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Effects of black seed and thyme leaves dietary supplements against malathion insecticide-induced toxicity in experimental rat model

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ABSTRACT

Objectives: Malathion is a widely used pesticide in the Kingdom of Saudi Arabia against pests that infest dates, and it causes severe damage to humans and animals. We evaluated the possible defensive effect of dietary supplementation with powdered black seeds (PBS) and powdered thyme leaves (PTL) against malathion-induced toxicity in a rat model.

Methods: Rats were divided into 4 equal groups: healthy control group, malathion-intoxicated group, malathion-intoxicated rats fed diets supplemented with PBS group, and malathion-intoxicated rats fed diets supplemented with PTL group. about I mL aliquot of each blood sample was kept in an EDTA-containing tube, and used immediately to measure different hematological parameters, including hemoglobin, hematocrit, red blood cells, leukocytes and platelets. Signs of toxicity were recorded daily. After sacrifice, internal organs (liver, kidneys) were removed and washed in cold saline and used to obtain gross findings and perform biochemical serum analysis.

Results: Malathion-induced toxicity was shown by altered hematological parameters, increasing serum lipid parameters, oxidative stress and inflammatory biomarkers, and kidney and liver damage. However, dietary supplementation with either PBS or PTL reversed the toxic effect of malathion by ameliorating lipid parameters, antioxidant enzyme levels, and proinflammatory cytokine levels. Moreover, both supplements exhibited a protective effect against liver and kidney damage.

Conclusion: Dietary supplementation with either PBS or PTL reduced malathion toxicity through different antioxidant and anti-inflammatory mechanisms.

1. Introduction

Pesticide consumption has been increasing rapidly in the Kingdom of Saudi Arabia (KSA), with increased agricultural development. Saudi Arabia is the biggest date producer in the world, and insects cause severe damage to the crop, so various insecticides are used to protect the dates (Saggu et al., 2016).

Malathion (MAL) is an organophosphate insecticide used in Saudi Arabia to reduce the motile stages of fruit and vegetable insects. Harmless pesticides with minor residual deposits have always been preferred because of the large amount of dates consumed by Saudi residents (Kamel et al., 2007). Acute or chronic exposure to MAL may affect health and cause metabolic disorders, oxidative stress, immunotoxicity, inflammation, and hepatotoxicity (Mantovani et al., 2008). Malathion exhibits minimum toxicity and is converted to malaoxon after human absorption or ingestion, but malaoxon is more toxic and responsible on health risks, such as severe, acute, and chronic human and animal toxicity (Yarsan and Cakir, 2006). The liver is a great target for reducing malathion toxicity possibly because of its importance in the homeostasis of glucose and lipids and the production of associated enzymes. A medicinal herb is a plant that changes physiological and pathological processes and prevents or treats diseases (Al-Khalaf and Ramadan, 2013). There has been an increase in the use of medicinal plants because of their availability without a prescription, low cost, and fewer side effects (Grandjean and Landrigan, 2014).

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Black seed (*Nigella sativa*) is a plant used in traditional medicine because of its remedial ability in different health problems (El-Saleh et al., 2004). Black seed has been shown to be beneficial in treating dysentery, bronchial asthma, obesity, gastritis, hypertension, and headache (Shirpoor et al., 2009). Thyme (*Thymus vulgaris L.*) is a herb used as a spice, as a flavoring in tea, and as a herbal medicine. In addition, thyme leaves are used in food for preservation, aroma, and flavor and is added to fish and meat. Thyme possesses different useful properties, such as antimicrobial, antiseptic, anthelmintic, bactericidal, and antioxidant activities, and recently was considered to be a natural alternative to synthetic antioxidants. It is also rich in iron, manganese, calcium, and vitamin K (Sasaki et al., 2005). In this study, different biochemical parameters were examined to evaluate the lipid profile, antioxidant stress, and inflammatory variations in rats intoxicated with MAL and to clarify the possible insect defensive benefits of powdered thyme and its effects on reducing toxicity and on biochemical alterations after MAL administration in a rat model.

2. Material and methods

2.1. Chemicals

Malathion was purchased from the Agrochemical Market in the KSA, and black seed and thyme were obtained from local markets in the KSA.

2.2. Animals

Sixty male albino rats, weighing 200 ± 10 g were used in this experiment and divided into 4 groups (15 rats each). The rats were acclimatized for 1 week and maintained under standard laboratory conditions. The rats received basal diets prepared according to AIN-93 (Reeves et al., 1993) and water *ad libitum*. The animal procedures were conducted according to the Ethics Committee of King Fahd Medical Research Center.

2.3. Animal groups

After the acclimation period, the rats were divided into 4 groups (15 animals each) as follows:

G1 (C): Healthy rats served as the control group
G2 (MAL): Rats were orally intoxicated with MAL pesticide at a dose level of 100 mg/kg body weight daily for 30 days according to Hazarika et al. (2003).
G3 [MAL + powdered black seeds (PBS)]: Rats were intoxicated with malathion pesticide and fed diets supplemented with PBS at a concentration of 5% according to Szczerbinska et al. (2012).
G4 [MAL + powdered thyme leaves (PTL)]: Rats were intoxicated with MAL pesticide and fed diets supplemented with PTL at a concentration of 5% according to Hanna et al. (2014).

2.4. Clinical examination

Throughout the experimental period, signs of toxicity were recorded daily. Changes in the rats' weights were recorded weekly. At the end of the experimental period, the rats were sacrificed by ether inhalation, dissected, and then blood samples were collected, centrifuged, and kept at −20 °C until biochemical analysis. Internal organs (liver, kidneys) were removed and washed in cold saline and then used to obtain gross findings and perform biochemical analysis.

2.5. Hematology

1-ml aliquot of each blood sample was kept in an EDTA-containing tube and used immediately to measure different hematological parameters, including hemoglobin (Hb), hematocrit (HCT), red blood cells (RBCs), leukocytes (WBC), and platelets (PLT).

2.6. Biochemical serum analysis

To assess liver injury, activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total protein, and albumin were determined by kits from BioVision Inc. (California. USA). Lipid peroxidation (malondialdehyde) (MDA), catalase (CAT) activity, reduced glutathione (GSH), and superoxide dismutase (SOD) activity were assessed using kits from Kamiya Biomedical Co. (Seattle, WA, USA). Biomarkers of kidney function, including creatinine, urea, and uric acid, and lipid profile parameters, including high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglycerides (TGs) were measured using kits from BioVision Inc. (California. USA). According to the manufacturer's instructions. Serum acetylcholinesterase (AchE) and paraoxonase-1 (PON-1) activities were determined colorimetrically using commercial kits from Abcam (Cambridge, MA, USA).

2.7. Determination of serum cytokines

Tumor necrosis factor-alpha (TNF-α) and interferon gamma (IFN-γ) were determined using enzyme-linked immunosorbent assay kits from Kamiya Biomedical Co.

2.8. Statistical analyses

Statistical analyses were performed using SPSS software version 20.0 (IBM Corp., Armonk, NY, USA) and one-way analysis of variance ANOVA.

3. Results

As shown in Table 1, malathion administration altered blood parameters, significantly decreased Hb, HCT, and platelet levels ($p \leq 0.01$), and significantly increased RBC and WBC counts ($p \leq 0.01$) relative to those in the control; in contrast, dietary supplementation with either PBS or PTL (G3 and G4) restored these levels.

Table 2 shows that malathion administration (G2) induced liver damage, as shown by the significant increase in liver enzyme markers relative to those in control rats ($p \leq 0.01$). In addition, comparing results of G3 and G4 showed the efficiency of powdered thyme leaves in restoring liver function enzymes as compared to G3 supplemented powdered black seeds.

In Table 3, MAL caused a significant decrease in serum total proteins and albumin ($p < 0.01$). A significant increase in serum creatinine and urea levels ($p < 0.01$) and a significant reduction in uric acid concentration were observed in the MAL group ($p < 0.01$) relative to the healthy group. This effect was reversed in the G3 (PBS) and G4 (PTL) groups.

The harmful effect of MAL on oxidative status is shown in Table 4 as the oxidative damage caused by altered oxidative enzyme levels in G2 relative to that in the healthy group. In contrast, dietary supplementation with PBS (G3 group) and PTL (G4 group) restored these enzyme levels.

Table 5 shows the variations in the lipid profile parameters in rats. Serum TC, TG, and LDL-C levels were significantly increased

Table 1
Effect of administration of PTL or black seeds on Hb, HCT, RBC, WBC, and platelet levels.

Rat groups	Hb (g/dL)	HCT (%)	RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Platelets (10^3/mm^3)
Group 1 Control Healthy	14.7 ± 1.20[a]	42.2 ± 3.50[a]	4.10 ± 0.9[a]	9.75 ± 1.7[a]	501.0 ± 26.2[a]
Group 2 Malathion (MAL)	8.6 ± 1.19[b]	27.9 ± 2.99[b]	8.60 ± 1.0[b]	12.8.0 ± 1.2[b]	368.7 ± 11.6[b]
Group 3 MAL + powdered black seeds	12.6 ± 1.4[c]	39.2 ± 1.22[c]	5.50 ± 0.9[c]	10.92 ± 1.3[c]	478.5 ± 14.6[c]
Group 4 MAL + powdered thyme leaves	12.3 ± 1.9[c]	38.1 ± 2.20[c]	5.68 ± 1.1[c]	10.72 ± 1.6[c]	470.1 ± 11.5[c]

Data are expressed as the mean ± SD; means that have similar letters are not significant at $p \leq 0.01$.

Table 2
Effect of administration of PTL or black seeds on serum liver functions of malathion-intoxicated rat groups.

Rat groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Group 1 Control Healthy	42.44 ± 1.17[a]	61.93 ± 2.12[a]	52.63 ± 6.98[a]
Group 2 Malathion (MAL)	132.82 ± 2.62[b]	99.82 ± 9.20[b]	162.61b ± 7.43[b]
Group 3 MAL + powdered black seeds	57.63 ± 1.32[c]	72.66 ± 7.22[c]	63.20 ± 4.98[c]
Group 4 MAL + powdered thyme leaves	59.87 ± 1.93[c]	79.69 ± 8.42[c]	65.97 ± 4.54[c]

Data are expressed as the mean ± SD; means that have similar letters are not significant at $p \leq 0.01$.

($p < 0.01$), whereas the HDL-C level was significantly reduced ($p < 0.01$) in the MAL-treated group relative to those in the control group. There were no significant differences in the TC and HDL-C results between the PBS (G3)- and PTL (G4)-treated groups relative to those in the control (G1) group, which demonstrated that this treatment restored TC and HDL-C to normal levels.

Table 6 shows the improvement effect of PBS and PTL (G3 and G4) on AchE and PON-1 activities as compared with the activities in the MAL-fed group (G2). MAL increased cytokine levels significantly ($p < 0.01$) relative to the G1 rats. In addition, the results clearly revealed that supplementation with either PBS or PTL (G3 and G4 groups) improved serum TNF-α and IFN-γ and brought them to near normal levels when compared with the levels in the MAL-administered rats.

4. Discussion

Organophosphates are organic ester products of phosphoric or thiophosphoric acid that are used in the fields of medicine and agriculture. Medicinal plants are the main sources for the identification of possible anti-inflammatory and anticoagulant constituents. Numerous plant extracts and phytochemicals have been examined and shown to have potential anti-inflammatory and anticoagulant activities (Wang et al., 2013).

Hematological studies are considered useful in ecological and physiological studies in order to understand the relationship of blood characteristics to the environment and are considered to be the main tools to select genetically resistant animals to certain diseases and environmental conditions (Mmereole, 2008). Changes in hematological parameters are often used to determine the health status of the body and to determine stress due to environmental, nutritional, and pathological factors (Afolabi et al., 2011). In this study, biological changes due to MAL toxicity caused hematological deterioration and cell damage manifested as reductions in Hb, HCT, and platelet counts, whereas the RBC and WBC counts increased, which indicated inflammation, relative to those in the

Table 3
Effect of administration of PTL or black seeds on serum kidney functions, total protein, and albumin of malathion-intoxicated rat groups.

Rat groups	Creatinine (mg/dL)	Urea (mmol/L)	Uric acid (μmol/L)	Total protein (g/dL)	Albumin (g/dL)
Group 1 Control Healthy	0.45 ± 0.02[a]	4.139 ± 0.34[a]	233.64 ± 14.5[a]	7.250 ± 0.21[a]	5.165 ± 0.22[a]
Group 2 Malathion (MAL)	2.04 ± 0.73[b]	16.317 ± 0.31[b]	153.91 ± 12.4[b]	5.076 ± 0.61[b]	3.322 ± 0.36[b]
Group 3 MAL + powdered black seeds	0.94 ± 0.03[c]	7.992 ± 0.33[c]	200.67 ± 9.1[c]	6.824 ± 0.58[c]	4.268 ± 0.20[c]
Group 4 MAL + powdered thyme leaves	0.85 ± 0.07[d]	6.993 ± 0.31[d]	192.45 ± 11.2[d]	7.059 ± 0.79[c]	4.767 ± 0.27[d]

Data are expressed as the mean ± SD; means that have similar letters are not significant at $p \leq 0.01$.

Table 4
Effect of administration of PTL or black seeds on markers of oxidative stress in liver tissues.

Rat groups	SOD (U/g tissue)	CAT (U/g tissue)	GSH (μg/g tissue)	MDA (nmol/g tissue)
Group 1 Control Healthy	382.32 ± 21.2[a]	2.25 ± 0.03[a]	1.48 ± 0.02[a]	33.21 ± 1.15[a]
Group 2 Malathion (MAL)	226.21 ± 13.1[b]	0.994 ± 0.01[b]	0.84 ± 0.04[b]	98.93 ± 5.05[b]
Group 3 MAL + powdered black seeds	311.57 ± 17.1[c]	1.57 ± 0.04[c]	1.17 ± 0.05[c]	62.22 ± 2.87[c]
Group 4 MAL + powdered thyme leaves	315.39 ± 15.01[c]	1.70 ± 0.05[c]	1.29 ± 0.04[c]	59.30 ± 2.07[d]

Data are expressed as the mean ± SD; means that have similar letters are not significant at $p \leq 0.01$.

Table 5
Effect of administration of PTL or black seeds on lipid profile parameters.

Rat groups	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Group 1 Control Healthy	3.51 ± 0.14[a]	1.04 ± 0.02[a]	1.29 ± 0.03[a]	1.73 ± 0.02[a]
Group 2 Malathion (MAL)	4.16 ± 0.23[b]	1.85 ± 0.04[b]	0.96 ± 0.10[b]	2.67 ± 0.06[b]
Group 3 MAL + powdered black seeds	3.77 ± 0.16[a]	1.47 ± 0.08[c]	1.16 ± 0.12b[a]	2.11 ± 0.06[c]
Group 4 MAL + powdered thyme leaves	3.67 ± 0.17[a]	1.61 ± 0.09[b]	1.13 ± 0.10[a]	2.18 ± 0.05[c]

Data are expressed as the mean ± SD; means that have similar letters are not significant at $p \leq 0.01$.

Table 6
Effect of administration of PTL or black seeds on serum acetylcholinesterase and paraoxonase-1 activities and serum tumor necrosis factor-alpha and interferon-gamma levels.

Rat groups	AchE (μmol/min/mL)	PON-1 (U/L)	TNF-α (pg/mL)	IFN-γ (IU/mL)
Group 1 Control Healthy	3.542 + 0.134[a]	292.12 + 42.26[a]	11.25 ± 2.31[a]	0.481 ± 0.054[a]
Group 2Malathion (MAL)	1.960 + 0.780[b]	152.97 + 22.53[b]	39.87 ± 5.44[b]	1.64 ± 0.151[b]
Group 3 MAL + powdered black seeds	3.247 + 0.131[a]	262.21 + 32.27[c]	16.45 ± 4.95[c]	0.476 ± 0.106[a]
Group 4 MAL + powdered thyme leaves	2.821 + 0.194[c]	260.31 + 29.39[c]	18.84 ± 3.5[c]	0.675 ± 0.118[c]

Data are expressed as the mean ± SD; means that have similar letters are not significant at $p \leq 0.01$. AchE: acetylcholinesterase; PON-1: paraoxonase-1; TNF-α: tumor necrosis factor alpha; IFN-γ: interferon-gamma.

healthy controls. Higher WBC counts in the present study indicated that the pesticides had a toxic effect on the blood and that the body's immune system tried to overcome the toxicants (Barger, 2003). Elevated WBC counts might be a pathological response to disease due to MAL intoxication. High RBC levels indicate the possibility of renal cell carcinoma or kidney cancer, pulmonary fibrosis, or polycythemia caused by MAL and cypermethrin intoxication. Reductions in Hb and HCT could be due to the increased activity of bone marrow and deficiency of some hemopoietic factors. As blood platelets play a major role in blood clotting, the decrease in platelet counts in the MAL-fed group suggests that the clot-formation process continued in response to injury, which was reflected by an excessive loss of blood (Purves et al., 2004). The results of the groups treated with PBS and PTL indicated great improvement compared to the results in the untreated MAL group. Thyme has an anticoagulation effect and prevents platelet accumulation, thrombosis, and atherosclerosis (Al-Khalaf, 2013).

In this study, we assessed the supposed resultant oxidative stress in experimental rats. Malathion induced a significant elevation in liver enzyme activities and reduction in total protein and albumin, which reflected hepatotoxicity. Moreover, the elevation in both creatinine and urea levels indicated kidney damage. Disturbances have been previously (Kamath and Rajini, 2007) detected in numerous types of organophosphorus pesticides poisons, fenthion, and dimethoate, and the detected increase in serum ALP activity was caused by elevated plasma membrane permeability. Furthermore, serum levels of uric acid have been previously shown to be affected by different organophosphorus compounds that affect uric acid reabsorption in nephron proximal tubules (Reyes, 2003).

Dietary supplementation with PBS and PTL attenuates the toxic effect of MAL, as indicated by the serum AST, ALT, and ALP levels, which indicated their hepatoprotective efficacy and demonstrated the membrane-stabilizing activity of PTL. *N. sativa* (black seeds) protect hepatocytes against tertbutyl-induced toxicity as demonstrated by reduced levels of ALT and AST (Daba and Abdel-Rahman, 1998). Treatment of MAL-intoxicated rats with PTL markedly reduced the elevated levels of transaminases in serum toward normal levels. On the other hand, dietary supplementation with PBS and PTL prevented kidney dysfunctions induced by MAL, as shown by their normalization effects on serum creatinine and urea levels.

The reduction in serum albumin was attributed to the alteration of free amino acid metabolism, synthesis in the liver, and reduction in protein synthesis or increased proteolytic activity or degradation; whereas PTL treatment prevented the reduction in serum protein level and increased serum total protein. Moreover, co-administration of thyme has been shown to alleviate the harmful effects induced by MAL by improving kidney functions (Hajimehdipoor et al., 2010).

Oxidative stress in MAL-treated male rats was manifested by increasing MDA, with reduction of CAT, total SOD, and GSH. Pesticides increase cellular membrane peroxidation by various mechanisms, including direct initiation by free radicals produced by chemical metabolism and indirect initiation by the production of reactive oxygen species through their metabolism (Sharma et al., 2005). The present study findings are consistent with those of other studies showing that organophosphorus administration caused an imbalance in the antioxidant status in liver and kidney tissues (Kalender et al., 2005).

The results of this study indicate that PBS and PTL significantly reduced the toxic effects of MAL by altering the hepatic antioxidant enzyme activities. Consequently, it is considered to be a potential hepatoprotective agent in conditions of organophosphate poisoning. Aqueous extracts of thyme are rich in total phenolic content and have radical scavenging activity. Thyme provides an efficient protective mechanism in response to reactive oxygen species because it scavenges free radicals, which is one of the major antioxidant mechanisms that underlies the inhibition of the chain reaction of lipid peroxidation (Hamzawy et al., 2012). The antioxidant property of PBS has been found to be a potential liver protectant against fibrosis, cirrhosis, and hepatic damage induced by CCL4 (Mahmoud et al., 2002). Moreover, Kanter et al. (2005) studied the effect of black seed on lipid peroxidation and the antioxidant defense system and stated that the volatile oil of black seed normalized serum antioxidant enzyme levels.

The elevation in serum TC levels observed in the present study may have been caused by the blockage of liver bile ducts that led to cholestasis thus stimulate catecholamines, which accelerates lipolysis and fatty acid production (Slotkin et al., 2005). Dietary supplementation by either PBS or PTL could increase serum HDL and thus accelerate cholesterol removal from peripheral tissue to the liver for catabolism and excretion (Jokanovic, 2009). The hypolipidemic effect of black seed has been demonstrated previously in experimental animals. Those studies reported that black seed had a favorable effect on TG and lipoprotein patterns in normal rats (Dahri et al., 2005).

Ramchoun et al. (2012) analyzed the aqueous extracts of different plants for their hypocholesterolemic and antioxidant activities and concluded that all extracts possess considerable antioxidant activities, but only thyme extract exhibited a potent hypolipidemic capacity. A study by Shattat et al. (2010) revealed that an aqueous extract of thyme leaves significantly suppressed the elevated blood concentrations of TGs, which was because this extract was able to restore the catabolism of TGs and stimulate the lipolytic activity of plasma lipoprotein lipase.

An enzyme of marked interest is PON-1 because of its ability to modulate organophosphate toxicity. This enzyme, which possesses arylesterase and lactonase activities, hydrolyzes the active metabolites (oxons) of several organophosphate insecticides and nerve agents (El-Nekeety et al., 2011). In toxicity caused by organophosphates, the decrease in PON-1 activity may be because of increased oxidative stress and consequent cell injury. Paradoxically, the enzyme is also susceptible to inactivation by oxidative stress (Nguyen and Sok, 2003). John et al. (2001) demonstrated that the phenolic compounds in thyme can increase the efficiency of enzymes involved in the antioxidant defense system and boost the body's immune cells.

Our results agreed with those of the study by Hariri et al. (2010) showing that malathion directly increased the expression of proinflammatory cytokines (TNF-α and IL-10). Mostafalou et al. (2012) examined the effects of MAL on rat inflammatory responses. Malathion was administered by gavage at different doses, and it was found that 50 mg/kg MAL significantly elevated proinflammatory cytokine expression. Lasram et al. (2014) reported that MAL (200 mg/kg) increased IFN-γ in the liver in rats. Modulation of TNF-α and IFN-γ was observed in both groups treated with either PBS or PTL. The anti-inflammatory effect of black seed and its major component, thymoquinone, has been shown in numerous studies (Shirpoor et al., 2009). A study by Juhás et al. (2008) claimed that the presence of carvacol, which is the main component of thyme, possessed an anti-inflammatory effect in paw edema induced by carrageenan application.

In conclusion, our study results showed that dietary supplementation with either PBS or PTL reduced MAL toxicity through different antioxidant and anti-inflammatory mechanisms.

Declaration of Competing Interest

Authors declare that no conflict of interest.

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