Ginger extract protects metalaxyl-induced histomorphological and histochemical alterations in testes of albino mice

Saber A. Sakr and Somya Y. Shalaby

ABSTRACT

Metalaxyl is a benzenoid fungicide used to control soil-borne fungal diseases on fruits, cotton, soybean, ornamental and grasses. Ginger (Zingiber officinale) is example of botanicals which showed many pharmacological effects. The present study investigated the protective effect of ginger extract on metalaxyl-induced testicular abnormalities in mice. Treating animals with metalaxyl caused significant decrease in diameters and germinal epithelial heights of the seminiferous tubules. Histological results revealed that the spermatogenic cells were degenerated and exfoliated in the lumen of the tubules. Inhibition of spermatogenesis was recorded after 6 weeks of treatment with metalaxyl. Histochemical results showed decrease of carbohydrates and total proteins in the testicular tissue. Moreover, metalaxyl led to increase of sperm head abnormalities. Co-administration of aqueous extract of ginger improved the histological as well as the histochemical alterations induced by metalaxyl. This may be attributed to the antioxidant properties of ginger constituents.

Keywords: Metalaxyl, Testis, Ginger, Histology.

INTRODUCTION

In recent years environmental contamination with pesticides represents one of the problems of the region as well as world-wide importance. The presence of these toxic chemicals were recorded in water, air, house dust and in the tissues of non-occupationally exposed people, particularly in the adipose tissue, blood and urine (Reisinger et al., 2006). Metalaxyl is a benzenoid fungicide used to control soil-borne fungal diseases on fruits, cotton, soybean, ornamental and grasses (Sukul and Spiteller, 2000). On the other hand, metalaxyl showed hazardous effects in mammalian animals. Hrelia et al. (1996) reported that metalaxyl has cytogenetic effects on human and animal chromosomes in vitro. Experimental studies in mice demonstrated that liver is the primary target for metalaxyl-treated animals (Walker and Keith, 1992). Sakr and Lamfon (2005) reported that metalaxyl induced histological and biochemical alterations in the liver of albino mice. Paolini et al. (1996) indicated the cocarcinogenic potential of metalaxyl in Swiss albino mice. Sakr and Abdel-Samie (2008) reported that metalaxyl induced apoptosis and bax expression in hepatocytes of mice. Dasgupta et al. (2011) reported that residues of buprofezin, chlorpyriphos, metalaxyl, and myclobutanil were detected in incurred grape and wine samples. Denisia et al. (2007) found that imidacloprid and metalaxyl separately or in combination induced in vitro micronucleus formation and sister-chromatid exchange induction in human lymphocytes and in vivo micronucleus induction in polychromatic erythrocytes of the rat bone-marrow. Recently, metalaxyl was found to...
induce nephrotoxicity in mice (Sakr et al., 2011).

Medicinal plants play an important role in pharmacology and medicine for many years. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs (Ogbera et al., 2010). Ginger (Zingiber officinale) is an example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally useful part (Mascolo et al., 1989). Many studies were carried out on ginger and its pungent constituents, fresh and rhizome. One of the most popular uses of ginger is to relieve the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Gilani and Rahman, 2005). Ginger extracts showed different pharmacological effects such as anti-platelet, anti-oxidant, anti-tumour, anti-rhinoviral, anti-hepatotoxicity and anti-arthritis effect (Fisher et al., 1991; Sharma et al., 1994; Kamtchouing et al., 2002). Ginger was found to have hypocholesterolaemic effects and cause decrease in body weight, glucose in blood, serum total cholesterol and serum alkaline phosphatase in adult male rats (Bhandari et al., 2005).

The present study was undertaken to investigate the effect of metalaxyl on testes of mice and the possible protective effect of ginger aqueous extract.

MATERIALS AND METHODS

Animals

Sexually mature male albino mice (Mus musculus) weighing 25 ± 5 g were purchased from the breeding center of experimental animals at Helwan University, Helwan, Egypt. The animals were kept in the laboratory under constant temperature (25 ± 1°C) for at least one week before and along the period of the experimental work. They were maintained on a standard rodent diet composed of 55% corn starch, 20% casein, 15% corn oil, 5% salt mixture and 5% vitaminized starch (Egyptian Company of Oils and Soap Kafr-Elzayat, Egypt). Water was available ad libitum.

Preparation of ginger aqueous extract

Ginger (Z. officinale Roscoe) rhizomes were purchased from the local market at Shebin El-kom, Egypt. One kilogram fresh ginger rhizome was cleaned, washed under running tap water, cut into small pieces, air dried and powdered. 125 g of this powder were macerated in 1000 ml of distilled water for 12 h at room temperature and were then filtered. The concentration of the extract is 24 mg/ml. Each animal in the present study was orally given 1 ml of the final aqueous extract (Kamtchouing et al., 2002).

Experimental design

All the experiments were done in compliance with the guide for the care and use of laboratory animals (National Research Council, 1985). Animals (eighty) were equally divided into 4 groups.

Group 1: Animals of this group were orally given metalaxyl by gastric intubation at a dose level of 1/10 LD₅₀ (130mg/kg body weight) three times per week for continuous 6 week (Sakr and Lamfon, 2005).

Group 2: Animals in this group were given the same dose of metalaxyl given to animals of group 1 followed by 1 ml of final aqueous extract of ginger (24 mg/ml) three times weekly for 6 weeks. This dose of ginger was selected according to Sakr et al. (2011).

Group 3: Animals of this group were orally given ginger at the same dose level of group 2.

Group 4: This group is preserved as normal control.

Histological and morphometrical examination

Ten rats were sacrificed from treated and control groups after 3 and 6 weeks. Their testes were excised. For histological study, testes were fixed in Bouin’s fluid, dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin wax. Sections of five micrometers thickness were cut and stained with haematoxylin and eosin for histological examination. The diameter and germinal epithelial height of seminiferous tubules were measured from the spermatogonial tubules on the inner surface of the basement membrane through the most advanced cell types lining the lumens of the tubules. All data were obtained from 10 random microscopic fields per animal at X 100 objective.

Histochemical studies

For histochemical study, testes of the different animal groups were fixed in Carnoy’s fluid. Periodic acid Schiff’s reaction (Kiernan, 1981) was used for demonstration of polysaccharides. Total proteins were detected using the mercury bromophenol blue method (Pearse, 1972).

Sperm head abnormalities test

The sperm suspension was obtained from animals by cutting the caudal epididymis of a testis in few drops of mammalian saline. The sperm suspension was spread on clean glass slides. Sperm smears were dried in air and incubated at 50°C overnight. The sperms were fixed in methyl alcohol and stained with haematoxylin and eosin. 1000 sperms were examined for each animal directly under microscope to detect the morphological abnormalities in head region.

Statistical Analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student’s “t” test using Minitab 12 computer program (Minitab Inc., State Collage, PA). Values of P < 0.05 were considered statistically significant

RESULTS

Morphometric results

The morphometric changes in the diameter of seminiferous tubules and their epithelial height in different animal groups are shown in Figure1, a & b. Treating mice with metalaxyl for 6 weeks showed significant decrease (p < 0.05) in the diameter...
of the tubules and their epithelial heights. On the other hand, animals treated with metalaxyl and ginger extract showed highly significant increase in the diameter of seminiferous tubules and their epithelial heights in comparison with metalaxyl group. No significant changes were recorded in the diameter of the tubules or the epithelial height in ginger-treated mice compared with control animals.

**Histological observations**

Figure (2a) showed histological picture of testis of control mouse. No histological alterations were observed in animals treated with ginger extract. Testes of animals treated with metalaxyl for 3 weeks exhibited a distinct histological difference when compared with control. In testes of these animals, large numbers of seminiferous tubules appeared irregular with intertubular hemorrhage (Fig. 2b) and most of the spermatogonia were observed with cytoplasmic vacuolization (Fig. 2c). These histopathological alterations were more obvious in animals treated with metalaxyl for 6 weeks. In these animals, the seminiferous tubules were more affected, the spermatogenic cells were degenerated and exfoliated in the lumen of the tubules (Fig. 3a). There was marked decrease in the number of spermatogenic cells and the sperm bundles were absent in most tubules (Fig. 3b). Examination of testes of animals treated for 3 weeks with metalaxyl and ginger extract revealed less prominent histopathological changes when compared with the group treated with metalaxyl for the same period. Most of the seminiferous tubules were compact with each other. The spermatogenic layers appeared somewhat normal. Advanced degree of improvement was seen in testes of animals treated for 6 weeks with metalaxyl and ginger extract. Most of the seminiferous tubules restored its normal structure (Fig. 3c).

**Histochemical observations**

Polysaccharides

PAS-positive materials appeared in tunica albuginea as well as in the intertubular connective tissue of testes of control mice and in those given ginger extract. The spermatogenic cells exhibited weak reaction while the sperms showed strong reaction (Fig. 4a). Testes of mice treated with metalaxyl revealed a decrease of PAS-positive materials. This decrease started after 3 weeks of treatment and reached its maximum after 6 weeks. In these specimens, tunica albuginea, the boundaries of the seminiferous tubules as well as the intertubular connective tissue had a weak PAS-positive materials (Figs. 4b). A more or less normal polysaccharides contents were illustrated after treatment with metalaxyl and ginger (Fig. 4c).
**Total proteins**

The total proteins appeared in the testicular tissues of control rats as deeply stained granules inside the nuclei and cytoplasm of all spermatogenic cells. The tunica albuginea, intertubular connective tissue as well as the boundaries of seminiferous tubules showed strong reaction (Fig. 5a). Animals treated with metalaxyl for 3 weeks showed a noticeable decrease in the proteinic content of the spermatogenic cells in both the nucleus and the cytoplasm. The treatment 6 weeks showed large number of degenerated spermatogenic cells which contained diffused proteins (Fig. 5b). After treatment with metalaxyl and ginger, the spermatogenic cells and the other testicular elements restored some of their protein content but it still had a diffused appearance (Fig. 5c).

**Sperm head abnormalities**

The sperm is formed of head and tail regions. The head region is characterized by its basophilic affinity to haematoxylin stain and it is elongated in shape.

![Figures 3, 4, and 5](image1.png)  
**Fig.3.** Photomicrographs of cross section from testis showing (a): seminiferous tubules of metalaxyl-treated mouse showing exfoliated germ cells (arrow heads) X400, (b): reduction of spermatogenic cells X 400,(c): seminiferous tubules of metalaxyl- ginger treated mouse with increase of spermatogenic cells and normal spermatozoa (SZ), X 400.  
**Fig.4.**Seminiferous tubules of (a) a control mouse showing strong PAS-positive reaction in boundaries and in the intertubular connective tissue, (b) a mouse treated with metalaxyl showing marked decrease of PAS-positive materials ,(c) a mouse treated with metalaxyl and ginger showing nearly normal content of PAS-positive materials,(PAS, X400).  
**Fig.5.** Seminiferous tubules of (a) a control mouse showing strong protein contents in all layers of spermatogenic cells, Leydig cells, and sperms head ,(b) a mouse treated with metalaxyl showing decrease of total proteins ,(c) a mouse treated with metalaxyl and ginger showing improvement of protein materials (bromophenolblue, X 400).  
![Fig 6](image2.png)  
**Fig (6) showing (A): normal sperm with head, hook (H) and tail, (B): banana shape head, (C): hummer shape head. (X1000).**

The base of the head (the point of attachment with the tail region) is thicker than the diameter of the tail and the head tip is sharply bent into a hook-shape. The abnormal heads of the sperm had many shapes. It have banana, without hook, amorphous in shape or hummer shape (Fig. 6). Figure (7) showed that there is no
significant difference in mean of abnormal sperm head between control and ginger groups. Treatment with metalaxyl induced significant (P<0.05) increase in sperm abnormalities after 6 weeks. Combined treatment with metalaxyl and ginger significantly reduced the abnormal sperm heads.

DISCUSSION

Results obtained in the present study indicated that metalaxyl induced many histological and histomorphological alterations in testicular tissue of mice. It also increased sperm head abnormalities. These results indicated the antispermatogenic adverse effect of metalaxyl. Similar results were also observed in testes of some mammalian animals exposed to various fungicides. Khan and Sinha (1994) reported that there was a decrease in sperm count and a higher frequency of sperm with aberrant head morphology in mice exposed to mancozeb. Kackar et al. (1999) also observed that mancozeb caused histopathological changes in gonads of male rats after chronic exposure. These changes include a significant increase in testes and decrease in epididymis weights, degeneration in seminiferous and epithelial tubules with loss of sperm. Sakr and Okdah (2004) studied the effect of benomyl fungicide on the testis of albino mice. Their results showed a degeneration of the spermatogenic cells, absence of sperm bundles and a significant reduction in the diameter of the seminiferous tubules and the height of the germinal epithelium. Vinclozolin fungicide was found to have anti-androgenic effects on spermatogenesis in rat testis (Kubota et al., 2003). Blystone et al. (2007) reported that the fungicide prochloraz induced malformations in androgen-dependent tissues in male rats when administered during sex differentiation. Yu et al. (2009) reported that carbendazim has adverse effects on spermatogenesis, resulting in reduced fertility in male rats. They added that the apoptosis rate and Bax expression were significantly raised, while the expression of Bcl-2 significantly decreased.

Histochemical results revealed that metalaxyl induced reduction of polysaccharides as well as total proteins in testicular tissue of mice. Similar observations were reported by Sakr and Okdah (2004) in testes of mice intoxicated with benomyl. Ivanova and Izmirova (1977) reported that oral administration of the fungicide, maneb inhibited protein synthesis in testes and liver of rats. The fungicide, mancozeb was found to decrease protein content in testis, thyroid and adrenal of rat (Nicolaou, 1982). Ksheerasagar and Kaliwal (2010) observed that treatment with mancozeb caused significant decrease in the levels of proteins and glycogen in testes of mice. Reduction in protein content in testes of metalaxyl-treated animals might be due to either arrested metabolism in the testes or to usage of proteins to build up new cells or enzymes to reduce the stress. It has been speculated that the decrease in proteins could be attributed to disruption of lysosomal membranes under the effect of various toxicants leading to liberating their hydrolytic enzymes in the cytoplasm and resulted in marked lysis and dissolution of the target materials. This result confirmed that of Awasthi et al. (1984) who found elevated lysosomal enzymatic activity accompanied by a decrease in protein and nucleic acids contents in response to organophosphate insecticide with release of nucleases and proteases affecting nucleic acids and protein metabolism.

Oxidative stress is defined as a disruption of the equilibrium between pro- and antioxidant systems. An imbalance between pro-oxidant [reactive oxygen species (ROS)] and antioxidant mechanisms in cells cause oxidative stress. However, antioxidative defence system, which consists of superoxide dismutase, catalase and glutathione peroxidase offer protection to cells against ROS (Cerutti, 1985). Excess generation of ROS in cells is known to damage DNA, lipids and proteins resulting in several biological effects ranging from alterations in signal transduction, gene expression, mutagenesis and apoptosis (Halliwell and Gutteridge, 1990). Sakr and Lamfon (2005) indicated that there was a significant increase in the oxidative stress, malondialdehyde which is lipid peroxidation marker and a significant decrease in the level of serum antioxidant enzyme, catalase activity in metalaxyl-treated mice. Kaloyanova et al. (1991) reported that the oxidative stress is the principle manifestations of metalaxyl-induced toxicity. Banks and Soliman (1997) recorded increase in serum hydroperoxides and decrease in reduced glutathione after benomyl toxicity in rats. The authors added that the in vivo toxicity of benomyl may be associated with oxidative stress. According to Calviello et al.(2006) fungicides-induced damage is closely associated with increase in lipid peroxidation and the decrease in the antioxidant enzymes. Sakr (2007) found that mancozeb fungicide induced a significant decrease in the serum antioxidant superoxide dismutase and an increase in malondialdehyde which is lipid peroxidation marker in albino rats. The testicular toxicity recorded in the present study may be due to the oxidative stress resulted from metalaxyl or its metabolite.
Concerning the effect of ginger, present study indicated that ginger improved the histological and histochemical alterations induced by metalaxyl in testes of mice. Similarly, Sakr and Badawy (2011) reported that ginger extract improved testicular damage induced by metiram fungicide in mice. Qureshi et al. (1989) reported that ginger significantly increased the sperm mortality and sperm contents in the epididymis and vas deference without producing any spermatotoxic effect. Kamthoving et al. (2002) observed that Z. officinale significantly increased weight of testes, the serum testosterone level and epididymal α-glucosidase activity in rats. Amin and Hamza (2006) demonstrated that Z. officinale extract reduced the extent of cisplatin-induced sperm abnormality, enhanced sperm motility and testicular damage by increase the activities of testicular antioxidants. Khaki et al. (2009) reported that administration of ginger significantly increased sperm percentage viability, motility and serum total testosterones in rats. Ginger rhizome was found to overcome reproductive toxicity of gentamicin and induced spermatogenesis through the elevation of testosterone levels (Zahedi et al., 2010).

Hafez (2010) reported that intake of ginger roots as a drink may be beneficial for diabetic patients who suffer from sexual impotency as their extracts induce antiandibiotic activity and enhance male fertility in diabetic rats. Morakinyo et al., (2008) indicated that extract of Z.Officinale possesses pro-fertility properties in male rats which might be a product of both its potent antioxidant properties and androgeic properties. Nassiri et al. (2009) reported that treating diabetic rats with ginger for twenty consecutive days significantly increased sperm motility and viability and decreased lipid peroxidation. Co-administration of aqueous ginger extract with arsenite was found to protect against adverse change in the reproductive organ weight, attenuate the decrease in sperm functions, enhance plasma reproductive hormones level along with increased antioxidants activities and reduced peroxidation (Morakinyo et al., 2010).

It was reported that the mechanism of protection of ginger is related to its antioxidant properties. Siddaraju and Dharmesh (2007) reported that ginger extracts exhibited free radical scavenging, inhibited lipid peroxidation, DNA protection and reduced power abilities indicating strong antioxidant properties. It is concluded from the present study that the protective effect of ginger extract against testicular damage induced by metalaxyl may be attributed to its antioxidant properties.

REFERENCES


