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RESEARCH ARTICLE

GINGER PROTECTS RATS AGAINST CADMIUM-INDUCED HEPATOTOXICITY.

***Taghred M. Saber[1] and Haytham A. Ali[2].**

1. Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
2. Department of Biochemistry, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

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Corresponding Author

Taghred M. Saber.

Abstract

Cadmium (Cd) is a major environmental and industrial pollutant that can induce a wide range of toxicological effects in humans and experimental animals. Ginger is a widely consumed spice known to possess several pharmacological effects. The present study investigated the protective role of ginger against Cd-induced hepatotoxicity in rats. Twenty-four male rats were divided into four groups: group I served as a control, group II was orally administered ginger (100 mg/kg b.wt), group III received oral cadmium chloride ($CdCl_2$) at a dose of 15 mg/Kg b.wt, and group IV was co-treated with 15 mg/Kg b.wt $CdCl_2$ and 100 mg/kg b.wt ginger daily for 4 weeks. Cd administration elicited liver damage that was indicated by remarkable increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase(LDH). Also, Cd treatment resulted in a significant increase in MDA concentration and significant decreases in reduced glutathione (GSH) level and activity of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) in liver. However, co-treatment with ginger alleviated all these toxic effects. This study concluded that ginger efficiently ameliorated Cd-induced hepatotoxicity in rats.

Introduction:-

Cadmium (Cd) is one of the most toxic industrial and environmental pollutants. It is of worldwide concern due to its very long half-life in humans in addition to its many industrial uses in batteries, electroplating, plastic, pigments and fertilizers. Thus, human intoxication occurs via occupational and environmental exposure (Klaassen et al., 2009; Satarug and Moore, 2004). Non-occupational exposure to Cd primarily results from air pollution, smoking and consumption of Cd- contaminated food and water (Waisberg et al., 2003).

Cd toxicity induces severe damage to several organs such as liver (Karadeniz et al., 2009), kidney (Veljkovic et al., 2012), testis (Oguzturk et al., 2012) in humans and animals. Cd exerts its toxic effects through induction of oxidative stress by generating reactive oxygen species (ROS) and disturbing the antioxidant defense system (Masso et al., 2007; Wang et al., 2004). Therefore, there is a growing interest toward the application of natural antioxidants such as quercetin (Prabu et al., 2010), vitamin E and vitamin C (Karabulut-Bulan et al., 2008) in mitigation of Cd toxicity.

Ginger, Zingiber *officinale* Roscoe, is a widely used spice all over the world belonging to Zingiberaceae family. It is rich in bioactive polyphenolic compounds such as zingerone, shogaols, gingerols and curcumene (Baliga et al., 2011). Ginger possesses numerous medicinal properties like antioxidant (Stoilova et al., 2007), anti-inflammatory (Young et al., 2005) and anti-cancer (Shukla and Singh, 2007) effects. Additionally, ginger has hepatoprotective effects againist different toxicants such as mercuric chloride (Vitalis et al., 2007), carbon tetrachloride and

acetaminophen (Ajith et al., 2007). To our knowledge, There are scanty publications regarding the mitigating effect of ginger on Cd-induced hepatotoxicity. Therefore, the objective of present study was to evaluate the potential protective effect of ginger against hepatotoxicity induced by Cd in rats through estimation of serum hepatic marker enzymes, antioxidant defense system assay and histopathological examination of the liver.

Materials and Methods:-

Chemicals and reagents:-

Cadmium chloride ($CdCl_2$) was obtained from El-Gomhoria Chemical Co. (Zagazig, Egypt). Ginger power was purchased from Mepaco-Medifood Co. (Inshas, Sharkiya, Egypt). Cadmium chloride ($CdCl_2$) was obtained from El-Gomhoria Chemical Company (Zagazig, Egypt). Ginger and other chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA).

Experimental design:-

Twenty-four healthy adult male Sprague-Dawley rats weighing 200 ± 10 g were obtained from the Animal Research Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were acclimated to laboratory conditions for 2 weeks before the start of the experiment. Rats were kept in metal cages during experimental period and maintained on 12 h light-darkness cycle with a controlled temperature (21 - 24 °C) and a relative humidity (50 - 60%) and given a standard diet and water ad libitum. All animals were treated in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals, and the protocols were approved by the ethics of animal use in research committee, Cairo University. The animals were divided into four groups (six per group). Group I (control group) received no treatment. Group II (ginger-treated group) was gavaged with ginger dissolved in distilled water as a suspension at a dose of 100 mg/kg b.wt daily for 4 weeks (Ognjanovic et al., 2008). Group III (Cd-treated group) was orally administered $CdCl_2$ dissolved in distilled water at a dose of 15 mg/kg b.wt daily for 4 weeks (Ognjanovic et al., 2008). Group IV (Cd + ginger-treated group) was simultaneously treated with $CdCl_2$ (15 mg/kg b.wt) and ginger (100 mg/kg b.wt) at the same mentioned route and duration.

Sample collection:-

At the end of the experiment, blood samples were collected from the median canthus of rats. The samples were allowed to clot overnight at room temperature and then centrifuged at 3000 rpm for 10 min for separation of serum. The serum samples were stored at -20°C until used for estimation of serum hepatic marker enzymes.

Rats were anesthetized using diethyl ether and then sacrificed by cervical dislocation. The livers were excised and perfused with ice cold saline. Specimens from the liver were preserved at -20°C until their subsequent use in assessment of antioxidant status. Other liver specimens from all groups were preserved in 10% neutral-buffered formalin for histopathological examination.

Estimation of serum hepatic marker enzymes:-

ALT, AST and LDH activities were estimated in serum using commercial kits (Biodiagnostic Company, Dokki, Giza, Egypt).

Assessment of hepatic antioxidant status:-

Liver specimens were homogenized (10% w/v) in potassium phosphate buffer solution (pH 7.4) using a tissue homogenizer. Samples were then centrifuged at 3000 rpm for 15 min. The supernatant was used to determine the oxidative stress parameters. MDA concentration (a marker of lipid peroxidation) was measured according to Ohkawa et al. (1979). This assay relies on the formation of thiobarbituric acid reactive substances (TBARs). GSH concentration was assayed according to Beutler et al. (1963). GPx, CAT and SOD activities were determined according to the methods described by Gross et al. (1967), Aebi (1984) and Nishikimi et al. (1972) respectively.

Histopathological examination:-

The preserved liver specimens in 10 % neutral buffered formalin were processed, stained with hematoxylin and eosin (H&E) dyes and examined under a light microscope.

Statistical analysis:-

The data were expressed as the mean ± standard error (SE). The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by the post-hoc Duncan's test for comparison between different

experimental groups. This was performed using IBM SPSS Statistics computer software (version 21). P-values < 0.05 were considered statistically significant.

Results:-

Effect of Cd and co-treatment with ginger on liver function:-

The Cd-treated group showed a significant increase in serum ALT, AST and LDH activities when compared with the control group. The group co-treated with Cd and ginger depicted a significant decrease in these enzymes when compared with Cd-treated one (Fig. 1).

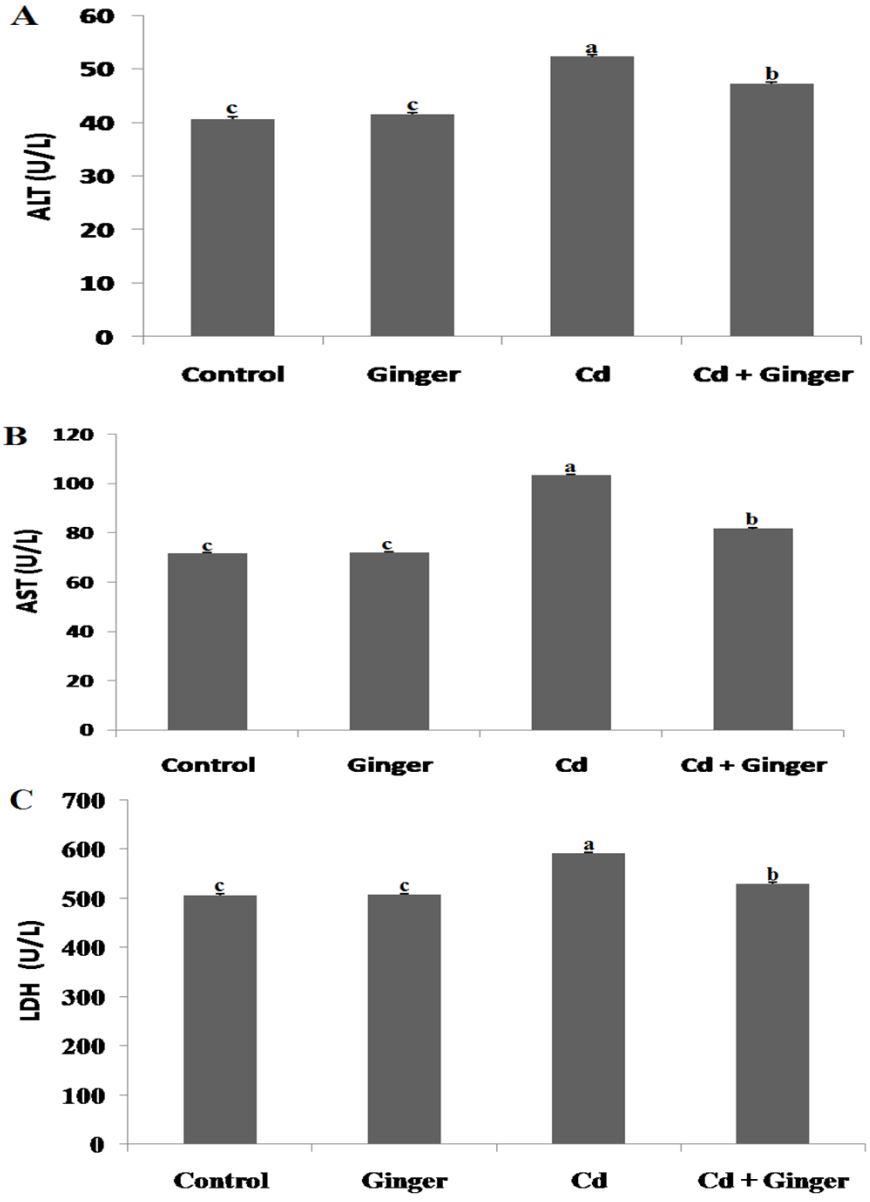

Fig.1:- Effect of ginger on serum ALT (A), AST (B) LDH (C) activities in Cd-treated rats. Bars with different letters were significantly different ($p < 0.05$) (Mean ± SE, n = 6).

Effect of Cd and co-treatment with ginger on oxidative stress parameters:-

The hepatic MDA concentration was significantly increased in Cd-treated rats when compared with the control group. The co-treated rats with Cd and ginger showed a significant decrease in hepatic MDA level when compared with Cd-treated group. The GSH concentration and GPx, CAT and SOD activities were significantly decreased in liver of rats treated with Cd when compared with the control group. Co-administration of ginger with Cd significantly increased GSH concentration and GPx, CAT and SOD activities in comparison with Cd-treated group (Table 1).

Table 1:- Effect of ginger on MDA and GSH concentrations and antioxidant enzyme activities (GPx, CAT and SOD) in the liver of Cd-treated rats (Mean ± SE) (n = 6).

Parameters	Groups			
	Control	Ginger	Cd	Cd + Ginger
MDA (nmol/g tissue)	60.66 ± 0.40^{c}	60.99 ± 0.23^{c}	112.24 ± 1.18^{a}	74.68 ± 0.23^{b}
GSH (µg/g tissue)	12.01 ± 0.17^{a}	11.67 ± 0.23^{a}	4.74 ± 0.09^{c}	8.84 ± 0.16^{b}
GPx (U/g tissue)	1.74 ± 0.04^{a}	1.75 ± 0.03^{a}	1.06 ± 0.05^{c}	1.47 ± 0.02^{b}
CAT (µmol/H_2O_2 decomposed/mg tissue)	69.62 ± 0.32^{a}	70.12 ± 0.08^{a}	45.25 ± 0.46^{c}	59.75 ± 0.35^{b}
SOD (U/g tissue)	6.28 ± 0.04^{a}	6.27 ± 0.02^{a}	20.57 ± 0.26^{c}	10.13 ± 0.18^{b}

Means within the same row that had different superscripts were significantly different ($p < 0.05$).

Histopathological investigation of the liver:-

The liver of control and ginger-treated groups showed normal hepatocytes and sinusoidal architectures (Fig 2A). The liver of Cd-treated rats revealed numerous areas of coagulative necrosis represented by pyknosis and karyolysis and widespread hemorrhage was noticed (Fig 2B). Also, severe congestion of hepatic blood vessels and sinusoids was seen (Fig 2C). The lesions were mostly alleviated in the liver of Cd + ginger-treated group. Normal appearance of hepatocytes and focal congestion of hepatoportal blood vessels were observed (Fig 2D).

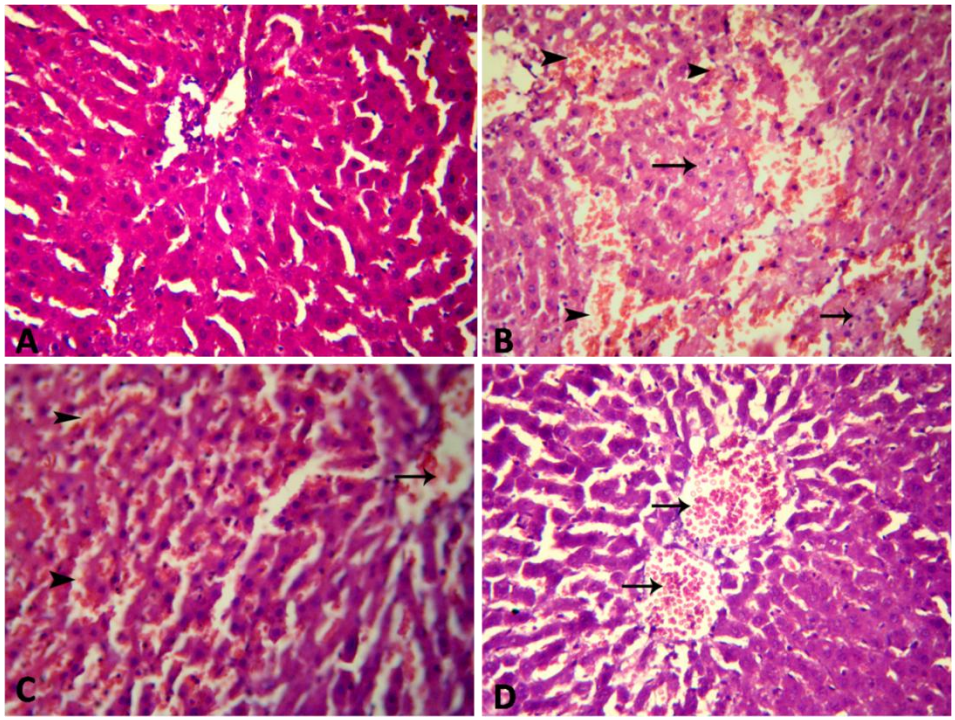

Fig.2:- Photomicrographs of liver sections of control and experimentally treated rats stained with H&E under light microscopy. A; Control and ginger-treated groups showing normal hepatocytes and sinusoidal architectures (400x). (B, C); Cd-treated group showing: B; Coagulative necrosis (arrow) and widespread hemorrhage (arrowheads). C; Severe congestion of hepatic blood vessels (arrow) and sinusoids (arrowheads). (400x). D; Normal hepatic parenchyma with focal congestion of hepatic blood vessels (arrows). (400x).

Discussion:-

Cd induces a wide range of toxicological effects and biochemical alterations representing serious hazards to health (Jarup and Akesson, 2009). In the present study, Cd administration induced hepatic dysfunction as evidenced by a significant increase ($p < 0.05$) in serum hepatic marker enzymes in Cd-treated rats when compared with the control group. Increased ALT and AST serum activities are vital parameters to detect the liver damage (Honda et al., 2010). LDH is an intracellular enzyme and its elevation in serum indicates cell death or damage (Kim et al., 2001). These findings were concordant with previous reports (Prabu et al., 2012; Renugadevi and Prabu, 2010; Rogalska et al.; 2011). The increment of these enzyme activities in serum after Cd exposure could be attributed to the fact that Cd causes structural and functional destruction to the cell membrane and increased the membrane permeability resulting in leakage of hepatic enzymes into the blood (Renugadevi and Prabu, 2010).

Our results showed that Cd elicited hepatic oxidative damage that was indicated by increased lipid peroxidation (MDA) and by a significant ($p<0.05$) decline in GSH level and antioxidant enzyme activities (GPx, CAT and SOD) in liver of Cd-treated rats in comparison with control group. These results were in agreement with previous studies (Prabu et al., 2012; Renugadevi and Prabu, 2010).

Oxidative stress is the main mechanism for Cd-induced hepatotoxicity in spite of its inability to directly generate free radicals. It indirectly generates a variety of radicals like superoxide and hydroxyl radicals through displacement of iron and copper from different intracellular sites leading to increasing of the ionic iron and copper concentrations which in turn induces oxidative stress through Fenton reaction (Yiin et al., 1999). Moreover, the impairment of antioxidant defense system is considered as a crucial event in Cd-induced toxicity (Rikans and Yamano, 2000). Most of the antioxidant enzymes become inactive following exposure to Cd due to direct binding of this metal to the active site of the SH group-containing enzymes or the displacement of metal co-factors from the active sites (Casalino et al., 2000).

Interestingly, the oral administration of ginger to Cd-treated rats alleviated Cd-induced hepatic dysfunction through a significant ($p<0.05$) lowering of serum hepatic marker enzymes. In similar reports, ginger ameliorated liver injury induced by carbon tetrachloride, acetaminophen (Ajith et al., 2007) and mercuric chloride (Vitalis et al., 2007) in rats. Furthermore, simultaneous treatment with ginger attenuated Cd-induced hepatic oxidative damage, as evidenced by a significant ($p<0.05$) reduction of lipid peroxidation. In addition, ginger significantly restored the reduction in GSH concentration and CAT, SOD and GPx activities. On the same context, ginger mitigated hepatic oxidative damage induced by bromobenzene (El-Sharaky et al., 2009) and ethanol (Mallikarjuna et al., 2008). The hepatoprotective effect of ginger may be attributable to the presence of Polyphenolic compounds, flavonoids and vitamin C which contribute to its antioxidant properties (Oboh et al., 2012). Besides, ginger prevents free radicals production, scavenges free radicals and chelats prooxidant transition metals such as iron. Additionally, Gingerols and shogaols, the major bioactive flavonoids in ginger, suppress ROS accumulation in the cells (Dugasani et al., 2010).

Our biochemical findings were corroborated with the histopathological findings of the liver. The histopathological examination of liver in Cd-treated rats showed extensive damage and structural abnormalities. Our results were consistent with former studies (Karadeniz et al., 2009; Rogalska et al., 2011). The concurrent treatment with ginger mitigated these pathological lesions. These findings were substantiated with previous reports showing that ginger conferred histological protection against liver damage caused by acetaminophen (Ajith et al., 2007) and mancozeb (Abdel-Aziz, 2010).

Conclusion:-

This study concluded that ginger exerted significant protective effect against Cd-induced hepatotoxicity in rats by enhancing antioxidant enzyme activities, GSH level, and liver function and reducing lipid peroxidation. This effect may be due to its antioxidant and free radical scavenging activity. Our results emphasized the use of ginger as a spice in different foods.

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