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Protective effects of a ginger (*Zingiber officinale* Roscoe, *Zingiberaceae*) extract on the liver and heart tissues in the bile duct-ligated rats

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Objective. Cholestasis is a disorder with accumulation of bile acids in the liver that can lead to toxicity and impairment in liver function and heart. In current study, we investigated the protective effect of ginger extract on the liver and heart damages in cholestatic rats.

Methods. Thirty-six male Wistar rats were randomly divided into 6 groups (n=6). Cholestasis was induced in the rats by ligating the bile ducts. The animals were treated with different doses of methanol extract of ginger (100, 200, 400 mg/kg/day) for 28 days. Serum factors including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin, and the oxidative stress indices, glutathione (GSH), malondialdehyde (MDA), reactive oxygen species (ROS), and total antioxidant capacity in the liver and heart tissues were measured. Histopathological changes were also investigated.

Results. The results showed that ligating the bile ducts in rats was associated with an increase in the serum factors including ALT, AST, ALP, and bilirubin. It also increased the amount of ROS, MDA, and decreased the GSH and total antioxidant capacity. On the other hand, severe histopathological changes were seen in the liver and heart tissues. The results show that ginger extract reduced the amount of serum factors, modulated the antioxidant indices and improved the histopathological changes.

Conclusions. According to the results of this study, it can be concluded that the administration of ginger extract can be a new strategy to treat the complication of liver and heart damage caused by cholestasis. The protective effects of ginger against cholestasis and its complications may be mediated by the antioxidant activity of its components.

Keywords: bile duct ligation, cholestasis, ginger, oxidative stress indices

Cholestasis is an acute or chronic hepatobiliary situation, in which the formation, secretion or flow of bile is damaged (Sheibani et al. 2020). Cholestasis can be intrahepatic or extrahepatic. Absence of bile duct obstruction indicates intrahepatic cholestasis, while the presence of bile duct obstruction indicates extrahepatic cholestasis. It may be difficult to differentiate intrahepatic and extrahepatic cholestasis (Ludwig et al. 1988; Dhalla et al. 2000; Sherlock et al. 2002; Freitas et al. 2006; Kathi et al. 2020). Extrahepatic cholestasis occur science a mechanical or structural abnormality exists between the bifurcation of the hepatic duct and the ampulla of vater. Extrahepatic obstruction can result from masses, stones, strictures, cysts or biliary

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flukes. Intrahepatic cholestasis occurs when bile flow is attenuated or absent due to an abnormality of bile secretion or flow within the liver. This may result from hepatocyte dysfunction or abnormalities within the intrahepatic biliary tract (Pollock and Minuk 2017). Bile acids in cholestasis cause damage through various possible mechanisms, including oxidative stress, apoptosis, necrosis, inflammation, and membrane changes (Erlinger 2014).

Although it is believed that the accumulation of hydrophobic bile acids, in cholestatic diseases, plays an important role in liver damage by apoptosis or necrosis of hepatocytes (Attili et al. 1986), but the exact mechanisms involved in the toxicity of these bile acids are not well known. In addition, the increase and accumulation of bile acids in the systemic circulation during cholestatic liver diseases may cause severe toxicity to other organs such as endothelial damage in the kidney, lung, and heart (Dold et al. 2010). These toxic bile acids, in contact with the cells of the digestive system, may promote cancer through indirect mechanisms such as oxidative stress (Venturi et al. 1997; Bernstein et al. 2005).

Accumulation of these bile acids can also have destructive effects on the cells of the placenta and fetus (Marin et al. 2005; Perez et al. 2006). The acute damage to various organs can occur after severe and chronic liver failure (cirrhosis) (Chayanupatkul and Liangpunsakul 2014). The heart is one of the organs that are affected in liver cirrhosis and chronic liver failure. The complication of cardiomyopathy related to cirrhosis has been clinically known for years (Gaskari et al. 2006; Chayanupatkul and Liangpunsakul 2014). On the other hand, there is no suitable pharmacological treatment to prevent, treat or alleviate this complication.

Natural products and their compounds have attracted consideration in last years for treatment of diseases in studies. Ginger (*Zingiber officinale* Roscoe, *Zingiberaceae*) is one of them that has been used as a food spice all over the world. The ginger rhizome has been applied in traditional herbal medicine and has various health promoting potential effects. Ginger contains active phenolic compounds that have potential effects in several of diseases containing degenerative disorders, digestive health, cardiovascular disorders, diabetes mellitus, and cancer. Additionally, it has anti-inflammatory, hypolipidemic, antioxidant, and hepatoprotective properties (White 2007; Ali et al. 2008; Nicoll and Henein 2009). In the current study, we investigated the protective effect of ginger extract on the liver and heart damages in cholestatic rats.

Materials and Methods

Animals. A total of 36 male Wistar rats weighting 200–250 g purchased from Lorestan University of Medical Sciences (Khorramabad, Iran) was used. All animals were housed at a stable room temperature (22±2 °C) with humidity (50%±10%) and an automatically controlled 12-h light and dark cycle. Feed and water were provided ad libitum. The animals were acclimatized to the environment for 1 week before the initiation of the experiment. All the experiments in this study were approved by the Animal Ethics Committee (IR.LUMS.REC.1402.067).

Experimental design. Bile duct-ligation (BDL) surgery was performed on the animals, and 28 days after the surgery, the animals were divided into six groups (n=6/group): group I (Sham) that only surgery was performed without BDL. The animals with the BDL were randomly divided into four groups (II–V). The group II (BDL) received no ginger extract and the groups III–V (BDL) received ginger extract at the doses of 100, 200, and 400 mg/kg/day, respectively, orally via gavage for 28 days and have been designated as (BDL+oZ100, BDL+oZ200, and BDL+oZ400, respectively). The group VI (BDL, oZ400) received ginger extract 400 mg/kg/day without surgery. All the experiments were performed based on the guidelines for the care and use of laboratory animals.

Preparation of the methanol extract of ginger. The methanol extraction of ginger was prepared by the maceration method as described previously (Sharif and Bennett 2016). To maximize the extraction yield while minimizing the extraction cost, 80% methanol was used in a sample-solvent ratio of 1 g/20 ml. Ten grams of ginger was weighed and placed into a bottle, adding 200 ml of methanol and covering with an aluminum foil before placing on an orbital shaker at 200 rpm for 8 h. After the solvent extraction, the solution was filtered through a cotton-wrapped muslin cloth with an additional 100 ml of the same fresh solvent into a conical flask before filtering through a filter paper. The methanol in the ginger extract was then evaporated using a rotary evaporator under reduced pressure at 40 °C, while the trace amounts of solvent were evaporated by blowing nitrogen gas over the extracts. The final extract was stored in the refrigerator at 4 °C. Each solvent extraction yielded 23% of its starting sample. The extract was constituted to a solution of known concentration using distilled water and administered according to body weight and group treatment doses (Sharif and Bennett 2016; Li et al. 2022).

Bile duct-ligation. To induct a model of cholestasis, the rats were anesthetized by injecting of 10 mg/kg of xylazine and 70 mg/kg of ketamine intraperitoneally. A median incision was done and the common bile duct was identified. Both ends of the common bile duct were ligated and the middle part of ligatures was cut. In the sham group, a middle incision was made without BDL.

Blood and tissue sampling. At the end of treatment, all animals were anesthetized and the blood samples were collected from the abdominal aorta and centrifuged at 3000 g for 10 min to prepare the serum and stored at -20 °C. The collected serum samples were used to measure biochemical factors. The tissue samples were also taken from the liver and heart for histological investigations and oxidative stress tests.

Biochemical and enzyme studies. For biochemical and enzyme assays, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) enzymes and bilirubin levels were determined in serum sample. AST and ALT in the samples were estimated based on Reitman and Frankel method (Reitman 1957). ALP activity was determined based on the optimized method as recommended by the German Society for Clinical Chemistry. The total bilirubin was estimated by method of Evelyn and Malloy (1938).

Histological study. After 6-week treatment, following induction of anesthesia, the liver and heart tissues were separated, washed with 0.9% sodium chloride, and fixed in 10% formalin. The paraffin blocks were prepared and sections of 5-µm thickness were cut using rotary microtome. All the slides were stained with hematoxylin and eosin dyes. Finally, the histological changes were evaluated under the light microscope.

Image analysis. A minimum of 10 fields for each slide was evaluated and assigned a severity score on a scale of none (0), mild (+), moderate (++), and severe (+++) damages. Digital images were analyzed by a semi-quantitative scoring system (Image J software, Java based application for analyzing images).

Electrocardiography. Electrocardiography (ECG) was done before sacrificing the animals using a Power Lab data acquisition system. After the rats were under anesthesia, electrodes were placed under the skin of the left and right front paws and the tail of the animal. The unipolar leads were placed in the anterior part of the chest. The electrophysiological function of the heart recorded to the evaluation of ECG markers including HR, P-R interval, QRS interval, QTc and QT intervals.

Echocardiography. At the end of the experiment following the induction of anesthesia, echocardiography examination was performed by laying the rat on its left side and using an echocardiography device equipped with a 7.5 MHz transducer. The left ventricular end diastolic dimension (LVEDd) left ventricular end-systolic dimension (LVESd), shorting time (St), and septal dimension (SEPd) were measured.

Liver and heart oxidative stress assessment. Liver and heart tissue homogenates were prepared. Reactive oxygen species (ROS), glutathione (GSH), and malondialdehyde (MDA) levels, and total antioxidant capacity were measured using the commercial kits, according to the protocol that were described in references. The Bradford protein quantification assay was used to standardize the samples based on protein content.

Statistical analysis. Statistical analysis was done by IBM SPSS Statistics (V26) and the results were reported as mean+SD. The comparison between different groups was done using one-way analysis of variance (ANOVA) statistical test, and $p<0.05$ was considered as a significant level.

Results

The effect of ginger extract on the body weight, liver, and heart of cholestatic rats. At the end of the experiment, animals showed no significant change in the weight of body in different groups (Figure 1A). On the other hand, the measurement of liver and heart weight showed a significant ($p<0.001$) increase in the BDL group compared to the sham group. The heart weight also showed a significant ($p<0.001$) increase in the BDL+oZ200 group compared to the sham group. It has been shown that the use of ginger extract in the other groups significantly ($p<0.001$) decreased the liver and heart weights in comparison to the BDL group (Figures 1B,C). Liver weight significantly ($p<0.05$) increased in the BDL+oZ400 group compared to the BDL+oZ100 group (Figure 1B). The results presented in Figure 1C indicate that heart weight also significantly ($p<0.05$) increased in the BDL+oZ200 group compared to the BDL+oZ100 group. Additionally, heart weight significantly ($p<0.05$) decreased in the BDL+oZ400 and oZ400 groups compared to the BDL+oZ200 group (Figure 1C).

The effect of ginger extract on electrocardiography and echocardiogram parameters in cholestatic rats. Table 1 illustrates the effects of ginger extract on ECG. Results showed that QTc significantly increased in the BDL+oZ200 ($p<0.01$)

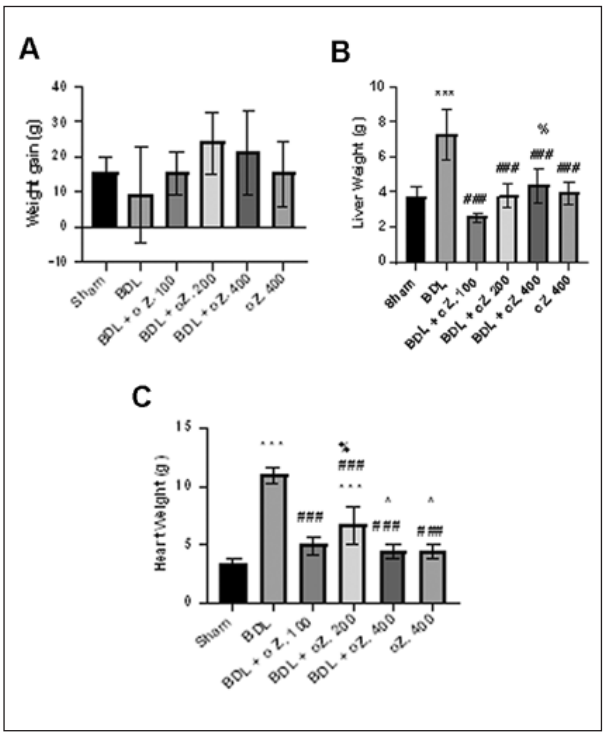

Figure 1. The effect of the ginger extract on the body (**A**), liver (**B**), and heart (**C**) weight gain in cholestatic rats. The data are displayed as mean±SD for 6 animals/each group. ***p<0.001 vs. sham group; ###p<0.001 vs. BDL group; %p<0.05 vs. BDL+oZ100 group; ^p<0.05 vs. BDL+oZ200 group. Abbreviations: BDL - bile duct-ligation.

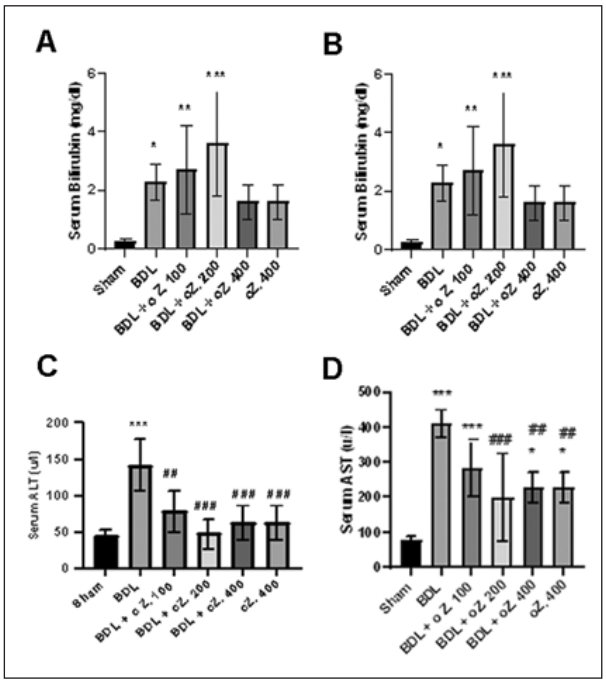

Figure 2. The effects of ginger extract on the level of bilirubin (**A**), ALP (**B**), ALT (**C**), and AST (**D**) in the serum of cholestatic rats. The data are presented as mean±SD for 6 animals/each group. *p<0.05 vs. sham group; **p<0.01 vs. sham group; ***p<0.001 vs. sham group; ###p<0.001 vs. BDL group; ##p<0.01 vs. BDL group. Abbreviations: ALP – alkaline phosphatase; ALT – alanine aminotransferase; AST – aspartate aminotransferase; BDL – bile duct-ligation.

Table 1
The effect of ginger extract on ECG parameters in cholestatic animals

Treatment	HR	P_R interval (ms)	QRS interval (ms)	QTc (ms)	QT interval (ms)
Sham	187.87±29.87	327.61±58.85	19.25±4.55	143.38±25.34	81.16±10.82
BDL	251.62±66.57	254.60±55.11	19.39±6.05	116.64±37.42	59.59±24.25
BDL+oZ100	222.52±14.53	265.58±20.46	17.89±0.53	146.22±28.21	75.61±14.62
BDL+oZ200	187.77±69.03	403.87±252.33	21.34±9.67	178.45±48.94##	117.04±68.82#
BDL+oZ400	242.33±29.13	246.98±26.28	16.95±2.03	163.23±14.49#	80.62±6.32^
oZ400	210.83±26.05	281.85±30.14	17.67±1.86	98.29±19.34%^^^!	52.59±12.30

Data are presented as mean±SD for 6 animals/each group. #p<0.05 vs. BDL group; ##p<0.01 vs. BDL group; %p<0.05 vs. BDL+oZ100 group; ^p<0.05 vs. BDL+oZ200 group; ^^^p<0.001 vs. BDL+oZ200 group; !p<0.05 vs.BDL+400 group. Abbreviations: BDL – bile duct-ligation; BDL+oZ100, BDL+oZ200, and BDL+oZ400 – the groups of BDL rats that received ginger methanol extract at the doses of 100, 200, and 400 mg/kg/day, respectively; oZ400 – the group of rats that received ginger methanol extract at a dose of 400 mg/kg/day; ECG – electrocardiography.

and BDL+oZ400 (p<0.05) groups compared to the BDL group. It also significantly decreased in the oZ400 group compared to the BDL+oZ100, BDL+oZ200, and BDL+oZ400 groups, (p<0.05), (p<0.001), and (p<0.05), respectively. Additionally, in the BDL+oZ200 group, administration of ginger extract significantly increased QT interval (p<0.05) compared to the BDL group. The QT interval also significantly decreased (p<0.05) in BDL+400 group compared to the BDL+oZ200 group (Table 1).

Table 2 presents the effects of different doses of ginger extract on echocardiogram parameters

in cholestatic rats. The results show that LVEDd increased in all the groups compared to the sham group, but the increase was significant ($p<0.01$) only in the BDL+oZ400 group. Also, administrations of ginger extract significantly increased LVEDd ($p<0.01$) in the BDL+oZ400 group compared to the BDL+oZ100 and BDL+oZ200 groups. Additionally, LVESd increased in all the groups compared to the sham group, the increase was significant ($p<0.001$) in the BDL+oZ400 group. The treatment with the ginger extract significantly increased LVESd in the BDL+oZ400 group compared to the BDL ($p<0.01$), BDL+oZ100 ($p<0.001$), and BDL+oZ200 ($p<0.01$) groups. Administrations of ginger extract not significantly increased St in the BDL groups, but St significantly increased in the oZ400 group compared to the BDL and BDL+oZ100 groups ($p<0.001$, $p<0.01$, respectively). Furthermore, the ginger extract had no significant effect on the SEPd in the cholestatic rats. The SEPd significantly increased ($p<0.05$) in the oZ400 group compared to the BDL+oZ100 group (Table 2).

The effects of ginger extract on biochemical factors in serum of cholestatic rats. After 28 days of treatment, the measurement of serum enzyme levels including ALT, AST, ALP, and serum bilirubin was done in all the groups (Figure 2). Serum bilirubin significantly increased in the BDL, BDL+oZ100 and BDL+oZ200 groups compared to the sham group, ($p<0.05$, $p<0.01$ and $p<0.001$, respectively) (Figure 2A). It has also been demonstrated that administration of the ginger extract significantly increased ($p<0.01$) the level of serum ALP in the BDL+oZ100 group compared to the sham group. This increase was not significant in the other groups (Figure 2B). Serum ALT level significantly increased ($p<0.001$) in the BDL group compared to the sham group and the administration of ginger extract significantly decreased in the BDL+oZ100 group ($p<0.01$) and the other treatment groups ($p<0.001$) (Figure 2C). Serum AST level significantly increased in the BDL ($p<0.001$), BDL+oZ100 ($p<0.001$), BDL+oZ400 ($p<0.05$), and oZ400 ($p<0.05$) groups compared to the sham group. Treatment with the ginger extract significantly decreases serum AST levels in the BDL+oZ200 ($p<0.001$), BDL+oZ400 ($p<0.01$), and oZ400 ($p<0.01$) groups compared to the BDL group (Figure 2D).

The effects of ginger extract on the oxidative stress markers in the liver tissue of the cholestatic rats. The measurements of oxidative stress markers in the liver tissue showed a significant ($p<0.001$) increase in the thiobarbituric acid reactive substances (TBARS) and ROS formation, and a significant ($p<0.001$) decrease in the GSH level and total antioxidant capacity in the liver of the cholestatic rats compared to the sham group (Figure 3). Figure 3A shows that ginger extract significantly elevated total antioxidant capacity in the BDL+oZ100 group ($p<0.01$) and the other treatment groups ($p<0.001$) compared to the BDL group. The oZ400 group also presented a significant increase compared to the BDL oZ100 ($p<0.001$), BDL+oZ200 ($p<0.01$), and BDL+oZ400 ($p<0.001$) groups. Figure 3B indicates that ROS formation significantly increased in the BDL ($p<0.001$) and BDL+ oZ100 ($p<0.01$) groups compared to the sham group. Administration of ginger extract significantly ($p<0.001$) decreased ROS formation in all the treatment groups compared to the BDL group. It also significantly reduced ($p<0.01$) in the oZ400 group compared to the BDL+oZ100 group. The Figure 3C

Table 2
The effect of ginger extract on echocardiogram parameters in cholestatic animals

Treatment	Left ventricular diastolic dimension (m/m; FI)	Left ventricular end-systolic dimension (m/m)	Shortening time (m/m)	Septal dimension (m/m) equivalent
Sham	2.2±0.21	4.2±0.95	0.10±0.018	1.33±0.08
BDL	3.1±0.89	5.1±0.85	0.08±0.004	1.26±0.11
BDL+oZ100	2.5±0.13	4.3±0.45	0.09±0.008	1.06±0.19
BDL+oZ200	2.4±0.50	4.7±0.98	0.11±0.013	1.08±0.09
BDL+oZ400	4.2±0.77$^{**\%\%\wedge\wedge}$	7.6±0.76$^{***\#\#\%\%\%\wedge\wedge}$	0.11±0.011	1.35±0.22
oZ400	3.1±1.06	5.8±1.66	0.130±0.023$^{\#\#\#\%\%}$	1.39±0.17$^{\%}$

The data are presented as mean±SD for 6 animals in each group. ***p<0.001 vs. sham group; **p<0.01 vs. sham group; $^{\#\#\#}$p<0.001 vs. BDL group; $^{\#\#}$p<0.01 vs. BDL group; $^{\%\%}$p<0.01 vs. BDL+oZ100 group; $^{\%}$p<0.05 vs. BDL+oZ100 group; ^^p<0.01 vs. BDL+oZ200 group. Abbreviations: BDL – bile duct-ligation rats; BDL+oZ100, BDL+oZ200, and BDL+oZ400 – the groups of BDL rats that received ginger methanol extract at the doses of 100, 200, and 400 mg/kg/day, respectively; oZ400 – the group of rats that received ginger methanol extract at a dose of 400 mg/kg/day.

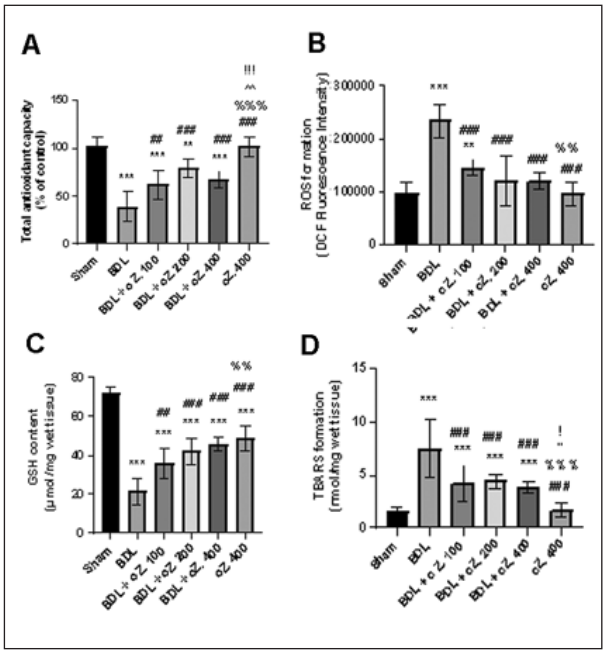

Figure 3. The effects of the ginger extract on total antioxidant capacity (**A**), ROS formation (**B**), GSH content (**C**), and TBARS formation (**D**) in the liver tissue of cholestatic animals. Data are presented as mean±SD for 6 animals/each group. ***p<0.001 vs. sham group; p<0.01 vs. sham group; ###p<0.001 vs. BDL group; ##p<0.01 vs. BDL group; %%%p<0.001 vs. BDL+oZ100 group; %%p<0.01 vs. BDL+oZ100 group; ^^p<0.01 vs. BDL+oZ200 group; ^p<0.05 vs. BDL+oZ200 group; ^^p< 0.01 vs. BDL+oZ400 group; ^p<0.05 vs. BDL+oZ400 group. Abbreviations: BDL – bile duct-ligation; GSH – glutathione; ROS – reactive oxygen species; TBARS – thiobarbituric acid reactive substances.

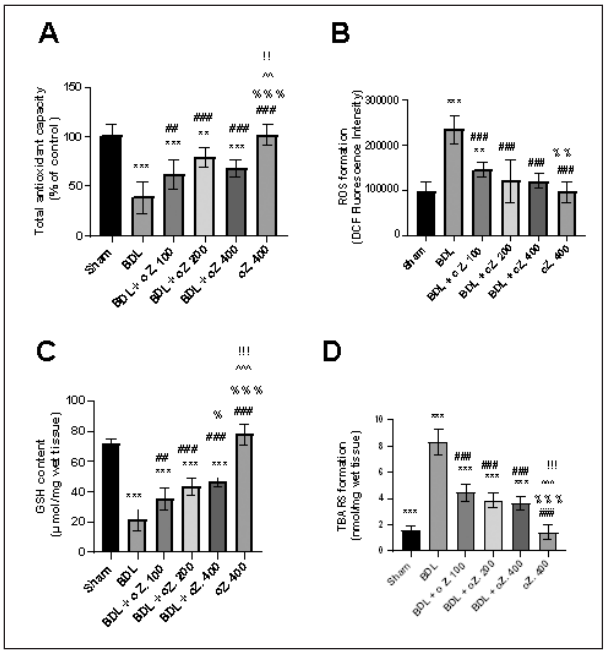

Figure 4. The effects of ginger extract on total antioxidant capacity (**A**), ROS formation (**B**), GSH content (**C**), and TBARS formation (**D**) in the heart tissue of cholestatic animals. Data are presented as mean±SD for 6 animals/each group. ***p<0.001 vs. sham group; **p<0.01 vs. sham group; ###p<0.001 vs. BDL group; ##p<0.01 vs. BDL group; %%%p<0.001 vs. BDL+oZ100 group; %%p<0.01 vs. BDL+oZ100 group; ^^p<0.01 vs. BDL+oZ200 group; ^p<0.05 vs. BDL+oZ200 group; ^^p<0.01 vs. BDL+oZ400 group; ^p<0.05 vs. BDL+oZ400 group. Abbreviations: BDL – bile duct-ligation; GSH – glutathione; ROS – reactive oxygen species; TBARS – thiobarbituric acid reactive substances.

illustrates that GSH content significantly (p<0.001) reduced in all groups compared to the sham group. Treatment with ginger extract significantly increased GSH content in the BDL+oZ100 group (p<0.01) and the other treatment groups (p<0.001) compared to the BDL group. Additionally, GSH levels significantly increased in the oZ400 group (p<0.01) compared to the BDL+oZ100 group. Figure 3D shows that TBARS formation significantly increased (p<0.001) in all BDL groups compared to the sham group. The ginger extract significantly (p<0.001) decreased TBARS formation in all treatment group compared to the BDL group. The oZ400 group also presented a significant decrease in TBARS formation compared to the BDL+oZ100 (p<0.001), BDL+oZ200 (p<0.05), and BDL+oZ400 (p<0.05) groups.

The effects of ginger extract on the oxidative stress markers in the heart tissue of the cholestatic rats. The measurements of the oxidative stress markers in the heart tissue are shown in Figure 4. Figure 4A shows that total antioxidant capacity significantly reduced in all the BDL groups (p<0.01 for BDL+oZ200 group and p<0.001 for other BDL groups) compared to the sham group. Administration of ginger extract significantly elevated total antioxidant capacity in the BDL+oZ100 group (p<0.01) and the other treatment groups (p<0.001) compared to the BDL group. Also, the oZ400 group presented a significant increase in total antioxidant capacity compared to the BDL+oZ100 (p<0.001), BDL+ oZ200 (p<0.01), and BDL+oZ400 (p<0.01) groups. Figure 4B indicates that ROS formation significantly increased in the BDL and BDL+oZ100 groups (p<0.001 and p<0.01, respectively) compared to the sham group. Administration of ginger extract significantly (p<0.001) decreased ROS formation in all the treatment groups compared to the BDL group. Also, ROS formation in the oZ400 group significantly reduced (p<0.01) compared to the BDL+oZ100 group. Figure 4C illustrates that GSH content significantly (p<0.001) reduced in all

BDL groups compared to the sham group. Treatment with ginger extract significantly increased GSH content in the BDL+oZ100 group ($p<0.01$) and the other treatment groups ($p<0.001$) compared to the BDL group. Additionally, the BDL+oZ400 group shows a significant ($p<0.05$) increase in GSH content compared to the oZ100 group. The GSH content significantly increased in the oZ400 group compared to the BDL+oZ100 ($p<0.001$), BDL+oZ200 ($p<0.001$), and BDL+oZ400 ($p<0.001$) groups. Figure 4D demonstrates that TBARS formation significantly increased ($p<0.001$) in all the BDL groups compared to the sham group. Ginger extract significantly ($p<0.001$) decreased TBARS formation in all treatment group compared to the BDL group. Also, the oZ400 group presented a significant decrease in TBARS formation compared to the BDL+oZ100 ($p<0.001$), BDL+oZ200 ($p<0.001$), and BDL+oZ400 ($p<0.001$) groups.

The effects of ginger extract on histological changes in liver tissue of cholestatic rats. The evaluation of histological changes in liver tissue of cholestatic rats showed moderate changes in focal necrosis and bile duct proliferation, and severe changes in portal inflammation in the BDL group (Figure 5 and Table 3). Rats of the BDL+oZ100 group showed mild changes in focal necrosis and portal inflammation. Also, the BDL+oZ200 and BDL+oZ400 groups showed mild changes in portal inflammation and bile duct proliferation (Figure 5 and Table 3).

The effects of ginger extract on histological changes in heart tissue of cholestatic rats. As seen in Figure 6, the obstruction of the bile duct in rats caused severe changes in inflammation, myofibrillar degeneration, and vacuolar degeneration in cardiac tissue in the BDL group. It also showed moderate changes in fibrosis and loss of transverse striations and mild changes in myophagocytosis in cardiac tissue of the BDL group. On the other hand, treatment of animals with the ginger methanol extract at a dose of 100 mg/kg of presented moderate changes in inflammation, myofibrillar degeneration, myophagocytosis, and fibrosis and mild changes in loss of transverse striations and vacuolar degeneration. In the BDL +oZ200 and BDL+oZ400 groups, moderate changes in fibrosis and mild changes in myofibrillar

Table 3
Grading of pathological liver tissue damage in cholestatic rats and investigating the effect of administration of ginger extract on these damages

Treatment	Sham	BDL	BDL+oZ100	BDL+oZ200	BDL+oZ400	oZ400
Confluent Necrosis	0	0	0	0	0	0
Focal Necrosis	0	++	+	0	0	0
Portal Inflammation	0	+++	+	+	+	0
Bile duct proliferation	0	++	–	+	+	0

None (0), mild (+), moderate (++), severe (+++). Abbreviations: BDL - bile duct-ligation rats; BDL+oZ100, BDL+oZ200, and BDL+oZ400 - the groups of BDL rats that received ginger methanol extract at the doses of 100, 200, and 400 mg/kg/day, respectively; oZ400 - the group of rats that received ginger methanol extract at a dose of 400 mg/kg/day.

Table 4
Grading of pathological heart tissue damage in cholestatic animals and investigating the effect of administration of ginger extract of on these damages

Treatment	Sham	BDL	BDL+oZ100	BDL+oZ200	BDL+oZ400	oZ400
Necrosis	0	+++	+	0	0	0
Inflammation	0	+++	++	+	0	0
Loss of transverse striations	0	++	+	0	0	0
Myofibrillar degeneration	0	+++	++	+	+	0
Myophagocytosis	0	+	++	++	+	0
Fibrosis	0	++	++	++	++	0
Vacuolar degeneration	0	+++	+	+	0	0

None (0), mild (+), moderate (++), severe (+++). Abbreviations: BDL - bile duct-ligation rats; BDL+oZ100, BDL+oZ200, and BDL+oZ400 - the groups of BDL rats that received ginger methanol extract at the doses of 100, 200, and 400 mg/kg/day, respectively; oZ400 - the group of rats that received ginger methanol extract at a dose of 400 mg/kg/day.

degeneration have been shown. Additionally, the BDL+oZ200 group showed mild changes in inflammation and vacuolar degeneration, and moderate changes in myophagocytosis. The BDL+oZ400 group showed mild changes in myophagocytosis. To see the degree of cardiac tissue changes in cholestatic animals, refer to Table 4.

Discussion

The results of our study showed a significant increase in serum bilirubin, AST, ALT, and ALP levels in BDL group. These changes indicate liver damage (Tolunay et al. 2021) and have been previously reported in the BDL model (Geenes et al. 2014). Treatment with ginger extract significantly prevented the increase of these markers in the cholestatic rats. Rahimlou et al. (2016) have investigated the effect of ginger supplement on the non-alcoholic fatty liver disease. They have found that ginger supplementation led to a significant decrease in ALT, gamma-glutamyl transferase (GGT), inflammatory cytokines, insulin resistance index, and degree of steatosis (More and Aithal 2006). Hepatoprotective activity of ginger extract against hepatotoxicity induced by CCl4 has also been reported (Mbaveng and Kuete 2017).

The ginger extract significantly reduced the glutamate pyruvate transaminase (GPT) and the glutamate oxaloacetate transaminase (GOT) enzymes (Mbaveng and Kuete 2017). The extract of the ginger rhizome significantly reduced the serum SOD level and with a higher dose of 300 mg/kg caused a significant decrease in plasma SOD, liver MAD, serum AST, and an increase in plasma proteins (Mbaveng and Kuete 2017). The ginger reduced the prostaglandin synthesis by inhibiting cyclooxygenase 1 and 2. The extract of this plant also inhibited the production of leukotrienes by inhibiting 5-lipoxygenase (Bischoff-Kont and Furst 2021). The ginger compounds including ginger dione and shoagol have similar pharmacological effects to non-steroidal anti-inflammatory drugs and inhibited the metabolism of arachidonic acid and ultimately the synthesis of prostaglandin. Therefore, it acts as a more effective anti-inflammatory agent

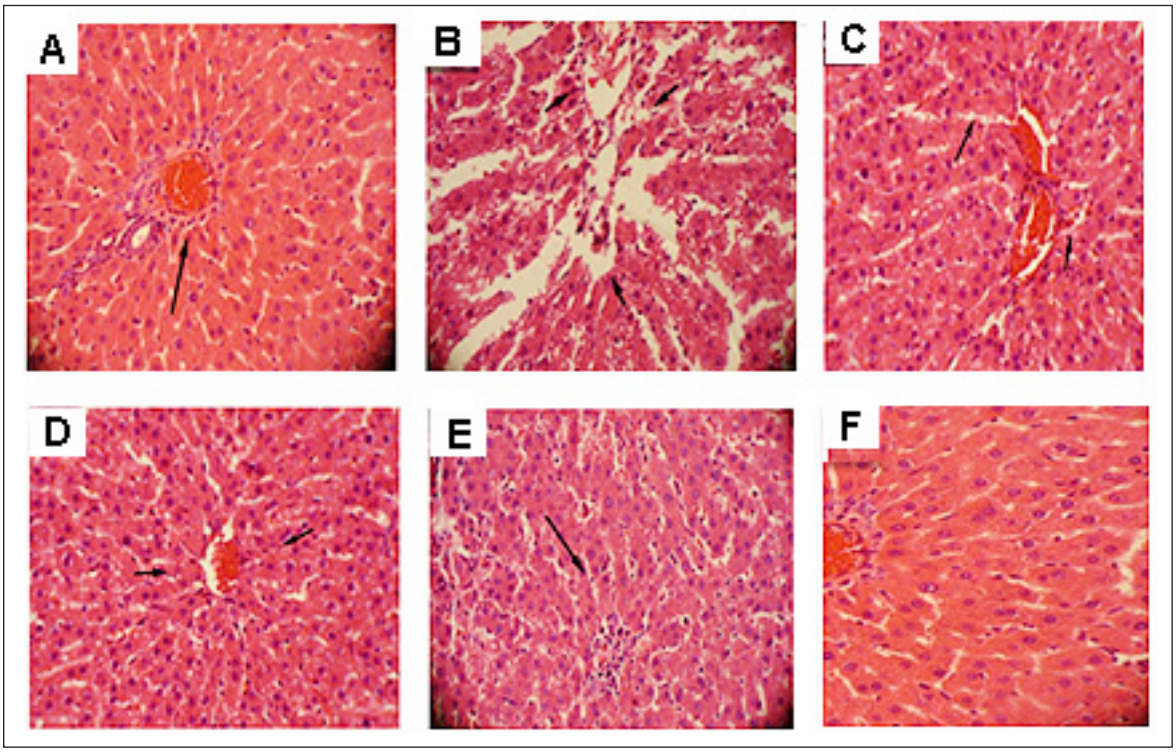

Figure 5. Histopathological changes of liver tissue in cholestatic rats (28 days after BDL surgery) and the effect of ginger extract administration. (The black arrow shows the necrosis of hepatocytes and the accumulation of inflammatory cells in the port space). (**A**) Sham group; (**B**) BDL group; (**C**) BDL+oZ100; (**D**) BDL+oZ200; (**E**) BDL+oZ400; (**F**) oZ400. To observe the degree of liver tissue changes in cholestatic animals, refer to Table 3. Abbreviations: BDL – bile duct-ligation; oZ100, oZ200, oZ400 – the groups of rats that received ginger extract at the doses of 100, 200, and 400 mg/kg/day, respectively.

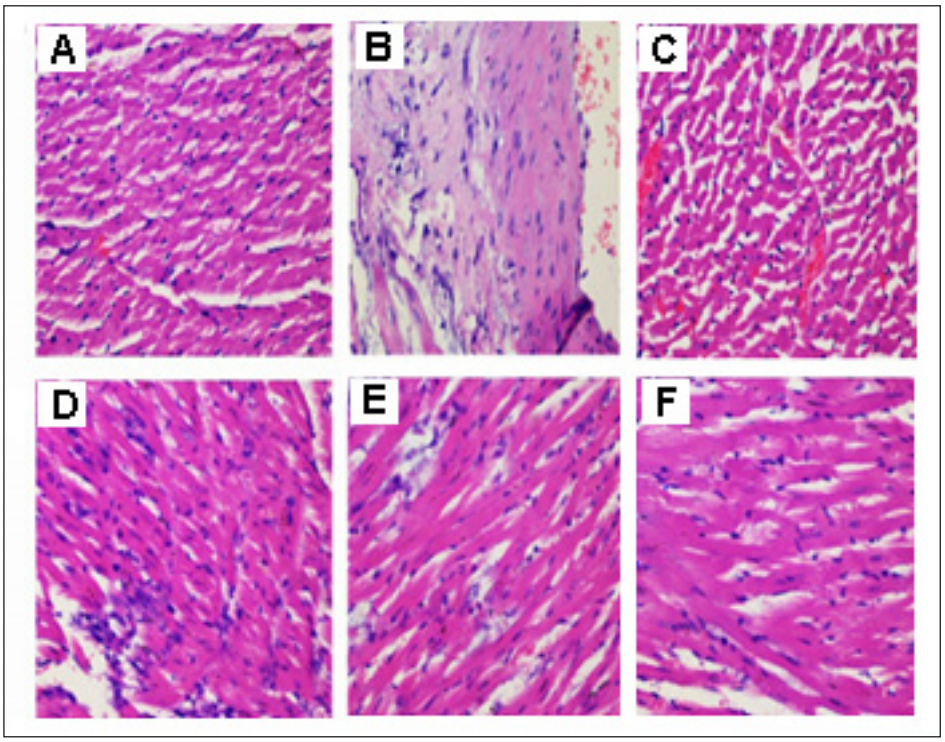

Figure 6. Histopathological changes of heart tissue in cholestatic rats (28 days after BDL surgery). (**A**) Sham group; (**B**) BDL group; (**C**) BDL+oZ100 group; (**D**) BDL+oZ200 group; (**E**) BDL+oZ400 group; (**F**) oZ400 group. To observe the degree of liver tissue changes in cholestatic rats, refer to Table 4. Abbreviations: BDL – bile duct-ligation; oZ100, oZ200, oZ400 – the groups of rats that received ginger extract at the doses of 100, 200, and 400 mg/kg/day, respectively.

than conventional anti-inflammatory drugs with fewer side effects (Meshram et al. 2021).

Our results showed that cholestasis in the rats leads to decrease in glutathione reserves and antioxidant capacity. On the other hand, it increases in MDA activity and ROS formation. The available evidence shows that the accumulation of bile acids in the liver increases the sensitivity of this tissue to other damaging factors such as oxidative stress, which causes the progress of steatosis towards steatohepatitis, fibrosis, and cirrhosis. Considering the relationship between the oxidative stress and tissue inflammation (Masarone et al. 2018), the present study confirms that cholestasis can cause oxidative damage in the liver tissue of BDL rats. The induction of oxidative stress is also confirmed by the MDA increase and disturbance in the amount of antioxidant enzymes in the liver of cholestatic rats.

In previous studies, the role of hydrophobic bile acids in the pathogenesis of cardiomyopathy related to cholestasis has been mentioned. Hydrophobic bile acids are cytotoxic agents that affect different intracellular targets including mitochondria (Rolo et al. 2003). DNA is another important target affected by bile acids (Martinez-Diez et al. 2000). It has been proven that cytotoxic bile acids can cause oxidative stress, inflammatory responses, mitochondrial disorder, and fibrosis during cholestasis (Li et al. 2017). Oxidative stress is closely related to inflammatory responses, mitochondrial disorders, and tissue fibrosis (Bomzon et al. 1997). Oxidative stress and related events such as lipid peroxidation, reduction of cellular antioxidant activities and damage to cellular proteins are effective factors in the development of liver fibrosis and ultimately liver failure and heart damage caused by cholestasis. It seems that increased levels of cytotoxic molecules including hydrophobic bile acids play a major role in cholestatic-cirrhotic related to oxidative stress (Mormone et al. 2011).

Bile acids have adverse effects on cell that have also been proven (Mustafa et al. 1969). These molecules increase the production of ROS in cell mitochondria (Brookes et al. 2004; Ott et al. 2007). Mitochondria are primarily involved in the formation of intracellular ROS. Fault of mitochondrial function significantly increases the formation of ROS. Therefore,

mitochondrial dysfunction and oxidative stress are related together. GSH reserves play a major role in maintaining organelle homeostasis (Garcia-Ruiz and Fernandez-Checa 2006). A high level of GSH helps to the proper functioning of the mitochondrial organelle and energy metabolism (Garcia-Ruiz and Fernandez-Checa 2006). It has also been shown that increased GSH stores can protect cells from toxins (Garcia-Ruiz and Fernandez-Checa 2006). On the other hand, some studies have shown that a high level of GSH regulates mitochondrial permeability (Chernyak 1997). Mitochondrial permeability plays an important role in cell death. GSH is transported from the cytoplasm to the mitochondrial intermembrane space by various transporters and transferred to the mitochondrial matrix subsequently. Therefore, changes in cytoplasmic GSH can lead to mitochondrial GSH stores.

Impairments in the cellular GSH synthesis have previously been reported in various tissues of cholestatic animals (Lash 2006; Huang et al. 2009; Copple et al. 2010). The reduction of tissue GSH levels during cholestasis is mainly related to impairments in the synthesis of this peptide (Yang et al. 2009). In this study, the antioxidant activity of the ginger extract was also investigated by measuring the MDA content in the liver and heart tissues, and the activity of the antioxidant enzymes. The cholestasis causes an increase in the MDA content in the liver and heart tissues and a decrease in the activity of antioxidant enzymes in comparison with the sham group. The administration of the ginger methanol extract significantly improved the antioxidant defense system in a dose-dependent manner in cholestatic rats. These results show that the imbalance between the level of oxidative stress and the formation of antioxidants can result in cholestasis and ginger extract can improve this pathological process.

The increase in the amount of ROS in patients with liver abnormalities may have a destructive effect on membrane lipids and cardiomyocyte fibrotic destruction by collagen accumulation between myocyte cells (Akashi et al. 2010) and cause also an inflammatory response (Ciutac and Dawson 2021). The increase of bile acids may increase the ROS level in cardiac myocytes and the high amount of ROS induces oxidative stress and lipid peroxidation leading to the production of cytotoxic cardiomyopathy (Ciutac and Dawson 2021). Additionally, as a result of ischemia-reperfusion injury, the amount of MDA increases, which indicates oxidative stress (Wei et al. 2011). MDA, which is used as a marker for oxidative stress damage, is one of the products obtained from lipid peroxidation by ROS (Nayki et al. 2018).

Another factor investigated in this research was the evaluation of histopathological changes in the liver and heart tissues. In this study, the loss of hepatocytes was observed in a large amount, which could be due to the high concentration of bile salts after duct obstruction or anemia due to the decrease in blood flow in the portal vein (Galle et al. 1990). Research showed that the decrease in portal blood flow is observed in extrahepatic obstruction (Galle et al. 1990). This reduction can lead to a decrease in the amount of tissue oxygen and cause tissue damage (Glaser et al. 2010). It can also cause oxidative stress in hepatocytes and intracellular organelles, which have toxic effects by bile salts and bilirubin.

In the present study, necrosis was observed, which can be due to the reduction of blood flow due to obstruction and plays an important role in the death of hepatocytes. Gradual increase in the amount of necrosis was also observed, which probably indicates the spread of damage with a decrease in blood flow. Galle et al. (1990) have shown that in the liver damage during the cholestasis, the proliferation of clanangiocytes, proliferation and expansion of bile ducts, expansion of sinusoids and arteries can be observed. The proliferation and expansion are necessary to maintain homeostasis in the biliary tree and secretory function during cholestasis syndrome.

Maintaining the autocrine and paracrine mechanisms is important to regulate the homeostasis created by proliferation of bile ducts (Wadie et al. 2021). Obstruction of the bile duct causes significant expansion in the upper bile ducts and the accumulation of bile and high-pressure lead to a multiplication and doubling of the ducts. These tortuous ducts reduce the speed of bile flow and increase the formation of sediments that lead to the obstructing of ductules (Nayki et al. 2018). Obstruction can also cause fibrosis, which eventually leads to cirrhosis. In this study, the biochemical results were confirmed by histopathological findings. We showed that cholestatic rats revealed a high degree of liver damage. The histopathological evaluations also showed the beneficial effects of ginger methanolic extract in cholestatic rats.

The results of recent studies on medicinal plants have shown that some medicinal plants, like many chemical drugs, have harmful side effects on human cells and animal models (Nateghpour et al. 2014). In the current study, in the groups receiving the ginger extract, there were reported no side effects and histological changes in the liver and heart tissues, which,

of course requires further research with higher doses. The animal study investigating the effect of ginger on liver diseases in mice has shown that ginger significantly reduced pathological changes in the liver tissue (Huang et al. 2014).

On the other hand, the investigation of the survival has shown that the administration of ginger extract increased the survival in the experimental groups compared to the control positive one by reducing the ROS formation and MDA activity and increasing the glutathione reserves and antioxidant capacity in the investigated tissues.

Since the ginger plant has a large number of antioxidants and flavonoids, it shows protective effects. Therefore, ginger consumption increases the activity of antioxidant enzymes and removes free radicals. Fourty types of antioxidant compounds have been identified in the ginger plant (Nakatani 2000). All species of ginger have antioxidant properties (Chen et al. 2002). The ginger can be effective like ibuprofen and mefenamic acid in the treatment of primary dysmenorrhea (Ozgoli et al. 2009). The high antioxidant activity and chemical compounds of the ginger plant have been reported in many studies (Ojewole 2006).

One of the effective compounds of ginger is 6-gingerol, which inhibited the production of nitric oxide in macrophages in a dose-dependent manner (Ghasemzadeh et al. 2010). It also significantly decreased the enzyme nitric oxide synthase (iNOS) in lipopolysaccharide of macrophages (Ippoushi et al. 2003). It can be concluded that oxidative stress is an important factor in many diseases including liver and cardiac disorders. Methanolic extract of ginger can be a new strategy in the treatment of cardiac and liver damages due to its high antioxidant effects. Discovering its exact mechanism requires further studies in this field.

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