Role of antioxidant and anti-inflammatory of Ginger (Zingiber officinal Roscoe) against metalaxyl induced oxidative stress in rats.

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A B S T R A C T

The purpose of this study was carried to evaluate the protective and anti-inflammatory effect of ginger against metalaxyl toxicity induced liver damage and oxidative stress in rats. Forty two male albino rats were divided into three equal groups. Group I (normal group): rats administered distilled water. Group II (metalaxyl exposed group): rats received metalaxyl 1/10 LD_{50} (130 mg/kg b.wt) orally three times per weeks for 8 weeks. Group III (metalaxyl + ginger treated group): rats received metalaxyl (130 mg/kg b.wt) and treated with ginger (100 mg/kg b.wt/day/orally). The obtained results showed significant increase in serum hepatic function enzymes (ALT, AST and ALP) activities, hepatic tissue L-MDA levels and inflammatory biomarkers (MPO and IL-6) in metalaxyl exposed rats. However, activities of liver tissue antioxidant enzymes (SOD, CAT) and GSH concentration were markedly decreased. Administration of ginger with metalaxyl exposed rats caused significant improvement of all previous parameters towards its normal ranges. These results suggested that, ginger treatment may have a protective effect against liver damage and oxidative stress in rats through free radical scavenging and anti-inflammatory activity as well as regenerating endogenous antioxidant defense system mechanisms.

Key words: Metalaxyl, ginger, oxidative stress, rats.

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1. INTRODUCTION

Metalaxyl is a systemic benzenoid fungicide belonging to the most widely known member of the amide group. It is used in foliar spray mixtures for tropical and subtropical crops, as a soil treatment for the control of soil-borne pathogens, and as a seed treatment to control downy mildews, fungal diseases on fruits, soybeans, cotton, peanuts, ornamentals and grasses (Ding et al., 2012). The problems resulting from metalaxyl come from their high residual level in agriculture crops especially vegetables cultivated under greenhouse conditions and other components of environment (Pattanasupong et al., 2004). Metalaxyl exposure leads to abnormal haematological and biochemical activities

induce oxidative stress and an observable toxicity (Al-Amoudi, 2012).

To control the level of ROS and to protect cells under stress conditions, mammalian tissues contain several enzymatic catalase (CAT) and superoxide dismutase (SOD), and non-enzymatic antioxidants (reduced glutathione) that scavenge ROS. Because of continuous exposure of pesticides, the level of these endogenous antioxidants decreases leading to accelerated cell damage (Ojo et al., 2014). Antioxidants, both from natural and synthetic sources have proved to be strongly effective to control the bulk of free radicals production, to inhibit its undesirable effects, as well as to support the organism antioxidant and detoxifying mechanisms (Martins et al., 2016). Ginger is one of the most commonly used spices around the world and a traditional medicinal plant that has been widely used in Chinese and Unani-Tibb medicines for several thousand years (Rong et al., 2009). Ginger is example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally useful part (Mascolo et al., 1989). The pharmacological effects of ginger and its pungent constituents, fresh and dried rhizome were investigated. Among the effects demonstrated are antioxidant, anti-platelet, anti-tumour, anti-hepatotoxicity, anti-arthritic and anti-diabetic effect (Kamtchouing et al., 2002; Islam and Choi, 2008). Ginger extract pretreated rats attenuated in a dose-dependent manner, carbon tetrachloride and acetaminophen-induced increases in the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in serum (Yemitan and Izegbu, 2006).

The anti-inflammatory properties of ginger have been known and valued for centuries, and many scientific studies have validated this beneficial effect of ginger and its phytochemicals in several study models (Grzanna et al., 2005). Ginger and its phytochemicals are also shown to decrease the levels of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interlukin-6 (IL- 6), and to reduce the elevated expression of nuclear factor kappa-B (NF-κB) (Aggarwal and Shishodia, 2004). Also, administering ginger increased the activities of SOD, CAT, glutathione peroxidase (GPx), reduced glutathione (GSH) in the mouse liver, and thereby, reduces the hepatotoxicity effects (Verma and Asnani, 2007). This study was to investigate the possible beneficial effect of ginger against deleterious effect of metalaxyl intoxication in adult male rats through investigation of liver functions, inflammatory markers, oxidative stress biomarkers and enzymatic antioxidant status.

2. Materials and methods

2.1. Experimental animals:

Forty-two white male albino rats of 4-5 weeks old and weighing 150 – 200 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The rats were fed on constant ration and fresh, clean drinking water was supplied ad-libitum. All rats were acclimatized for minimum period of 15 days prior to the beginning of study.

2.2. Chemicals and antioxidant:

All chemicals were of analytical grade and obtained from standard commercial suppliers .The antioxidant and chemicals used in the present study were:

a- Metalaxyl: Metalaxyl [*N*-(2,6-

Dimethylphenyl)-*N*-(methoxyacetyl)-DL-alanine methyl ester], 98% technical grade was obtained from Zhejiang Heben Pesticide & Chemicals Co., Ltd. China. It was dissolved in 430 µl DMSO and 5.577 ml Propylene glycol, freshly prepared and administered orally three times per weeks at a dose of 130 mg/kg b.wt (1/10 of LD_{50}) (Sakr and Lamfon, 2005).

b- Ginger: ginger was purchased from Aktin Chemicals, Inc. company (Nature connecting health), Chengdu, China., it was dissolved in distilled water and administered orally to rats at a dose level of (100 mg/kg b.wt) once daily for 8 weeks (Abdel-Azeem *et al.*, 2013).

c- Other chemicals used in this study were of the highest purified grades available purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt.

2.3. Experimental design:

After acclimatization to the laboratory conditions, the animals were randomly divided into three groups (14 rats each) placed in individual cages and classified as follow:

Group I (normal control group): Rats received no drugs, served as control non-treated for all experimental groups.

Group II (metalaxyl exposed group): Rats received metalaxyl at a dose level of 1/10 LD_{50} (130 mg/kg b.wt) orally three times per weeks for 8 weeks.

Group III (metalaxyl + ginger treated group): Rats received metalaxyl (130 mg/kg b.wt) orally three times per weeks and treated daily with ginger (100 mg/kg b.wt/ orally) for 8 weeks.

2.4. Sampling:

2.4.1. Blood samples:

About 5 ml of blood samples were collected by ocular vein puncture from all animal groups two times along the duration of experiment at 4 and 8 weeks in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was taken by automatic pipette and received in dry sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis. All sera were analyzed for determination of the following parameters: AST, ALT and ALP.

2.4.2. Tissue samples:

About 0.5 g of liver tissue specimen was taken two times from each groups of rats after had been sacrificed at 4 and 8 weeks from the onset of rats exposed to metalaxyl. The specimens were immediately removed and washed several times with saline and blotted between two damp filter papers, weighed and stored at -20°C for subsequent biochemical analyses.

2.4.2.1. Liver tissue for biochemical analysis

Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of the following parameters: L-MDA, MPO, SOD and CAT.

0.2 g of liver tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed and incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m then the clean supernatant was removed and used for determination of

GSH concentration.

2.4.2.2. Liver tissue for molecular gene expression

About 0.5 g of liver tissue put in eppendorf tubes and were immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for determination of IL-6 level.

2.5. Biochemical analysis

Serum ALT, AST were determined according to the method described by Schumann et al., (2002) and serum ALP activity was determined enzymatically according to EL-Aaser and EL-Merzabani, (1975). Liver tissue L-MDA, MPO, SOD, CAT and GSH were determined according to the method described by Mesbah et al., (2004), Bradley et al., (1982), Kakkar et al., (1984), Xu et al., (1997) and Patterson and Lazarow, (1955) respectively. Moreover, the mRNA expression level of IL-6 was determined by real-time quantitative polymerase chain reaction (real- time qPCR) analysis in liver of rats. Target gene was normalized with β –actin by used the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6. Statistical analysis:

The results were expressed as mean ± SE using SPSS (13.0 software, 2009) program. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p<0.05$.

3. RESULTS

The obtained data presented in table (1) revealed that, metalaxyl intoxicated rats showed significant increase in serum ALT, AST and ALP activities all over the periods of the experiment when compared to normal control group.

Ginger treatment to metalaxyl intoxicated male rats caused a significant decrease in elevated serum ALT, AST and ALP activities when compared with metalaxyl toxic group.

The obtained data presented in table (2) revealed that, metalaxyl intoxicated rats showed significant increase in liver tissue L-MDA, MPO and significant up-regulation of IL-6 all over the periods of the experiment when compared to normal control group.

Ginger treatment to metalaxyl intoxicated male rats caused a significant decrease in elevated liver tissue L-MDA, MPO and a significant down-regulation IL-6 gene expression when compared with metalaxyl toxic group.

The obtained data presented in table (3) revealed that, significant decrease in CAT activity was observed in metalaxyl intoxicated rats after four weeks followed by a non-significant decrease after eight weeks of the experiment associated with a significant decrease in SOD and GSH all over the period of experiment compared to control.

Ginger treatment to metalaxyl intoxicated male rats caused a non-significant increase in CAT activity after four weeks followed by a significant increase after eight weeks of the experiment associated with a significant increase in SOD and GSH all over the period of experiment when compared with metalaxyl toxic group.

Table (1): Effect of ginger administration on serum ALT, AST and ALP activities in metalaxyl intoxicated male rats (U/L).

Parameters	ALT (U/L)		AST(U/L)		ALP(U/L)	
Exp. groups	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks
Group I: Normal control	29.00 ±1.53^{d}	27.67±3.18^{d}	54.00±0.58^{e}	57.00±1.53^{d}	138.33±3.84^{d}	178.67±6.98^{d}
Group Π : Metalaxyl group	56.33±4.09^{a}	59.33±3.28^{a}	117.67±1.86^{a}	121.00±0.58^{a}	301.33±8.69^{a}	317.67±9.13^{a}
Group III: Metalaxyl + Ginger	40.00±0.58^{bc}	42.33±1.45^{b}	74.00 ± 1.53^{c}	85.67 ± 2.60^{b}	219.33±6.74^{b}	182.33±2.33^{d}

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (2): Effect of ginger administration on liver tissue L-MDA, MPO and IL-6 levels in metalaxyl intoxicated male rats.

Parameters	L-MDA (mmol/ g tissue)		MPO (μ/mg protein)		Fold change in IL-6 gene expression	
Exp. groups	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	4 weeks
Group I: Normal control	4.08±0.01^{d}	4.46±0.05^{c}	0.05±0.001^{d}	0.08±0.002^{d}	1.00±0.07^{d}	1.00±0.08^{d}
Group Π : Metalaxyl group	7.64±0.13^{a}	8.37±0.07^{a}	0.54±0.009^{a}	0.69±0.010^{a}	10.63±0.25^{a}	15.45±0.36^{a}
Group III: Metalaxyl + Ginger	4.99 ± 0.18^{c}	5.84 ± 0.47^{b}	0.18 ± 0.004^{c}	0.21 ± 0.005^{c}	3.61 ± 0.17^{c}	2.45 ± 0.16^{c}

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Table (3): Effect of ginger administration on liver tissue SOD, CAT activities and GSH concentration in metalaxyl intoxicated male rats.

Parameters	SOD (u/g.tissue)		CAT (mmol/g.tissue)		GSH (ng/g.tissue)	
Exp. groups	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks
Group I: Normal control	44.37±2.19^{bc}	48.15±3.02^{b}	1.14±0.02^{a}	1.17±0.03^{bc}	4.40 ± 0.18^{ab}	4.77 ± 0.14^{a}
Group Π : Metalaxyl group	31.14±2.55^{d}	37.56±2.03^{c}	1.03±0.03^{b}	1.07±0.01^{c}	3.36 ± 0.07^{c}	3.88 ± 0.03^{b}
Group III: Metalaxyl + Ginger	50.50 ± 3.01^{b}	53.88 ± 2.29^{b}	1.12 ± 0.04^{b}	1.62 ± 0.13^{a}	4.47 ± 0.15^{ab}	5.00 ± 0.07^{a}

Data are presented as (Mean ± S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

4. DISCUSSION

Metalaxyl intoxicated rats showed a significant increase in serum ALT and AST and ALP activities all over the periods of the experiment when compared with normal control group. These results are nearly similar to those reported by Al-Amoudi, (2012) who reported that, a significant increase in transaminase ALT and AST levels in the sera of metalaxyl-treated mice after four weeks of administration when compared with normal group. It was reported that transaminases were considered to be a more sensitive measure in evaluating liver function and damage. The elevations in serum levels of these enzymes were mostly attributed to acute hepatocellular damage or extra-hepatic obstruction, or both (Sherlock, 1981). The levels of some important biochemical parameters in serum are used as diagnostic markers of hepatic damage. One of the most sensitive and dramatic indicators of hepatocyte damage is the release of intracellular enzymes, such as transaminases and serum alkaline phosphatase. The elevated activities of these enzymes are signal of cellular leakage and the loss of the functional integrity of the cell membranes in liver which are always associated with hepatonecrosis (Naik and Panda, 2008; Howell et al., 2014). Elevated activities of AST and ALP were found to be related to damage in the liver and the change in hepatic functions (Kanbur et al., 2009). This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation, indicating a necrosis and inflammatory reactions (Sidhu et al., 2014).

Treatment with ginger in metalaxyl intoxicated rats significantly reduced elevated serum ALT, AST and ALP activities when compared with metalaxyl intoxicated rats. These findings are in harmony with results of Badr et al., (2016) who indicated that, ginger

extract treatment leads to significantly decrease in sera ALT and AST activities compared to tumor induced mice. The aforementioned effects clearly indicate that ginger may offer protection by stabilizing the cell membrane in metalaxyl-induced hepatic damage, which is in agreement with previous studies (Lebda et al., 2013; Jabran et al., 2015). The treatments with ginger offered hepatoprotective effect, and ginger may be protected against oxidative hepatocytes damage that reduced lipid peroxidation in liver. The possible mechanism by which ginger exhibited significant protection against metalaxyl- induced hepatotoxicity may be because of its antioxidant effect.

Presented findings showed that, treatment with metalaxyl in rats exhibited a significant increase in liver tissue L-MDA, MPO and significant up-regulation of IL-6 when compared with normal group control. Similarly, Ahmed and Nasr, (2015) found that, administration of imidacloprid insecticide caused significantly increased in brain tissue MPO activity when compared with normal rat. Malondialdehyde is the end point of lipid peroxidation process which might be defined as an oxidative deterioration of polyunsaturated lipids (Dar et al., 2013). According to Calviello, et al., (2006) fungicides-induced damage is closely associated with increase in lipid peroxidation and the reduced in the antioxidant enzymes. The precise mechanism of metalaxyl-induced toxicity is unclear. Metalaxyl may be metabolized to a reactive metabolite which may initiate a chain reaction with respect to lipid peroxidation and other tissue damaging effects Therefore, it is suggested that tissue injury induced by metalaxyl is mediated by depletion of antioxidants and elevation of lipid peroxidation (Lamfon, 2011). Increased intracellular ROS production exceeding the antioxidant defense capacity of the cell caused lipid peroxidation and generalized oxidative damage to all mitochondrial components (Franco *et al.,* 2009).

The observed increase of liver tissue MPO activity leads to stimulation of pro-inflammatory cytokine expression. MPO activates neutrophils and promotes their recruitment leading to an enhanced pro-inflammatory immune response (Klinke et al., 2011), suggests that, as with other pesticides, metalaxyl may mediate its effect through the NF-kB pathway in the chronic phase of inflammation. NF-kB mediates the cellular responses to a wide variety of extracellular stress stimuli (Jung et al., 2014), and up-regulates the expression of cytokines, such as TNF-α, IL-6 and inducible nitric oxide synthase (iNOS) to initiate inflammatory responses and apoptosis (Nakamoto and Kanai, 2014). As well as activate them to produce oxidants including superoxide derived nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) and endocytose bacteria carried through the portal circulation (Moore et al., 2013). NADPH may stimulate, in hepatocytes, the production of ROS (Bhogal et al., 2010) that cause DNA damage, induce apoptosis, the expression of genes involved in the synthesis of pro-inflammatory cytokines. INOS may stimulate hepatocyte toxicity by increasing the production of nitric oxide (Czaja, 2014).

Treatment with ginger in metalaxyl intoxicated rats significantly reduced elevated liver tissue L-MDA, MPO and IL-6 level when compared with metalaxyl intoxicated rats these results came in accordance with the recorded data of, Lebda et al., (2013) who revealed that, supplementation of ginger 1% together with paracetamol significantly decreased hepatic L-MDA content in rats as compared paracetamol group. Ginger, which behaves as free radical scavenger and a potent antioxidant can decrease L-MDA level perturbed by metalaxyl in rat liver, as observed in this study (Kikuzaki

and Nakatani, 2006). The findings of this study suggest that ginger could attenuate oxidative stress by decreasing the lipid peroxidation in metalaxyl-treated liver. This result was in agreement with the results of (Sakr et al., 2011) who indicated that ginger contains a higher content of flavonoids with high antioxidant activity. The inhibition of peroxidative damage evidenced by reduced L-MDA level and the elevation of antioxidant enzymes in the ginger-treated rats was also in concomitance with previous findings concluded that ginger have protective effect against metalaxyl induced liver toxicity. This effect may be mediated by free radicals scavenging activity of ginger (Lamfon, 2011).

Myeloperoxidase is regarded as not only an index of inflammation but also as an index of oxidative damage. In the present study, ginger significantly decreased the elevated liver MPO activity suggesting that inhibition of neutrophil infiltration might be mechanism by which ginger achieves its anti-inflammatory effect. Ginger treatment cause a decrease in the elevated liver MPO activity, this finding is in accordance with those of Dileep et al., (2016) who reported that, ginger treatment significantly decrease colon MPO activity as compared to acetic acid treated rats group. The suppression of neutrophil infiltration, as indicated by the decrease of MPO activity may be because of the anti-inflammatory effects of ginger extract subsequently reduction of MPO activity could further lead to block in iNOS synthesis and ROS production (Ko et al ., 2005). Furthermore, ginger extract is shown to decrease of NF-κB levels, and to inhibit the release of IL-6, interlukine-8 (IL-8), and TNF-α from lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells (Gaus et al., 2009).

The obtained results demonstrated that, a significant decrease in liver tissue CAT, SOD and GSH in metalaxyl treated rats. Similarly, Abolaji et al., (2017) who documented that, exposure to insecticide chlorpyrifos alone, fungicide carbendazim alone and their co-treatment resulted in significant decrease in antioxidant enzymes (SOD, CAT) and GSH level in the liver, kidney, and spleen when compared with the control rat. Under oxidative stress, GSH is depleted by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation (Cathcart, 1985). Decreased serum glutathione level has been observed due to metalaxyl oral treatment in rats (Hashem, 2012). SOD is enzyme that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide. Thus, they constitute the first line of defense against ROS (Almeida et al., 2005). Sakr and Abel-Samie, (2008) found that, mancozeb fungicides induce a significant decrease in the serum antioxidant superoxide dismutase. the decrease in SOD activity may be because of the suppression of SOD synthesis due to a genetic defect, leak of SOD out of cell due to increase in the production of oxygen radical causing cell membrane damage and inactivation of SOD by increased peroxide level in cell (Suzuki et al., 1991).

The obtained results showed that, treatment with ginger in metalaxyl intoxicated rats significantly increased the reduced liver tissue CAT, SOD and GSH levels. Similarly, Badr et al., (2016) who suggested that, ginger extract treatment leads to significantly increase serum SOD and CAT activities compared to tumor induced mice. Tissues have a variety of defense mechanisms, including the enzymatic SOD scavenger and the non-enzymatic GSH systems against the oxidative injuries (Tirkey et al., 2005). Zingiber officinale has long been used in conventional medicine for treatment of various diseases (Hanafy, 2010). Ginger contains active phenolic compounds such

gingerol, shogoal and paradol that have antioxidant effects (Jeyakumar et al., 1999). There are more than 50 antioxidants isolated from rhizomes of ginger (Masuda et al., 2004). The isolated antioxidants are divided into two groups; diarylheptanoids and gingerol related compounds. The nonvolatile fraction of the dichloromethane extract of ginger rhizomes showed a powerful antioxidant activity. The fraction was purified by chromatographic techniques to provide eight diarylheptanoids and five gingerol related compounds (Kikuzaki and Nakatani, 2006). Between them, 12 compounds showed higher antioxidant activity than α- tocopherol. The activity was probably dependent upon side chain structure and substitution patterns on the benzene ring (Balachandran et al., 2006). These phenolic antioxidants may conserve the antioxidant enzymes and increase the SH-containing compounds including glutathione (Lebda et al., 2012).

Ginger is strong antioxidant substance and may [illegible]on (Ali et al. and improve the activities of the hepatic antioxidant enzymes (Ramakrishna et al., 2015). Ginger may exert its protective actions against metalaxyl-induced hepatic damage in rats possibly through its antioxidant and anti-inflammatory mechanisms. The results elevate the possibility of ginger being considered as one of the component of the regular diet of the people in the areas, where they may have chances of exposure to pesticides toxicity.

5. CONCLUSION

The present study demonstrated that, administration of ginger relieved actions and harmful effects caused by exposure to toxic metalaxyl fungicide. Pesticide toxicity affected different organs mainly liver and these occurred through affected in several parameters. Metalaxyl caused significant increase in serum AST, ALT, ALP and liver tissue L- MDA, MPO and IL-6, however, a significant reduce in liver tissue SOD, CAT and GSH. Ginger treatment in metalaxyl intoxicated rats relieved all previous parameters towards its normal range with best result after 8 weeks. So, these results confirm the strong antioxidant, anti-inflammatory and cytoprotective effects of ginger in metalaxyl toxicity.

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