Evaluation The Effect of The Aqueous Extract of Green Tea on Renal Toxicity Induced by-Methomyl in Experimental Animals Model

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ABSTRACT

Methomyl (MET) [N-methyl N-(methylcarbamoyloxy) thioacetimide] [C\textsubscript{8}H\textsubscript{10}N\textsubscript{2}O\textsubscript{5}S] is considered as one of the most important carbamate (oximes) pesticides that is extensively utilized around the world. Carbamate compounds were found to create an alteration in biochemical parameters and affect the oxidative status of the body through producing free radicals. Herbal medicines derived from plant extracts are useful in treatment of a variety of clinical disease, they are considered as natural antioxidants that have a protective role against toxicities. The purpose of the present investigation was to assess the ability of green tea extract (GTE) to protect kidney against methomyl induced toxicity in experimental animals. The insecticide caused significant elevation in urea, creatinine, NF-kB and malondialdehyde levels, and marked decrease in the levels of superoxide dismutase, reduced glutathione and glutathione -S-transferase. Alterations in these biochemical markers occurred were referred to kidney damage and the oxidative stress induced by MET. Co-administration of GTE of higher concentration (1.5%) in combination with MET brought the tested biochemical parameters to their normal levels. The study introduced novel findings regarding to the protective effect of GTE and their mixture against MET-induced toxicity in female albino mice.

Keywords: Green tea, methomyl; oxidative stress, Kidney, insecticides

INTRODUCTION

The synthetic carbamates comprise the third major group of pesticides used worldwide in agriculture (West and Marnett 2006). They possess fast action on target pests and have a relative short life span in the environment (Kaur and Sandhir, 2006). It is known that carbamates can stimulate the formation of reactive oxygen species (ROS) and thus alterations in antioxidant enzymes, resulting in oxidative stress (Ott et al., 2007). Lipid peroxidation (LPO) is considered as one of the important molecular mechanisms through which carbamate can induce toxicity (Heikal et al., 2013, Trachantong et al., 2017, Mansour et al., 2019).

Methomyl (MET) [N-methyl N-(methyl carbamoyloxy) thioacetimide; C\textsubscript{8}H\textsubscript{10}N\textsubscript{2}O\textsubscript{5}S] is considered as an oxime carbamate insecticide which found to be highly toxic to all living organisms. It is found to have vital role in controlling insect pests on different types of field crops as fruits, vegetables, and grains, in different parts of the world (Kidd and James, 1991, Meng et al., 2019). MET was classified according to WHO (2005) as a highly hazardous (class 1B) compound (Mansour et al., 2019). It inhibits acetylcholinesterase activity producing cholinergic over stimulation and neuromuscular dysfunction and may cause coma and death at high doses (Moser et al., 2010). In mammals, metabolic pathway of MET undergoes via conjugation with glutathione producing amercaptoic acid derivative (MAD) through replacement of the S-methyl groups, which can be eliminated by liver and kidney (Hinchman et al., 1998).

Failure to remove MAD from blood cells may cause nephrotoxic diseases. Moreover, MET may be hydrolyzed producing S methyl- N-hydroxy thioacetimide. MAD is rapidly broken down in the blood to carbon dioxide, and rising carbon dioxide production average may lead to hypoxic respiratory failure (Mansour et al., 2019).

Pesticides have toxicity mechanism that include oxidative stress, where many trials were done to determine dietary compounds that have the ability to support the antioxidant system so as to oppose the action of oxidative stress that cause injury through biochemical parameters like lipids, proteins and nucleic acids (Sakr et al., 2018).

Normally, cells can fight oxidative stress through non-enzymatic and enzymatic pathways ensuring cellular redox homeostasis (Givertz et al. 2001). This includes non-enzymatic glutathione (GSH) and the enzymes: superoxide dismutase (SOD), glutathione peroxidases (GPx) and catalase (CAT). Disturbance of cellular oxidation-reduction balance by the decrease of antioxidant GSH or the inhibition of the antioxidant enzymes that support the formation of ROS (Mansour, et al., 2009). This leads to cellular dysfunction and associated programmed cell death (apoptosis). Apoptosis is found to be initiated by caspase-3 activation, resulting in DNA fragmentation, degeneration of nuclear protein, condensation of chromatin, formation of apoptotic bodies and finally cell Death (Chen, et al., 2014, Kaleem et al. 2016, Meng et al., 2019).

In this respect, different types of clinical disease can be treated by herbal medicines isolated from plant extracts, so there is an interest in protecting role of natural antioxidants against toxicities caused chemically

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(Mansoureh et al., 2019). An observed increase in scientific research for protection and treatment of several diseases by using green tea due to its main catechin polyphenols (Mandel et al., 2006; Heikal et al., 2013). Green tea extract (GT) was found to have health properties as antioxidants and free radicals scavenger properties (Crespy and Williamson, 2004; Heikal et al., 2011, Mansoureh et al., 2019). It is known that green tea contains several tea polyphenols, the most important of them, green tea catechins (GTCs) which represent about 30–40% of the solids that can be extracted from dried green tea leaves. GTCs have the ability to cause an increase blood level of antioxidant enzymes, that leading to protection through scavenging of ROS such as superoxide, hydrogen peroxide (H2O2), and hydroxyl radicals. GTCs have proved its ability to overcome the free radicals produced by oxidative toxic compound in the environment and thus, reduce cytological damage mediated by toxicant, DNA damage, cancer, and programmed cell death (Chen et al., 2017).

Therefore, this research was designed to study the effect of aqueous extract of green tea against renal toxicity and oxidative stress caused by widely used pesticides methomyl in female swiss albino mice as experimental animals.

MATERIALS AND METHODS

Chemicals

The chemicals were purchased from Sigma Aldrich & co.

Preparation of green tea extract:

Green tea was purchased from the local market of herbs and medicinal plants as dry packages and scientifically identified at the herbarium of NRC, Cairo, Egypt. Aqueous solutions of GTE equivalent to 1.5% and 0.75% (w/v) were used according to Ibrahim et al. (2015) and adopted as follows. Approximately 15 g of GT leaves was soaked in 1 L of boiled distilled water for 5 min with occasional swirling. The prepared solutions were filtered and distributed into glass bottles, and each contained 300 ml of fluid. Such bottles will be placed in specific experimental animal cages (1 bottle/cage) as their only source of drinking fluid. Over the experimental period (14 days), the tested fluids were freshly prepared daily and the actual consumed fluid was measured.

Experimental design:

Experimental animals (30 adult female albino mice) weighing about 20 to 25 g obtained from the Animal Breeding House, the National Research Centre (NRC), Dokki, Cairo, Egypt, were left for one week for acclimation. They were randomly distributed to the different groups (i.e. n=5 per group). They were maintained in clean cages with free access to water ad libitum, in rooms where the temperature and humidity under control, under a 12-hr light-dark cycle. All practical steps with animals were proceeding strictly depending on guidelines approved by the Institutional Animal Ethics Committee, Mansoura University.

A total of 30 Swiss female mice were divided into 6 groups that is five animals/group (G). First group (G1) served as negative control and received water only for 14 days. Groups G2&G3 served as positive control were treated with green tea extract in a dose of 1.5% (w/v) and 0.75% (w/v) respectively, as the only drinking fluid for 14 days. Groups G4, G5, and G6 injected intravenously with sub lethal doses of methomyl i.e.5 mg/kg b.wt. i.p. in the next day G4&G5 were treated with green tea extract in a dose of 1.5% (w/v) and 0.75% (w/v) respectively, as the only drinking fluid for 14 days.

Blood and organs’ collection:

After 14 days of the experiment, all animals were sacrificed by cervical dislocation. Blood samples were collected in a centrifuge tubes to separate serum. They were kept at 4°C and centrifuged at 1000 rpm for 30 min. The sera obtained were utilized for the investigation of biochemical parameters, urea and creatinine as a kidney function test, and nuclear factor NF-kappa-B assay.

Kidneys were dissected out and washed in physiological saline. Small pieces of kidney were kept in formalin of concentration of 10% for histopathological studies. The kidney homogenate was applied for the estimation of parameters, superoxide dismutase, glutathione peroxidase, reduced glutathione and (MDA) as a marker for lipid peroxidation.

Preparation of Kidney Homogenate:

Fresh tissue specimens were used for preparation of homogenate. The tissue specimens were washed with isotone tris EDTA buffer, 3.029 gm of 0.1 M tris (hydroxymethylaminomethane, 1.022 gm of 0.07 M sodium chloride (ADWIC) and 0.47 gm of 0.005 M EDTA. Then, they were dissolved in 250 ml of distilled water and the PH was adjusted at 7.5 by using 1N HCl. Then, the cell suspension was centrifuged at 1800 rpm for 10 mins. If supernatants were contaminated with blood, it was then subjected to haemolysis with filtered tap water for 10 mins. After centrifugation and aspiration of the supernatant, the cell is fixed in ice-cold 96-100% ethanol (BDH) in approximately 1 ml for each sample, and stored indefinitely in a refrigerator.

Biochemical analyses:

Concentration of urea (mg/dl) was measured in sera at 550 nm according to Fawcett and Scott (Fawcett and Scott, 1960). Creatinine (mg/dl) was measurable in sera at 495nm according to Bartels and Bohmer (Bartels and Bohmer, 1972). LPO (ng/mg) was determined in terms of MDA, which is a marker of LPO at 534 nm according to Satoh (Satoh, 1978). The SOD (EC1.15.1.1) activity (U/mg) was measured at 560 nm according to the method of Nishikimi et al., (Nishikimi et al., 1972). GST (EC 2.5.1.18) activity (U/mg) was measured according to Habig et al. (1974). GSH level (mg/g) was estimated using a colorimetric technique as mentioned by Ellman (1959), modified by Jollow et al. (1974). Nuclear factor NF-kappa-B (NF-kB) activity assay (ng/mg) was measured in sera at 450 nm using ELISA Kit (Cat No. MBS260718) according to the method followed by Gilmore [1999].

Histological studies

The samples were taken from kidney from female swiss albino mice of different groups and fixed in formalin of concentration of 10% for 24 h. samples were washed in tap water and then dried in ascending grades of ethyl alcohol. Specimens were cleared in xylene and embedded in paraffin bees at 56°C in hot air oven for 24 h. Paraffin blocks were prepared for sectioning at 4-μm thickness by slide microtome. The tissue sections obtained were
collected on glass slides, deparaffinized and stained by hematoxylin for 20 min and eosin (1%) for 10 min. Slides were examined under light microscope according to Bancroft and Stevens. (Bancroft and Stevens, 2008). The histopathology was carried out in the Pathology Department, Faculty of Veterinary Medicine, Mansoura, Egypt.

Statistical Analysis:
Data collected were expressed as mean ± SE and results were considered significantly different if p < 0.05. All the data were analyzed using SPSS/22 student software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by LSD.

RESULTS AND DISCUSSION

Results:
In our study, some biochemical parameters, kidney function tests, as well as antioxidant enzymes in female swiss albino mice that received the different tested treatments (Fig. 1, 2, 3, 4 and Tab. 1).

Highly significant increase (P<0.0001) in the concentration of creatinine has been obtained in MET treatments, compared with control value (1.05 mg/dl). Co administration of GTE with the low and high doses (1.5 %, 0.75%) has resulted in non-significantly different values. Co administration of GTE with the low dose (0.75%) with MET has resulted in significant elevated different values (P<0.001). However, co administration of GTE with the high dose (1.5%) with MET has resulted in no significant different values (Fig. 1).

Urea concentration in the control group recorded 63.25 mg/dl. Highly significant elevation (P<0.01) in urea concentrations has been obtained in MET treatments and GT of dose (0.75%) with MET. Co-administration of GT of dose (1.5 %) with MET restored urea concentration to the normal levels (Fig. 2).

Nuclear Factor NF-κB activity in control group recorded 5.55ng/mg. Highly significant elevation (P<0.01) in NF-κB activity has been obtained in MET treatments and GT of dose (0.75%) with MET. Co-administration of GT of dose (1.5 %) with MET restored NF-κB activity to the normal levels (Fig. 3).

Lipid peroxidation (LPO), in terms of MDA, recorded 7.40 ng/mg tissue for the control group. In comparison, MET alone caused high significant elevation (P<0.01) as well as GTE (0.75 %) with MET. Co-administration of GTE (1.5%) with MET caused improvement (Table 1).

The activity of SOD in the control group recorded 12.58 U/mg tissue. In comparison, MET alone caused high significant decrease (P<0.01) in SOD activity. On contrast, high significant elevation (P<0.01) in SOD activity was found in case of groups treated with GTE and GTE (1.5%) with MET (Table 1).

The concentration of Reduced Glutathione (GSH) in the control group recorded 2.16 U/mg. In comparison, high significant elevation (P<0.01) was found in groups treated with GTE (1.5%) and GTE (0.75%, 1.5%) with MET (Table 1).

Glutathione S-transferases (GST) activity recorded 0.31 ng/mg in control group. In comparison, high significant decrease (P<0.01) was found in groups treated with MET, and high significant elevation (P<0.01) was found in groups treated with GTE (1.5%) and GTE (0.75%, 1.5%) with MET (Table 1).

Table 1. The effect of green tea extract on the level of lipid peroxidation, glutathione, glutathione-S-transferase and SOD activities in green tea and methomyl-treated groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>LPO (ng/mg)</th>
<th>SOD (U/mg)</th>
<th>GSH (ng/mg)</th>
<th>GST (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7.40±0.25</td>
<td>2.16±0.23</td>
<td>0.31±0.03</td>
<td></td>
</tr>
<tr>
<td>G 1.5%</td>
<td></td>
<td>6.92±0.31</td>
<td>4.50±0.39</td>
<td>0.41±0.04</td>
<td></td>
</tr>
<tr>
<td>G 0.75%</td>
<td></td>
<td>8.26±0.59</td>
<td>14.40±0.43</td>
<td>0.31±0.04</td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td></td>
<td>22.46±0.80</td>
<td>8.88±0.47</td>
<td>1.45±0.20</td>
<td>0.18±0.04</td>
</tr>
<tr>
<td>G 1.5%+ME</td>
<td></td>
<td>7.66±0.21</td>
<td>25.04±0.38</td>
<td>7.28±0.24</td>
<td>0.48±0.03</td>
</tr>
<tr>
<td>G 0.75%+ME</td>
<td></td>
<td>10.36±0.50</td>
<td>13.26±0.40</td>
<td>1.95±0.28</td>
<td>0.20±0.01</td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± SE. Each group consists of five animals.
- (*) significant, P<0.05 for effect on treated groups. Statistical significance: compared with control group throughout vertical columns.

Fig.1 . The effect of Green tea extract and Methomyl on creatinine level in blood (expressed as mg/dl) as kidney function test.

![Fig.1](image1)

Fig.2 . The effect of Green tea extract and Methomyl on Urea level in blood (expressed as mg/dl) as kidney function test.

![Fig.2](image2)
Kidney sections from group received methomyl showed severe congestion, tubular degeneration, and necrosis. Meanwhile, kidney sections from group received methomyl + Green tea 1.5% showed mild tubular degeneration and necrosis. Kidney sections from group received methomyl + Green tea 0.75% show very mild congestion.

**Discussion:**

This study was an attempt for assessment the toxicity of MET on some biochemical and histopathological parameters in female albino mice and to test the possible protective effect of leaf extracts of Green tea in different concentrations against toxicity of the insecticide MET.

Treatment of mice with Methomyl resulted in induction of renal toxicity as shown by elevation of renal markers like urea and creatinine, as well as Nuclear Factor NF-κB (NF-κB) (Fig. 1, 2, 3). Groups treated with Green tea extract especially of high concentration (1.5%) plus Methomyl led to significant reduction in the concentrations of urea, creatinine, and NF-κB.

Kidney plays an important role in detoxification of xenobiotics. Kidney toxicity was shown by an observed increase in the levels of creatinine and urea in the sera of mice treated with MET. It is responsible for the excretion of metabolic waste products and toxins such as urea, creatinine and uric acid (Gounden and Jialal, 2019). Creatinine is a waste product of creatine metabolism that is cleared from blood by glomeruli and is excreted in the urine, whose measurement provides a useful index of kidney function (Hood, 1980). According to Gilman et al. (Gilman, 1991), the elevation of urea could be attributed to an increase of nitrogen retention and/or owing to corrupted renal function. A marked significant increase in serum urea level was shown in severe defect of glomerular filtration (Kaneko, 1989). These results are in agreement with those obtained before (Eissa and Zidan, 2010, Mansour and Mossa, 2010, Djeffal et al., 2015, Sakr et al., 2018, and Mansour et al., 2019).

The NF-κB transcription factors family found to have vital important in many functions inside cells, like initiation and propagation of inflammatory and immune responses (Fearn et al., 2017). Previous studies found that NF-κB has important role in the pathogenesis of renal inflammation caused by infection, injury, or autoimmune factors (Zhang and Sun, 2015, Song et al., 2019). Methomyl was found to induce the reactive oxygen species (ROS) production, causing oxidative stress in the tissues and thus, chronic permanent damage in kidney and their functions [Mansour et al., 2017C]. Body respond to infections and tissue damages through induction of inflammation, which is characterized by vasodilation and recruitment of leukocytes, plasma proteins and fluid to the affected tissue (Ashley et al., 2012, Zhang and Sun, 2015, SONG et al., 2019).

The present research proved that administration of MET to mice caused an elevation in the LPO as expressed by the increase in MDA level (Table 1). These obtained results agree with those obtained in several studies on MET (Djeffal et al., 2015, Mansour et al., 215; 2017a&b, Trachantong et al., 2017). The MET cause an increase in MDA level which might be due to the conjugation of the insecticide or its metabolites to the polysaturated fatty acids, which might lead to high production of ROS (Gutteridge and Halliwell, 2000, Sakr et al., 2018, and Mansour et al., 2019).

In the present study, methomyl administrations led to a significant decrease in SOD, GSH content and GST activities in the renal cells of mice (Table 1). Antioxidants,
which are divided into enzymatic and non-enzymatic components, are the participants in the ROS scavenging pathways (Hasanuzzaman et al., 2012).

SOD is the most important of antioxidants enzymes, which provides the first line of defense against ROS (Sudheer and Kaliwal, 2010). It catalyzes the destruction of the superoxide radicals, through dismutation of O$_2^-$ to H$_2$O$_2$ and O$_2$ (Tuteja, 2015 & Saibi and Brini, 2018). Carbamates are known to diminish activities of SOD and to elevate ROS. These obtained results of the present investigation agree with several studies, MET was reported to decrease the activity of SOD (Mansour et al., 2012, 2015, 2017b and 2019).

GST is considered as a cytoplasmic enzyme that belongs to family of detoxifying enzymes. It is found at a high level in the renal tubular cells, and leaks out with damage (Treacy et al., 2019). It is characterized by high substrate specificities which catalyzes the binding of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms (Hayes et al., 2005, Lee-Hilz et al., 2006, Djefal et al., 2015). A marked decrease in GST activity in mice treated methomyl was abstained, this may be due to the decrease in GSH content and glutathione dependent enzyme systems that protect against oxidative stress (Ashour et al., 2017).

Thus these enzymes act to protect the biological systems against damage caused by oxidative stress (Bhattacharjee and Sil, 2006). Oxidative damage results when the concentration of reactive oxygen species generated exceeds the antioxidant capacity of the cell (Sies, 1991). Therefore, there was observed decrease in these enzymes activities in Methomyl treated groups that result from oxidative stress damage in renal tissues.

GSH level was decreased in groups treated with Methomyl. GSH is considered as an antioxidant, which provides protections against free radicals and toxic compounds; GSH redox cycles play an important role in antioxidant defenses inside the cells and are essential for the tissues to protect themselves against the ROS damage, in addition, it also acts as an essential cofactor for antioxidant enzymes including GPx and GST (Sharma et al., 1997, Ashour et al., 2017). Increase in GSH levels because of the recycling of other antioxidants such as vitamin E and C (Tsukamoto et al., 2002, Waheed, and Mohammed, 2012). In the present study, observed depletion in GSH level might be due to the decrease in the activity of GSH-Px, thus it may be related to the oxidative stress generated in renal tissues of Methomyl treated groups (Mansour et al., 2019).

The observed histopathological effects in the MET treated groups corroborated with biochemical alterations, and agreed with those previously reported for the same insecticide and have attributed to generation of ROS (Mansour et al., 2017a). Co-administration of the tested Green tea extracts especially of concentration 1.5% has improved the cell architecture the examined tissues to a pronounced extent. This might be due to the antioxidant capacity of Green tea (Haque et al., 208, Coimbra et al., 2006, Mansour et al., 2019).

CONCLUSION

The insecticide MET, can induce renal dysfunction, oxidative stress, and histopathological changes in renal tissues of mice. The observed alterations occurred and were referred to the oxidative stress induced by MET. Co-administration of GTE with MET brought the tested biochemical parameters to their normal levels. This was referred to the ability of the tested herbal extracts to scavenge the ROS, which may be generated owing to exposure to MET. Therefore, GTE administration could be effective against MET induced renal toxicity caused by oxidative stress.

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REFERENCES


