Ameliorating effect of green tea, sage, and their mixture against methomyl-induced physiological, biochemical, and histopathological alterations in male rats

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Background and objectives
Methomyl (MET) [S-methyl N-(methylcarbamoyloxy) thioacetimidate; $C_5H_{10}N_2O_2S$] is one of the most important carbamate (oximes) insecticides that is extensively used around the world. Carbamate compounds are known to cause an alteration in biochemical parameters and affect the oxidative status of the body through producing free radicals. Many antioxidants have been used to ameliorate the toxic effect of pesticides, but the search for such compounds will always be an urgent need to achieve the optimum degree of amelioration. For this purpose, the present study was designed to evaluate the ameliorating effect of green tea extract (GTE) (*Camellia sinensis*), sage extract (SE) (*Salvia officinalis*) and their mixture (GTE+SE) against MET-induced toxicity in male rats.
Materials and methods
A total of 60 rats (*Rattus norvegicus*) were divided into 12 groups: one negative control group (water); three positive control groups (GTE, SE, and GTE+SE) as the sole drinking source throughout the experimental duration (28 days); two groups administered MET orally at a dose equivalent to the acceptable daily intake and 10× acceptable daily intake; two groups were specified for GTE with MET two doses; two groups for SE with MET two doses; and two groups for GTE+SE with MET two doses. At the end of the experiment, blood samples were collected for measuring biochemical parameters for liver and kidney, as well as antioxidant enzymes.
Results and conclusion
The insecticide caused high elevation in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, urea, creatinine and malondialdehyde levels, and high decline in the levels of butyrylcholinesterase, superoxide dismutase, and total antioxidant capacity. Alterations in these biochemical markers occurred in a dose-dependent manner and were referred to the oxidative stress induced by MET. Co-administration of GTE or SE in conjunction with MET brought most of the tested biochemical parameters to their normal levels, but the mixture (GTE+SE) resulted in superior ameliorating effects as compared with each of the individual extracts. The study introduced novel findings regarding to the protective effect of GTE, SE, and their mixture against MET-induced toxicity in rats.

Keywords:
amelioration effects, green tea (*Camellia sinensis*), methomyl; oxidative stress, sage (*Salvia officinalis*)

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Introduction

The synthetic carbamates comprise the third major group of pesticides used worldwide in agriculture [1]. They possess fast action on target pests and have a relatively short life span in the environment [2]. It is possible that carbamates may induce oxidative stress through generation of reactive oxygen species (ROS) and alterations in antioxidant enzymes [3]. It is documented that lipid peroxidation (LPO) is one of the molecular mechanisms of carbamate-induced toxicity [3].

Methomyl (MET) [S-methyl N-(methylcarbamoyloxy) thioacetimidate; $C_5H_{10}N_2O_2S$] is an oxime carbamate insecticide produced by Du Pont since 1966. It is used extensively for controlling insect pests on a variety of field crops including fruits, vegetables, grains, and cotton in different parts of the world [4]. The WHO [5] has classified MET as a highly hazardous (class 1B) compound. It inhibits acetylcholinesterase activity producing cholinergic overstimulation and neuromuscular dysfunction and may cause coma and death at high doses [6]. In mammals, metabolic pathway

of MET undergoes via conjugation with glutathione producing a mercapturic acid derivative (MAD) through replacement of the S-methyl groups, which can be eliminated by liver and kidney [7]. Failure to remove MAD from blood cells may cause nephrotoxic diseases. Moreover, MET may be hydrolyzed producing S-methyl-N-hydroxy thioacetimidate. MAD is rapidly broken down in the blood to carbon dioxide, and rising carbon dioxide production averagemay lead to hypoxic respiratory failure [7].

It is well recognized that induction of oxidative stress is one of the main mechanisms of pesticides and other xenobiotics that may generate ROS in the cells [8]. It has been reported that MET-induced oxidative damage and LPO in rat erythrocytes [9]. Moreover, it influenced the mixed-function oxidase and created abnormal liver functions in rats [10]. To alleviate toxic hazards of these ROS, several substances, including essential mineral elements, such as selenium (Se) [11–14] and zinc (Zn) [13,14], were used in our previously published work on experimental animals. Several vitamins were also tested for their ameliorating effects against toxicity of MET in experimental animals. For example, vitamin E has shown potential protective effect against MET-induced reproductive toxicity in female rats treated during gestation [15]. Vitamin C, Se, and both were used to protect against MET-induced tissue oxidative stress in adult rats [16]. On the contrary, the hot aqueous extract of chamomile (*Matricaria chamomilla*) flowers was found to protect against renal toxicity induced by MET in male albino mice [17].

In recent years, several studies have focused on the bioavailability of phenolic compounds and flavonoids in certain plants, owing to their pharmacological activity in the protection and treatment of many diseases. Fortunately, these compounds are widely distributed in various plants forming a part of the human diet [18]. Green tea (GT), *Camellia sinensis* (Family: Theaceae), is one of the most favorite beverages around the world and is known as a safe and nontoxic medicinal plant. Therefore, its therapeutic properties have been studied extensively [19,20]. GT contains polyphenolic compounds of high antioxidant capacities. They are present in large quantities in GT and called catechins (e.g. epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate) [21]. The biological activity of these phytochemicals as antioxidants, anti-aging, anti-cancer, and anti-inflammatory agents is well documented [20,22–24]. These phytochemicals have the attendance to react with oxygen-free radicals and other macromolecules to neutralize the generated free radicals and/or to initiate useful biological effects [18]. It was suggested that drinking GT has beneficial effects, by reducing the development of oxidative stress, thus protecting the individual from oxidative stress diseases [25].

Green tea extracts (GTEs) have been tested against toxicity – induced by certain xenobiotics in experimental animals. As examples, co-administration of GTE in conjunction with lead (Pb) was found to ameliorate liver toxicity [26] and arsenic-induced biochemical toxicity and LPO in rats [27], as well as renal dysfunction in male rats [28]. GTE also possess a hepatoprotective effect in rats exposed to ketamine (an anesthetic and analgesic drug) [29]. According to Ibrahim *et al.* [30], GTE was found to ameliorate the hepatotoxicity and apoptosis induced by copper nanoparticles in rats. Moreover, GTE was found to protect male Wistar rats against hepatotoxicity and pathological alterations induced by acetaminophen [31]. To the best of our knowledge, there are no studies on the ameliorative effect of GTE on MET-induced toxicity in experimental animals.

Sage (*Salvia officinalis* L.; Family: Lamiaceae) is known to have a wide range of biological activities, such as antioxidant [32], antibacterial [33], hypoglycemic [34], and anti-inflammatory [35] properties. Its antidiabetic effects have been reported by several investigators [36–38]. The identified constituents in this plant include rosmarinic and phenolic acids, carnosic compounds, and flavonoids or their derivatives [32,39,40].

The available data in the literature have focused on the usefulness of sage in protection against diseases owing to its antioxidative effects. For examples, the methanolic extract of sage extract (SE) showed protective effect against genotoxicity through its antioxidant property in rats [41]. Osman and Abd El-Azime [42] found that SE could modulate radiation-induced oxidative stress and enzyme activities in the brain of rats. The authors referred such improvement to its antioxidant constituents. Anita *et al.* [43] reported the protective effect of *S. officinalis* against *Aspergillus parasiticus* aflatoxin-induced hepatotoxicity in rats. To the best of our knowledge, however, there are no studies on the ameliorative effect of sage on MET (or any other pesticide) in experimental animals.

From the aforementioned considerations, the present study was undertaken to provide an overview on the effect of MET on some physiological, biochemical, and

histopathological parameters in male rats and to evaluate the ameliorative effect of GT and sage leave extracts, as well as the mixture of both herbs.

Materials and methods

Chemicals and reagents

Lannate (MET) 90% SP, the commercial formulation of MET [S-methyl N-(methylcarbamoyloxy) thioacetimidate; $C_5H_{10}N_2O_2S$], was obtained from the company for Agro Chemicals (CAM, Mohandseen, Giza, Egypt). 2-Thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol) was purchased from Merck Serono, Egypt. Dibasic and monobasic sodium phosphates were obtained from Alliance Bio (Irvine, California, USA). The reagents used in biochemical measuring for liver enzymes, kidney functions, and oxidative status were purchased from Biodiagnostic Company (Dokki, Giza, Egypt).

Animals

Healthy male albino rats of the Wistar strain (*Rattus norvegicus*), 60 days of age and with average weights of 105±15 g, were obtained from the Animal Breeding House, the National Research Centre (NRC), Dokki, Cairo, Egypt, and maintained in clean plastic cages in the laboratory animal room (23±2°C) of 12/12 h daily light-dark cycle. The animals were fed a standard pellet (wheat, barley, wheatfeed, de-hulled extracted toasted soya, soya protein concentrate, macro minerals, soya oil, whey powder, amino acids, vitamins, micro minerals) diet and had free access to water for 1 week acclimatization period before experimentation. The experimental work on rats was performed with the approval of the Animal Care and Experimental Committee, NRC, Cairo, Egypt, and in accordance with the guidance for care and use of laboratory animals [44].

Preparation of green tea and sage extracts

Both GT and sage were 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% (w/v) were used according to Ibrahim *et al.* [30] 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 aqueous solutions of SE equivalent to 1.5% (w/v) were prepared exactly similar to that followed with GTE. On the contrary, aqueous extracts containing both GT and sage were prepared by using 0.75-g GT leaves and 0.75-g sage leaves/1 l of boiled distilled water. 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 sole source of drinking fluid. Over the experimental duration (28 days), the tested fluids were freshly prepared daily and the actual consumed fluid was measured.

Experimental design

A total of 60 male rats were divided into 12 groups, that is five animals/group (G). G1 represented negative control (water only), and G2, G3, and G4 were administrated GTE, SE, and their mixture (GTE +SE), respectively, as the sole drinking fluid. Two groups of rats were administered MET orally, but one (G5) at a dose equivalent to the acceptable daily intake value (ADI; 0.027 mg a.i./kgbw/day) [4] and the other (G6) at the 10× ADI value (0.27 mg a.i./kgbw/day). Rats of G5 and G6 were allowed to drink water freely. Groups 7 and 8 were given GTE+MET as of G5 and G6, correspondingly. G9 and G10 were given SE +MET as of G5 and G6, following the same order. G11 and G12 were given a mixture of GTE and SE +MET as of G5 and G6, correspondingly. Over the experimental duration (28 days), the daily food consumption was measured, and the animals were weighed weekly to modify MET dose accordingly to the new recorded weight.

Blood and organs' collection

At the end of the experiment (28 days), the final body weights were recorded and blood samples were taken from the facial artery of each animal under ether anesthesia and added to nonheparinized centrifuge tubes to separate serum. This was performed by centrifugation at 3500 rpm (600*g*) for 10 min at 4°C using Hereaeus Labofuge 400 R, Kendro Laboratory Products GmbH (Japan) [9]. The sera were kept in a deep freezer (−20°C) until analyzed. Then, the animals were killed by cervical dislocation, and the liver, kidney heart, spleen, and testes were removed and weighed. Small pieces of liver and kidney were kept in 10% formalin for histopathological studies. Other pieces of liver (1 g tissue+1 ml phosphate buffer, pH 7.4) were homogenized for 1 min and centrifuged at 4500 rpm for 10 min at 4°C (Ref). The supernatant was withdrawn in clean tubes and kept in a deep freezer (−20°C) until analyzed for malondialdehyde (MDA) and superoxide dismutase (SOD).

Biochemical analyses

The measurements of biochemical parameters were performed on Shimadzu UV-VIS Recording 2401 PC (Japan). Spectrophotometer at the specified

wavelengths and in accordance to the pamphlet instructions given by the manufacturers, and in light of the published methods [9]. The activities of aspartate aminotransferase (AST) (EC.2.6.1.1) and alanine aminotransferase (ALT) (EC.2.6.1.2) were determined according to the method described by Reitman and Frankel [45] at 546 nm, expressing the enzyme's activity in terms of U/L. Alkaline phosphatase (ALP) (EC.3.1.1) was measured in sera at 510 nm (U/l) according to Belfield and Goldberg [46]. Butyrylcholinesterase (BuChE) activity (U/l) (EC.3.1.1.8), was measured at 405 nm using the method followed by Knedel and Böttger [47]. Concentration of urea (mg/dl) was determined in sera at 550 nm using the method of Fawcett and Scott [48]. Creatinine was measurable at 495 nm according to Bartels and Bohmer [49] in terms of mg/dl. LPO was determined in terms of MDA, which is a marker of LPO according to Satoh [50] at 534 nm, expressing concentration of MDA in terms of nmol/g tissue. The SOD (EC1.15.1.1) activity was measured at 560 nm and expressed in terms of μg/g tissue [51]. The determination of total antioxidant capacity (TAC) was performed colorimetrically according to Koracevic *et al.* [52] at 505 nm in terms of mmol/l.

Oxidative stress and amelioration analysis

According to Mansour and Gamet-Payrastre [53], alteration in the levels of biochemical parameters, owing to pesticide treatments, could be determined by calculating the percentage of change in pesticide-treated groups relative to untreated control groups to estimate how much deviation than normal values can be attributed to pesticide treatments:

$$\%\text{of change} = \frac{\text{Treatment value} - \text{Control value}}{\text{Control value}} \times 100.$$

On the contrary, the 'amelioration index' (AI) could be estimated by comparing the results of a given biochemical parameter in the groups of a pesticide +antioxidant agent (e.g. herbal extract, here) with the results of the control group of the herbal extract to assess the ameliorative effect of GTE, SE, and their mixture. As AI approaches 1, the amelioration reaches a high degree of normalization to the control value [53]:

$$\text{AI} = \frac{\text{Treatment value(Pesticide + Herbal extract)}}{\text{Control value of herbal extract}}.$$

Histological studies

Autopsy samples were taken from the liver and kidney from rats of different groups and fixed in 10% formalin saline for 24 h. Washing was done in tap water and then dehydrated 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 obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin stain. Two slides were prepared for each animal; each slide contained two sections for each organ. Ten field areas for each section were selected and examined for histopathological changes under light microscope according to Banchroft *et al.* [54] at ×40 magnification. The histopathology was carried out in the Pathology Department, NRC, Cairo, Egypt.

Statistical analysis

The data were analyzed by using GraphPad Prism 5 Demo and expressed as means±SE. Paired samples (*t*) test was used to compare the data of the control with those of treatments, where *P* value less than 0.05 and *P* value less than 0.01 were considered for significant and high significant differences, respectively. *P* value more than 0.05 meant no significant difference.

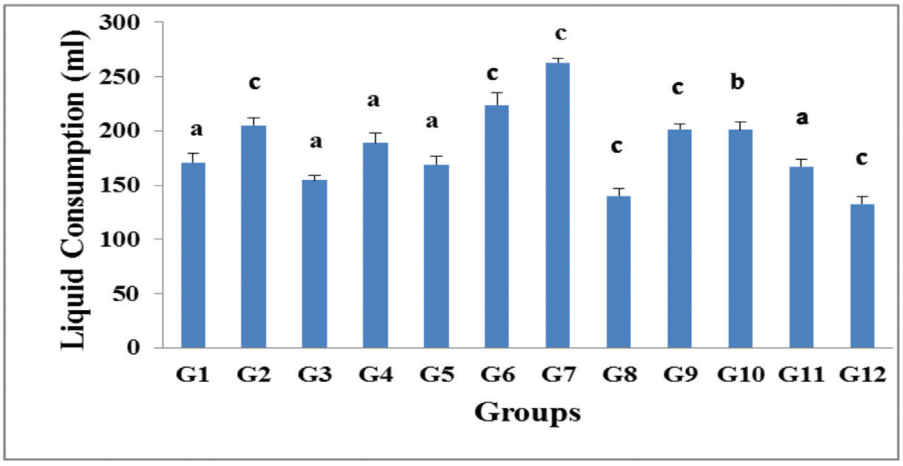
Results

Drinking solutions and food consumption

Over the experimental duration (28 days), the fluid consumption per one experimental group (five rats) ranged between 132.4 ml (G12: MET at 10× ADI +GTE+SE) and 263.0 ml (G7: MET at ADI+GTE). The consumed GTE (205 ml) was significantly ($P<0.01$) higher than that of water (171 ml), and those of SE and GTE+SE showed no significant differences as compared with water alone (Fig. 1). It appeared that rats treated with the higher dose of MET consumed more amount of water (223.6 vs. 168.9 ml), whereas MET at lower dose (G7) consumed more amount of GTE than that consumed by MET at the higher dose (G8) and both groups showed high significant increase in water consumption compared with control group. Similar trend was observed for the groups treated with MET+GTE+SE (G11 and G12). On the contrary, both MET groups (G9 and G10) consumed equal amounts of SE nearly (Fig. 1).

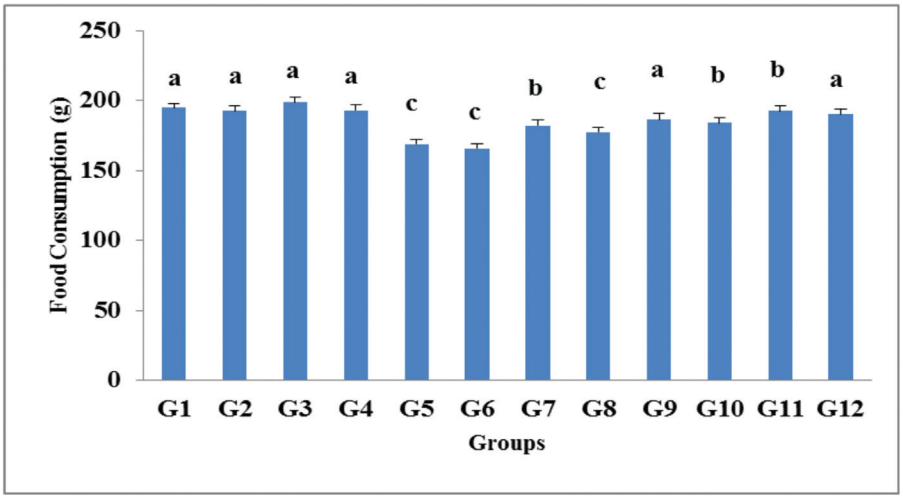
Figure 2 demonstrates food consumption of the experimental rats over the experimental duration (28 days). The quantity of food consumed/group ranged between 165.8 g and (G6: MET at 10× ADI) and 198.9 g (G3: SE). However, there were no significant differences between the groups of rats that received fluids free of MET as the sole drinking source.

Figure 1



Consumption of drinking solutions by male rats (*Rattus norvegicus*) treated with methomyl (MET), with and without green tea extract (GTE), sage extract (SE), and their mixture, over the experimental duration (28 day). Group description: G1=control (water); G2=green tea; G3=sage; G4=green tea+sage; G=ET at ADI+water; G=ET at 10× ADI+water; G=ET at ADI+green tea; G=ET at 10× ADI+green tea; G=ET at ADI+sage; G10=MET at 10× ADI+sage; G11=MET at ADI+green tea+sage; G12=MET at 10× ADI+green tea+sage. Statistical significance: compared with G1 throughout vertical columns, the superscripts a, insignificant ($P \geq 0.05$); b, significant difference ($P \leq 0.05$); and c, high significant difference ($P \leq 0.01$); $n=5$. ADI, acceptable daily intake.

Figure 2



Consumption of food by male rats (*Rattus norvegicus*) treated with methomyl (MET), with and without green tea extract (GTE)and sage extract (SE), over the experimental duration (28 day). Group description: G1=control (water); G2=green tea; G3=sage; G4=green tea+sage; G=ET at ADI+water; G=ET at 10× ADI+water; G=ET at ADI+green tea; G=ET at 10× ADI+green tea; G=ET at ADI+sage; G10=MET at 10× ADI+sage; G11=MET at ADI+green tea+sage; G12=MET at 10× ADI+green tea+sage. Statistical significance: compared with negative G1 throughout vertical columns, the superscripts a, insignificant ($P \geq 0.05$); b, significant difference ($P \leq 0.05$); and c, high significant difference ($P \leq 0.01$); $n=5$. ADI, acceptable daily intake.

Quantities of food consumed by both MET dose groups were significantly ($P<0.01$) lower than those consumed by control groups. GTE with MET at lower dose (G7) consumed more quantity of food (181.9 g) than that consumed by GTE with MET at the higher dose (G8: 177.5 g), and both groups showed high significant decrease in food consumption than control group (195 g). Generally, there were little differences in food consumption among the other MET groups received either SE or GTE+SE (Fig. 2).

Body and organ's weights

The mean final body weight of control rats (G1: 287.4 g) recorded insignificant differences as compared with the values recorded for G4 (GTE +SE), G7 (MET at ADI+GTE), G8 (MET at 10× ADI), G9 (MET at ADI+SE), and G11 (MET at ADI+GTE+SE). The mean final body weight for the rest of treatments showed different levels of significance ($P<0.05$ and 0.01), as compared with control values (Table 1).

Table 1 Relative organ weights of male rats (*Rattus norvegicus*) treated with methomyl, with and without green tea extract, sage extract, and their mixture

Treatment (groups)	Final body weight (g)	Liver (%)	Kidneys (%)	Heart (%)	Spleen (%)	Testes (%)
G1	287.4±6.62[a]	2.84±1.52[a]	0.64±0.36[a]	0.28±1.38[a]	0.36±2.52[a]	0.95±0.66[a]
G2	259.3±7.34[b]	2.641±1.06[a]	0.68±0.57[a]	0.29±0.06[a]	0.39±0.25[a]	0.95±1.23[a]
G3	336.4±8.47[c]	2.73±0.58[a]	0.68±1.02[a]	0.30±1.22[a]	0.39±0.79[a]	0.97±0.76[a]
G4	284.2±12.32[a]	2.81±1.53[a]	0.66±1.70[a]	0.28±0.05[a]	0.38±3.65[a]	0.96±0.57[a]
G5	233±7.41[c]	4.92±1.46[c]	1.72±0.06[c]	0.46±0.72[c]	0.54±0.95[c]	1.15±2.35[c]
G6	216.6±5.94[c]	5.14±1.03[c]	1.86±0.32[c]	0.48±1.85[c]	0.59±0.04[c]	1.46±0.83[c]
G7	255.3±15.18[a]	3.60±1.23[b]	0.86±0.90[c]	0.32±0.09[b]	0.42±1.87[b]	1.05±2.36[b]
G8	220.4±6.60[a]	3.76±1.50[b]	0.89±1.02[c]	0.36±1.84[b]	0.50±1.37[c]	1.35±0.05[c]
G9	318.2±13.05[a]	3.65±0.68[b]	0.85±1.33[c]	0.38±1.22[b]	0.44±0.26[b]	1.08±1.09[b]
G10	241.7±5.03[c]	3.90±1.05[b]	0.91±1.20[c]	0.40±0.63[c]	0.53±1.64[c]	1.38±0.64[c]
G11	296.2±13.70[a]	2.93±0.6[a]	0.69±0.42[a]	0.30±2.60[a]	0.39±2.20[a]	0.98±2.33[a]
G12	268±6.08[c]	3.02±1.34[b]	0.72±0.48[b]	0.32±1.04[b]	0.42±3.69[a]	1.03±3.48[b]

Group description: G1=control (water); G2=green tea; G3=sage; G4=green tea+sage; G=ET at ADI+water; G=ET at 10× ADI+water; G=ET at ADI+green tea; G=ET at 10× ADI+green tea; G=ET at ADI+sage; G10=MET at 10× ADI+sage; G11=MET at ADI+green tea +sage; G12=MET at 10× ADI+green tea+sage. ADI, acceptable daily intake; MET, methomyl. Statistical significance: compared with G1 throughout the vertical columns, the superscripts were as follows: [a]Insignificant ($P \geq 0.05$). [b]Significant difference ($P \leq 0.05$). [c]Highly significant difference ($P \leq 0.01$); $n=5$.

The relative organ weights for liver, kidneys, heart, spleen, and testes from control rats that received fluids free of MET as the sole drinking source (e.g. G1–G4) showed insignificant differences between their values. Except for the relative weight of liver from G11 (MET at ADI+GTE+SE), which equaled 2.93%, the values recorded for the rest of treatments were significantly higher at *P* value less than 0.05 and *P* value less than 0.01 levels. Similar trend was obtained for the relative weights of kidneys, heart and testes. The relative weights of spleen in rats treated with the two doses of MET+GTE+SE (G11 and G12) recorded 0.98 and 1.03%, respectively, for the low and high doses of MET, which are values of insignificant difference than that obtained for control (0.95%) (Table 1).

Biochemical parameters

Table 2 presents the results of some biochemical parameters related to liver and kidney functions, as well as antioxidant enzymes in male rats that received the different tested treatments. Generally, the groups of rats allowed to drink fluids free of MET (e.g. G1–G4) showed results of insignificant differences between them. The rest of treatments showed some differences according to the type of drinking fluid with MET. The activity of AST in control rats (G1) recorded 62.12 U/l, that for MET at ADI (G5) and MET at 10× ADI (G6) recorded 76.42 and 86.78 U/l, respectively, achieving high significant differences ($P<0.01$). MET at ADI+GTE (G7), MET at ADI +SE (G9), and both low and high MET doses+GTE +SE (G11 and G12) recorded AST activities very close to that of control results. The obtained AST values for MET at 10× ADI+GTE (G8) and MET at 10× ADI +SE (G10) were significantly higher ($P<0.05$) than that of control value.

The activity of ALT (in both doses of MET) was significantly higher ($P<0.01$) than control value (24.98 U/l). Both doses of MET either with GTE or SE recorded ALT activities higher than that of control at *P* value less than 0.05 level. Both doses of MET+GTE+SE (G11 and G12) recorded ALT activities insignificantly different than that of control results (Table 2).

ALP activity in the control treatment recorded 92.88 U/l, a value which was highly significantly lower ($P<0.01$) than the values recorded for MET treatments (373.40 and 441.70 U/l, respectively, for MET at ADI+water and MET at ADI 10× +water). Co-administration of GTE and/or SE limited such differences to a pronounced extent (Table 2).

MET alone or in conjunction with the tested fluids caused severe decline in BuChE activity, except in the case of giving GTE+SE with the low dose of MET (G11), where the activity of BuChE was 7684 U/l, achieving insignificant difference when compared with the respective control value (7636 U/l) (Table 2).

Highly significant elevation ($P<0.01$) in urea concentrations has been obtained in MET treatments. Compared with control value (39.34 mg/dl), co-administration of GTE, SE, and GTE+SE with the low dose of MET has resulted in nonsignificantly different values. However, there were significant differences ($P<0.05$) in the treatments of MET at 10× ADI with the tested extracts (Table 2).

Table 2 Some biochemical parameters (mean±SE) of liver enzymes, kidney functions, and antioxidant enzymes in male rats (*Rattus norvegicus*) treated with methomyl, with and without green tea extract, sage extract, and their mixture

Groups	AST (U/l)	ALT (U/l)	ALP (U/l)	BuChE (U/l)	Urea (mg/dl)	Cr (mg/dl)	MDA (nmol/g tissue)	SOD (μg/g tissue)	TAC (mmol/l)
G1	62.12±0.56[a]	24.98±0.11[a]	92.88±1.75[a]	7630±9.11[a]	39.34±0.81[a]	0.66±1.65[a]	4.16±0.78[a]	314.7±1.86[a]	1.30±1.48[a]
G2	61.34±0.13[a]	25.08±0.10[a]	92.40±0.65[a]	7626±5.36[a]	37.94±1.47[a]	0.67±1.32[a]	4.15±1.37[a]	310.6±0.88[a]	1.31±0.53[a]
G3	60.64±0.11[a]	25.50±0.05[a]	91.40±0.70[a]	7635±2.33[a]	39.56±0.53[a]	0.65±1.06[a]	4.14±1.69[a]	319.1±0.80[a]	1.31±0.57[a]
G4	62.84±0.25[a]	25.66±0.05[a]	90.62±1.27[a]	7636±8.01[a]	40.54±1.57[a]	0.67±1.36[a]	4.15±0.77[a]	317.7±1.86[a]	1.33±0.64[a]
G5	76.42±0.40[c]	74.42±0.37[c]	373.4±1.89[c]	5285±38.70[c]	54.40±1.19[c]	1.32±0.18[c]	7.09±0.48[c]	205.0±1.23[c]	0.28±1.38[c]
G6	86.78±0.54[c]	85.34±0.24[c]	441.7±3.81[c]	4763±15.14[c]	58.26±1.03[c]	1.65±0.48[c]	7.43±1.15[c]	199.0±1.45[c]	0.23±1.47[c]
G7	65.32±0.21[a]	55.58±0.30[b]	107.6±0.52[b]	8530±34.12[c]	42.74±0.53[a]	0.64±3.19[a]	6.73±0.65[c]	302.0±0.58[b]	1.22±1.08[b]
G8	72.08±0.24[b]	65.42±0.19[b]	260.8±2.15[b]	6471±32.78[c]	47.54±0.77[b]	0.66±1.58[a]	6.33±1.06[c]	281.0±1.13[b]	0.72±0.16[b]
G9	68.94±0.32[a]	46.54±0.48[b]	112.3±1.81[b]	6822±46.51[c]	42.62±2.64[a]	0.62±3.12[a]	6.93±0.66[c]	247.7±0.75[c]	1.13±1.63[b]
G10	73.46±0.32[b]	51.86±0.46[b]	123.8±1.13[b]	6041±59.31[c]	48.52±0.39[b]	0.65±1.23[a]	6.55±0.73[c]	235.8±1.05[c]	0.27±1.37[c]
G11	62.84±0.25[a]	27.88±0.17[a]	95.02±0.83[a]	7684±21.32[a]	38.88±1.38[a]	0.67±0.24[a]	5.86±0.32[b]	313.9±0.67[a]	1.30±0.49[a]
G12	60.90±0.25[a]	34.44±0.42[a]	110.3±1.31[b]	7479±41.32[c]	39.88±0.59[b]	0.68±0.34[a]	4.11±0.55[a]	296.3±1.19[b]	1.29±0.56[a]

Group description: G1=control (water); G2=green tea; G3=sage; G4=green tea+sage; G=ET at ADI+water; G=ET at 10× ADI+water; G=ET at ADI+green tea; G=ET at 10× ADI+green tea; G=ET at ADI+sage; G10=MET at 10× ADI+sage; G11=MET at ADI+green tea +sage; G12=MET at 10× ADI+green tea+sage. ADI, acceptable daily intake; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BuChE, butyrylcholinesterase; MDA, malondialdehyde; MET, methomyl; SOD, superoxide dismutase; TAC, total antioxidant capacity. Statistical significance: compared with G1 throughout vertical columns, the superscripts were as follows: [a]Insignificant ($P \geq 0.05$). [b]Significant difference ($P \leq 0.05$). [c]High significant difference ($P \leq 0.01$); $n=5$.

Creatinine concentration in the control group recorded 0.66 mg/dl. Highly significant elevation ($P<0.01$) in creatinine concentrations has been obtained in MET treatments. Co-administration of the tested extracts restored creatinine concentration to the normal levels (Table 2).

LPO, in terms of MDA, recorded 4.16 nmol/g tissue for the control group. In comparison, MET alone at both doses caused highly significant elevation ($P<0.01$). Co-administration of the tested extracts with MET caused some improvement, especially in the rats that received GTE+SE treatments (Table 2).

The activity of SOD in the control treatment recorded 314.70 μg/g tissue. Severe/drastic decline ($P<0.01$) was obtained in MET and MET+SE treatments. Co-administration of GTE+SE with low dose of MET normalized SOD level (Table 2).

The activity of TAC in the control treatment was accounted to 1.30 mmol/l. MET alone and MET at 10× ADI+SE treatments caused highly significant decline ($P<0.01$), whereas co-administration of GTE+SE with the two doses of MET normalized the TAC levels (Table 2).

Evaluation of oxidative stress and amelioration effects

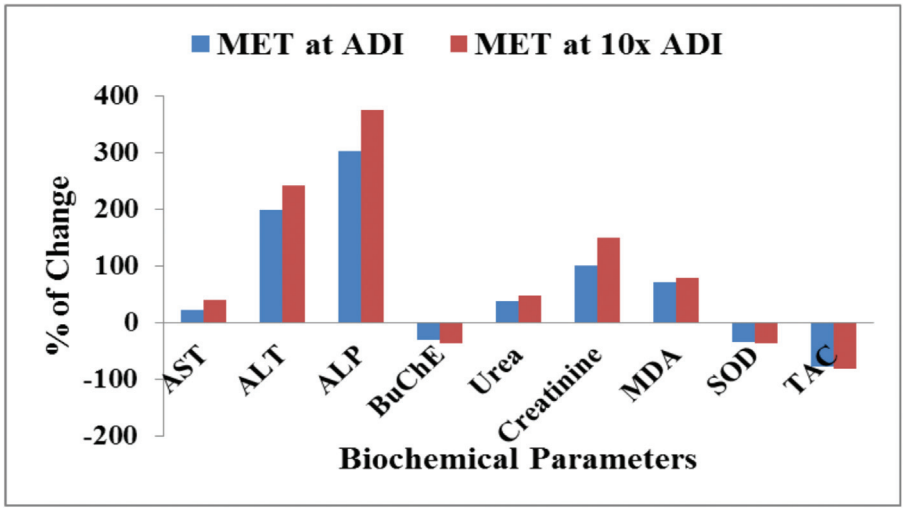
The percentage of changes in some biochemical parameters in male rats treated with two different doses of MET is illustrated in Fig. 3. Deviation of the biochemical parameters than normal control values, owing to exposure to MET, was varied greatly, based on the estimated biochemical parameter and the MET dose. Generally, changes were occurred in a dose-dependent manner, but differences between the levels were very little for some biochemical parameters (e.g. SOD, TAC, MDA, and BuChE). Changes in aminotransferases, ALP, and creatinine were the most pronounced. For instance, changes in ALP were accounted to 302 and 375%, respectively, for MET at ADI and MET at 10× ADI. Those for ALT were 198 and 241%, respectively (Fig. 3).

The capacity of the tested herbal extracts in ameliorating the oxidative stress, expressed in terms of AI, is presented in Table 3. For a number of the tested biochemical parameters, co-administration of GTE, SE, or GTE +SE has resulted in AI around 1.0, indicating optimum amelioration. Generally, both GTE and SE were more effective with the low MET doses, and GTE seemed to be more effective than SE. For both MET doses, co-administration of GTE+SE has resulted in AI values equaled ≈1.0 for a number of the tested biochemical parameters (e.g. AST, BuChE, urea, creatinine, SOD, and TAC). For a few number of biochemical parameters (e.g. ALT), the calculated AI approached or exceeded 2.0, for MET doses with GTE or SE (Table 3).

Histopathological studies

The results of histological examination of the liver of the experimental rats are illustrated in Fig. 4. Sections prepared from control water, GTE, and SE showed normal histological architecture represented by pronounced normal central vein, hepatocytes, sinsouids, portal area, and Kuppfer cells. In comparison, rats treated with MET showed congested portal vein, poorly indentified hepatocytes

Figure 3



Percent of change in biochemical parameters due to exposure of male rats (*Rattus norvegicus*) to methomyl (MET). Percent of change-=Treatment value–Control value/control value×100. Data of MET (G5 and G6) and control (G1) values refer to Table 2. ADI, acceptable daily intake; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BuChE, butyrylcholinesterase; MDA, malondialdehyde; MET, methomyl; SOD, superoxide dismutase; TAC, total antioxidant capacity.

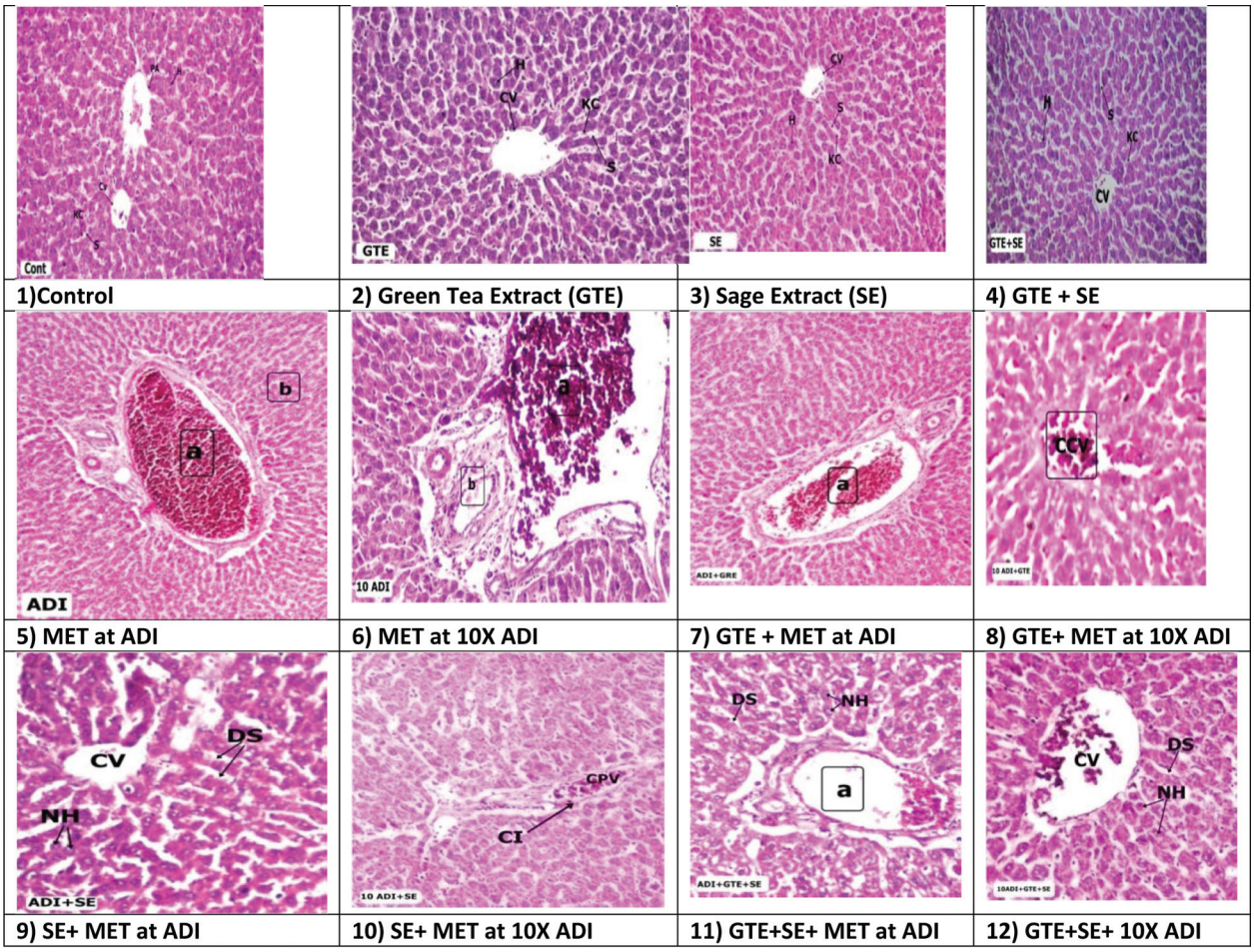
Table 3 Assessment of ameliorating effect of joint administration of green tea extract, sage extract, and their mixture with methomyl, based on measured biochemical parameters in male rats (*Rattus norvegicus*)

Treatments	Biochemical parameters[a]								
	AST (U/l)	ALT (U/l)	ALP (U/l)	BuChE (U/l)	Urea (mg/dl)	Creatinine (mg/dl)	MDA (nmol/g tissue)	SOD (μg/g tissue)	TAC (mmol/l)
MET at ADI+green tea (G7)	65.32	55.58	107.6	8530	42.74	0.64	6.73	302.0	1.22
Green tea (G2)	61.34	25.08	92.40	7626	37.94	0.67	4.15	310.6	1.31
Amelioration index[b]	1.1	2.2	1.2	1.1	1.1	1.0	1.6	1.0	0.9
MET at 10× ADI+green tea (G8)	72.08	65.42	260.8	6471	47.54	0.66	6.33	281.0	0.72
Green tea (G2)	61.34	25.08	92.40	7626	37.94	0.67	4.15	310.6	1.31
Amelioration index[b]	1.2	2.6	2.8	0.8	1.3	1.0	1.5	0.9	0.5
MET at ADI+sage (G9)	68.94	46.54	112.3	6822	42.62	0.62	6.93	247.7	1.13
Sage (G3)	60.64	25.50	91.40	7635.0	39.56	0.65	4.14	319.1	1.31
Amelioration index[b]	1.1	1.8	1.2	0.9	1.1	1.0	1.7	0.8	0.2
MET at 10× ADI+sage (G10)	73.46	51.86	123.8	6041.0	48.52	0.65	6.55	235.8	0.27
Sage (G3)	60.64	25.50	91.40	7635.0	39.56	0.65	4.14	319.1	1.31
Amelioration index[b]	1.2	2.0	1.4	0.8	1.2	1.0	1.6	0.7	0.2
MET at ADI+green tea+sage (G11)	62.84	27.88	95.02	7684.0	38.88	0.67	5.86	313.9	1.30
Green tea+sage (G4)	62.84	25.66	90.62	7636.0	40.54	0.67	4.15	317.7	1.33
Amelioration index[b]	1.0	1.1	1.0	1.0	1.0	1.0	1.4	1.0	1.0
MET at ADI+green tea+sage (G12)	60.90	34.44	110.3	7479.0	39.88	0.68	4.11	296.3	1.29
Green tea+sage (G4)	62.84	25.66	90.62	7636.0	40.54	0.67	4.15	317.7	1.33
Amelioration index[b]	1.0	1.3	1.2	1.0	1.0	1.0	1.0	1.0	1.0

ADI, acceptable daily intake; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BuChE, butyrylcholinesterase; MDA, malondialdehyde; MET, methomyl; SOD, superoxide dismutase; TAC, total antioxidant capacity. [a]Biochemical parameters refer to Table 2. [b]Amelioration index=(pesticide+plant extract value)/plant extract value (e.g. by dividing: G7 or G8/G2; G9 or G10/G3; G11 or G12/G4).

and sinsouids, but they were more pronounced in the high dose of MET. Light congestion in portal vein was seen in GTE+MET at ADI treatment, whereas congested central vein was manifested in the treatment of GET+MET at 10× ADI. Differentiated sinusoids and normal hepatocytes were observed in SE+MET at ADI, whereas cell infiltration around the portal area and congested central portal vein were characterized for SE+MET at 10× ADI treatment. Co-administration of GTE+SE with the two doses of MET showed normal hepatocytes and light differentiated sinusoids.

Figure 4



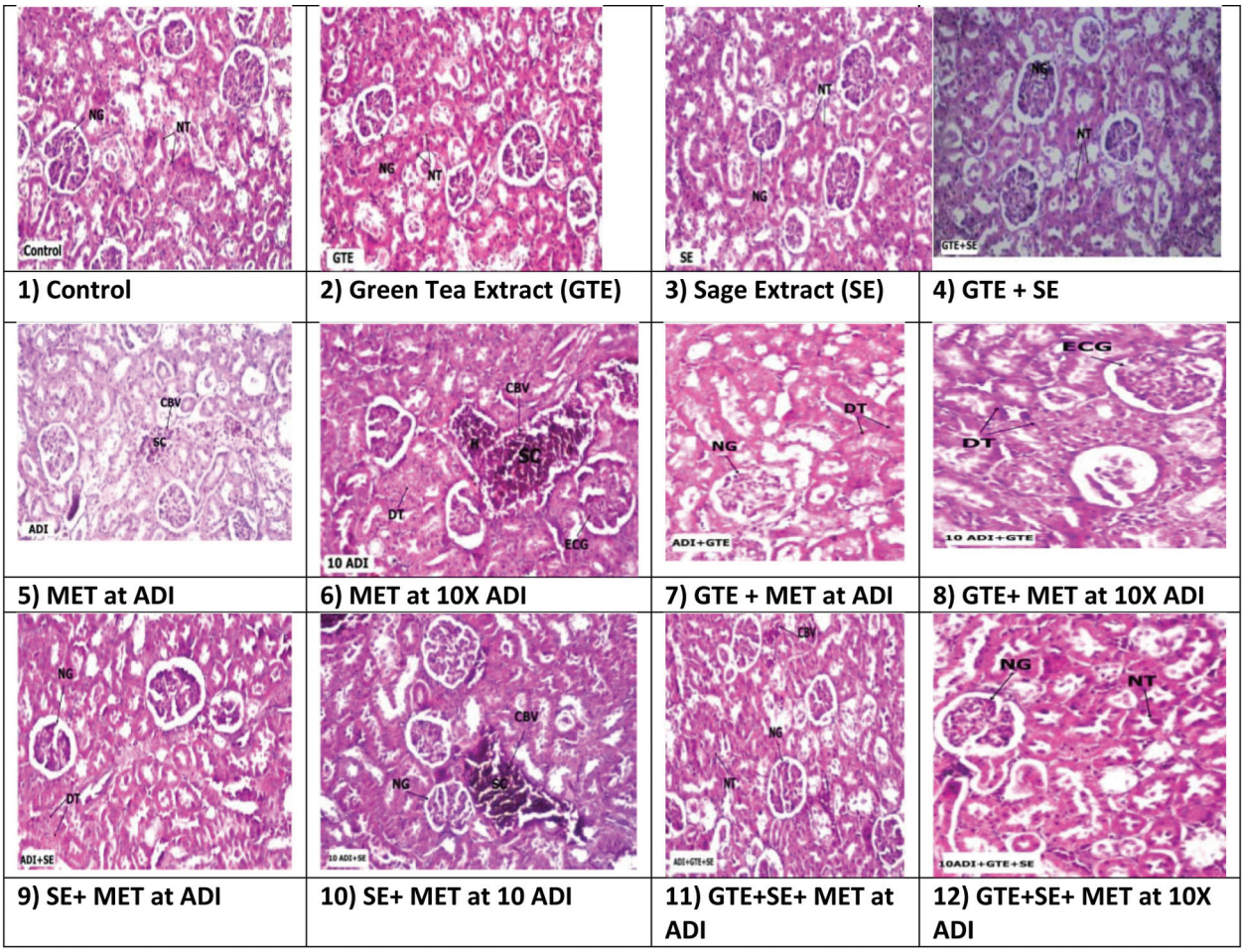
Histological examination of liver tissue for male rats (*Rattus norvegicus*) treated with methomyl (MET) at different doses with and without green tea extract (GTE) and sge extract (SE) extracts. Sections 1, 2, 3, and 4=control water, GTE, and SE, respectively; showing normal central vein (CV), normal hepatocytes (H), normal sinsouids (S), normal portal area (PA), and Kuppfer cells (KC). Sections 5 and 6=MET at ADA and MET at 10× ADI, respectively, showing congested portal vein (a), poorly indentified hepatocytes and sinsouids (b), but were more pronounced in the high dose of MET. Sections 7 and 8=GTE+MET at ADI and GET+MET at 10× ADI, respectively, showing light congestion in portal vein (a) (slide #7) and congested central vein (CCV) (slide #8). Sections 9 and 10=SE+MET at ADI and SE+MET at 10× ADI, respectively, showing differentiated sinusoids (DS), normal hepatocytes (NH) (slide #9), cell infiltration around the portal area (CI) congested central portal vein (CPV) (slide #10). Sections 11 and 12=at 10× ADI and GTE+SE+MET at ADI, respectively, showing normal hepatocytes (NH) and light differentiated sinusoids (DS) (slides #11 and 12). ADI, acceptable daily intake.

The results of histological examination for kidney of experimental rats are illustrated in Fig. 5. Sections prepared from control water, GTE, and SE, showed normal tubules and normal glomeruli. However, rats treated with MET at ADI showed sever congestion in central blood vessel, whereas those treated with MET at 10× ADI showed degenerated tubules, severe congestion in central blood vessel and expanded congested glomeruli. Normal glomeruli and degenerated tubules were seen in GTE+MET at ADI treatment, whereas expanded congested glomeruli and mild degenerated tubules were manifested in the treatment of GET+MET at 10× ADI. Normal glomeruli and degenerated tubules were observed in SE+MET at ADI, whereas normal glomeruli accompanied with mild congestion in central blood vessel were characterized for SE+MET at 10× ADI treatment. Co-administration of GTE+SE with the two doses of MET showed normal tubules, normal glomeruli, and light congestion in central blood vessel.

Discussion

The present study was an attempt to evaluate the toxicity of MET on some physiological, biochemical and histopathological parameters in male rats and to test the possible ameliorative effect of leaf extracts of GT, sage, and their combination against toxicity of the insecticide MET. The results of fluid consumption by the experimental rats revealed that GTE was more palatable than SE, but the drinking solution containing both extracts was more superior. This supports our assumption that preparation of a blend from the two

Figure 5



Histological examination of kidney tissue for male rats (*Rattus norvegicus*) treated with methomyl (MET) at different doses with and without green tea extract (GTE) and sage extract (SE). Sections 1, 2, 3, and 4=control water, GTE, and SE, respectively, showing normal tubules (NT) and normal glomeruli (NG). Sections 5 and 6=MET at ADA and MET at 10× ADI, respectively, showing sever congestion (SC) in central blood vessel (CBV) (slide #5), degenerated tubules (DT), severe congestion (SC) in central blood vessel (CBV) and expanded congested glomeruli ECG) (slide #6). Sections 7 and 8=GTE+MET at ADI and GET+MET at 10× ADI, respectively, showing normal glomeruli (NG) and degenerated tubules (DT) (slide #7), expanded congested glomeruli (ECG) and mild degenerated tubules (DT) (slide #8). Sections 9 and 10=SE+MET at ADI and SE+MET at 10× ADI, respectively; showing normal glomeruli (NG) and degenerated tubules (DT) (slide #9), normal glomeruli (NG) and mild congestion (SC) in central blood vessel (CBV) (slide #10). Sections 11 and 12=at 10× ADI and GTE+SE+MET at ADI, respectively; showing normal tubules (NT), normal glomeruli (NG), and light congestion in central blood vessel (CBV) (slides #11 and 12). ADI, acceptable daily intake.

plant leaves may find acceptance as a drink containing two beneficial herbs. To the best of our knowledge, the literature offers no studies on the palatability of such blend as a drinking herbal beverage. Therefore, the health issues of such blend may need more investigations. On the contrary, it appeared that food consumption tended to be associated negatively with MET dosage, and co-administration of the studied extracts enhanced food consumption.

Alterations in body and absolute or relative organ's weights following exposure to MET have been documented in several studies [13,14,16,46]. Such alterations could be attributed to loss of appetite and decrease in food intake owing to disturbance in hormonal balance and/or direct cytotoxic effects of MET [55]. Although, the tested doses in the present study (≈0.03 and 0.3 mg/kgbw) might be considered very low as compared with the doses mostly used in previous investigations, these doses caused drastic decrease in the final body weight severely but increased the relative organs weights. Such results corroborated with Djeffal *et al.* [16] who reported decrease in final body weight and increase of relative weights of liver and kidney of male rats treated with MET in drinking water (8 mg/kgbw). Moreover, the present work results are supported by Mansour *et al.* [13], who reported similar trend for body and relative organ weights in male rats treated with different doses of MET (the lowest was nearly equaled to the 10× ADI of MET).

In toxicity tests, a variety of specific biochemical parameters are usually measured to evaluate

physiological and metabolic functions, which affect target organs and tissue injury [56]. The most widely measured parameters are AST, ALT, and ALP for hepatotoxicity and urea and creatinine for glomerular function [57]. Additionally, cholinesterase (ChE) assay is used to assess the cholinergic effects of organophosphorous and carbamate pesticides, as well as ChE inhibitors [6]. Carbamate pesticides, including MET, can induce oxidative stress in rat tissues, leading to excessive generation of free radicals that cause deleterious effects on various organs and erythrocytes [58]. The literature offers several studies on hepatorenal toxicities and oxidative stress of MET in rats [9,13,14].

Liver and kidney play an important role in detoxification of xenobiotics. The present work results revealed that MET treatment induced liver toxicity represented by the increase of ALT, AST, and ALP activities, which corroborated with previous studies [9,12–14]. The elevated levels of these enzymes indicate disturbance between the degree of oxidative stress and the antioxidant capacity [59]. ALT and AST are the most sensitive biomarkers that are directly implicated in the extent of hepatic damage and toxicity [60]. The serum ALP elevation following exposure to insecticides might be an evidence of damage in the hepatobiliary system [61].

Kidney toxicity was shown by an increase in the levels of creatinine and urea in the sera of male rats treated with MET. Creatinine is a waste product of creatinine metabolism, whose measurement provides a useful index of kidney function [62]. According to Gilman *et al.* [63], the elevation of urea could be attributed to an increase of nitrogen retention and/or owing to corrupted renal function. A significant increase in serum urea level was observed in severe defect of glomerular filtration [64].

BuChE, also known as pseudocholinesterase, was recognized as a hydrolyzing enzyme of choline esters. It is synthesized in hepatocytes and secreted into the blood [65]. In contrast to other serum enzymes involved in the assessment of liver function, the activity of BuChE decreases in liver dysfunction, owing to its reduced synthesis [66]. ChEs are specifically known as biomarkers of effect resulted from exposure to organophosphorous and carbamates [67]. The current results on MET are supported by the results of previous investigators [9,12–14].

SOD is an antioxidant enzyme that catalyzes the dismutation of superoxide free radicals [68]. It plays an important role in scavenging ROS and catalyzing the destruction of the superoxide radicals [69]. Carbamates are known to diminish activities of SOD and to elevate ROS in Leydig cells of rats [70]. In agreement with the results of the present investigation, MET was reported to decrease the activity of SOD in mice and rat livers [9,12–14].

The present study showed that administration of MET to rats caused an increase in the LPO as indicated by the increase in the level of MDA. These results are consistent with several studies on MET [9,12–14], and other carbamate pesticides, such as the fungicide benomyl [71], and the carbamate insecticides aldicarb [72], propoxur [73], and carbofuran [2] in rats. The MET-induced increase in MDA level might be owing to the conjugation of the insecticide or its degradation products (metabolites) to the polyunsaturated fatty acids, which might cause overproduction of ROS [74].

TAC considers the cumulative effect of all antioxidants present in blood and body fluids. The decreased level of serum TAC in MET-treated rats in the current study reflects a lower TAC. This is probably owing to the depletion of the antioxidant molecules, because of their consumption in the process of protecting cells against ROS generated by the insecticide MET [75].

The response of organisms to environmental pollutants is highly affected by the antioxidant defense [76]. The generated free radicals (atoms or molecules) have a very strong tendency to exist in a stable paired state. So, they pick up electrons from other atoms, converting them into secondary free radicals, thus, setting up a chain reaction, which can cause substantial oxidative stress. Such oxidative stress, if not regulated properly, may lead to damage in DNA, lipids, protein, and nucleic acids [77]. Owing to the high concentration of polyunsaturated fatty acids in the cells, LPO (as indicated by high elevation of MDA levels) is a major outcome of the free radical-mediated injury; thus, MDA levels could be considered an earlier diagnostic index in chemical toxicity [78].Generation of free radicals is expected to induce hepatorenal toxicity and oxidative stress [9]. Studies have shown that administration of mineral elements and vitamins can significantly decrease the range of tissue damages induced by MET [11–16]. Supplementation of such dietary antioxidants can be considered as the alternative method for chelation therapy [79,80]. Therefore, interest has been grown in elucidating the role of natural antioxidants as a strategy to prevent oxidative damage induced by various environmental pollutants, for example, pesticides [81]. In this respect, the hot

aqueous extract of chamomile (*M. chamomilla*) flowers was found to protect against renal toxicity induced by MET in male albino mice [17]. To the best of our knowledge, aqueous extracts of GT, sage, and their mixture have not yet been tested against MET-induced toxicity in rats. However, the antioxidant capacity of GTE against several toxic substances has been long recognized [26–29,31], as well as for SE [42,43,47].

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 [13]. Co-administration of the tested herbal extracts has improved the cell architecture of the examined tissues to a pronounced extent. Co-administration of GTE appeared to be more effective than SE. This might be attributed to the antioxidant properties of GTE [21,23,25] and SE [32,39,40]. On the contrary, co-administration of the herbal mixture (GTE +SE) showed superiority over each of the individual extract, suggesting 'potentiating effect' between both herbs. Such suggestion goes parallel with the powerful ameliorating effect of the mixture towards the estimated biochemical parameters in the present study.

The optimum AI obtained from applying the aforementioned formula, should theoretically be amounted to 1.0. This was achieved for a number of biochemical parameters that restored to normal levels (Table 3), such as creatinine and SOD by GTE+MET at ADI; creatinine by GTE+MET at 10× ADI; creatinine by SE+MET at ADI; creatinine by SE +MET at 10× ADI; and AST, BuChE, urea, creatinine, SOD, and TAC by both MET doses. AI values around 1.0, for example, 1.1 or 0.9, are considered also as indicative of high amelioration, but values lower than 0.5 may indicate little ameliorative effect [13]. For some biochemical parameters, the calculated AI approached or exceeded 2.0, which is a questionable result between 'superior improvement' and 'reversible effect.' Similar findings were recently reported for vitamin E with the organophosphorous insecticide chlorpyrifos [53], Zn with MET [13], Zn with MET and abamectin [14], and Se with the pyrethroid insecticide deltamethrin [82].

Conclusion

The insecticide MET, at extremely low doses, can induce hepatorenal dysfunction, oxidative stress, and histopathological damage in liver and kidney of male rats (strain). The observed alterations occurred in a dose-dependent manner and were referred to the oxidative stress induced by MET. Co-administration of GTE or SE in conjunction with MET brought most of 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. Surprisingly, co-administration of GTE and SE mixture gave superior ameliorating effects as compared with each of the individual extracts, suggesting 'potentiating effect' between both extracts. The study introduced novel findings regarding the protective effect of GTE, SE, and their mixture against MET-induced toxicity in rodents.

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Conflicts of interest

There are no conflicts of interest.

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