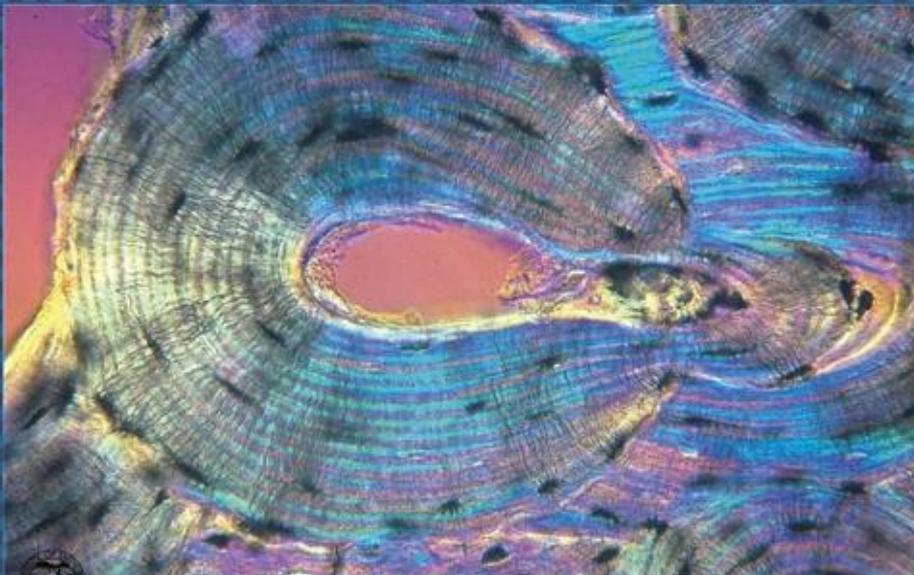




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Evaluation of the Possible Protective Role of Ginger on Sodium Valproate Induced Hepatotoxicity in Adult Male Albino Rat: A Biochemical, Histological, And Immunohistochemical Study

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ABSTRACT

Background: Sodium valproate (SVP) is a widely prescribed treatment for epilepsy and other neurological diseases. However, liver injury is a harmful side effect related to its usage. Oxidative stress, inflammation, and fibrosis seem to play a major role in SVP-induced hepatotoxicity.

Aim of the work: This work aimed, for the first time, to study the possible protective mechanisms of ginger (GN) in modulating the hepatotoxic effects of SVP in albino rats.

Material and methods: A total of 24 Sprague-Dawley adult male rats (180g - 220g) were divided into equal four groups: Group1 (control): received normal saline by gavage. Group2 (GN): received ginger (200mg/kg/day). Group3 (SVP): received SVP (300mg/kg/day). Group4 (GN+SVP): received combined ginger (200mg/kg/day) and SVP (300mg/kg/day). All medications were administrated daily for 14 days by gavage. At the assigned time the animals were sacrificed, the blood and tissue samples were collected and processed for biochemical, histological, and immunohistochemical studies.

Result: SVP treatment induced a significant increase in alanine transaminase, aspartate transaminase, and Malondialdehyde (ALT, AST, and MDA respectively), together with a significant reduction in the antioxidant enzyme Glutathione (GHS) indicated hepatic oxidative stress damage. Histological examination revealed loss of the normal hepatic architecture, extensive fibrosis identified by Masson's trichrome staining. Moreover, the SVP group showed a significant increase in tumor necrosis factor-alpha (TNF- α) and transforming growth factor-beta (TGF- β) Immunohistochemical expression. Co-treatment with GN significantly reversed the previous changes in the GN+SVP group.

Conclusion: In conclusion, ginger has hepatoprotective activity due to its anti-oxidant, anti-inflammation, and anti-fibrotic potentials.

INTRODUCTION

Sodium valproate (SVP) is frequently prescribed as an antiepileptic medication to treat epilepsy, convulsions, and migraines (Tolou-Ghamari Z and Palizban, 2015).

In spite of its medical effectiveness, dangerous complications such as hepatotoxicity were reported with SVP administration (Pourahmad *et al.*, 2012; Nahid *et al.*, 2017).

The mechanism of liver injury is not completely identified; however, SVP-induced liver toxicity includes several mechanisms including oxidative stress, inflammation, and fibrosis (Abdelkader *et al.*, 2020; Salimi *et al.*, 2020). Oxidative stress-related excessive reactive oxygen species (ROS) production and unbalanced antioxidant ability play an essential role in the SVP-related toxicity. SVP-induced inflammatory and immune reactions cause the release of inflammatory cytokines regulated by TNF- α . Several previous studies illustrated a significant increase in the TNF- α expression after SVP treatment (Nazmy *et al.*, 2017; Oztopuz *et al.*, 2020).

Hepatic fibrosis is a remarkable feature of SVP-induced hepatic injury. SVP was reported to increase the pro-fibrotic agents like transforming growth factor-beta family (TGF- β) and alpha-smooth muscle actin (α -SMA) (Gezginci-Oktayoglu *et al.*, 2016; Abdelkader *et al.*, 2020). TGF- β signaling plays an essential role in all stages of liver disease, from early hepatic injury, inflammation, fibrosis, to cirrhosis and cancer (Aly *et al.*, 2020).

Hepatic injury is a global problem. Although there are no reliable hepatic protective drugs in curative medicine, Herbal drugs provide a managing role for several hepatic disorders. Most herbal supplementations speed up the normal healing progression so it is critical to find an effective and preventive agent to manage various hepatic insults (Ramamurthy and Abarna, 2014).

Ginger (*Zingiber officinale*) is commonly used as a food spice all over the world. In addition, the herb extract has been traditionally used for

the treatment of various types of diseases such as rheumatism, diabetes, asthma, stroke, and nervous diseases (Tapsell *et al.*, 2006). The therapeutic efficacy of ginger extract is attributed to the presence of polyphenol compounds (6-gingerol and shogaols) which exhibit antioxidant, anti-inflammatory, and anti-tumor properties (Ali *et al.*, 2008). Several studies showed that ginger extracts can be used in the protection against various drug-induced hepatic toxicity such as cisplatin-induced liver injury (Attyah and Ismail, 2012), piroxicam-induced liver toxicity (Badawi, 2019), ciprofloxacin-induced hepatotoxicity (Hemieda *et al.*, 2019), CCl₄-induced hepatotoxicity (Abd-Elrhman *et al.*, 2020), and diethylnitrosamine-induced liver injury (Alsahli *et al.*, 2021).

Scanning of the literature revealed no information about the protective role of GN in SVP-induced hepatotoxicity. Based on the above information, the current study aimed to assess the SVP-induced hepatic injury and the possible protective effect of ginger in ameliorating liver function, histology, oxidative stress, inflammation, and fibrosis.

MATERIALS AND METHODS

Animals:

Twenty-four adult (10-12 weeks) male Sprague Dawley rats weighting 180-220gm were used in this experiment. The rats were housed two or three in a cage with free access to rodent diet and tap water. They were maintained under stable temperature (25 \pm 2 $^{\circ}$ C), humidity (55 \pm 5%), and a fixed dark/light cycle. All experiments will be carried out according to the recommendations of the guide for the care and use of laboratory animals of the National Institutes of Health.

Chemicals:

Sodium valproate (oral solution, 200mg/ml, Sanofi-Aventis) and ginger (tablet, 400mg, Mepaco-Medifood) were purchased from a local pharmacy.

Experimental Protocol:

- 1- Group (I), control non-treated group:** six rats received saline by gavage.
- 2- Group (II), GR-treated group:** six rats received ginger (200 mg/kg/day) by gavage for two weeks (Badawi, 2019).
- 3- Group (III), SVP-treated group:** six rats received sodium valproate (300 mg/kg/day) by gavage for two weeks (Shakya *et al.*, 2018).
- 4- Group (IV), GR+SVP treated group:** six rats received combined ginger (200 mg/kg/day) and sodium valproate (300 mg/kg/day) by gavage for two weeks

Scarification of Rats and Specimen's Collection:

On the 15th day, blood samples were obtained from the tail vein. after that, the rats were sacrificed by decapitation, the livers were dissected and divided. Parts of the liver were processed for GTH and MDA measurement in liver homogenate. Other parts were processed for paraffin sections for histological and immunohistochemical examination.

Liver Function Assessment:

For evaluation of biochemical markers of the liver, the blood was left to coagulate at room temperature. Then, the samples were centrifuged (20 min, 4000 rpm). The clear serum layer was removed and then stored (-80°C). Serum ALT and AST were measured as described by Nwosu *et al.* (2009).

Oxidative Stress Assessment:

The liver homogenates were utilized to detect the tissue levels of GSH and MDA as described by Koroglu *et al.* (2021).

Histological and Immunohistochemical Assessment:

The liver specimens were fixed in 10% formalin, prepared for paraffin sections (5 μ m), and stained with hematoxylin and eosin (H&E) and Masson's trichrome (Bancroft and Gamble, 2008; Chen *et al.*, 2017).

For the immunohistochemical technique, the paraffin sections (3 μ m) were rehydrated then treated with citrate buffer (10mM). Then, the sections were rinsed with PBS and treated with a solution of H₂O₂ (3%) for five minutes at room temperature in order to suppress the endogenous peroxidase. After washing with PBS, the sections were blocked with bovine serum albumin (5%) in Tris-buffered saline for two hours. They were then immunostained with the primary antibodies: anti-TNF- α antibody (1:100; cat. no. ab220210; Abcam, Cambridge, UK) (Wang *et al.*, 2019) and TGF- β 1 (1:100; cat. no. ab215715; Abcam, Cambridge, UK) (Wang *et al.*, 2020), and incubated overnight at 4 °C. After then, the slides were incubated with biotinylated IgG and then with streptavidin conjugated to horseradish peroxidase at 37°C for 30 minutes. The slides were finally stained with DAB and counterstained by hematoxylin.

Morphometric Study and Statistical Analysis:

Five non-overlapping fields (x400) were taken from each slide. The area percentage of blue-stained fibrous tissue in the Masson's trichrome stained sections and the brown pixels for TNF- α and TGF- β immunostain were measured using the NIH Image J program (National Institute of Health, Bethesda, MD).

The data were collected, coded, and analyzed using Statistical Package for Social Sciences version 22 (SPSS) software. Descriptive statistics were calculated in the form of mean \pm SD (Standard deviation). Comparison between different groups was tested using ANOVA (analysis of variance) followed by post-hoc Tukey test for multiple comparisons. The statistical significance was considered when $P \leq 0.05$.

RESULTS**I- Biochemical Results:****A) Liver Function:**

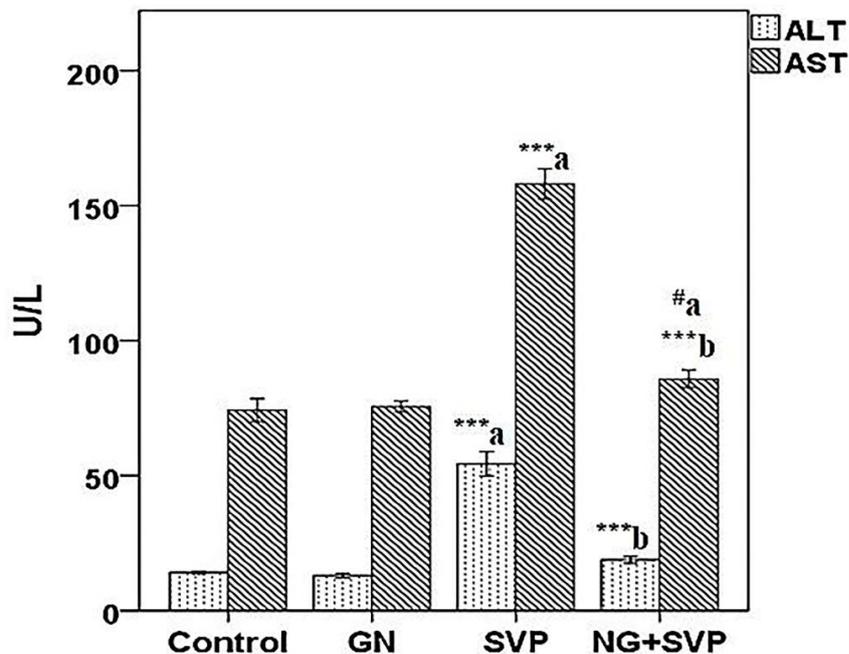
Assessment of the liver enzymes revealed a significant increase in the levels of ALT and AST of the SVP-treated group as compared

to the control group. The GN+SVP group showed a significant decrease in both ALT and AST to nearly the control level (Table 1, Histogram 1).

Table 1: Mean values of the liver enzymes (ALT, AST) and oxidative stress markers (GSH, MDA) in the studied groups.

	Control	GN	SVP	GN+SVP	P value
ALT (U/L)	14.12±0.6	12.95±0.88	54.36±4.51	18.8±1.18	P1= 0.000 P2= 0.000 P3= 0.154
AST (U/L)	74.29±4.29	75.54±2.12	158.05±5.57	85.81±3.33	P1= 0.000 P2= 0.000 P3= 0.033
GSH (nmol/mg protein)	52.69±1.25	55.73±0.66	41.94±2.83	48.55±1.62	P1= 0.000 P2= 0.008 P3= 0.082
MDA (nmol/g tissue)	18.75±1.47	17.32±0.88	29.51±1.36	23.35±0.91	P1= 0.000 P2= 0.001 P3= 0.006

The listed values are expressed as mean ±SD by utilizing ANOVA test. P1:P3 represent post-hoc Tukey test where **P1:** SVP versus control, **P2:** GN+SVP versus SVP, **P3:**GN+SVP versus control.

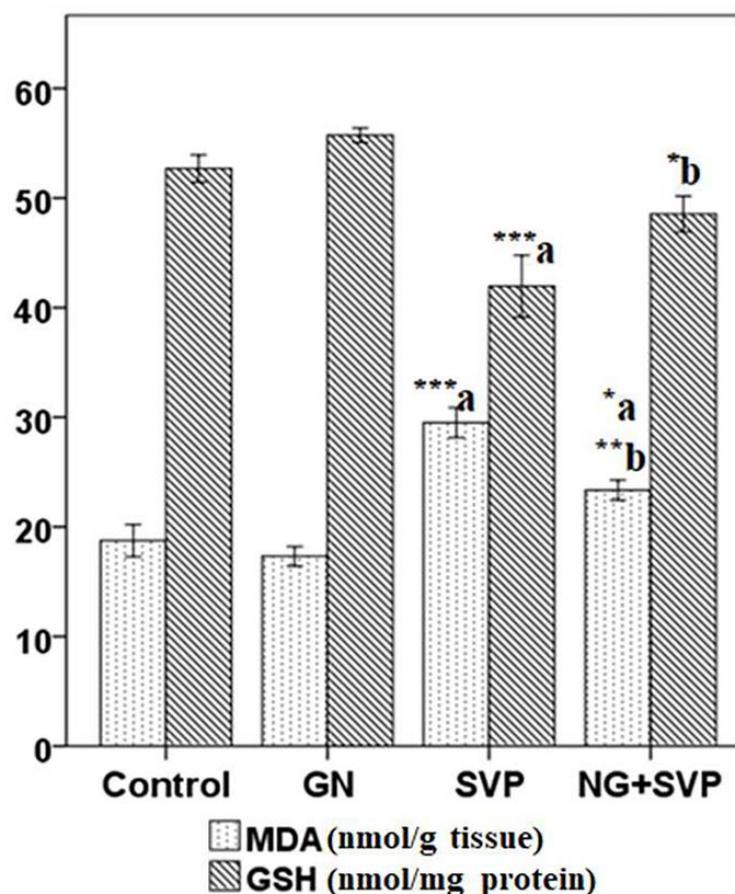


Histogram 1: Histogram showing the mean ALT and AST serum levels in different groups. Both ALT and AST are markedly increased in the SVP group as compared to the control group. The levels of these enzymes are considerably decreased in GN+SVP co-treated group as compared to the SVP group. **SVP**= sodium valproate, **GN**=ginger. #p<0.05, ***p<0.0001. **a:** versus control. **b:** versus SVP

B) Oxidative Stress:

Treatment with SVP resulted in a significant decrease in GSH anti-oxidant enzyme and an increase in MDA, a product of lipid peroxidation, indicating oxidative stress state. Co-

treatment with ginger caused a significant increase in the GSH and a decrease in the MDA levels in the GN+SVP group as compared to the SVP group (Table 1, Histogram 2).



Histogram 2: Histogram showing the mean tissue levels of GSH and MDA in different groups. The SVP-treated group illustrates a significant decrease in GSH and an increase in MDA levels compared to the control group. The levels of these oxidative stress markers are considerably reversed in GN+SVP co-treated group compared to the SVP group. SVP= sodium valproate, GN=ginger. * $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$. **a:** versus control. **b:** versus SVP.

II- Histological Results:

A) Haematoxylin and Eosin:

The H&E sections of the control and GN groups revealed nearly the same histological structures. Both groups showed that each hepatic lobule was formed of a central vein encircled by radiating cords of hepatocytes disjointed by blood sinusoids. The hepatocytes were polygonal in shape with acidophilic cytoplasm and vesicular nuclei, however, binucleated cells were also detected (Fig. 1A, 1B). The portal area was formed of a portal vein with a thin wall and large lumen and a bile duct that was lined by single cuboidal cells containing dark, rounded nuclei (Fig. 2A, 1B).

The SVP group illustrated a noticeable loss of normal hepatic architecture. There were hepatocytes with ill-defined borders, vacuolated cytoplasm, and darkly stained nuclei. Dilated central veins and inflammatory cell infiltration were also observed (Fig. 1C). The portal area showed marked cellular infiltration, dilated portal veins, bile duct proliferation, and increased thickness of the bile duct wall (Fig. 2C, 2D, 2F). These changes were greatly reversed in the rats of the GN+SVP group but some hepatocytes had slightly vacuolated cytoplasm and there was little inflammatory cellular infiltration in the portal area (Fig. 1D, 2F).

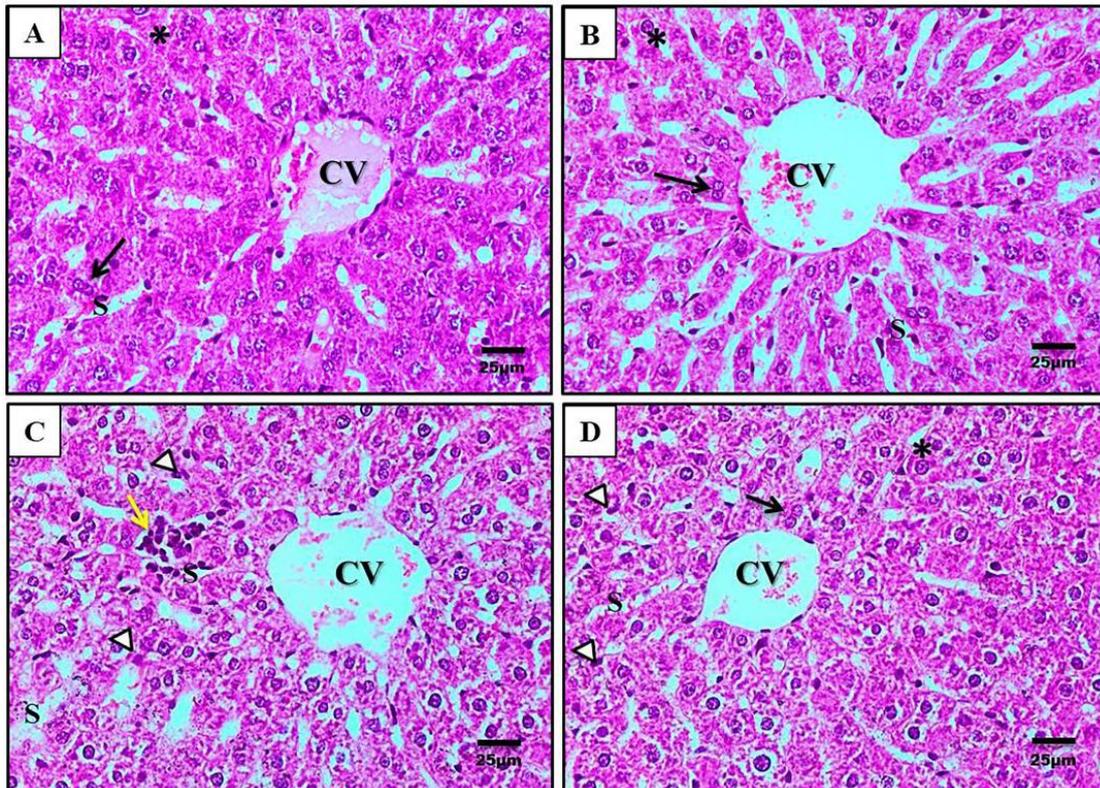


Fig. 1: **A, B):** Liver photomicrographs of control and GN-administrated rats respectively. Both of them illustrate normal hepatic lobules which are formed of central vein (CV) surrounded by radiating cords of hepatocytes. These cords are separated by blood sinusoids (S). The hepatocytes show vesicular nuclei (*) and acidophilic cytoplasm. Binucleated cells are also observed (black arrows). **C):** Liver Photomicrograph of SVP-treated rat showing dilated congested central vein (CV) and inflammatory cellular infiltration (yellow arrow). Some hepatic cells revealed cytoplasmic vacuoles (V), and pyknotic nuclei (arrowheads). **D):** Photomicrograph of GN+SVP co-treated rat illustrating improvement of the liver histology. There are normal central vein (CV), vesicular nuclei (*), and binucleated cells (black arrow). Some hepatocytes show vacuolizations (V) and pyknotic nuclei (arrowheads). (H&E; X400).

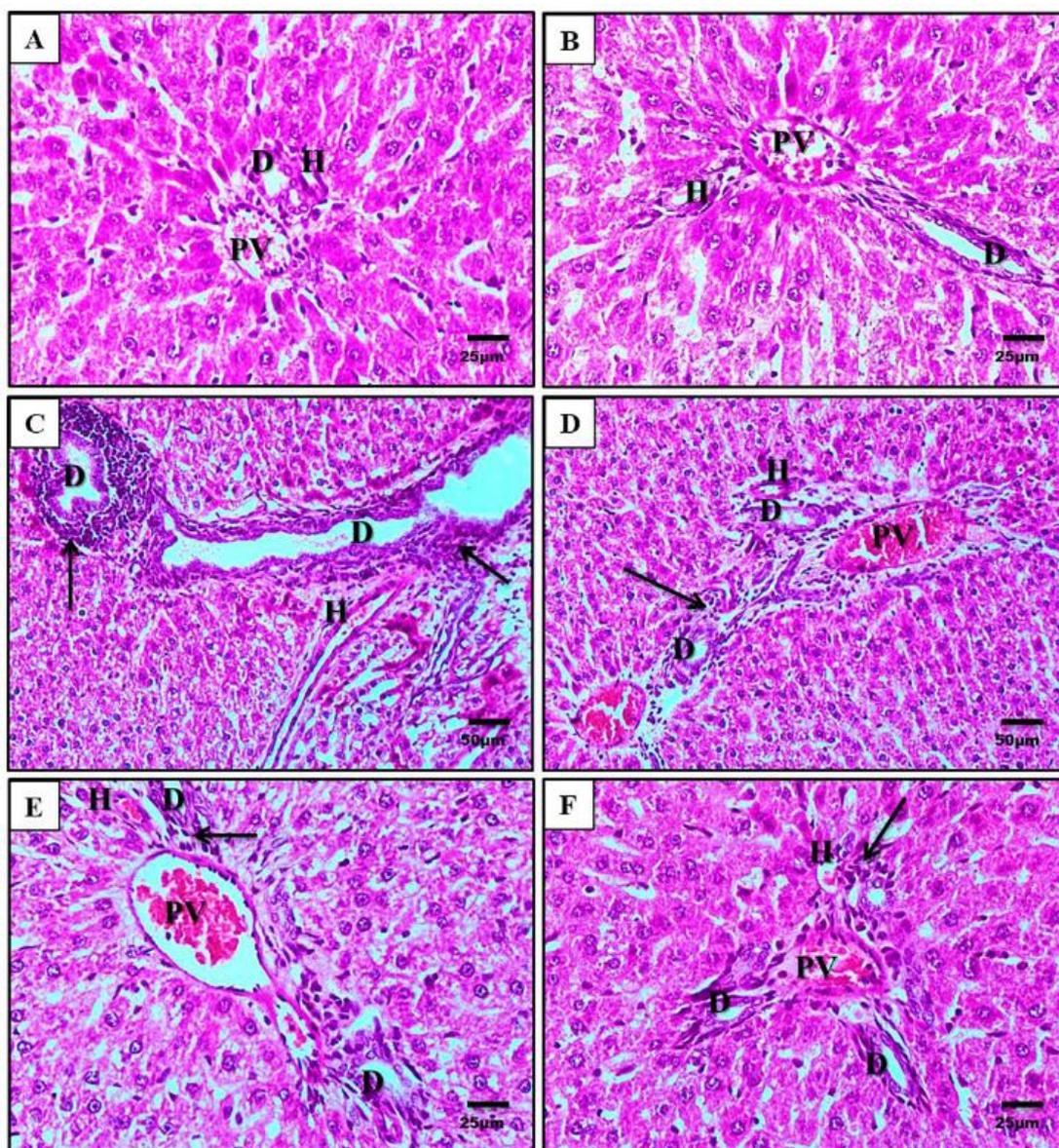


Fig 2: **A, B):** Liver photomicrographs of control and GN-administrated rats respectively. Both of them illustrate normal portal triads. Each triad contains portal vein (PV), bile duct (D), and hepatic artery (H). **C, D, E):** Liver photomicrographs of SVP-treated rats showing an increased wall thickness and duplication of the bile ducts (D), dilated congested portal veins (PV), and extensive inflammatory cellular infiltration (arrows). **F):** Photomicrograph of GN+SVP co-treated rat illustrating improvement of the liver histology. There is some congestion of the portal vein, bile duct (D) duplication, and minimal cellular infiltration (arrow). (H&E; A, B, E& F X400 – C&D X200).

B) Masson's trichrome

Examination of the Masson's trichrome-stained sections revealed trace blue-stained fibers in the control (Fig. 3A) and GN (Fig. 3B) groups mainly around the central vein and portal area. The SVP group illustrated a significant increase in the blue-

stained fibers as compared to the control group (Fig. 3C). Co-administration of GN significantly reduced the blue-stained area in the GN+SVP group (Fig. 3D) as compared to the SVP group, however it still significantly higher than the control group (Table 2, Histogram 3).

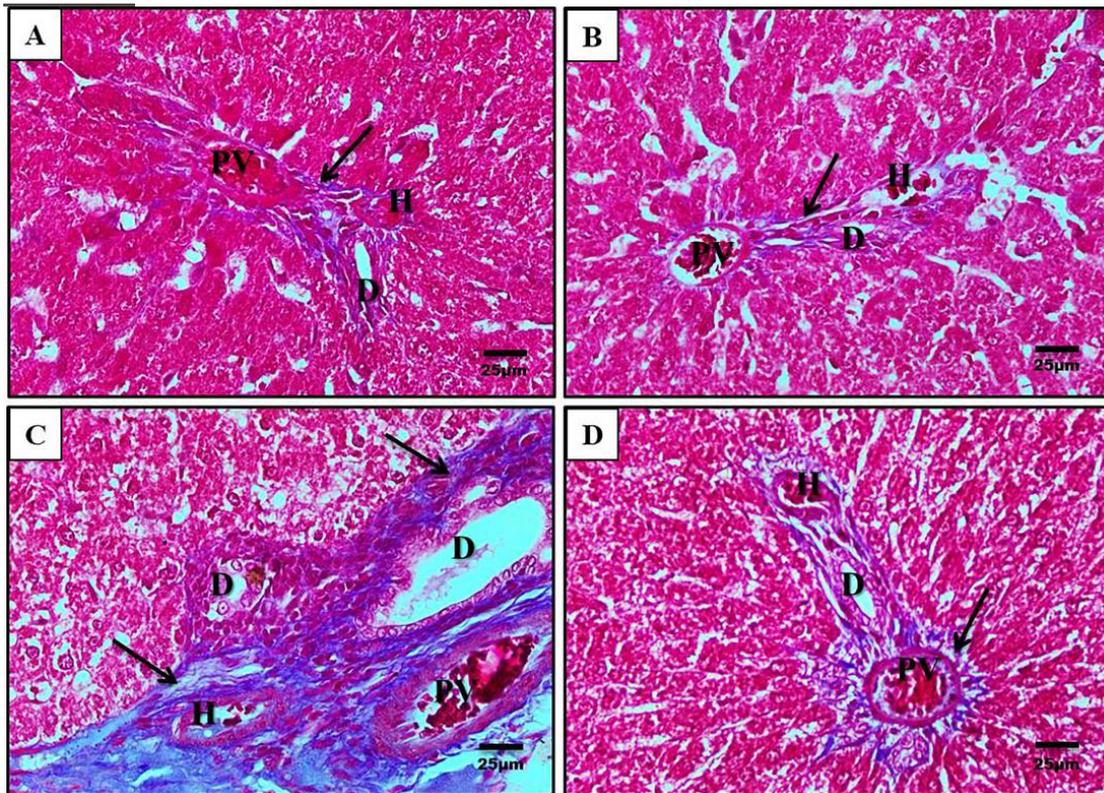
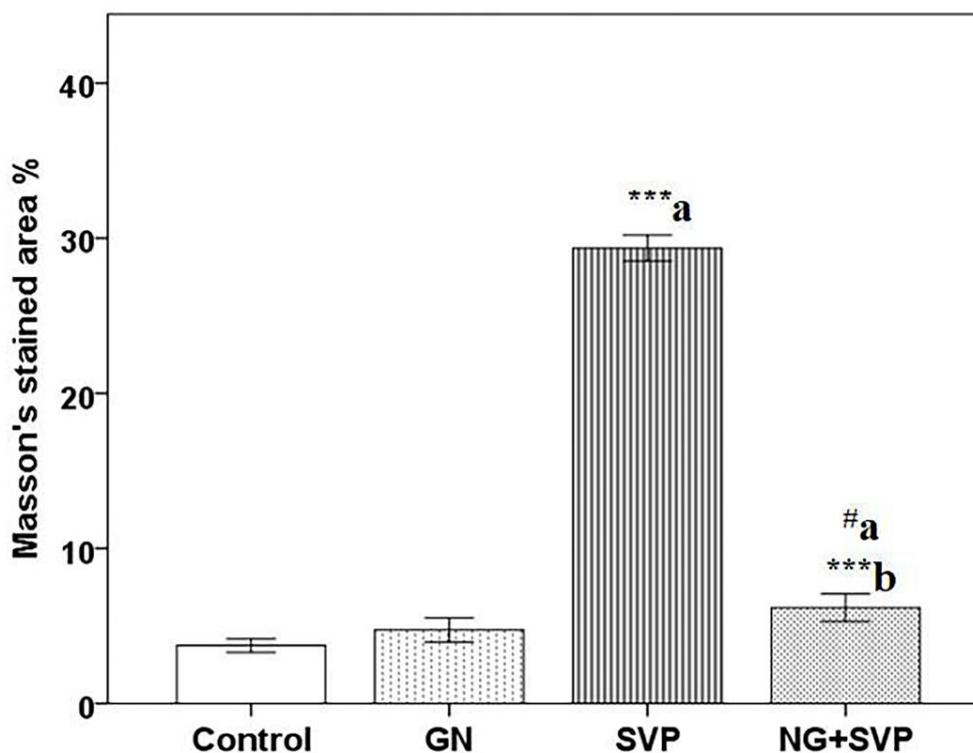


Fig 3: **A, B):** Liver photomicrographs of control and GN-administrated rats respectively. Both of them illustrate minimal blue-stained fibers mainly at the portal areas (arrows). **C):** Liver Photomicrograph of SVP-treated rat showing extensive fibrosis around the portal area (arrows). **D):** Photomicrograph of GN+SVP co-treated rat illustrating mild blue fiber staining around the portal area (arrow). PV = portal vein, D = bile duct, H = hepatic artery. (Masson's trichrome; X400).

Table 2: The mean Masson's trichrome, TNF- α , and TGF- β stained area % per field in all studied groups

	Control	GN	SVP	GN+SVP	<i>P value</i>
Masson's trichrome	3.74 \pm 0.45	4.75 \pm 0.78	29.36 \pm 0.84	6.17 \pm 0.89	P1= 0.000 P2= 0.000 P3= 0.019
TNF- α	0.78 \pm 0.09	0.7 \pm 0.04	13.37 \pm 1.44	4.69 \pm 1.01	P1= 0.000 P2= 0.000 P3= 0.003
TGF- β	0.57 \pm 0.09	0.74 \pm 0.07	8.03 \pm 0.94	2.77 \pm 0.48	P1= 0.000 P2= 0.000 P3= 0.004

The listed values are expressed as mean \pm SD by utilizing ANOVA test. P1:P3 represent post-hoc Tukey test where **P1:** SVP versus control, **P2:** GN+SVP versus SVP, **P3:**GN+SVP versus control.



Histogram 3: Histogram illustrating the mean Masson's trichrome stained area percentage in all studied groups. The SVP-treated group illustrates a significant increase in the blue-stained area compared to the control group. Co-treatment with GN+SVP significantly decreased the blue-stained area in comparison with the SVP group. SVP=sodium valproate, GN=ginger. # $p < 0.05$, *** $p < 0.0001$. **a:** versus control. **b:** versus SVP.

III- Immunohistochemical Results:

A) Immunostaining for TNF- α :

The TNF- α immunohistochemical staining showed a minimal cytoplasmic reaction in both control (Fig. 4A) and GN groups (Fig. 4B). The SVP group showed a significant increase in the TNF- α

immunohistochemical reaction (Fig. 4C) as compared to the control group. The GN+SVP group illustrated a significant decrease in the TNF- α immunohistochemical expression (Fig. 4D) as compared to the SVP group but still significantly higher than the control group (Table 2, Histogram 4).

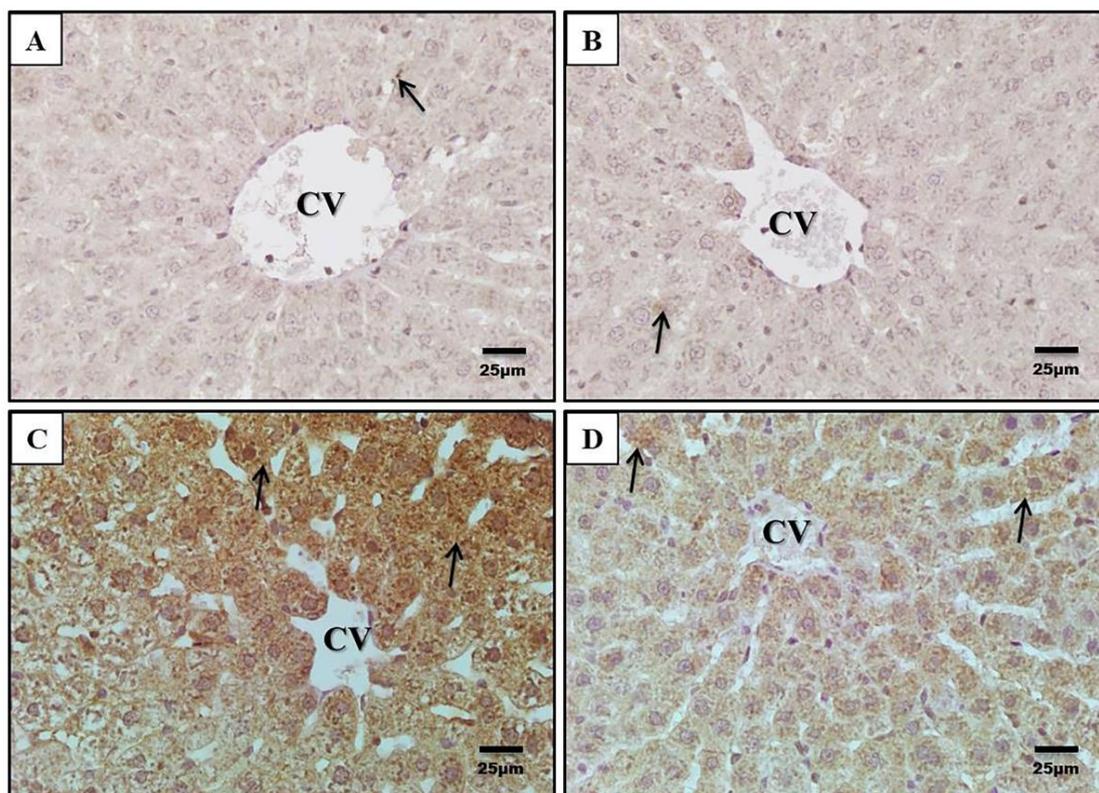
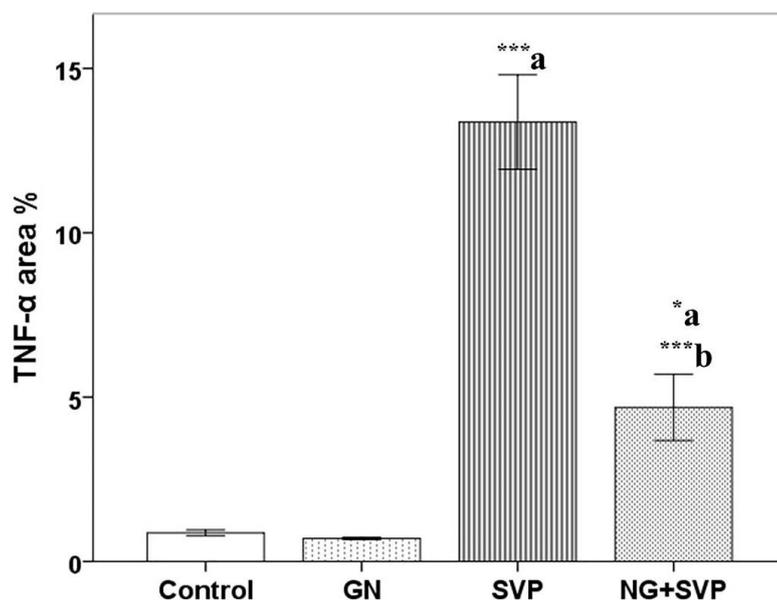


Fig. 4: **A, B):** Liver photomicrographs of control and GN-administrated rats respectively. Both of them illustrate minimal TNF- α immunohistochemical expression (arrows). **C):** Liver photomicrograph of SVP-treated rat representing marked positive TNF- α expression (arrows). **D):** Photomicrograph of GN+SVP co-treated rat illustrating moderate TNF- α expression (arrows). CV=central vein. (Immunohistochemistry for TNF- α with H&E counterstain; X400).



Histogram 4: Histogram illustrating the mean area percentage of TNF- α immunohistochemical stain in different groups. The SVP-treated group illustrates a significant increase as compared to the control group. Co-treatment with GN+SVP significantly reduced the TNF- α immunostained area in comparison with the SVP group. SVP= sodium valproate, GN=ginger. * $p < 0.01$, *** $p < 0.0001$. **a:** versus control. **b:** versus SVP.

B) Immunostaining for TGF- β :

The TGF- β immunohistochemical staining showed a very weak cytoplasmic reaction in both control (Fig. 5A) and GN groups (Fig. 5B). Treatment with SVP significantly increased the TGF- β expression (Fig.

5C) as compared to the control group. The GN+SVP group demonstrated a significant decrease in the TGF- β expression (Fig. 5D) as compared to the SVP group, however, it still significantly higher than the control group (Table 2, Histogram. 5).

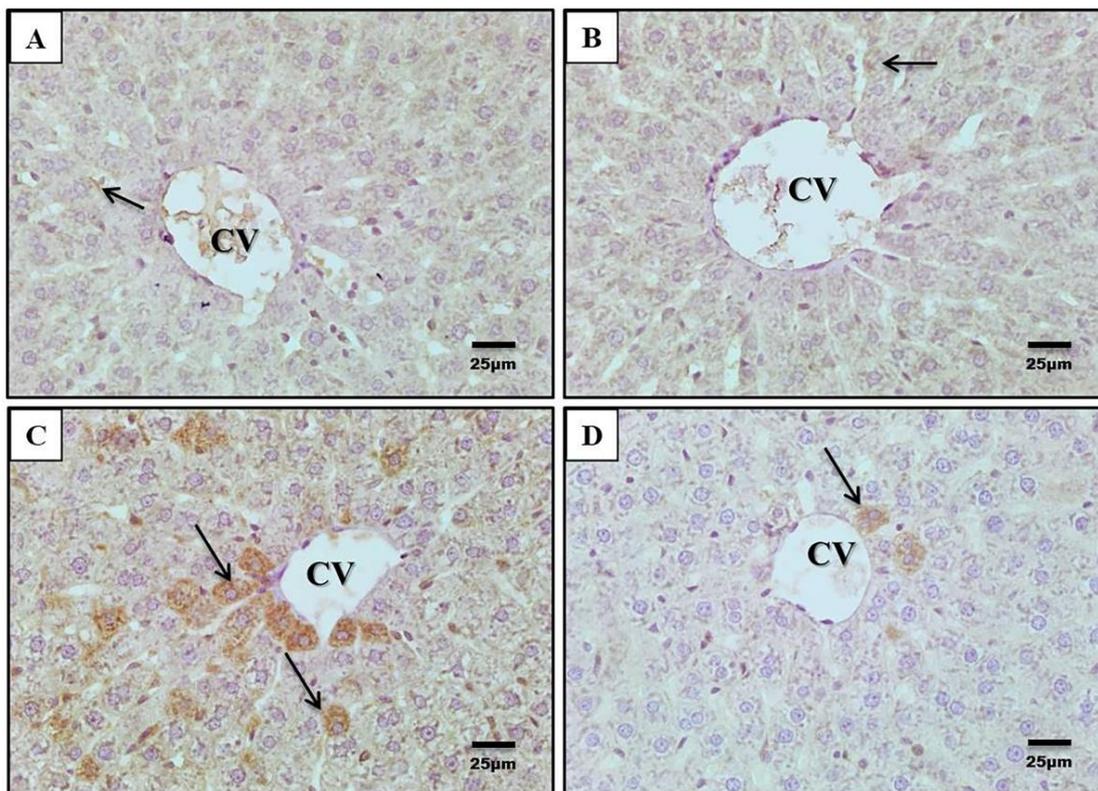
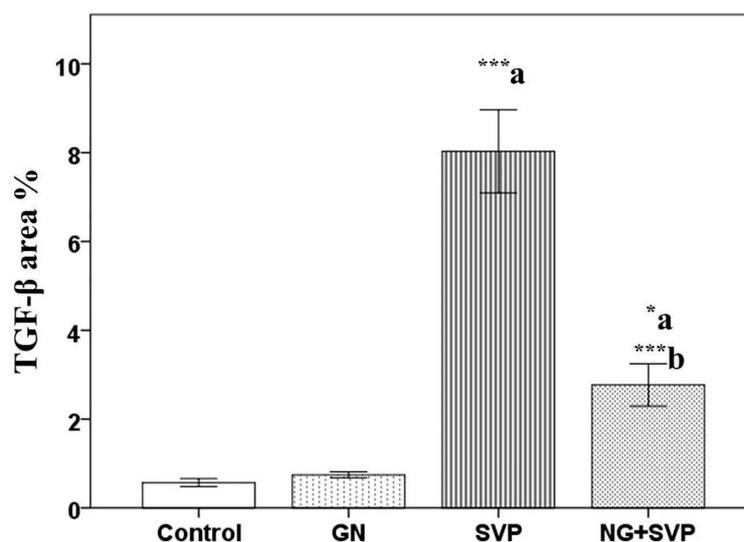


Figure 5: A, B): Liver photomicrographs of control and GN-administrated rats respectively. Both of them illustrate very weak TGF- β immunohistochemical expression (arrows). **C):** Liver photomicrograph of SVP-administrated rat demonstrating a strong positive TGF- β expression (arrows). **D):** Photomicrograph of GN+SVP co-treated rat illustrating mild TGF- β expression (arrow). CV=central vein. (Immunohistochemistry for TGF- β with H&E counterstain; X400).



Histogram 5: Histogram illustrating the mean area percentage of TGF- β immunohistochemical stain in all studied groups. The SVP-treated group illustrates a significant increase as compared to the control group. Co-treatment with GN+SVP significantly decreased the TGF- β immunostained area in comparison with the SVP group. **SVP**= sodium valproate, **GN**=ginger. * $p < 0.01$, *** $p < 0.0001$. **a**: versus control. **b**: versus SVP.

DISCUSSION

The liver is the primary organ of the metabolism and the breakdown of many anticonvulsants, so it is at risk for drug damage. Damage to the liver encompasses a range of hepatotoxic reactions, ranging from mild to temporary damage to fatal liver failure (Ahmed and Siddiqi, 2006). It is well known that 20% of valproate consumers may have an increase in liver enzyme levels (Omidipour et al., 2021). Recent studies illustrated that the inflammatory process is greatly implicated in the pathogenesis of hepatic injury oscillating from the early to the late-stage liver disease (Xiao et al., 2015).

The levels of ALT and AST are the most sensitive signs of hepatic cell injury because they are located inside the hepatocytes, and their leak is associated with cellular damage (Zeashan et al., 2009; Wang et al., 2015). In accordance with the previous studies (Abdelkader et al., 2020; Pirozzi et al., 2020; Omidipour et al., 2021), the current study illustrated a significant elevation in ALT and AST after SVP treatment. Co-administration GN significantly lowered the increased ALT and AST to nearly the control level

indicated its hepatoprotective effect. The protective effect of GN on the liver enzymes was proved in different hepatotoxic models (Badawi, 2018; Abd-Elrhman et al., 2020; Alsahli et al., 2021).

The exact mechanism of liver damage in SVP-induced hepatotoxicity is not fully known till now, but many mechanisms have been suggested such as oxidative stress formation, lipid peroxidation, glutathione depletion, increased apoptosis, and microvesicular hepatic steatosis (Gayam et al., 2018; Salimi et al., 2020). The balance disturbance between ROS and anti-oxidants induces biochemical and physiological dysfunctions (Mustafa et al., 2013; Gunata and Parlakpinar, 2020). The antioxidant enzymes such as glutathione (GSH), superoxide dismutase, and catalase protect the liver from oxidative injury (Kumar et al., 2012) since they change ROS into stable particles like O₂ and water (Uttara et al., 2009). Newly it was proved that SVP stimulates the formation of ROS (Ardianto et al., 2020).

Regarding the oxidative stress pathway, multiple studies on animal

models have suggested that SVP treatment is associated with an elevation in the liver level of an endogenous lipid peroxidation marker, and mitochondrial dysfunction (Omidipour *et al.*, 2021). Lipid peroxidation in biological membranes is one of the most common events that occur after the toxicity and oxidative stress in cells. The increased lipid peroxidation levels could damage the cell wall. GSH is one of the most important molecules in the cellular defence system against chemical reactions of toxic compounds and stress. Decreased GSH levels, which indicate GSH depletion, make cells more susceptible to chemical damage (Prakash *et al.*, 2008).

In accordance with the previous findings (Jamshidi *et al.*, 2020; Koroglu *et al.*, 2021), we illustrated a significant elevation in the MDA, and a reduction in the GSH levels in the SVP group indicated an oxidative stress state. Previous *in vivo* researches showed that SPV significantly increased the production of mitochondrial ROS and decreased reduced GSH levels in isolated rat hepatocytes (Jafarian *et al.*, 2013). The levels of these oxidative stress markers greatly reversed in the GN+SVP group proved the anti-oxidant property. Alsahli *et al.* (2021) found that GN could modulate the levels of MDA and GSH and attenuate the diethylnitrosamine-induced liver injury in rats through its high antioxidant properties and free radical scavenging ability that protects against the oxidative damage caused by free ROS.

Our results of hepatic affection and collagen deposition in the H&E and Masson's trichrome stains were in correspondence with Omidipour *et al.* (2021) findings who demonstrated hepatic lobular distortion in form of liver fibrosis, necrosis, and inflammation of hepatocytes in the rats treated with SVP (300 mg/kg/day) or higher doses. In another study, excess production of collagen, and hepatic lesions had occurred in the SVP treated

group (250 mg/kg/day) (Tanvir *et al.*, 2015). The reduction of collagen fibers deposition in the GN+SVP group was supported by Algardaby *et al.* (2016) who reported a significant amelioration in the hepatic fibrosis caused by CCl₄ after concomitant GN administration. Also, these results were more confirmed by those of Badawi (2018) who found an average deposition of few collagen fibers around the central vein and blood sinusoids in the animals co-treated with piroxicam and GN.

It is well known that hepatic cell injury follows the inflammatory process in the liver. In the event of hepatic injury, immune stimulation initiates nuclear translocation of NF- κ B with subsequent release of pro-inflammatory (IL-1 β , TNF- α) cytokines (Elsharkawy and Mann, 2007; Savastano *et al.*, 2015). The overexpression of pro-inflammatory cytokines increases the ROS and hereafter, oxidative stress which causes cell damage (Li *et al.*, 2016). In the present study, the TNF- α expression was significantly increased in the SVP group. In accordance with our result, Nazmy *et al.* (2016) found that the TNF- α level increase by 3.6-fold in the group treated with SVP (700 mg/kg/day). Moreover, Oztopuz *et al.* (2020) reported a two-fold increase in the liver TNF- α and IL-1 β gene expression of the SVP-administered group.

Natural products have been previously confirmed to decrease the level of the inflammatory marker and inhibit the subsequent pathogenesis (Rahmani *et al.*, 2019; Almatroodi *et al.*, 2020). GN was reported to reduce chronic liver and kidney inflammation in rats by reducing the pro-inflammatory cytokines (TNF- α and IL-1 β) expression (Mansour *et al.*, 2019; Abd-Elrhman *et al.*, 2020) which was matched with the current finding.

Inflammation is responsible for the progression of fibrosis (Eddy, 2000). Inflammation, caused by oxidative stress, is a crucial event in hepatic stellate cells (HSCs) activation

(Greenwel *et al.*, 2000). In chronic liver diseases, the HSCs are the main source of extracellular matrix (ECM) proteins leading to permanent fibrosis. Under normal conditions, these cells show little proliferative activity (Watanabe *et al.*, 2011). Numerous pro-inflammatory mediators released by damaged hepatic cells induce HSCs activation (Pinzani and Macias-Barragan 2010).

TGF- β is a pro-fibrotic mediator which elicits a fibrogenic response to inflammation. It stimulates the formation of ECM constituents, mesenchymal cell production, migration, and accumulation (Pohlers *et al.*, 2009). The current study reported a significant increase in the TGF- β expression in the SVP group which was confirmed by the previous study of Pirozzi *et al.* (2020) and Abd-Elrhman *et al.* (2020).

The SVP-induced fibrosis could be explained by the enhanced expression of TGF- β . TGF- β plays an essential role in enhancing collagen synthesis (Arias *et al.*, 2002). In the damaged liver, HSCs performed several changes as a response to pro-fibrogenic mediators such as TGF- β , connective tissue growth factor, and platelet-derived growth factor. During these phenotypic changes, HSCs acquire the ability to proliferate and produce an excessive amount of ECM proteins (Bulow *et al.*, 2007; Brenner, 2009).

Liver fibrosis is one of the major health concerns that resulted in significant morbidity and mortality (Sánchez-Valle *et al.* 2012). Until now, there is no medical-approved treatment for hepatic fibrosis. Several efforts have been made to prevent hepatic fibrosis by suppressing molecular pathways which are vital for the fibrogenesis pathway, as TGF- β and toll-like receptor-4 (TLR-4) (Bataller and Brenner 2005; Popov and Schuppan 2009). GN has been reported to inhibit molecular targets implicated in hepatic fibrosis as TLR-4 (Lee *et al.* 2012). In this study, the anti-fibrotic activity of GN was confirmed not only by the

reduction in Masson's trichrome staining but also by the significant reduction in the TGF- β immunohistochemical expression. The suppressive effect of GN on TGF- β expression was reported by previous researches in the CCl₄ liver fibrosis model (Puche *et al.* 2013; Algandaby *et al.*, 2016). Moreover, GN revealed an anti-fibrotic role against in vitro TGF- β -stimulated HSC-T6 cells and in vivo DMN-induced hepatic fibrosis (Cheong *et al.*, 2016). TGF- β is a basic mediator of HSCs activation. Consequently, the TGF- β inhibition signals could be used as a potential target for medical intervention in patients with hepatic fibrosis.

In addition, the anti-fibrotic property of GN could be related to its ability to prevent HSCs activation by lipid peroxidation inhibition and suppression of the inflammatory process in the early hepatic fibrosis. Also, it inhibits NF- κ B protein translocation into the nucleus and the expression of TNF- α and IL-1 β mRNA which regulate the expression of the inflammatory proteins. Furthermore, GN was reported to stimulate AMP-activated protein kinase (Li *et al.* 2013), which could suppress the production of NF- κ B-dependent mediators in TLR-4-stimulated cells (Zhao *et al.* 2008).

In addition, the ROS pathway is found to be correlated with HSCs activation and ECM proliferation causing accumulation of fibrous tissue (Algandaby *et al.*, 2016). The hepatoprotective effect of GN is postulated as it possesses antioxidant and anti-inflammatory properties as liver histology was remarkably improved in the GN added group. This effect could be explained partially by the down-regulation of the TNF- α /TGF- β signalling pathway.

Conclusion

In conclusion, our findings suggest that GN has a hepatoprotective activity due to its anti-oxidant, anti-inflammation, and anti-fibrotic potentials as it significantly improved

the liver function and oxidative stress state. This was confirmed by hepatocellular architecture study through H&E, Masson's trichrome, and immunohistochemical stains for TNF- α and TGF- β and oxidative stress markers. These results suggest that GN could be used as a liver supportive supplement in SVP-treated patients.

Ethical Approval: The experiments were approved by the Institutional Board Review and Ethics Committee of the Faculty of Medicine, Mansoura University.

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ARABIC SUMMARY

تقييم الدور الوقائي المحتمل للزنجيل على السمية الكبدية التي يسببها فالبروات الصوديوم في ذكور الجرذان البيضاء البالغة: دراسة كيميائية حيوية ونسجية وكيميائية مناعية

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المقدمة: يستخدم دواء فالبروات الصوديوم على نطاق واسع لعلاج الصرع والعديد من الاضطرابات العصبية الأخرى. ومع ذلك فإن إصابة الكبد هي أحد الآثار الجانبية الضارة المرتبطة بفالبروات الصوديوم. ويظهر أن الإجهاد التأكسدي والالتهاب والتليف يلعبون أدوارًا مهمة في السمية الكبدية التي يسببها فالبروات الصوديوم.

هدف الدراسة: صممت الدراسة الحالية لتقييم التأثيرات السمية الكبدية لفالبروات الصوديوم على ذكور الجرذان البيضاء، ولتقييم الدور الوقائي المحتمل للزنجيل في تلافي هذا التأثير.
المواد والطرق المستخدمة: قسمت 24 من ذكور الجرذان البالغة التي يتراوح وزنها بين (180 جم - 220 جم) إلى أربع مجموعات متساوية عشوائياً: المجموعة 1 (المجموعة الضابطة): تلقت المحلول الملحي. المجموعة: عولجت بزنجيل بجرعة (200 مجم / كجم / يوم). المجموعة 3: عولجت بفالبروات الصوديوم بجرعة (300 مجم / كجم / يوم). المجموعة 4: عولجت بالزنجيل بجرعة (200 مجم / كجم / يوم) مع فالبروات الصوديوم بجرعة (300 مجم / كجم / يوم). واعطيت جميع الأدوية يوميًا لمدة 14 يومًا بأنبوب فموي. وفي الوقت المحدد تم التضحية بالجرذان، وجمعت عينات الدم والكبد وعولجت للتحليل البيوكيميائية والنسجية والكيميائية المناعية.

النتائج: تسبب العلاج بفالبروات الصوديوم في ارتفاع كبير في إنزيمات الكبد (ALT و AST)، وإحدى علامات الإجهاد التأكسدي (MDA) مع انخفاض كبير في إنزيم مضادات الأكسدة (GHS) الذي يشير إلى الإجهاد التأكسدي الكبدي. وكشف الفحص الهستولوجي عن فقدان لبنية الكبد الطبيعية، وتليف واسع النطاق صبغ بصبغة ماسون ثلاثية الألوان. علاوة على ذلك، أظهرت المجموعة التي عولجت بفالبروات الصوديوم زيادة كبيرة في كل من تعبير TNF- α و TGF- β المناعيين. ولكن العلاج بفالبروات الصوديوم مع الزنجيل أظهر انعكاساً في التغييرات السابقة بشكل كبير.

الاستنتاج: لوحظ أن الزنجيل يمكنه حماية الكبد وذلك لكونه مضاداً للأكسدة والالتهابات والتليف.