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Effect of Ginger and Turmeric Extracts Enhanced Formula on Non-Alcoholic Fatty Liver Induced by Oxy-Tetracycline in Wistar Rats

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Abstract

Non-alcoholic fatty liver disease (NAFLD)represents a significant health burden globally, with increasing prevalence rates parall eling the rise in obesity and metabolic syndrome. Turmeric and ginger are natural substances with antioxidant, anti-inflammatory and other medicinal properties. This study investigates the therapeutic effects of ginger and turmeric ethanolic extracts individually and after enhancement of bioavailability through the addition of phospholipids and piperine on oxytetracycline-induced fatty liver. The effect of these extracts was compared to the effect of fenofibrate (hypolipidemic medication). The experiment was conducted using thirty-five Wistar rats, divided into seven groups (five rats per group). Group 1: (the Negative control group) was fed on the basal diet only and the other six groups were fed on basal diet and intraperitoneal oxytetracycline injection (120 mg/kg/body weight daily) for three consecutive days to induce non-alcoholic fatty liver induction. followed by different treatments. Group 2: (The positive control group) was fed basal diet + intraperitoneal oxytetracycline injection (120 mg/kg/body weight daily) for three consecutive days only. Group 3: was treated with (100 mg/kg) of Fenofibrate. Groups 4 and 5: were treated with (500 mg/kg) of ginger and turmeric ethanolic extracts, individually. Groups 6 and 7: were treated with the mixture consisting of (500 mg/100 mg/3 mg) of ginger and turmeric ethanolic extracts individually, phospholipids, and piperine.The extracts for all treated groups will be administered orally through a gastric tube day after day for 30 days.Serum liver function, serum and hepatic lipid profile and histological changes were assessed. The results indicated that all groupstreated with ginger and turmeric enhanced formulation through piperine and phospholipids showed a significant decrease in liver enzyme activities, bilirubin total, direct and indirect, triglycerides, total cholesterol, LDL-cholesterol and VLDL. At the same time, HDL-cholesterol has significantly increased especially in theturmeric-enhanced formula. Results suggest that the enhanced bioavailability formulations of turmeric and ginger exhibit promising therapeutic potential comparable to fenofibrate in mitigating NAFLD.

Keywords: Non-alcoholic Fatty Liver Disease, Ginger, Turmeric, Phospholipid, Piperine, Oxytetracycline, Fenofibrate.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver disorders ranging from simple steatosis (fat accumulation in the liver) to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. With its rising prevalence and lack of specific pharmacotherapies, NAFLD represents a pressing public health concern. Understanding the pathophysiology of NAFLD is crucial for developing effective treatments and preventive strategies.The liver plays a pivotal role in lipid metabolism. In NAFLD, there is an imbalance between lipid acquisition and disposal. Increased FFAs uptake, enhanced de novo lipogenesis, and decreased beta-oxidation contribute to lipid accumulation in hepatocytes. Impaired very-low-density lipoprotein (VLDL) secretion also exacerbates hepatic steatosis [2].Oxidative stress is

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a key factor in the progression from simple steatosis to NASH. The accumulation of FFAs in the liver leads to increased mitochondrial beta-oxidation, which produces reactive oxygen species (ROS). Excessive ROS generation overwhelms the antioxidant defences, leading to oxidative damage of lipids, proteins, and DNA [3]. The importance of antioxidants in mitigating oxidative stress and inflammation against human diseases has been extensively reviewed [4].This oxidative stress triggers inflammatory pathways, contributing to liver injury and fibrosis.Chronic inflammation is a hallmark of NASH. Hepatocyte injury and death release damage-associated molecular patterns (DAMPs) that activate Kupffer cells, the liver's resident macrophages. Activated Kupffer cells produce pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β, which exacerbate inflammation and liver injury [5]. Additionally, the activation of the NF-κB pathway plays a crucial role in maintaining the inflammatory response in NASH.Persistent inflammation and oxidative stress in NASH lead to the activation of hepatic stellate cells (HSCs), which play a central role in fibrosis. Activated HSCs produce extracellular matrix components, leading to scar tissue formation and fibrosis. Over time, extensive fibrosis disrupts liver architecture and function, progressing to cirrhosis [6].Cirrhosis significantly increases the risk of hepatocellular carcinoma (HCC). Chronic liver injury and regeneration create a pro-tumorigenic environment. Genetic and epigenetic alterations in hepatocytes, along with the inflammatory microenvironment, contribute to the development of HCC in patients with NAFLD [7].

Oxytetracycline, a broad-spectrum antibiotic, is widely used in medicine, but its hepatotoxic effects are well-documented. The mechanism by which oxytetracycline induces non-alcoholic fatty liver disease (NAFLD) involves multiple pathways, including oxidative stress, mitochondrial dysfunction, and lipid metabolism dysregulation. Oxytetracycline-induced NAFLD in animal models shares histological and biochemical similarities with human NAFLD, making it a suitable model for investigating potential therapeutic interventions [8].

Traditional treatments for NAFLD include lifestyle modifications such as diet and exercise, as well as pharmacological interventions like the use of insulin sensitizers and lipid-lowering agents [9]. Fenofibrate, a peroxisome proliferator-activated receptor-alpha (PPAR-α) agonist, is commonly used to treat hyperlipidemia and has shown efficacy in alleviating NAFLD in the preclinical and clinical studies and being a commonly used medication [10]

In addition to conventional treatments, there is growing interested in the use of natural products for the management of NAFLD such asturmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) ethanolic extracts. These botanicals contain bioactive compounds such as gingerols, shogaols, paradols (in ginger), and curcuminoids (in turmeric), which have long been revered for their extensive therapeutic properties, like anti-inflammatory, antioxidant,and hepatoprotective properties and are deeply rooted in traditional medicine [11,12]. However, their clinical application is limited by poor bioavailability due to rapid metabolism, low solubility and poor absorption [13].Consequently, the beneficial effects of ginger and turmeric may not be fully realized when consumed in their natural form. To address this limitation, researchers have explored various strategies to enhance the bioavailability of gingerols and curcuminoids.Among these, using adjuvants such as phospholipids and piperine has shown promise [14;15].

Phospholipids, particularly lecithin, improve the solubility and absorption of bioactive compounds, while piperine, a compound found in black pepper, inhibits metabolic enzymes, thereby increasing the bioavailability of gingerols and curcuminoids [16;17].

The study aims to enhance the therapeutic efficiencyagainst NAFLD of the ethanolic extracts of ginger and turmeric by combining these extracts with phospholipid and piperine. Also, the effects of these extracts and theircombinationon blood, hepatic biochemistry and hepatic histopathology compared to Fenofibrate.

2. Materials and Methods

2.1. Martials

The chemical compounds, Oxytetracycline was obtained from the Arabco-med pharmaceutical company, Obour City Industrial area, Cairo, Egypt. and Fenofibrate was obtained from Mina pharm for pharmaceutical and Chemical Industries Co. (10th Ramadan - Egypt) and Piperine was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phospholipids were obtained from Amoun for Pharmaceuticaland Chemical Industries Co. Obour City Industrial area, Cairo, Egypt. Other chemicals and solvents used throughout this study were of analytical grade.

2.2. Methods:

2.2.1. Preparation of plant extracts:

Five hundred grams of turmeric (Curcuma longa) rhizomes or ginger (Zingiber officinale) roots that were obtained from Agricultural Seeds, Spices and Medicinal Plants Co., Al-Azhar St., Cairo, Egypt extracted twice with 2:1 of ethanol (70%) for 24 hours at room temperature (25°C), and the samples were filtered after each extraction. The solvent was removed from the extracts with a vacuum rotary evaporator at 40°C to obtain crude plant extracts. The dried extracts were weighed and stored at –20°C according to [18] for ginger and according to [19] for turmeric.

2.2.2. Experimental design:

A total of 35 male Wistar albino rats, 100g, average body weight were divided into 7 groups, each consisting of 5 rats that were obtained from the National Research Center, Dokki, Giza, Egypt.

The rats were kept under normal laboratory conditions. Fed on a basal diet formulated and consisting of corn starch 70%, casein 10%, corn oil 10%, salts mixture 4%, vitamin mixture 1% and cellulose 5% [20].For one week as an adaptation period before starting the experiment.

All rats had free access to water and the basal diet throughout the experimental period in all groups. These rats were maintained at 23 ± 2 °C with 50 % ± 10 % moisture.

According to the types of plant extracts mixtures. Rats were divided into 7 groups each group containing five rats and six groups of them were injected Intraperitoneally with oxytetracycline (120 mg / Kg body weight for three consecutive days to induce NAFLD induction as follows:

1- Negative control group: rats received basal diet only.
2- Positive control group rats were injected Intraperitoneally with oxytetracycline (120 mg / Kg body weight for three consecutive days.
3- group 3: was fed on the basal diet and fenofibrate (100mg/kg).
4- group 4 received ginger ethanolic extracts (500 mg/ Kg body weight)
5- group 5 received turmeric ethanolic extracts (500 mg/ Kg body weight).
6- group 6: rats received a mixture of ginger ethanolic extract, phospholipid, and piperine (500mg, 100 mg,and 3 mg/Kg body weight).
7-group 7: rats received a mixture of turmeric ethanolic extract, phospholipid, and piperine (500mg, 100 mg,and 3 mg/Kg body weight).

The extracts were received in all treated groups orally through gastric tube day after day for 30 days. These experimental animals were treated according to the ethics committee by the Faculty of Agriculture, Ain Shams University.TheEthics Committee approved this animal study, which was conducted in conformity with international guidelines for the care and welfare of laboratory animals [Approval no. 9-2024-05]

2.2.3. Blood and sample collection:

At the end of the experiment, the rats were anaesthetized with ether and blood samples were collected from the retro-orbital vein of all the animals by a capillary tube. Serum was prepared from the collected blood and kept in the freezer for biochemical analysis. After blood collection and under anaesthesia with ether the rats were euthanasia and the liver was removed and divided into two sections, one of them was prepared [21]and kept in the freezer for hepatic biochemical assays, and the other section was stored in 10% formalin for histopathological assessment. Blood biochemical assayswere determined using reagent kits including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [22], bilirubin total and direct [23], alkaline Phosphatase (ALP) [24], Gamma-Glutamyl transferase (GGT) [25], total protein [26], total Cholesterol [27], High-Density Lipoprotein-Cholesterol HDL [28], Low-Density Lipoprotein-Cholesterol LDL [29], Triglycerides [30], hepatic total cholesterol and total triglycerides were determined using reagent kits after liver was homogenized with isopropanol according to the method described by [27 and 30].Kits were obtained from The Egyptian Company for Biotechnology, Obour city Industrial area, block 20008 pieces 19A. Cairo, Egypt. Histological investigations for hepatic tissue [31].

2.2.4. Statistical analysis:

The data were presented as means ± SE of five replicates and subjected to one-way ANOVA. The means of different treatments were compared using Duncan's multiple range test at $p \leq 0.05$. Statistical analyses were performed using SPSS statistical software (IBM SPSS Statistics, version 30) [32].

3. Results.

3.1. Blood biochemical assays.

3.1.1. Liver function

Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP) and the levels of bilirubin total, bilirubin direct, bilirubin in-direct and total protein were presented in Table (1).

The normal values in the negative control group were recorded as follows: ALT (29.60 U/L), AST (39.10 U/L), GGT (21.2 U/L), ALP (96.6 U/L) Bilirubin total (0.39 mg/dl), Bilirubin direct (0.15 mg/dl), Bilirubin indirect (0.23 mg/dl) and total protein (7.10 g/dl).

Treated by oxytetracycline to induced non-alcoholic fatty liver (NFLD) in the positive control group showed significant increase ($P<0.05$) in serum ALT (293.40), AST (304.40 U/L), GGT (80.80 U/L), ALP (256.60 U/L), Bilirubin total (2.24 mg/dl) and Bilirubin indirect (0.86 mg/dl). In addition to, a significant ($P<0.05$) decrease in serum total protein (4.37 g/dl) and an insignificant ($P<0.05$) increase in Bilirubin direct (0.83 mg/dl) when compared with the normal values in the negative control group.

After induction of fatty liver with oxytetracycline followed by treatment with Fenofibrate day after day for 30 days there was insignificant ($P<0.05$) change in serum ALT (274.20 U/L), AST (287.20 U/L) and total protein (4.62 mg/dl) while, there was a significant ($P<0.05$) increase in Bilirubin total (2.58 mg/dl), Bilirubin direct (1.09 mg/dl), Bilirubin indirect (1.49 mg/dl) and ALP (256.60 U/L). In addition to a significant ($P<0.05$) decrease in GGT (29.80 U/L).

Ginger and turmeric groups after 30 days on treatment on their extracts day after day showed a significant ($P<0.05$) decrease but not reach normal values in ALT (84.40 and 132.60 U/L), AST (95.40 and 142.00U/L), GGT (54.40 and 65.20 U/L), Bilirubin total (0.79 and 0.89 mg/dl), Bilirubin direct (0.23 and 0.29 mg/dl), Bilirubin indirect (0.56 and 0.60 mg/dl) an total protein (6.18 and 6.05 g/dl) while,there was an insignificant ($P<0.05$) decrease in ALP (146.80 and 176.00 U/L)when compared with the negative control group.

Enhanced formula of ginger and turmeric groups specially in turmeric enhanced formula group recorded a significant (P<0.05) decrease in ALT (64.40 and 57.20 U/L), AST (74.80 and 67.20 U/L), GGT (37.06 and 35.88 U/L), ALP (141.60 and 119.80 U/L), Bilirubin total (0.60 and 0.51 mg/dl), Bilirubin direct (0.22 and 0.21 mg/dl), Bilirubin indirect (0.37 and 0.29 mg/dl) and total protein (6.54 and 6.66 g/dl) when compared with the negative control group.

Table (1) Effect of Oxytetracycline (Positive control), Fenofibrate, Ginger, Turmeric, Ginger-enhanced formula (Ginger + PIP + PL) and Turmericenhanced formula (Turmeric + PIP + PL) on the levels of ALT, AST, GGT, ALP, Bilirubin total, direct, indirectand total protein.

	Negative Control	Positive Control	Fenofibrate	Ginger	Turmeric	(Ginger + PIP + PL)	(Turmeric + PIP + PL)
ALT (U/L)	$29.60^{e}\pm$ 2.54	$293.40^{a}\pm$ 3.95	$274.20^{a}\pm$ 18.85	$84.40^{c}\pm$ 5.56	$132.60^{b}\pm$ 5.82	$64.40^{cd}\pm$ 1.02	$57.20^{d}\pm$ 3.05
AST (U/L)	$39.10^{e}\pm$ 2.72	$304.40^{a}\pm$ 6.48	$287.20^{a}\pm$ 26.43	$95.40^{c}\pm$ 6.65	$142.00^{b}\pm$ 7.87	$74.80^{cd}\pm$ 1.49	$67.20^{d}\pm$ 3.47
GGT (U/L)	$21.2^{f}\pm$ 3.00	$80.80^{a}\pm$ 4.46	$29.80^{e}\pm$ 3.54	$54.40^{c}\pm$ 1.12	$65.20^{b}\pm$ 3.03	$37.06^{d}\pm$ 0.53	$35.88^{de}\pm$ 0.43
ALP (U/L)	$96.6^{d}\pm$ 6.77	$135.20^{c}\pm$ 3.42	$256.60^{a}\pm$ 28.38	$146.80^{c}\pm$ 2.32	$176.00^{b}\pm$ 10.10	$141.60^{c}\pm$ 3.90	$119.80^{cd}\pm$ 4.02
Bilirubin Total (mg/dl)	$0.39^{d}\pm$ 0.02	$1.24^{b}\pm$ 0.25	$2.58^{a}\pm$ 0.40	$0.79^{c}\pm$ 0.03	$0.89^{bc}\pm$ 0.01	$0.60^{cd}\pm$ 0.01	$0.51^{cd}\pm$ 0.01
Bilirubin Direct (mg/dl)	$0.15^{b}\pm$ 0.02	$0.38^{b}\pm$ 0.10	$1.09^{a}\pm$ 0.39	$0.23^{b}\pm$ 0.02	$0.29^{b}\pm$ 0.01	$0.22^{b}\pm$ 0.01	$0.21^{b}\pm$ 0.01
Bilirubin Indirect (mg/dl)	$0.23^{e}\pm$ 0.01	$0.86^{b}\pm$ 0.19	$1.49^{a}\pm$ 0.10	$0.56^{cd}\pm$ 0.04	$0.60^{c}\pm$ 0.00	$0.37^{de}\pm$ 0.01	$0.29^{e}\pm$ 0.01
Total Protein (g/dl)	$7.10^{a}\pm$ 0.17	$4.37^{e}\pm$ 0.21	$4.62^{e}\pm$ 0.12	$6.18^{cd}\pm$ 0.03	$6.05^{d}\pm$ 0.06	$6.54^{bc}\pm$ 0.09	$6.66^{b}\pm$ 0.13

The data are presented as means ± SE calculated of five replicates. Different letters refer to significant differences at (P<0.05).

3.1.2. Lipid profile.

As shown in Table (2) data inthe positive control group there was a significant (P<0.05) increase in the levels of total cholesterol (322.00 mg/dL), triglyceride (5332.40 mg/dL), LDL (181.92 mg/dL), VLDL (106.48 mg/dL), total cholesterol / HDL (R1) (9.72) and LDL/HDL (R2) (5.54) while HDL level recorded a significant decrease (33.60 mg/dL) and after treated with fenofibrates day after day for 30 days there was a significant (P<0.05) reduction in the level of Total cholesterol (165.2 mg/dl), triglyceride (285.20 mg/dL), LDL (51.36 mg /dL), VLDL (57.04 mg/dL), total cholesterol / HDL (R1) (2.89) and LDL/HDL (R2) (0.88), while HDL levels was significant (P<0.05) increase (56.80 mg/ dL). On the other hand, ginger and turmeric groups showed insignificant reduction on the results of total cholesterol (157.60 and 169.40 mg/dL), triglyceride (261.60 and 310.60 mg/dL), HDL (36.00 and 34.60 mg/dL) LDL (69.28 and 72.68 mg/dL), VLDL (52.32 and 62.12 mg/dL), total cholesterol / HDL (R1) (4.41,4.91) and LDL/HDL (R2) (1.95,2.10) while enhanced formula of ginger and turmeric groups specially in turmeric enhanced formula group recorded significant (P<0.05) reduction (134.40 and 131.00 mg/dL) in total cholesterol, (188.2 and 154.60 mg/dL) in triglyceride, (54.86 and 57.60 mg/dL) in LDL, (37.64 and 30.92 mg/dL) in VLDL, total cholesterol / HDL (R1) (3.21 and 3.08), LDL/HDL (R2) (1.31,1.35) and significant increase (41.91 and 42.48 mg/dL) in HDL when compared with normal control group (96.80 mg/dL) in total cholesterol, (85.20 mg/dL) in triglyceride, (43.00 mg/dL) in HDL, (36.76 mg/dL) in LDL, (17.04 mg/dL) in VLDL, total cholesterol / HDL (R1) (2.25) and LDL/HDL (R2) (0.86).

Table (2) Effect of Oxytetracycline (Positive control), Fenofibrate, Ginger, Turmeric, Ginger-enhanced formula (Ginger + PIP + PL) and Turmeric enhanced formula (Turmeric + PIP + PL) on the levels of Total cholesterol, triglyceride, HDL, LDL, VLDL, R1 (Total Cholesterol/HDL) and R2 (LDL/HDL).

	Negative Control	Positive Control	Fenofibrate	Ginger	Turmeric	(Ginger + PIP + PL)	(Turmeric + PIP + PL)
Total Cholesterol (mg/dl)	$96.80^{e} \pm 3.72$	$322.00^{a} \pm 23.38$	$165.2^{bc} \pm 18.04$	$157.60^{bcd} \pm 15.50$	$169.40^{b} \pm 16.36$	$134.40^{cd} \pm 3.20$	$131.00^{d} \pm 3.18$
Triglyceride (mg/dl)	$85.20^{d} \pm 1.24$	$532.40^{a} \pm 29.44$	$285.20^{b} \pm 20.41$	$261.60^{b} \pm 5.85$	$310.60^{b} \pm 28.54$	$188.20^{c} \pm 6.06$	$154.60^{c} \pm 1.63$
HDL (mg/dl)	$43.00^{b} \pm 0.94$	$33.60^{c} \pm 1.85$	$56.80^{a} \pm 3.88$	$36.00^{c} \pm 1.41$	$34.60^{c} \pm 1.12$	$41.90^{b} \pm 0.85$	$42.48^{b} \pm 0.75$
LDL (mg/dl)	$36.76^{c} \pm 2.26$	$181.92^{a} \pm 30.41$	$51.36^{bc} \pm 14.03$	$69.28^{bc} \pm 15.65$	$72.68^{b} \pm 16.67$	$54.86^{bc} \pm 3.87$	$57.60^{bc} \pm 3.18$
VLDL (mg/dl)	$17.04^{d} \pm 0.24$	$106.48^{a} \pm 7.60$	$57.04^{b} \pm 5.27$	$52.32^{b} \pm 1.51$	$62.12^{b} \pm 7.36$	$37.64^{c} \pm 1.56$	$30.92^{c} \pm 0.42$
R1 (Total Cholesterol/HDL)	$2.25^{d} \pm 0.11$	$9.72^{a} \pm 1.15$	$2.89^{d} \pm 0.18$	$4.41^{bc} \pm 0.53$	$4.91^{b} \pm 0.54$	$3.21^{cd} \pm 0.13$	$3.08^{d} \pm 0.07$
R2 (LDL/HDL)	$0.86^{c} \pm 0.10$	$5.54^{a} \pm 1.16$	$0.88^{c} \pm 0.20$	$1.95^{bc} \pm 0.47$	$2.10^{b} \pm 0.49$	$1.31^{bc} \pm 0.11$	$1.35^{bc} \pm 0.7$

The data are presented as means ± SE calculated of five replicates. Different letters refer to significant differences at ($P<0.05$).

3.2. Hepatic biochemical assays:

Table (3) shows that, the positive control group recorded a significant ($P<0.05$) increase in the levels of hepatic total cholesterol (12.96 mg/g) and hepatic triglyceride (56.12 mg/g) and after being treated with fenofibrates day after day for 30 days there was a significant ($P<0.05$) reduction in the level of hepatic total cholesterol (9.16 mg/g) and liver triglyceride (25.28 mg/g). On the other hand, ginger and turmeric groups showed a significant reduction but not reach normal on the results of hepatic total cholesterol (7.55,8.52 mg/g), hepatic triglyceride (34.06,44.28 mg/g), while the enhanced formula of ginger and turmeric groups recorded a significant ($P<0.05$) reduction (6.83,6.31 mg/g) in hepatic total cholesterol, (25.14,24.8 mg/g) in hepatic triglyceride, when compared with the negative control group (5.30 mg/dl) in hepatic total cholesterol, (12.62 mg/dl) in hepatic triglyceride.

Table (3) Effect of Oxytetracycline (Positive control), Fenofibrate, Ginger, Turmeric, Ginger-enhanced formula (Ginger + PIP + PL) and Turmeric enhanced formula (Turmeric + PIP + PL) on the levels of Hepatic Total Triglyceride and Hepatic Total Cholesterol.

	Normal Control	Negative Control	Fenofibrate	Ginger	Turmeric	(Ginger + PIP + PL)	(Turmeric + PIP + PL)
Hepatic Total Triglyceride (mg/g)	12.62a ± 0.23	56.12a ± 1.52	25.28d ± 2.21	34.06c± 1.58	44.28b ± 1.06	25.14d ± 0.99	24.8d ± 2.04
Hepatic Total Cholesterol (mg/g)	5.30e± 0.07	12.96a ± 0.65	9.16b ± 0.24	7.55c± 0.20	8.52b ± 0.20	6.83d ± 0.15	6.31d ± 0.04

The data are presented as means ± SE calculated of five replicates. Different letters refer to significant differences at ($P<0.05$).

3.3. Histological investigations for hepatic tissue.

Liver sections of the negative control group showed that the liver parenchyma is organized as hepatic lobules in which hepatocytes are arranged in anastomosing and branching cords radiating around the central vein and are separated by blood sinusoids. Hepatocytes are large cuboidal or polyhedral epithelial cells, with large, round central nuclei and eosinophilic cytoplasm (Fig. 1A). Each lobule has peripheral portal canals with few fibrous connective tissues containing three interlobular structures that comprise the portal triad: a venule branch of the portal vein; an arteriole branch of the hepatic artery; and one

or two small bile ducts lined with cuboidal epithelium (Fig. 1B). In the positive control group administration of oxytetracycline resulted in the portal and periportal histopathological changes. Portal areas showed congestion of portal vein, abundant inflammation and fibrosis (periportal fibrosis) (Figs 2A & 2B). Periportal hepatocytes showed well-circumscribed tiny vacuolations of lipid droplets in their cytoplasm with centrally located nuclei (microvascular steatosis) (Fig. 2B). Focal areas of inflammation between plates of hepatocytes appeared away from the portal areas (Fig. 2A).

In the group administration of Fenofibrate, ameliorated the microvascular steatosis and the hepatocytes appeared to have homogenous acidophilic cytoplasm, however, few hepatocytes showed deeply stained nuclei (Fig. 3A & 3D). On the other hand, it resulted in severe fibrosis and inflammatory reaction in the periportal areas as well as bridging fibrosis between portal areas (Fig. 3A & 3C). Moreover, focal, and diffuse areas of inflammatory infiltration appeared in between hepatocytes' plates (Figs.3B & 3D).

In ginger group administration of ginger alone after oxytetracycline showed many inflammatory cells and fibrosis (periportal fibrosis), however the surrounding hepatocytes showed arrangement and morphology comparable to the control (Fig. 4A & 4B). The periportal hepatocytes had acidophilic cytoplasm and centrally located vesicular nuclei comparable to the control group (Fig. 4B). In the turmeric group administration of turmeric alone after oxytetracycline resulted in the persistence of abundant fibrous tissue and inflammatory cells (periportal fibrosis). Moreover, focal inflammatory areas were still present in between plates of hepatocytes, however, no lipid droplets appeared inside the cytoplasm of hepatocytes (Figs. 5A & 5B).

The addition of phospholipids and piperine to ginger in ginger enhanced formula group resulted in few fibrous tissue and connective tissue cells in the portal canals. Also, the surrounding hepatocytes showed homogenous acidophilic cytoplasm arranged in branching cords separated by blood sinusoids comparable to the control (Figs. 6A &6B).

The addition of phospholipids and piperine to turmeric in turmeric enhanced formula group resulted in periportal fibrosis in the form of abundant fibrous tissue and inflammatory cells. On the other hand, the hepatocytes in liver parenchyma showed histological structure comparable to the control (Figs. 7A & 7B).

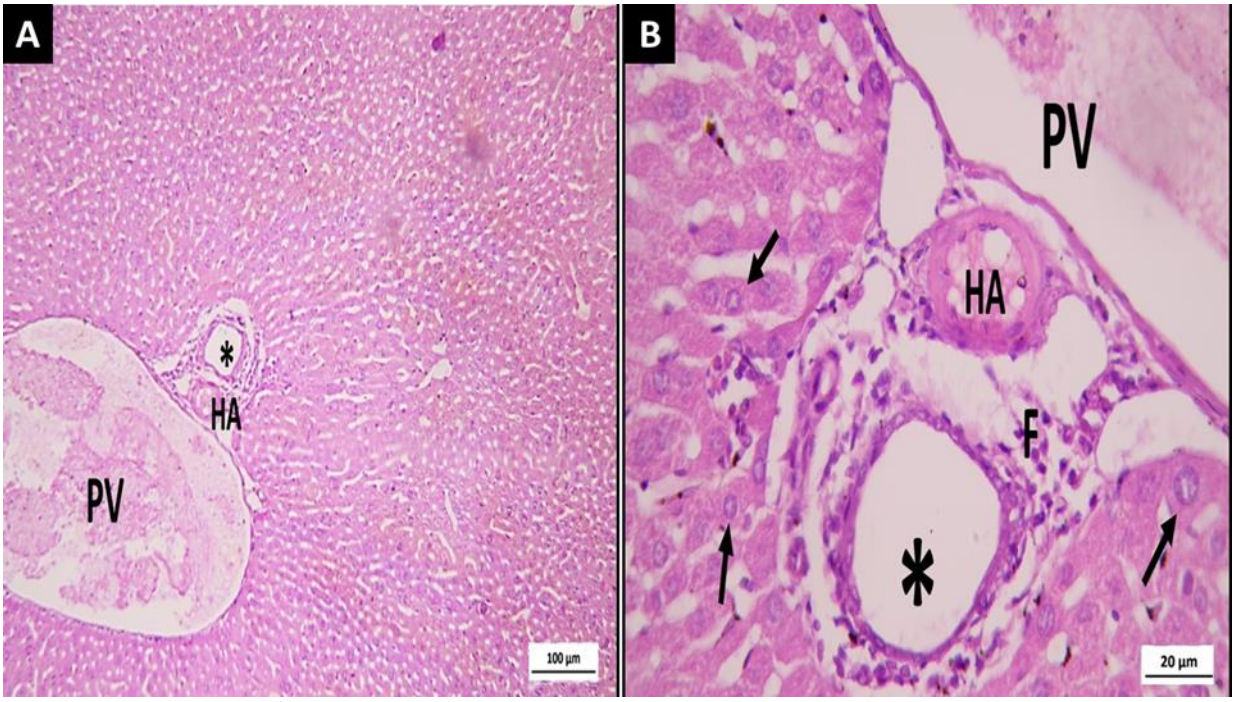

Fig.1: A photomicrograph of a liver section of a rat from the negative control group showing a portal canal containing a portal triad: a large branch of the portal vein (PV, a branch of the hepatic artery (HA) and a bile duct (*). The surrounding hepatocytes are arranged in branching and anastomosing plates of acidophilic cells separated by blood sinusoids (Fig. 1A, H&E X100). A higher magnification shows a portal canal containing a portal triad: a large branch of the portal vein (PV), a branch of the hepatic artery (HA) and a bile duct lined by simple cuboidal cells (*). The portal canal is surrounded by minimal fibrous tissue and connective tissue cells (F). The surrounding hepatocytes appear as polyhedral cells having homogenous acidophilic cytoplasm and vesicular nuclei (↑) (Fig. 1B, H&E X 400).

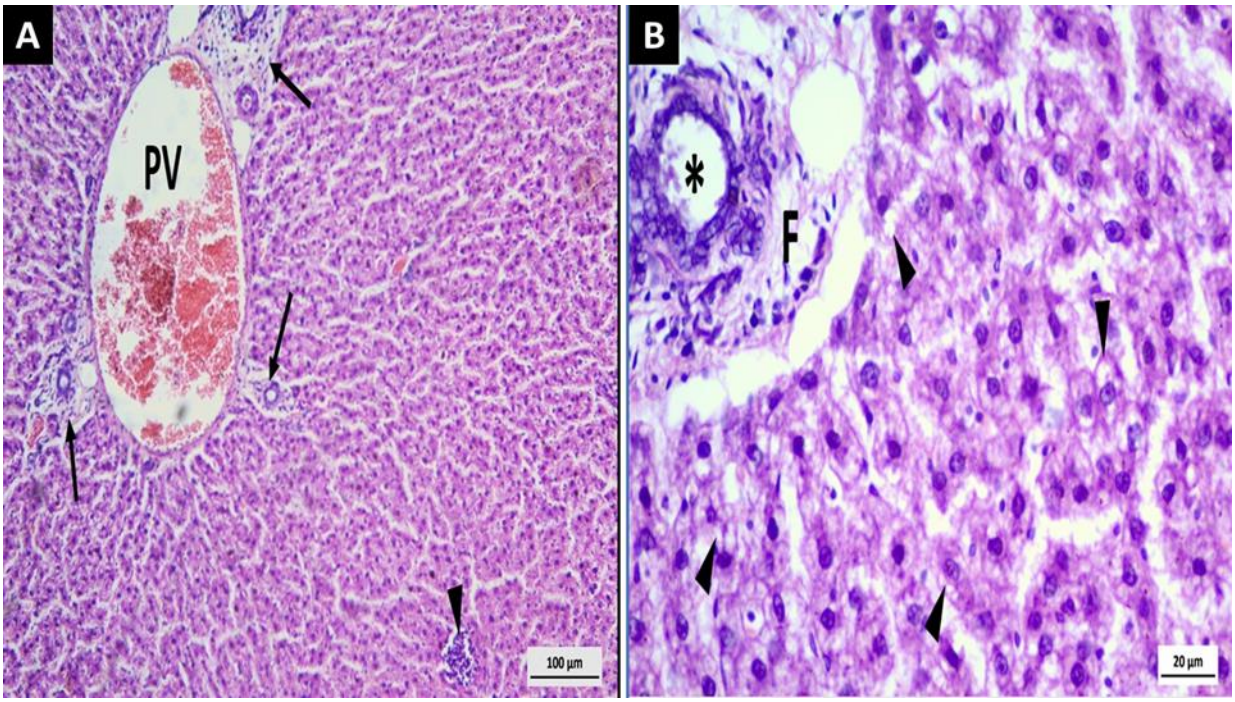

Fig. 2: A photomicrograph of a liver section of a rat from the positive control group showing a portal canal having congested large branch of portal vein and bile ducts surrounded with infiltrating inflammatory cells and fibrosis (↑). Focal areas of inflammation (▲) are present in between plates of hepatocytes (Fig. 2A, H&E X100). A higher magnification shows a portal canal containing a bile duct lined by cuboidal epithelial cells (*) and surrounded by fibrous connective tissue with abundant infiltrating inflammatory cells (F) (periportal fibrosis). The periportal hepatocytes show tiny vacuolations of lipid droplets in their cytoplasm (▲), and the nucleus is located centrally (microvascular steatosis). (Fig. 2B, H&E X400).

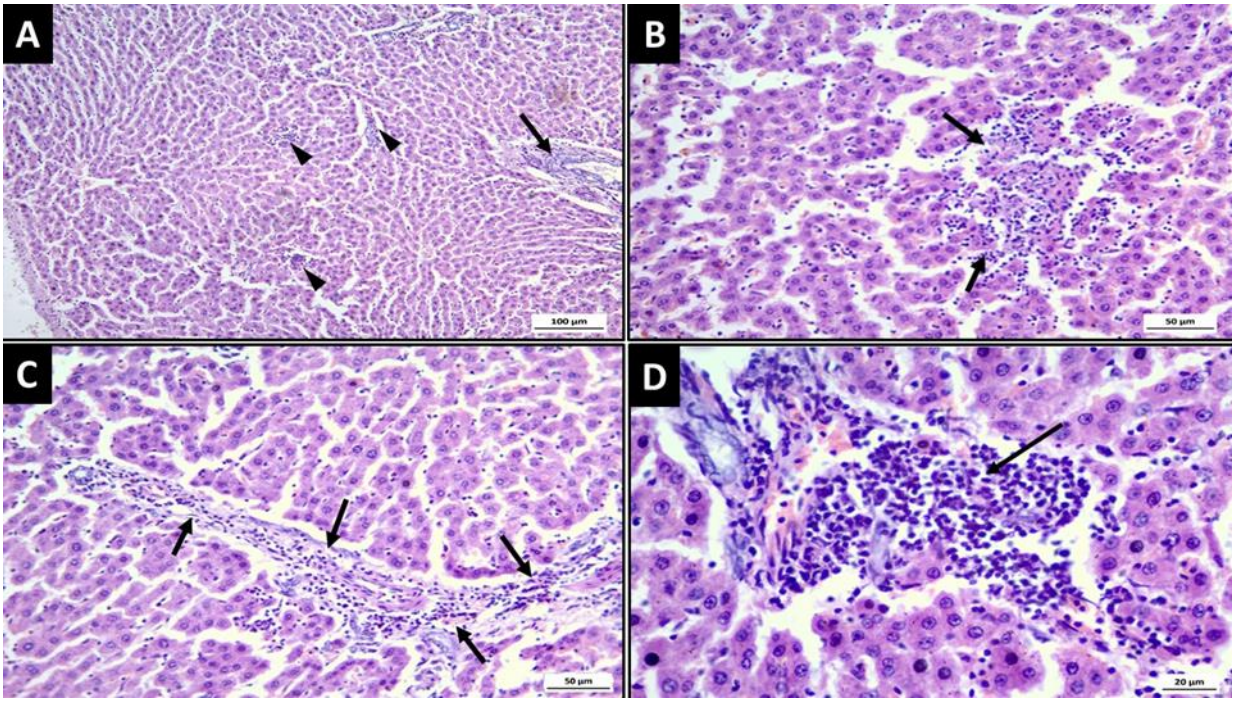

Fig.3: A photomicrograph of a liver section of a rat from the Fenofibrate group showing a portal canal having surrounded by abundant fibrous tissue and inflammatory cells (↑). Infiltrating inflammatory cells are seen scattered in between hepatocytes and accumulated in focal areas of the hepatic tissue. The surrounding hepatocytes shows arrangement and morphology comparable to the control (▲) (Fig. 9A, H&E X100). Scattered inflammatory infiltrate in between hepatocytes affecting a wide area (Fig. 9B, H&E X 200). Fibrous tissue infiltrated by abundant inflammatory cells extending between portal areas (bridging fibrosis) (↑) (Fig. 9B, H&E X 200). A portal canal highly infiltrated by inflammatory cells which extends in between the surrounding hepatocytes (↑). Most of the surrounding hepatocytes are comparable to the control. (Fig. 9D, H&E X 400).

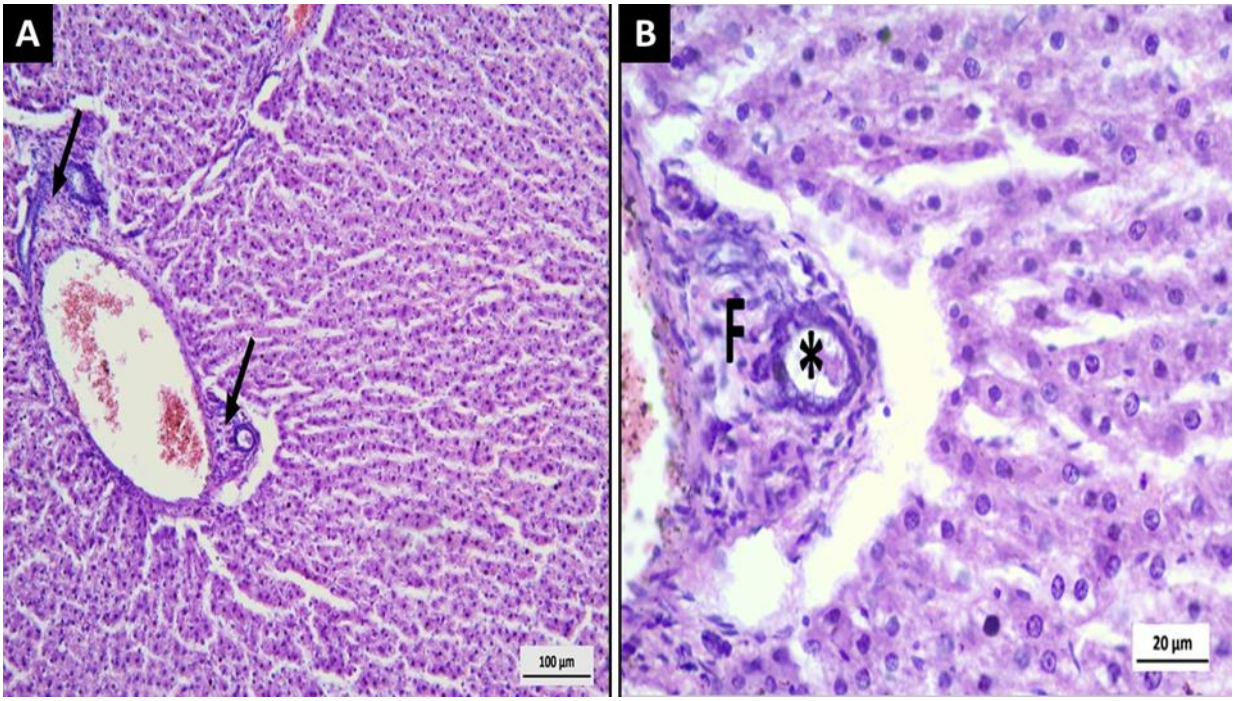

Fig. 4: A photomicrograph of a liver section of a rat from theginger group showing a portal canal having large branch of portal vein and bile ducts surrounded with infiltrating inflammatory cells and fibrosis (↑). The surrounding hepatocytes show arrangement and morphology comparable to the control (Fig. 3A, H&E X100). A higher magnification shows a portal canal containing a bile duct lined by cuboidal epithelial cells (*) and surrounded by fibrous connective tissue with abundant infiltrating inflammatory cells (F) (periportal fibrosis). The periportal hepatocytes have acidophilic cytoplasm and centrally located vesicular nuclei comparable to the control group. (Fig. 3B, H&E X400).

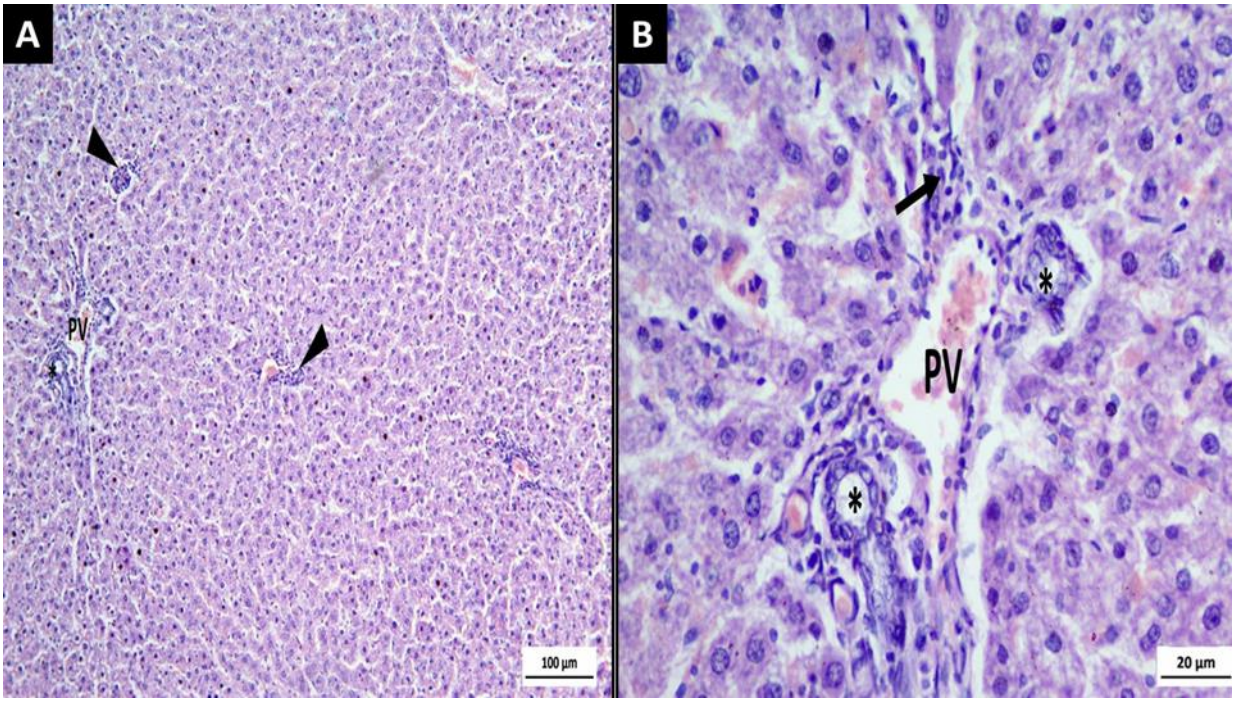

Fig.5: A photomicrograph of a liver section of a rat from theturmeric group showing a portal canal containing a branch of portal vein (PV), and bile ducts (*) surrounded with few fibrous tissue and abundant inflammatory cells (↑). Focal accumulation of inflammatory cells appears between hepatocytes (▲) (Fig. 8A, H&E X100). A higher magnification shows minimal fibrous tissue with abundant inflammatory cells (↑) surrounding the portal canal containing bile duct lined by cuboidal cells (*) and portal vein (PV). Most of the surrounding periportal hepatocytes show arrangement and morphology comparable to the control group (Fig. 8B, H&E X400).

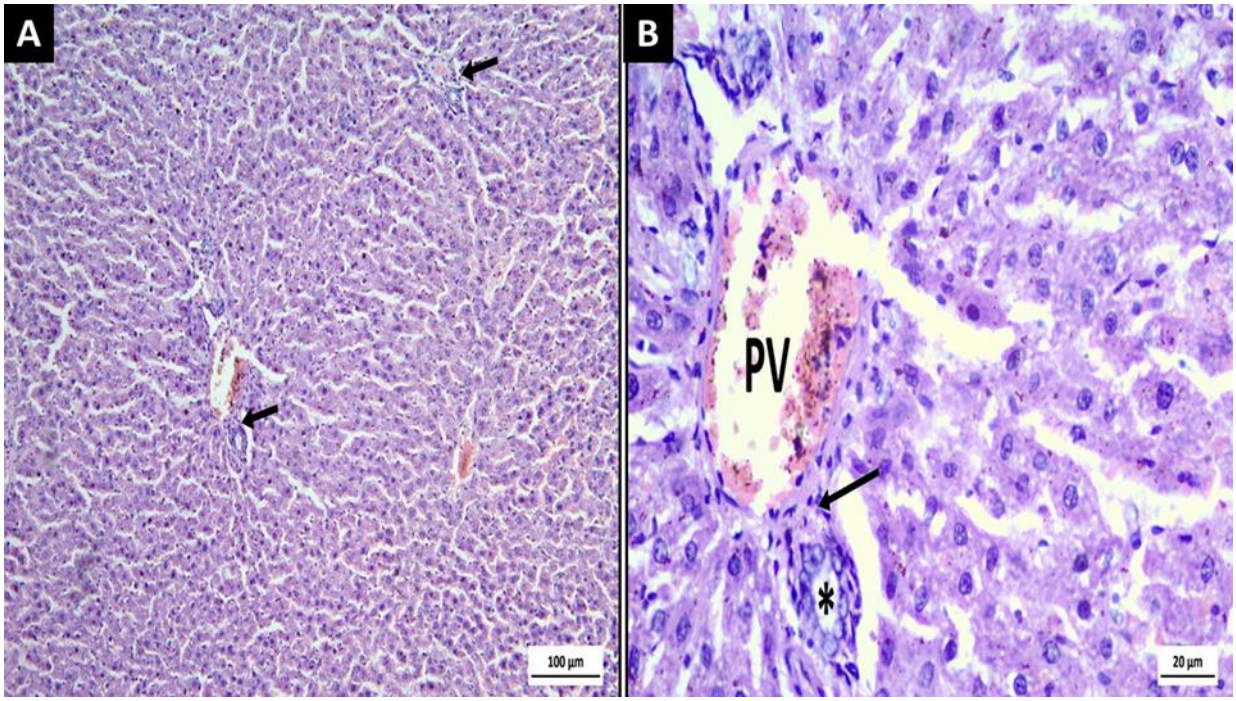

Fig. 6: A photomicrograph of a liver section of a rat from ginger enhanced formula by phospholipid and piperine group showing a portal canal having a branch of portal vein and bile ducts surrounded few fibrous tissue and inflammatory cells (↑). The surrounding hepatocytes show arrangement and morphology comparable to the control (Fig. 4A, H&E X100). A higher magnification shows very minimal fibrous tissue and inflammatory cells (↑) surrounding the branch of the portal vein (PV) and bile duct (*). The surrounding periportal hepatocytes show arrangement and morphology comparable to the control group (▲) (Fig. 4B, H&E X400).

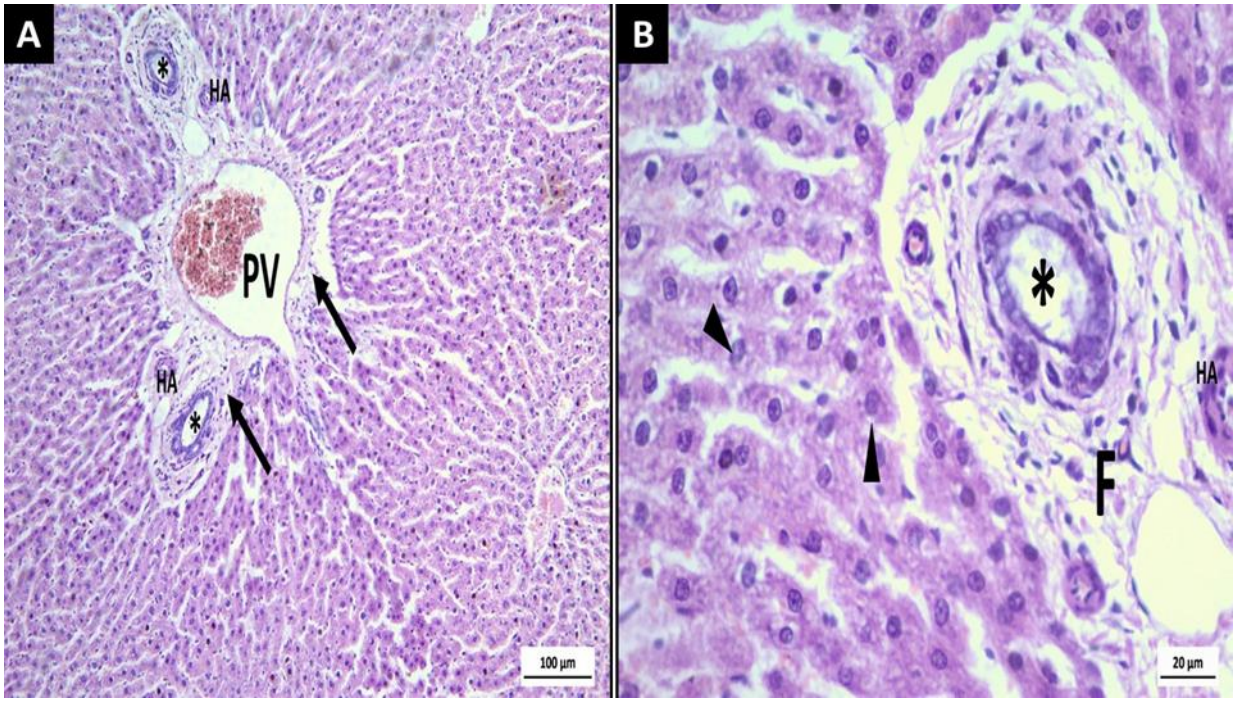

Fig.7: A photomicrograph of a liver section of a rat from turmeric enhanced formula by phospholipid and piperine shows a portal canal containing a large branch of portal vein (PV), hepatic artery (HA) and bile ducts (*) surrounded with abundant fibrous tissue and inflammatory cells (↑) (Fig. 7A, H&E X100). A higher magnification shows fibrous tissue with inflammatory cells (F) surrounding the portal canal containing the bile duct lined by cuboidal cells (*) and a branch of the hepatic artery (HA). Most of the surrounding periportal hepatocytes show arrangement and morphology comparable to the control group (▲) (Fig. 7B, H&E X400).

4. Discussion

Turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) have long been revered for their medicinal properties, including anti-inflammatory, antioxidant, and hepatoprotective effects. Curcumin, the active compound in turmeric, and gingerol, the principal bioactive component in ginger, have shown significant potential in treating various conditions, including non-alcoholic fatty liver disease (NAFLD) [11;12].

The present study investigated the effect of ginger and turmeric ethanolic extracts enhanced formula via phospholipid and piperine on NAFLD- Induced by oxytetracyclinecompared to Fenofibrate in Wistar rats.

Estimation of the activity of ALT, AST, GGT, ALP, Bilirubin and total protein are good markers of assessment of liver function.

Our results finding that, treated rats with oxytetracycline for three consecutive days were associated with a highly a significant increase in activities of serum ALT, AST, GGT and ALP. In addition to a significant decrease in total protein synthesis and a significant increase in bilirubin total, direct and in-direct. This agrees with A previous study demonstrating that injected oxytetracycline intraperitonially for three consecutive days led to induce of fatty liver, necrosis and inflammation. These histological changes were associated with the high significant increase in the levels of ALT, AST and GGT. This significant increase may be due to impairs mitochondrial function by disrupting the electron transport chain, leading to reduced ATP production and increased ROS generation. This mitochondrial damage exacerbates oxidative stress and promotes hepatocyte apoptosis, a key feature in the progression of NAFLD [33].

Additionally, antibiotics like oxytetracycline can inhibit mitochondrial respiration and reduce cellular energy status, further contributing to liver injury [34].

Moreover, oxytetracycline-induced NAFLD involves inflammatory responses. The oxidative stress and lipid accumulation activate Kupffer cells (resident liver macrophages), leading to the production of pro-inflammatory cytokines such as TNF-α and IL-6. these cytokines further exacerbate liver inflammation and promote the progression of NAFLD to more severe forms, such as non-alcoholic steatohepatitis (NASH) [35].

The group of rats that were treated with fenofibrate showed an insignificant decrease in AST, ALT, GGT and ALP activities while total protein, bilirubin total and direct were significantly increased which is in agreement with a previous observations demonstrated that fenofibrate treatment can increase the levels of Bilirubin total and direct in indirect way due to increased risk of gallstone formation (cholelithiasis). These results may be due to, enhance the catabolism of triglyceride-rich lipoproteins, leading to increased hepatic cholesterol uptake. This process elevates the cholesterol content in bile, which can precipitate and form gallstones. Elevated cholesterol saturation in bile is a primary factor in the pathogenesis of cholesterol gallstones [36;37;38].

In this study, the groups that were treated with ginger and turmeric extracts showed a significant decrease in serum transaminases AST, ALT, GGT and ALP activities. We further demonstrated a significant decrease in bilirubin total, direct and in-direct in addition to increase in total protein level.These results agreed with a previous study that attributed these effects to the anti-inflammatory effects of gingerol, the primary active compound in ginger and curcumin in turmeric extract that inhibits the nuclear factor kappa B (NF-κB) pathway, which plays a crucial role in the expression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. By downregulating these cytokines, curcumin and gingerol helps reduce liver inflammation and prevent the progression of NAFLD [39;40].

Additionally,gingerolandcurcuminare strong antioxidant thatincreases the levels of superoxide dismutase (SOD), catalase, and glutathione peroxidase, thereby reducing oxidative damage in hepatocytes [41;42]

Moreover, a previous studies found that ginger and turmeric extract was shown to reduce hepatic apoptosis by downregulating the expression of pro-apoptotic proteins such as Bax and upregulating anti-apoptotic proteins such as Bcl-2. This protective effect helps in maintaining liver function and integrity in NAFLD conditions [43;44]. While this study focused on the liver, it's important to note that gut microbiota dysbiosis is increasingly recognized as a potential contributing factor to NAFLD. The gut microbiome can influence inflammation and metabolic processes. Nanotechnology offers promising strategies to manipulate the gut microbiome, including the use of nanoparticles to target specific bacterial species within biofilms [2].

Despite this improvement in results, it was slight, and the results did not reach normal rates, this is consistent with earlier studies [46;47] that stated despite the well-documented therapeutic potential of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*), a significant challenge in their clinical application is their poor bioavailability. This limitation is primarily due to their low aqueous solubility, rapid metabolism, and poor absorption in the gastrointestinal tract.

Additionally, this agrees with a previous study observed that less than 10% of orally administered gingerols were detected in the plasma of human subjects. This rapid metabolism is a major barrier to achieving therapeutic concentrations of gingerols in the bloodstream [44].

Moreover,a previous study demonstrated that the peak plasma concentrations of curcumin achieved after oral administration were very low, even at high doses. The study highlighted that curcumin's poor absorption, rapid metabolism, and systemic elimination contribute to its low bioavailability [46].

In our study we found that, the groups that treated with ginger and turmeric enhanced formula thru phospholipid and piperine shows a significant decrease in serum transaminases AST, ALT, GGT and ALP activities. We further demonstrated a significant decrease in bilirubin total, direct and in-direct in addition to increase in total protein level and reached to the normal values in all assays specially in turmeric enhance formula group. This is confirmedwith a previous study demonstrating that the combination of curcumin with phospholipid co-administered with piperine significantly improved the

bioavailability and therapeutic effects of curcumin in animal models. The study concluded that the dual approach of using piperine and phospholipids could be a highly effective strategy for enhancing the bioavailability of poorly soluble bioactive compounds [48].

Additionally, this role may be due to Piperine's ability to inhibit metabolic enzymes such as cytochrome P450 enzymes, particularly CYP3A4, which is involved in the metabolism of many drugs and bioactive compounds. By inhibiting these enzymes, piperine reduces the rate at which compounds like curcumin and gingerols are metabolized, thereby increasing their plasma concentration and duration of action [49].Piperine also inhibits P-glycoprotein, a membrane transporter that pumps foreign substances out of cells. By inhibiting this transporter, piperine increases the intracellular concentration of bioactive compounds, enhancing their absorption and retention in the body [50].In addition to, interact with the lipid bilayer of intestinal cells, altering membrane dynamics and improving the uptake of compounds like curcumin and gingerols [51].

Moreover, a previous studies demonstrated that phospholipids can encapsulate hydrophobic compounds, improving their solubility in aqueous environments. This encapsulation protects the bioactive compounds from degradation in the gastrointestinal tract and enhances their stability during transit [52]. In addition to,facilitate the formation of micelles or vesicles that can fuse with cell membranes, promoting the direct transfer of encapsulated compounds into cells. This mechanism significantly enhances the cellular uptake and bioavailability of compounds like curcumin and gingerols [53].

In this study, intraperitoneally injection with oxytetracycline to rats for three consecutive days resulted in a highly significant increase in serum triglycerides, total cholesterol, LDL-cholesterol and VLDL, while HDL-cholesterol was significantly decreased. Also, showed a highly significant increase in hepatic total cholesterol, and hepatic triglycerides.In addition to induced acute pathological changes in the liver including accumulated tiny lipid droplets in their cytoplasm (microvascular steatosis) and the portal areas showed portal fibrosis and inflammation. Moreover, focal inflammatory areas were present in between hepatocytes' plates.
This result agrees witha previous study [54] that concluded to oxytetracycline affects lipid metabolism, leading to hepatic steatosis and disrupting the normal regulation of lipid synthesis and degradation pathways. These results may be due to increases the expression of sterol regulatory element-binding proteins (SREBPs), which are key transcription factors that regulate lipid biosynthesis, while simultaneously inhibiting the activity of peroxisome proliferator-activated receptor alpha (PPARα), a critical regulator of fatty acid oxidation. This imbalance results in the accumulation of triglycerides and other lipids in hepatocytes, a hallmark of NAFLD [55].
Our results show that, treated rats with fenofibrate led to a significant decrease in serum triglycerides, total cholesterol, LDL-cholesterol and VLDL, while HDL-cholesterol was significantly increased. Also, showed a highly significant decrease in hepatic total cholesterol, and hepatic triglycerides.This result agrees with a previous study demonstrated that, Fenofibrate exerts its primary effects by activating PPAR-α, a nuclear receptor that regulates lipid metabolism. Upon activation, PPAR-α modulates the expression of genes involved in fatty acid oxidation, lipoprotein metabolism, and inflammation [56].

In our study, the rats that were treated with ginger and turmeric ethanolic extract alone shows a decrease in serum triglycerides, total cholesterol, LDL-cholesterol and VLDL, while HDL-cholesterol was insignificantly increased and did not reach the normal range.
This result agrees with a previous study reported that despite the proven efficacy of ginger and turmeric in reducing lipids through regulating lipid metabolism a significant challenge in their clinical application is their low bioavailability. This obstacle is mainly due to their poor aqueous solubility, rapid metabolism, and low absorption in the gastrointestinal tract. It's important to note that gut microbiota dysbiosis is increasingly recognized as a potential contributing factor to NAFLD. The gut microbiome can influence inflammation and metabolic processes. Nanotechnology offers promising strategies to manipulate the gut microbiome, including the use of nanoparticles to target specific bacterial species within biofilms [3]. This emerging area of research could open new avenues for developing more effective natural therapies for NAFLD which is crucial for the management of NAFLD.
A previous studies reported that ginger and turmeric effect in reducing triglyceride accumulation in the liver may be due to the dual action of modulating lipid synthesis and degradation pathways that express sterol regulation. SREBPs, which are involved in lipid biosynthesis, regulate peroxisome proliferator-activated receptor alpha (PPARα), a master regulator of fatty acid oxidation. [57,58,59,60,61].

This investigation also agrees in this study with the results of the groups that were treated with ginger and turmeric-enhanced formula through piperine and phospholipids which showed a highly significant decrease in serum triglycerides, total cholesterol, LDL-cholesterol and VLDL. At the same time, HDL-cholesterol was significantly increased especially in turmeric enhanced formula Which is attributed and proven in the previous studies [61,62,63,64,48] As explained previously in this study to the role of piperine and phospholipids in enhancing absorption and protection from hepatic metabolism enzymes degradation. The results in histological investigation were in the same direction.

5. Conclusions

Our finding provide evidence that ginger and turmeric extracts enhanced formula by phospholipids and piperine represents a promising strategy for improving its therapeutic efficacy in mitigating oxytetracycline-induced NAFLD in rats and turmeric enhanced formula effect is superior to Fenofibrate and the other ethanolic extracts in this study.Further studies are warranted to elucidate the underlying mechanisms and evaluate long-term safety and efficacy in clinical settings.

6. Conflicts of interest

"There are no conflicts to declare".

7. Formatting of funding sources

Not applicable

8. Acknowledgments

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