahydro	C10H12O2	164	25 .26	2.94
4	1(4H)-Naphthalenone, 4a,5,8,8a-tetrahydro-4-hydroxy-, (4à,4aá,8aá)-	C10H12O2	164	25 .46	1.53
5	2-Aminoethanethiol hydrogen sulfate (ester)	C2H7NO3S2	157	47 .77	1.07
6	Oleic acid	C18H34O2	282	47 .82	0.94
7	Estra-1,3,5(10)-trien-17 á-ol	C18H24O	256	48 .15	0.69
8	9-Octadecenoic acid (z)-	C18H34O2	282	54 .68	1.15
9	Phenol, 2,2'-methylenebis[6-(1 ,1-dimethylethyl)-4-m ethyl	C23H32O2	340	60 .70	8.46
10	13,16-octadecadienoic acid, methyl ester	C19H30O2	290	62 .63	0.70
11	7-Methyl-Z-tetradecen1-ol acetate	C17H32O2	268	63 .30	2.07
12	Methyl 4-acetylhydroxypalmitate	C19H36O4	328	68 .82	1.66
13	9-Octadecenoic acid (z)-	C18H34O2	282	69 .77	0.87
14	3-Oxo-20-methyl11-à-hydroxycon anine-1,4-diene	C22H31NO2	341	70 .34	0.84
15	9-Octadecenami de	C18H35NO	281	70 .98	16.44
16	Cholestan-3-ol, 2-methylene-, (3á,5à)-	C28H48O	400	72 .35	0.93
17	4H-1-BENZOPYRAN -4-ONE, 2-(3,4-DIMETHOXYP HENYL)-3,5-DIHYDR OXY-7-METHOXY	C18H16O7	344	74 .49	2.44

18	2-HYDROXY-3-[(9E)-9-OCTADECENOYLOXY]PROPYL (9E)-9-OCTADECENOATE #	C39H72O5	620	77.55	1.58
19	Ethyl iso-allocholate	C26H44O5	436	79.88	1.45
20	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydr oxy-7-methoxy	C18H16O7	344	80 .3	1.62
21	1-Heptatriacotanol	C37H76O	536	82.70	0.96
22	Oleic acid	C18H34O2	282	84.27	0.26
24	Ethyl iso-allocholateethyl iso-allocholate	C26H44O5	436	86.18	1.09

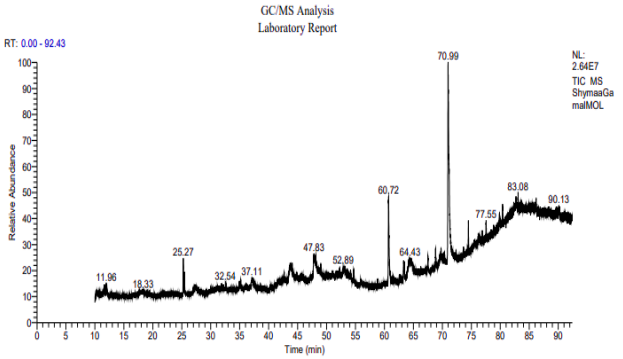

Figure 1: Total ion chromatogram of GC-MS analysis of MOL extract

3.2. Effect of $NaNO_2$ and MOL on liver enzymes (biochemical examination):

The biochemical analysis revealed significant alterations in liver enzyme activities following $NaNO_2$ and MOL administration. As presented in **Table 2**, rats exposed to $NaNO_2$ exhibited a marked elevation in serum levels of ALT, AST, ALP, and GGT compared to the control group (***P < 0.001), indicating pronounced hepatic injury. In contrast, rats treated with MOL extract showed a remarkable reduction in these enzyme levels, approaching values similar to the control group. These findings suggest that MOL administration effectively alleviates $NaNO_2$-induced hepatotoxicity and supports its protective and therapeutic role in maintaining liver function

Table 2: Effect of $NaNO_2$ and MOL on liver enzymes of male rats
Values are expressed as mean ± SD (n = 7). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control group; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001 vs. $NaNO_2$ group; NS = non significant.

Parameters	Control	$NaNO_2$	MOL	$NaNO_2$+ MOL	MOL+ $NaNO_2$
ALT (U/L)	32.46±0.571	68.37±0.704***	34.51±0.501^{NS}	28.10±0.43$^{NS,\#\#\#}$	36.66±0.97$^{NS,\#\#\#}$
AST (U/L)	68.71±0.533	105.80±0.489***	76.66±0.386^{NS}	82.74±0.4$^{NS,\#\#\#}$	79.03±0.6$^{NS,\#\#\#}$
ALP (U/L)	66.00±0.541	114.46±0.299***	66.07±0.956^{NS}	55.20±0.648$^{NS,\#\#\#}$	60.95±0.76$^{NS,\#\#\#}$
GGT (U/L)	3.96±0.085	5.05±0.251***	3.66±0.237^{NS}	3.98±0.070$^{NS,\#\#\#}$	4.14±0.15$^{NS,\#\#\#}$

3.2. Effect of $NaNO_2$ and MOL extract on liver enzymes (histological examination):

Histological examination of liver sections stained with hematoxylin and eosin (H&E) revealed marked differences among the experimental groups (Figs. **2** and **3**). In the normal control group (G**1**; Fig. **2**), the liver showed normal histological architecture with well-arranged hepatic cords radiating from the central vein (CV) toward the portal area (PA). Hepatocytes appeared polygonal with rounded vesicular nuclei and acidophilic cytoplasm. The blood sinusoids (thin spaces) were normally distributed between hepatic cords (thick black arrows). The portal area contained a normal portal vein (PV), bile duct (BD), and hepatic artery (HA), all surrounded by minimal connective tissue.

In the $NaNO_2$-treated group (G**2**; Fig. **2**), liver sections displayed histopathological alterations characterized by widening of the central vein (CV), loss of normal hepatocyte arrangement, cytoplasmic vacuolation, and pyknotic or degenerated nuclei (dotted arrows). Blood sinusoids were compressed due to hepatocyte swelling, and the alterations were more evident around the portal areas, indicating hepatocellular degeneration and early necrotic changes.

In the Moringa oleifera (MOL)-treated group (Fig. **3**), liver architecture appeared normal, resembling that of the control group. Hepatocytes showed normal vesicular nuclei, intact cytoplasm, and well-defined hepatic cords surrounding the central vein (CV). The portal triads (PA) exhibited normal structures of the portal vein (PV), bile ducts (BD), and hepatic artery without inflammatory infiltration.

In the $NaNO_2$ + MOL co-treated group (Fig. **3**), liver sections revealed an almost normal hepatic architecture with mild cytoplasmic vacuolation and minimal sinusoidal dilatation. The portal areas showed slight inflammatory cell infiltration around PV and BD, indicating partial protective effects of Moringa oleifera extract against $NaNO_2$-induced damage.

In the MOL + $NaNO_2$ pre-treated group (Fig. 3), a marked improvement in liver structure was observed compared to the $NaNO_2$-treated group. Hepatocytes exhibited nearly normal morphology with only mild residual vacuolation (dotted arrows). The central vein (CV) and portal areas appeared normal, with minimal cellular infiltration, confirming the hepatoprotective potential of MOL pretreatment.

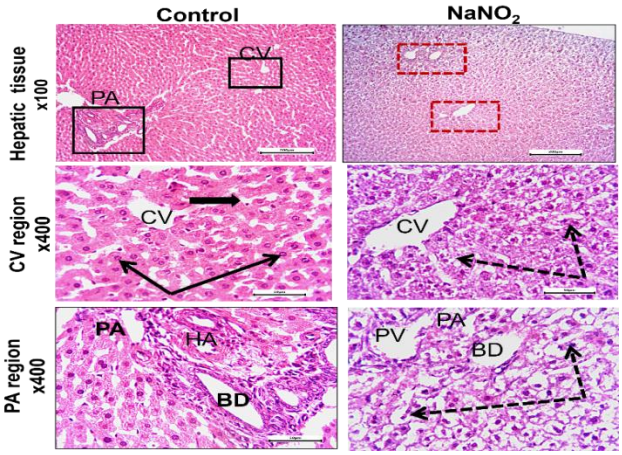

Figure 2: Histological sections of rat liver from central vein (CV) and portal area (PA) regions, stained with Hematoxylin & Eosin (H&E). Images were captured at ×100 and ×400.

G1: NC (Normal Control) showing normal hepatocyte plates with rounded vesicular nuclei (thin black arrows), normal central vein (CV), and thin blood sinusoids (thick black arrows) between liver cords. The portal area (PA) shows a normal portal vein (PV) and bile ducts (BD). **G2: $NaNO_2$-treated group** showing mild widening of the central vein (CV), hepatocyte swelling, loss of cell outline, and cytoplasmic vacuolation. Small degenerated hepatocyte nuclei are observed (dotted arrows). Blood sinusoids appear compressed by swollen cells, with changes more pronounced near the portal area (dotted arrows).

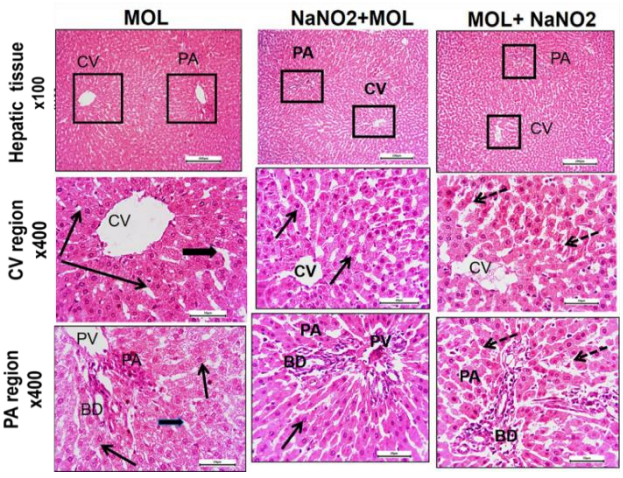

Figure 3: Histological of liver sections from different experimental groups stained with Hematoxylin and Eosin (H&E) and photographed at ×100 and ×400.

MOL group: Liver sections show normal hepatic architecture with central vein (CV), portal area (PA), and hepatocyte cords arranged radially around the CV. Hepatocytes exhibit normal vesicular nuclei (thin black arrows), normal sinusoids (thick arrows), and intact portal triads composed of portal vein (PV) and bile ducts (BD). **$NaNO_2$ + MOL group:** Hepatic tissues show nearly normal structure with mild cytoplasmic vacuolation and slight dilatation of some sinusoids (black arrows). The portal area displays mild inflammatory cell infiltration around PV and BD. **MOL + $NaNO_2$ group:** Sections show marked hepatic improvement compared with $NaNO_2$-intoxicated rats, with nearly normal hepatocytes and mild residual vacuolation (dotted arrows). Central vein (CV) and portal area (PA) appear normal with minimal cellular infiltration.

4. Discussion

4.1. Bioactive constituents of moringa oleifera and their biological activities:

Among the identified compounds, we found many valuable and influential compounds that participate in the value of MOL and support its effect, such as 9-Octadecenami de 9-Octadecenamide, which belongs to the primary amides class of fatty acyls with molecular weight 281, has previously been identified from Hildegardia barteri, and is reported to have antibacterial and antioxidant activity [23]. Also, Phenol, 2,2'-methylenebis [6-(1 ,1-dimethylethyl)-4-m ethyl is frequently used in Antibacteria1against pathogenic bacteria [24].

Although GC–MS analysis provided valuable insight into the major phytochemical constituents of the Moringa oleifera leaf extract, more advanced chromatographic and spectroscopic studies are needed to isolate and quantify the individual bioactive compounds responsible for its hepatoprotective effects.

4.2. Biochemical analysis:

$NaNO_2$ is commonly used in food preservation, yet it poses significant toxicological risks when consumed excessively or over prolonged periods [25]. The present study demonstrated that intraperitoneal administration of sodium nitrite to male rats induced marked hepatotoxic effects, as evidenced by significant increases in serum ALT, AST, ALP, and GGT levels. These enzymes are established biomarkers of liver function, and their elevation is a hallmark of liver injury.

The observed increase in ALT and AST suggests damage to hepatocytes, as these enzymes are primarily localized in the cytoplasm and mitochondria of liver cells. ALT is considered more liver-specific, while AST can also be found in cardiac and skeletal muscle. The parallel rise in both supports hepatocellular necrosis and loss of membrane integrity [26].

The elevation in ALP and GGT further indicates possible cholestasis or biliary tract dysfunction [27]. GGT, in particular, is a sensitive marker for oxidative stress-related liver damage and is often elevated in response to exposure to hepatotoxins [28].

The hepatotoxic effect of $NaNO_2$ can be attributed to its ability to generate RNS and ROS. These free radicals cause oxidative damage to cellular lipids, proteins, and DNA,

leading to lipid peroxidation, mitochondrial dysfunction, and apoptosis or necrosis of hepatocytes. In addition, $NaNO_2$ can be metabolized to nitrosamines, which are known to be carcinogenic and hepatotoxic [29].

The findings of our study are consistent with previous reports that have demonstrated sodium nitrite-induced oxidative stress and liver injury in experimental animals [30-32]. For instance, Fouad et al. (2017) reported similar increases in liver enzyme activities in rats treated with $NaNO_2$, accompanied by histopathological changes such as hepatocyte degeneration and inflammatory infiltration [33].

Treatment with MOL extract, whether administered therapeutically ($NaNO_2$ + MOL) or protectively (MOL + $NaNO_2$), significantly normalized liver enzyme levels and preserved hepatic histoarchitecture. The protective group showed the most robust histological recovery, with hepatocytes, central vein, and sinusoids appearing nearly indistinguishable from the control group.

These outcomes are consistent with prior research. For instance, Toppo et al. (2015) found MOL extract (500 mg/kg, oral for 28 days) significantly reduced cadmium-induced elevation of

ALT, AST, and ALP in rats, while improving SOD activity [34]. Similarly, Altaee et al. (2023) reported that MOL leaf extract mitigated paracetamol-induced increases in ALT, AST, ALP, total bilirubin, and TNF-α, while enhancing SOD and CAT levels [35]. Moreover, Singh et al.(2014) showed that MOL extract dose-dependently reversed CCl_4-induced elevations in SGOT, SGPT, GGT, ALP, and total bilirubin, while restoring serum protein and albumin levels [22].

The hepatoprotective effect of MOL extract is likely mediated by multiple mechanisms. Its high content of flavonoids (e.g., quercetin, kaempferol), vitamins (A, C, E), phenolics, and isothiocyanates underpins its antioxidant and free-radical scavenging capacity. These compounds can stabilize cell membranes, chelate pro-oxidant metals, and enhance endogenous antioxidant defenses (e.g., SOD, CAT, GPx), reducing lipid peroxidation and preserving cellular integrity.

4.3. Histological analysis:

The marked improvement observed in the liver architecture of the MOL + $NaNO_2$ pre-treated group compared with the $NaNO_2$ + MOL co-treated group indicates that Moringa oleifera exerts a stronger protective (prophylactic) effect when administered before toxic exposure rather than a curative one after damage has occurred. This enhanced protection may be attributed to the antioxidant and free radical scavenging properties of Moringa bioactive constituents, such as flavonoids, phenolics, and ascorbic acid, which can stabilize hepatocyte membranes and neutralize reactive oxygen species generated by sodium nitrite toxicity.

Pre-administration of Moringa likely allowed its phytochemicals to accumulate and activate the cellular defense mechanisms, including upregulation of antioxidant enzymes (e.g., SOD, CAT, and GPx) and inhibition of lipid peroxidation, thereby minimizing oxidative stress and cellular degeneration. These findings suggest that Moringa oleifera acts more effectively as a hepatoprotective agent when used as a preventive measure against nitrite-induced hepatotoxicity. These histopathological findings closely mirror those in other studies: for example, in acetaminophen-induced liver injury, MOL pre-treatment preserved liver architecture and normalized enzyme levels [36].

In future studies, we plan to further validate the present findings by performing dose–response and time-course experiments to determine the optimal therapeutic window of Moringa oleifera leaf extract. Additionally, isolation and characterization of the active phytoconstituents will be conducted to elucidate their individual and synergistic effects. We also aim to assess molecular mechanisms, including oxidative stress markers, apoptotic gene expression, and inflammatory signaling pathways. Expanding this research to include female rats and other models of hepatotoxicity will help confirm the generalizability and safety of Moringa oleifera as a potential hepatoprotective agent.

In summary, the data convincingly demonstrate that MOL extract exerts both protective and restorative effects against $NaNO_2$-induced liver injury in rats. The stronger effect observed in the protective regimen suggests practical implications: consumption of MOL as a functional food supplement may offer preventive benefits against hepatic oxidative challenges.

5. Conclusions:

The present study demonstrates that the aqueous extract of Moringa oleifera leaves exerts potential protective and therapeutic effects against sodium nitrite-induced hepatotoxicity in male albino rats. These effects may be attributed to the extract's antioxidant and anti-inflammatory activities. However, given the limitations of this experimental design and the use of a single dose level, the findings should be interpreted as preliminary evidence. Further comprehensive studies are required to isolate the bioactive constituents, explore the molecular mechanisms involved, and assess the safety and efficacy of Moringa oleifera in different experimental and clinical models.

CRediT authorship contribution statement:

Nagwa M. El-Sawi: Supervision, Conceptualization, Writing–manuscript. Amany M. Hamed: Supervision, Conceptualization, Formal analysis, Data curation, writing–original draft preparation, Writing–manuscript and editing. Shimaa G. Osman: Investigation, Formal analysis, Data curation, Writing–original draft preparation. Mahmoud Hefny Gad: Supervision, Conceptualization, manuscript editing. Soad Shaker: making the histological part. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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