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Potential protective effects of ginger and atorvastatin against diazinon-induced hepatotoxicity in rats: A comparative histopathological, immunohistochemical, and biochemical study

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ABSTRACT

One of the most widely used organophosphorus insecticides for agricultural use is diazinon (DZ). In this study, we aimed to investigate the ameliorative effect of ginger (GE) and atorvastatin (ATR) against DZ-induced hepatotoxicity in rats. Seven groups of 49 males were randomly created. Group 1 (saline); group 2 (GE group; 100 mg/kg/day orally); group 3 (ATR group; 20 mg/kg/ day orally); group 4 (DZ group; 20 mg/kg/ day orally); group 5 (DZ+GE); group 6 (DZ+ATR); and group 7 (DZ+GE+ATR) for 30 days. Liver enzymes (AST, ALT, and ALP) were significantly elevated in the serum of DZ-intoxicated rats, while albumin level was dropped. Also, DZ increased cholesterol, triglycerides, LDL-C and lowered HDL-C concentrations. Additionally, a significant elevation in hepatic malondialdehyde (MDA) was recorded. Additionally, the antioxidant indicators glutathione, superoxide dismutase (SOD), and hepatic catalase (CAT) were significantly lower in the DZ-treated rats. Also, the hepatic architecture was disturbed by DZ, and the liver caspase-3 expression was considerably elevated in DZ treated group compared to control group. In DZ-intoxicated rats and co-treated with GE and ATR, there were increases in antioxidant biomarkers, decreases in MDA, and improvement in serum hepatic enzymes levels. In conclusion, GE and ATR have significant protective effects on DZ- induced hepatotoxicity via their antioxidative and antiapoptotic properties.

1. INTRODUCTION

One of the most common types of pesticides used in agriculture to manage insect pests on livestock and crops is organophosphorus insecticides. They are a significant contributor to environmental pollution in various nations and are frequently found in air, water, vegetables, and soil (Abdel-Daim et al., 2016). The widespread use of organophosphates contributes to environmental pollution, poses health risks, and has negative effects on both humans and animals (Abdel-Daim et al., 2018). They cause oxidative stress, which is known to play a role in the aging process and the pathogenesis of many diseases (Abdel-Daim et al., 2020).

Diazinon (DZ) is one of the most popular organophosphorus insecticides used in agriculture, veterinary medicine, and public health. Males in particular are more likely than females to unintentionally consume or breathe in DZ due to the polluted environment (Al-Attar, 2015). The irreversible acetylcholinesterase inhibition is the most significant aspect of DZ toxicity, and at high doses, it can result in animal death. Significant changes in antioxidant enzyme activity are also induced by DZ toxicity, pointing to a potential role for reactive oxygen species (ROS) in DZ toxicity. Hematologic and hepatotoxicity are caused by these ROS's induction of oxidative damage (Al-Attar et al., 2017).

The use of natural products in reducing the impact of xenobiotics is reported in numerous recent literatures for complementary medicine and alternative therapies (Abdel-Daim et al., 2020). As a result, there is a growing interest in using natural products and medicinal plants to discover new pharmacologically active ingredients.

Ginger (Zingiber officinale) has antimicrobial and antifungal properties commonly used to treat nausea caused by travel and during pregnancy (Ahd et al., 2019). Polyphenol compounds are among the numerous bioactive antioxidants found in GE (Idris et al., 2019). The active component of ginger, gingerol, has been shown in numerous studies to have anti-inflammatory and analgesic properties (Zhang et al., 2022). Clinical and experimental data showed the anti-inflammatory, antihypercholesterolemic, antihyperlipidaemic, antiemetic and antitoxic properties of Z. officinale (Gholampour et al., 2017). However, the protective role of ginger against ferrous sulfate-induced liver

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and kidney injury has been investigated (Gholampour et al., 2017).

One of the statin medications most frequently prescribed for a variety of conditions is atorvastatin (ATR) (Demoz et al., 2019). ATR lowers LDL-cholesterol levels in patients with hyperlipidemia more effectively than other medications (Abdulwahab et al., 2021; Fan et al., 2022). ATR also has a variety of advantageous pharmacological effects, such as its anti-inflammatory and antioxidant properties, which manifest at low doses by inhibiting oxidative stress (Naeimi et al., 2017). ATR has demonstrated protective effects against cardiotoxicity (Acar et al., 2011), hepato-renal toxicity (El-Moselhy and El-Sheikh 2014), and testicular toxicities (Ramanjaneyulu et al., 2013). ATR also has neuroprotective properties through its antioxidant and anti-inflammatory effects (Mounier et al., 2021).

The goal of the current study was to evaluate the outcome of GE and/or ATR in ameliorating DZ-induced hepatic toxicity in rats.

2. MATERIAL AND METHODS

2.1. Chemicals

Diazinon-60® was purchased from Drug Pharmaceuticals, Cairo, Egypt. Ginger obtained from MEPACO Company, Abu Sultan, Ismailia, Egypt as 400 mg tables. Atorvastatin obtained from Delta Pharma Company, Egypt as 40 mg tablets.

2.2. Experimental design

Apparently healthy 49 Wister Albino male rats, which weigh between 185 and 200 g were obtained from Egyptian Organization for Biological Products and Vaccines. All animals underwent a seven-day acclimatization period at a constant temperature of 25°C, with a 12:12 h light/dark cycle, free access to water, and commercial pellets. Rats were randomly divided into seven groups; 7 rats each. Group (1) received saline solution (5 ml/kg orally). Group (2) GE (100 mg/kg/day; Yaghubi Beklar et al., 2021). Group (3) ATR (20 mg/kg/day; Lehnen et al., 2014). Group (4) intoxicated with DZ (20 mg/ kg/day; Danaei et al., 2019) to induce liver injury. Group (5) DZ (20 mg/ kg/day) +GE (100 mg/kg/day), Group (6) DZ (20 mg/ kg/day) +ATR (20 mg/kg/day) and Group (7) DZ (20 mg/ kg/day) +GE (100 mg/kg/day) +ATR (20 mg/kg/day) and all treatments were administered orally, once daily for 30 days. The study plan was approved by Institutional Ethical Committee, Faculty of Veterinary Medicine, Benha University (Approval No BUFVTM 04-08-22).

2.3. Blood sampling and processing

Rats were anaesthetized with isoflurane a day after the last treatment. Blood samples collected from the retro-orbital plexus were used to separate the serum (centrifugation at 1200 g for 15 min). The separated sera were stored at -20°C for subsequent biochemical analysis.

2.4. Serum biochemical analysis

Liver biomarkers AST and ALT (Reitman and Frankel, 1957), ALP (Tietz et al., 1983), and albumin level (Doumas et al., 1971) and lipid profile; cholesterol, Triglycerides were estimated by the methods of Siedel et al. (1983), Foster and Dunn (1973), respectively. HDL-C was estimated by the method of Warnick et al. (1985) and LDL (Friedewald et al., 1972) were determined in serum by specific kits (Biodiagnostic company, Egypt).

2.5. Hepatic antioxidants

The oxidative stress markers measured were MDA (Mihara and Uchiyama, 1978) , CAT (Aebi, 1984), SOD (Nishikimi et al. 1972), and GSH (Beutler et al. 1963) in hepatic tissue.

2.6. Histopathological studies

Small tissue specimens were collected from the liver of rats in all groups and immediately fixed in 10% neutral buffered formalin. Dehydrate next using ascending strengths of ethyl alcohol, clean in xylene, and then embedded in paraffin wax. 5 µm tissue paraffin were sectioned and stained with hematoxylin and eosin (H&E) staining according to Bancroft and Gamble (2008).

2.7. Immunohistochemical assessment of caspase 3

Deparaffinized liver sections were rehydrated and incubated in 3% H2O2 for 10 minutes. The sections were then exposed to a rabbit monoclonal anti-caspase-3 antibody for an hour at room temperature (Santa Cruz Biotechnology Inc., Dallas, TX, USA, 1:100 dilutions). The sections were incubated with goat anti-rabbit IgG for 30 minutes. Diaminobenzidine (DAB) was used for 10 minutes to see the immunoreaction.

2.8. Statistical analysis data

The recorded data were and expressed as mean±SE. Using the statistical program SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA), one-way ANOVA and Tukey's post hoc test for multiple group comparisons were used to analyze the data. Differences were considered statistically significant at ≤P 0.05.

3. RESULTS

3.1.Effect on biochemical and antioxidant parameters.

Administration of DZ induced hepatic damage evidenced by a substantial increase in AST, ALT, and ALP levels, while albumin level was dropped. When compared to DZ treated group, treatment with GE and ATR dramatically decreased the serum levels of AST, ALT, and ALP and raised albumin level. When compared to controls, DZ significantly raises levels of total cholesterol, TG, and LDL. GE plus ATR therapy significantly reduced lipid profile values compared to DZ treated group (Figure 1).

The liver tissue underwent oxidative damage as a result of DZ treatment, as shown by a significant increase in MDA levels and a decline in SOD activity, CAT, and GSH levels. Treatment with GE and ATR significantly reduced elevated MDA levels while also raising SOD activity, CAT, and GSH levels (Figure 2).

3.2. Histopathology.

Most examined livers of control group had normal histological architecture of hepatic lobules with apparently normal hepatocytes arranged in plates (Figure 3A). In GE, ATR-treated rats, most hepatocytes were nearly normal histologically. Few foci showed mild hepatocellular degeneration (Figure 3B-C). In DZ-intoxicated rats, severe multifocal hepatocellular degeneration (hydropic and ballooning degeneration), fatty change in the form of hydropic and ballooning degeneration and fatty change with focal areas (Figure 3D-F). Some hepatocytes were hypertrophic, and their cytoplasm was filled with amphophilic inclusions with loss of their nuclei. Vascular changes in DZ-intoxicated rats with occasional thrombosis of some portal blood vessels. Portal triads were severely hyperplasia with presence of newly formed bile ductules was prominent in many examined livers. Many hepatic sections showed significant cholangiofibrosis (Figure 3G). Multifocal mild to severe mononuclear inflammatory cellular aggregations were seen around bile ducts and among

hepatocytes (Figure 3H). Few syncytial cells were occasionally seen in many hepatic sections. In DZ-intoxicated rats co-treated with GE and or ATR, most rats showed mild to null hepatocellular degeneration (Figure 3I). No significant microscopic alterations were seen in the bile ducts and blood vessels in intoxicated rats co-treated with GE. In DZ-intoxicated rats co-treated with ATR, less ameliorative changes were observed in this group with persistent moderate to severe hepatocellular degeneration and necrotic hepatocytes in a few examined hepatic sections (Figure 3J). Mild bile ductal hyperplasia was also seen in some sections of this group. In DZ-intoxicated rats co-treated with both GE and ATR, the examined livers had similar findings to DZ+GE group where there was only mild hepatocellular degeneration with no significant histological changes in bile ducts and hepatic blood vessels (Figure 3K).

3.3. Immunohistochemical results.

There was minimal to null cleaved caspase 3 expressions (brownish coloration) in the hepatic sections of control, GE and ATR-treated rats (Figure 4A-C). The endothelial cells lining the sinusoids showed mild to moderate cleaved caspase 3 staining in these groups. In DZ-treated rats, multifocal areas of intense caspase-3 immuno-expression were recorded in hepatic parenchyma (Figure 4D-E). In these areas, apoptotic hepatocytes showed various amounts of cleaved caspase activity in form of diffuse or punctate cytoplasmic patterns. A few apoptotic hepatocytes with moderate caspase-3 staining were also seen around central veins and bile ducts in DZ+ATR treated rats (Figure 4F). A marked reduction in caspase 3 protein expression was seen in the hepatic parenchyma of rats in DZ+ATR, DZ+GE and DZ+GE+ATR-treated rats compared to DZ intoxicated rats (Figure 4G-H).

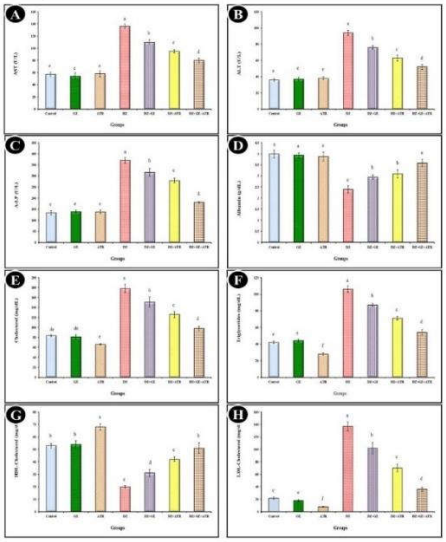

Fig (1). Effects of GE, ATR on serum hepatic biochemical parameters in DZ-intoxicated rats (n=7).

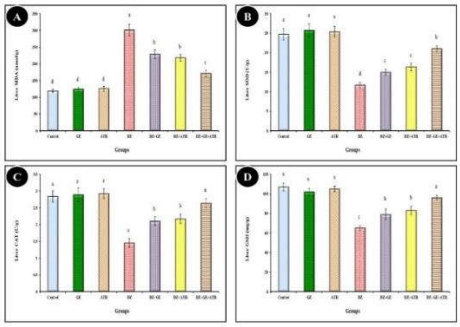

Fig (2). Effects of GE, ATR on hepatic antioxidants parameters in DZ-intoxicated rats (n=7).

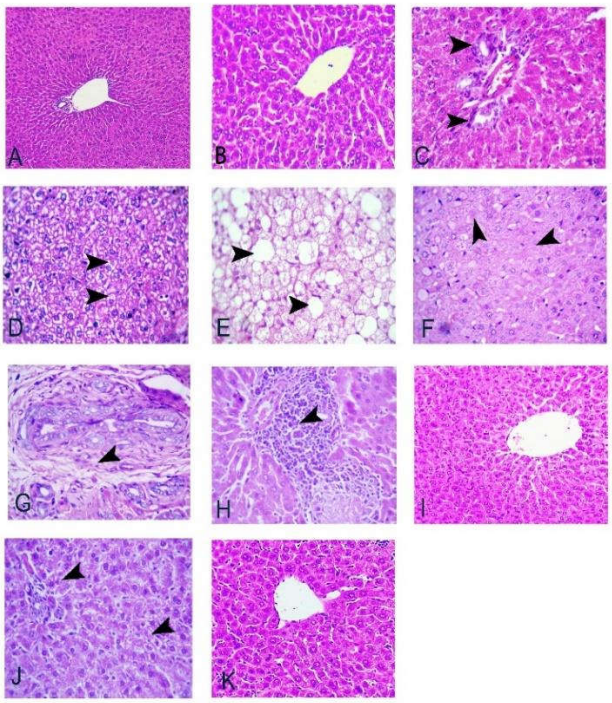

Fig (3). Histopathological micrographs of the liver showing; A-B) apparently normal hepatocytes in control (A) and GE (B) (100X). C) newly formed portal bile ductules (C) (arrowheads) in ATR treated group (200X). D-H) hepatocellular degeneration (D) (arrowheads, 200x), fatty change (E) (arrowheads, 200x) and necrosis (F) (arrowheads, 200X) with marked periductal fibrosis (G) (arrowhead, 200X) and mononuclear cellular infiltrations (H) (arrowhead, 200X) in the livers of DZ treated rats. I) No hepatocellular degenerative changes (I) (200X) in DZ+GE treated rats. J) Vacuolar degeneration of hepatocytes in DZ+ATR treated rats (J) (arrowheads, 200X). K) No significant microscopic changes in DZ+GE+ATR (K) (200X) treated rats.

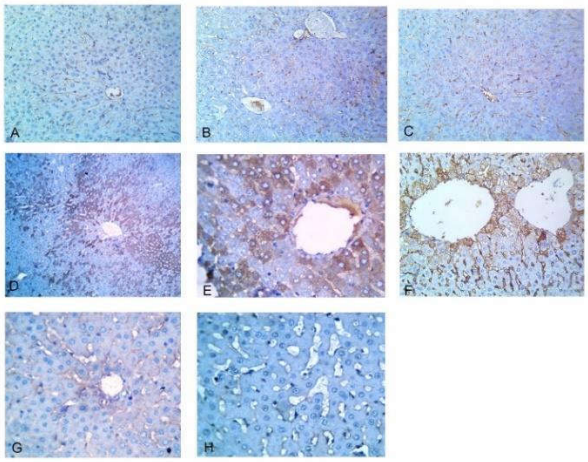

Fig (4) Photomicrographs of liver showing the immunohistochemical staining; (A–C) No cleaved caspase expression in hepatocytes in control (A) (100X), GE (B) (100X) and ATR (C) (100x) treated groups. (D-E) strong cytoplasmic staining of cleaved caspase 3 in the livers of DZ intoxicated rats (200X). (F) Weak to moderate cytoplasmic staining of cleaved caspase 3 in DZ+ATR treated rats (200X). G-H) Null cleaved caspase 3 protein expression in the livers of DZ+GE (G) and DZ+GE+ATR (H) treated rats (200X).

4- DISCUSSION

Organophosphorus insecticides may result in an increase in serum AST, ALP, and ALT activity (Sharma et al., 2005). Hepatic injury disrupted the transport function of the hepatocytes, and this led to altered membrane permeability, which caused enzyme leakage from cells (Fan et al., 2009). In previous studies, AST, ALT, and ALP activities of DZ-treated rats were significantly increased (Mossa et al., 2012; El-Demerdash and Nasr, 2014; Karimani et al., 2019). Furthermore, the obtained data suggested that supplementation of GE and ATR to DZ-intoxicated rats can alleviate the serum levels of AST, ALT, and ALP, indicating that exogenous antioxidants could protect liver function. AST and ALT elevations could potentially be attributed to

hepatocellular injuries (Ben Amara et al., 2011) and elevated ALP indicating biliary obstruction (Abdel-Daim et al., 2020).

The profiles of lipids and lipoproteins can be negatively impacted by Ops (Al-Attar et al., 2017). DZ increased cholesterol, triglycerides, LDL-C and lowered HDL-C concentrations. The results of the current study are consistent with those of other studies showed that OPs cause an increase in the level of total lipid, which are made up primarily of total cholesterol and triglycerides (Al-Attar 2015, AlAttar et al. 2017). Hepatocellular damage from oxidative stress-mediated cell membrane breakdown that results in cholesterol leakage into the blood may be related to hypercholesterolemia. Additionally, impaired cholesterol secretion into the bile resulted in stagnation of bile flow in bile ducts as a result of periportal cell loss (confirmed by elevated ALP) (Abdel-Daim et al. 2020), which ultimately caused an increase in total serum cholesterol in DZ-treated rats (Karimani et al., 2019). Increased adipocyte lipolysis carried on by DZ-induced insulin resistance can be used to explain the rise in total triglycerides. The liver serves as the primary site for the absorption, synthesis, and export of lipoproteins into the bloodstream, playing a key role in lipid metabolism. In contrast to HDL-C, which carries extra cholesterol from peripheral cells to the liver, LDL-C is the primary carrier of lipids from the liver to peripheral cells. TG and HDL levels in the blood are inversely correlated. As evidenced by the concurrent drop in total protein concentration, the decreased HDL blood concentration could be attributed to hyperlipidemia and decreased liver HDL synthesis, both of which increase the risk of atherosclerosis (Al-Attar et al. 2017).

The levels of AST, ALT, ALP, cholesterol, triglycerides, and LDL-cholesterol were significantly reduced after administration of ginger. Numerous studies demonstrated that the hepatoprotective properties of ginger against liver toxicity brought on by carbon tetrachloride, bromobenzene, and acetaminophen clearly support our findings (Yemitan and Izegbu, 2006; Ajith et al., 2007; El-Sharaky et al., 2009). Additionally, Rosuvastatin produced hepatoprotective effects in response to piroxicam-induced liver toxicity (Abdeen et al., 2019).

A significant drop in albumin levels, as seen in rats given DZ. Therefore, a decreased rate of protein synthesis in the liver caused by hepatotoxicity may be responsible for the loss of protein, particularly albumin (Al-Attar, 2015). After iron toxicity, ginger extract raised albumin levels, showing that it could improve liver functions (Gholampour et al., 2017).

Organophosphorus metabolism was primarily carried out in the liver, which also accumulated a significant amount of its metabolites (Messarah et al., 2013). Through the production of ROS that harms various membrane components. The elevated MDA concentrations and the decreased SOD, CAT, and GSH contents of the liver in this study indicated that DZ exposure induced oxidative stress in the rat liver, which would then cause membrane lipid peroxidation and cause liver damage. These findings were consistent with earlier research by Karimani et al. (2019), who hypothesized that increased lipid peroxidation may result from ROS produced by pyrethroid metabolism (Abdel-Daim et al., 2018). As a result, co-administration of GE and/or ATR after DZ treatment raised SOD, CAT, and GSH levels to normal levels and strengthened the body's antioxidant defense system. In fact, it has been documented that oxidative stress plays a role after exposure to OP (Esmailpour et al., 2022).

The histological findings demonstrated that DZ caused sever histopathological changes in the liver. These findings were in agreed with Sarhan and Al-Sahhaf (2011) and Zeinali et al. (2018). In the current study, rats that received GE and/or ATR along with DZ showed improvement in the form of preserved normal hepatic lobular architecture and unclogged veins. Additionally, we looked at caspases-3 in its activated state. Our research demonstrated that DZ activated caspase-3, which caused apoptosis in the hepatocytes of the rats given DZ. The final main implementer of apoptosis is known as caspase-3. It is in charge of splitting the essential cellular proteins, which causes the known morphological changes seen in apoptotic cells (Fischer et al., 2003). Many studies had shown the apoptotic effects of variable organophosphate compounds as Caughlan et al. (2004). Additionally, the activation of caspase-3 was significantly reduced in DZ+GE, DZ+ATR and DZ+GE+ATR treated groups to a level that was close to that of the control group.

5. CONCLUSION

Our results concluded that by safeguarding antioxidant enzymes and lowering lipid peroxidation, GE and ATR can be alleviating hepatic apoptosis and oxidative stress induced by DZ toxicity stress.

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