Original Paper

Evaluation of hepatoprotective and oxidative stress reducing activities of garlic and/or ginger against cisplatin-induced liver toxicity in rats

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ABSTRACT

Cisplatin is an anticancer drug with high efficiency against various types of tumors including head and neck, bladder, ovarian, lung, and testicular cancers. However, its use is limited due to its toxicity to various tissues including hepatotoxicity. Seven groups (n=10) of rats were used. Group I received normal saline, group II received garlic (200 mg/kg) and group III received ginger (310 mg/kg body weight) orally once per day for 21 consecutive days. Group IV received saline plus cisplatin on the 16th day (10 mg/kg, intraperitoneal). Group V received garlic plus cisplatin on the 16th day. Group VI received ginger plus cisplatin on 16th day and group VII received garlic and ginger plus cisplatin on 16th day. Cisplatin induced a significant (P<0.05) increase in hepatic serum biomarkers including AST, ALT, and ALP levels and a significant (P<0.05) decrease in ALB level. Moreover, cisplatin induced a significant (P<0.05) increase in MDA level and caused a significant (P<0.05) depletion in CAT, GSH and SOD levels in liver tissues. Furthermore, cisplatin induced severe histopathological alteration in liver tissue. Garlic and ginger have hepatoprotective and antioxidant activities, which may be beneficial for protecting against liver damage.

1. INTRODUCTION

Cancer is defined as the growth of cells in an uncontrolled manner that has damaged DNA expression, these cells then move through the tissues, shift to various parts of the body and enhances the growth of new blood vessels thus acquiring nutrients (Rana et al., 2017).

Cisplatin is one of the most efficient drugs used for the treatment of various solid tumors such as bladder, ovarian, lung and stomach cancers (Abdel-Daim et al., 2020). Cisplatin acts by interaction with purine bases of DNA in the cancer cells, preventing cells from replication and stopping their biological function, suppressing the growth of these malignant cells (Abd Rashid et al., 2020). However, cisplatin has limited use due to its toxic side effects including hepatotoxicity (Nasr and Saleh 2014; Abdullatif et al., 2017). The excessive production of mitochondrial reactive oxygen species (ROS) is the major mechanism of cisplatin-induced hepatotoxicity (Beagloo et al., 2019; Famurewa et al., 2020). The toxic effect of cisplatin can be limited by using a combination of cisplatin with various natural products that have antioxidant properties such as radical scavengers and enzyme inhibitors (Abd Elghaffar et al., 2016).

Garlic grows in a lot of countries of the world which has been used medicinally and as a dietary supplement. Organosulfur compounds such as allicin, diallyl disulfide, alliin, diallyl trisulfide, and diallyl sulfide, are the main active constituents of garlic (Abdel-Daim et al., 2020). The protective effect of garlic against cisplatin hepatotoxicity through the antioxidant and free radical scavenging activities (Nasr, 2014). Chemoprevention mechanisms of garlic include scavenging ROS, increased DNA repair, decreasing inflammation, suppression of proliferation and enhanced immunity (Aly et al., 2019). So, many studies were reported to discuss its protective action against the hepatotoxicity of several drugs (Nasr 2014; Oboma et al., 2018, Abdel-Daim et al., 2020)

Ginger is well known as a safe herbal medicine and one of the most widely consumed spices worldwide. Ginger contains numerous volatile oils and pungent phenol compounds known as gingerols, sesquiterpenoids, shogaols, also it has anthocyanin and tannin in its root bark. (Abd Rashid et al., 2021). Ginger has several beneficial effects and traditional uses in controlling hyperglycemia as well as hyperlipidemia, also ginger has immunomodulatory properties through inhibition of various inflammatory mediators e.g., prostaglandins and proinflammatory cytokines (Akbari et al., 2019). Ginger has hepatoprotective effect against liver toxicity induced by numerous substance such as carbon tetrachloride (Abd-Allah et al., 2016; Cheong et al., 2016; Mirazi et al., 2016; Oke et al., 2019), ferrous sulfate (Gholampour et al., 2017), lead acetate (Mohamed et al., 2015; Okediran et al., 2019) due to its antioxidant action.

This study was conducted to evaluate the protective and antioxidant activities of garlic and ginger against cisplatin-induced hepatotoxicity in a rat model.

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2. MATERIAL AND METHODS

2.1. Chemicals

Cisplatin was purchased from EIMC United Pharmaceuticals (Cairo, Egypt) as Unistin® (50 mg/50 ml) vial. Garlic was obtained from ATOS Pharma (Cairo, Egypt) as Tomex Plus tablets (300 mg dried garlic powder). Garlic tablets were crushed, dissolved in normal saline, and was administered at dose 200 mg/kg body weight according to Arafat et. al., (2021). Ginger was obtained from MEPACO Company (Sharquia, Egypt) as Ginger tablets (400 mg of ginger rhizome powder). Ginger tablets were crushed, dissolved in normal saline, and were administered at dose 310 mg/kg body weight according to Mohamed et. al. (2018). All kits for biochemical analysis were purchased from Bio Diagnostics Company (Giza, Egypt).

2.2. Experimental animals

Seventy Wister male albino rats, weighing between 160-200 gm were purchased from the Center of Laboratory Animals, Faculty of Veterinary Medicine, Benha University, Egypt and were kept in metal cages at a temperature of $36°C \pm 2°C$. Rats were given a commercial diet and and water *ad libitum.* They were left 14 days for acclimation before beginning the experiment. The study was approved by the Ethics Committee, Faculty of Veterinary Medicine, Benha University' with approval number (BUFVTM 05-01-23).

2.3. Experimental design

Seventy adult male rats were randomly divided into seven groups of 10 animals each as follows. Group I (control) received normal saline; Group II received garlic (200 mg/kg body weight according to Arafat et. al. (2021) and Group III received ginger (310 mg/kg body weight according to Mohamed et. al. (2018) orally, daily for 21 days. Group IV received normal saline daily, orally for 21 days, and on 16th day, rats were injected intraperitoneally with cisplatin (10 mg/kg body weight according to Abo El-Magd et. al. (2021) Group V received garlic orally for 21 days plus cisplatin (I.P on 16th day). Group VI received ginger for orally 21 days plus cisplatin (I.P on 16th day) and group VII received garlic and ginger orally for 21 days plus cisplatin (IP on 16th day).

2.4. Serum collection and tissue sampling

Twenty-four hours following the last dose, blood samples were collected from the medial canthus of the eye (Parasuraman et al., 2010). The blood sample was drawn without anticoagulants in sterilized tubes and the sera were separated and kept at -80 °C until used for the bioassays. The rats of all groups were euthanized by IP pentobarbital at a dose of 800 mg/kg (Zatroch et al., 2017), then the liver was immediately removed then washed. The first section of the liver was used in biochemical analysis to estimate the concentration of catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD) and malondialdehyde (MDA) as oxidative stress markers. The second part of the liver was kept for histological investigations.

2.5. Serum biochemical analysis

Serum ALT level were determined by special diagnostic kit (CAT. NO. AL 10 31 (45)) according to Reitman and Frankel (1957), Serum AST level were determined by special diagnostic kit (CAT. NO. AS 10 61 (45) according to Reitman Frankel (1957) and ALP level was evaluated according to the procedures of Tietz et al. (1983) by special diagnostic kit (CAT. NO. AP 10 20). While albumin was

determined according to Doumas et al. (1971) by specific diagnostic kit (CAT. No. AB 10 10). All commercial kits were obtained from Bio-Diagnostic Company, Giza, Egypt)

2.6. Tissue homogenate preparation for oxidative stress and antioxidant status evaluation:

The tissue was dissected and rinsed by a PBS solution (phosphate-buffered saline) consisting of 0.16 mg/ml heparin to separate any RBCs and curd. Tissues were homogenized by homogenizer (DAIHAN Scientific CO, Ltd. Korea HG-15A) using 5 ml of 5-10 ml buffer (i.e., 50 mM potassium phosphate, pH 7.5 1 mM EDTA) added each gram of tissue. Tissue homogenates aliquots were centrifuged in a cooling centrifuge (4000 rpm for 20 min) and then kept at -80ºC. Next, oxidative status was done by using SPECTROstar Nano (S/N 601-0450 BMG LABTECH GmbH 77799 Ortenberg /Germany) for determination of reduced-glutathione (GSH) activity (Sedlak and Lindsay 1968) by specific diagnostic kit (CAT. No. GR 25 11), catalase (CAT) activity (Aebi, 1984) by specific diagnostic kit (CAT. No. CA 25 17), superoxide dismutase (SOD) by specific diagnostic kit (CAT. No. SD 25 21) according to the procedures of Nishikimi et al. (1972) and malondialdehyde (MDA) according to Ohkawa et al. (1979) utilizing specific diagnostic kits (CAT. No. MD 25 29) get from the Laboratory Bio-Diagnostic Company Giza, Egypt.

2.7 Histopathological analysis

Liver tissue specimens (1-2 cm^3) were collected from rats in all groups and immediately fixed in 10 % formalin. The tissue was dehydrated using gradual concentrations of ethanol, cleared with xylene, and embedded in paraffin. The blocks were cut at a thickness of 5–7 μm. These sections were stained with hematoxylin and eosin (H&E) (Bancroft et al., 2013) and examined for histopathological changes.

2.8 Statistical analysis:

The statistical analysis was performed by the SPSS (Version 20; SPSS Inc., Chicago, USA) after the application of the Shapiro-Willk test for the determination of the normal distribution of the data. Comparison between groups was performed by one-way ANOVA and followed by Duncan's multiple range test. (Duncan, 1955) P-value less than or equal to 0.05 was considered significant.

3. RESULTS

Serum AST, ALT, and ALP levels were increased significantly ($P \leq 0.05$) in the cisplatin-intoxicated group compared to their levels in the control group. Serum albumin level was significantly ($P \leq 0.05$) decreased in cisplatin-treated groups in comparison to their normal levels in control rats. Rats intoxicated with cisplatin and pretreated with garlic or ginger showed a significant ($P \leq 0.05$) decrease in serum AST, ALT, and ALP activities. Also, cisplatin intoxicated rats and pretreated with garlic or ginger showed significant ($P \leq 0.05$) increase in albumin level as compared with cisplatin-intoxicated rats. The combination of garlic and ginger in cisplatin intoxicated rats induced significantly ($P \leq 0.05$) marked decrease in serum AST, ALT, and ALP levels and induced significantly ($P \leq 0.05$) marked increase in serum albumin level as compared with cisplatin-intoxicated rats and return to their normal level. The combination of garlic and ginger was more efficient than garlic and ginger independently.

In the present study, rats intoxicated with cisplatin showed a significant ($P < 0.05$) decrease in the hepatic tissue levels

of CAT, GSH and SOD with a significant increase (P<0.05) in hepatic MDA level by comparison to their normal levels in the control group. Pretreating cisplatin-induced hepatotoxicity rats with garlic or ginger decreased significantly (P ≤ 0.05) MDA level. Also, it significantly

increased CAT, GSH and SOD levels compared with the cisplatin-treated rats. Moreover, rats intoxicated with cisplatin and pretreated with the combination of garlic and ginger were more efficient than garlic and ginger alone in ameliorating oxidative stress (Fig.2).
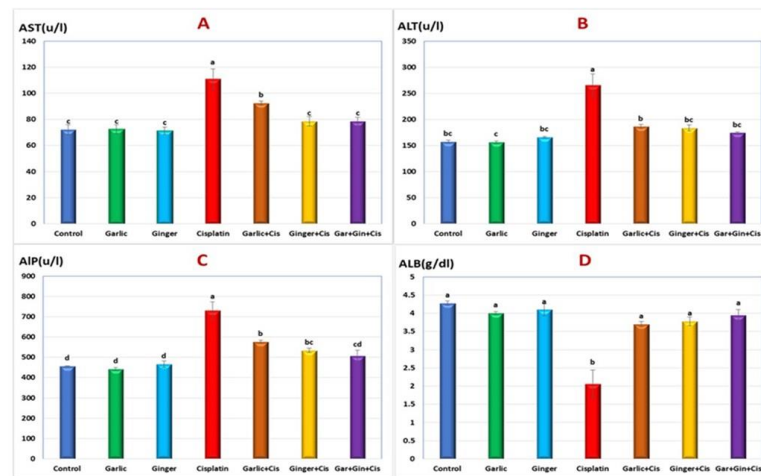

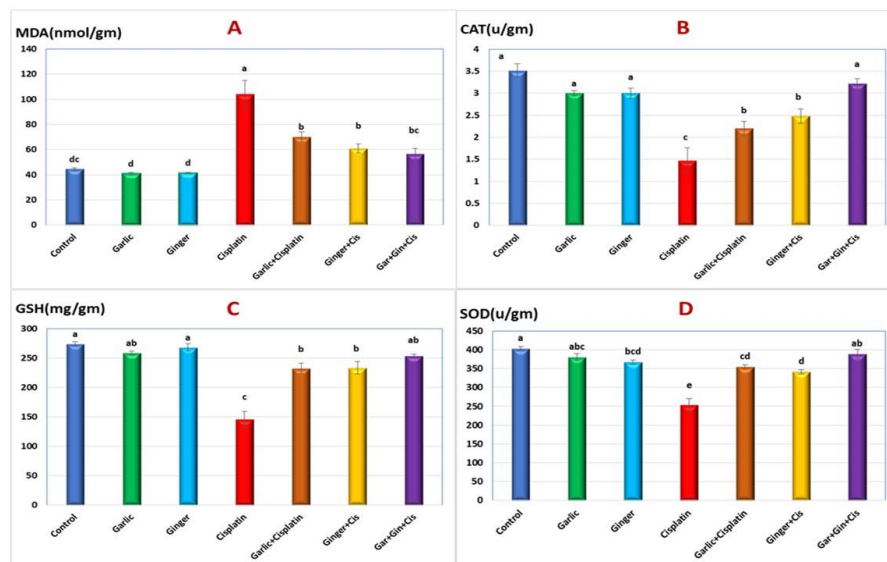Fig. 1 Serum ASL, ALT, ALP and ALB in control and treated groups. Data presented as mean ± SEM.

Fig. 2 Serum MDA, CAT, GSH and SOD in control and treated groups. Data presented as mean ± SEM.

In the present investigation, the histopathological examination of liver of rats in the control, garlic and ginger treated groups demonstrated normal architecture of liver tissue where polygonal hepatocytes with central vesicular nuclei were arranged in cords radiating from the central veins (Fig 3A, 3B and 3C). The examined liver of rats intoxicated with cisplatin showed hepatic congestion with multifocal areas of necrosis and massive vacuolar degeneration of hepatocyte (Fig3D). These degenerated hepatocytes displayed clear cytoplasm and pyknotic nuclei. In addition, focal mononuclear inflammatory cellular aggregation particularly, lymphocytes were found (Fig 3E). The examined liver of rats pretreated with garlic and intoxicated cisplatin revealed multifocal vacuolar degeneration of hepatocytes (Fig 3F). While the liver of rats pretreated with ginger and intoxicated with cisplatin showed mild congestion of central veins with focal vacuolar degeneration of hepatocytes (Fig 3G). Moreover, the liver of rats pretreated with both ginger and garlic and

intoxicated with cisplatin exhibited mild congestion of central veins with more or less normal hepatic cells (Fig 3H).

4. DISCUSSION

Hepatic toxicity is a serious adverse effect of cisplatin-induced chemotherapy. Reducing the potential side effects of cisplatin by garlic (Abdel-Daim et, al., 2020) and ginger pretreatment (Beagloo et. al., 2019) can be helpful during chemotherapy.

In the current study, rats intoxicated with cisplatin showed a significant increase in the serum levels of hepatic markers ALT, AST, and ALP and a significant decrease in the serum level of ALB in comparison to their normal levels. These results were harmonized with Mir et al., (2015) and Hassan et al., (2020). Furthermore, cisplatin-induced a significant elevation in MDA levels and a significant depletion in CAT, GSH and SOD activities which

explained that cisplatin administration induced marked oxidative stress. These results were consistent with Elkomy et. Al. (2020) In addition, cisplatin induced severe hepatic damage characterized microscopically by necrosis and degeneration of hepatocytes. These results came in harmony with Nasr and Saleh (2014).
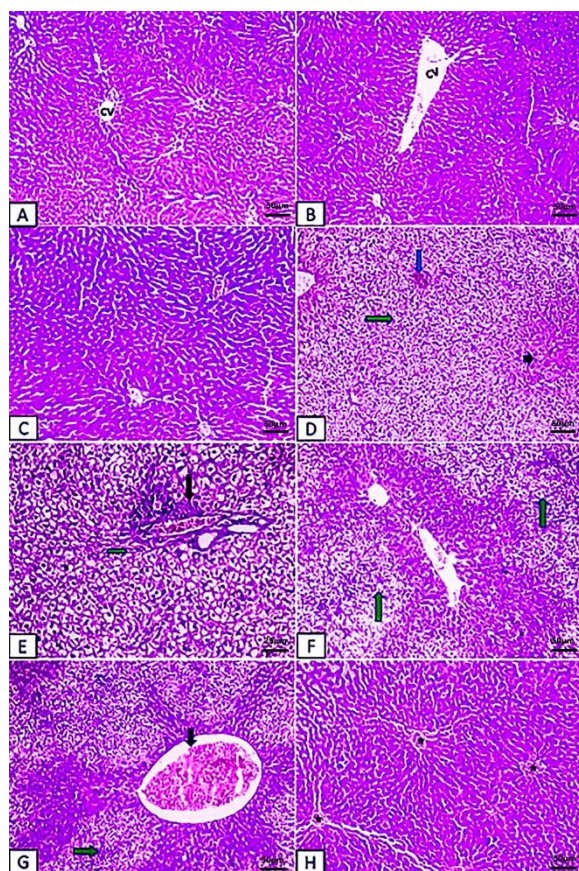

Fig. 3 Photomicrographs presented histopathological changes in liver sections of rats in (A): Control group, (B): Garlic and (C): Ginger showing normal architecture of liver tissue (D): Cisplatin group showing diffuse hydropic degeneration of hepatocytes,(E): congestion with perivascular lymphocytic cellular aggregation (F): Garlic + Cisplatin group showing   focal areas of hepatocellular degeneration (G): Ginger +cisplatin showing severe congestion and multi focal vacuolar degeneration of hepatocytes and (H): garlic +ginger +cisplatin showing   normal architecture of liver tissue with slight congestion of central veins.

The administration of garlic plus cisplatin restored the alterations in biochemical markers (ALT, AST, ALP, and ALB) to normal levels. In addition, garlic improved the antioxidant status in liver tissue through its antioxidant action by scavenging free radicals which were illustrated by the depletion of MDA level, and elevation in CAT, GSH and SOD levels. The obtained results agreed with Aly et al. (2019) and Abdel-Daim et al. (2020) Furthermore, the oral intake of garlic plus cisplatin significantly improved the microscopic picture of the liver comparing with cisplatin toxicity group. These findings were reported by El-Sebaey et al. (2018).

Rats treated with ginger plus cisplatin showed restoring of the serum hepatic biochemical markers including AST, ALT, ALP, and ALB to the corresponding levels in control group. These findings agreed with Mirazi et al. (2016) and Oke et al. (2019) Furthermore, ginger decreases malondialdehyde (MDA) level and increases reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). These results came consistent with Ezeasuka et al. (2015) and Beaglo et al. (2019). Administration of ginger extract improved the histological alterations induced by cisplatin. It can be owing to ginger having powerful antioxidant properties, which significantly

decreased oxidative stress leading to limiting of these pathological changes and recovery of normal physiological functions. These results agreed with Gholampour et. al. (2017), Ahd et. al. (2019) and Abd El-kader and Erfan (2021).

5. CONCLUSION

Cisplatin induced alterations of hepatic biochemical markers, oxidative stress system, and severe hepatic microscopic changes. However, pre-treatment of garlic or ginger ameliorates the hepatotoxic effect of cisplatin through its powerful antioxidant action.

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