Research in Molecular Medicine

Assessment of Ginger Extract's Antioxidative Properties on Diazinon and Malathion Toxicity in Lung Cells

Saeid Yaghubii[1], Mohammad Shokrzadeh[2], Yazdan Hasani Nourian[1], Hossein Rahmani[1], Hadi Esmaeili Gouvarchin Ghaleh[3], Alireza Shahriary[1*]

1. Chemical Injuries Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Department of Toxicology and Pharmacology, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
3. Applied Virology Research Center, Biomedicine Technologies Institute, Baqiyatallah University of Medical sciences, Tehran, Iran.

Use your device to scan and read the article online

Citation Yaghubii S, Shokrzadeh M, Hasani Nourian Y, Rahmani H, Esmaeili Gouvarchin Ghaleh H, Shahriary A. Assessment of Ginger Extract's Antioxidative Properties on Diazinon and Malathion Toxicity in Lung Cells. Research in Molecular Medicine. 2024; 12(1):23-30. https://doi.org/10.32598/rmm.12.1.1361.1

https://doi.org/10.32598/rmm.12.1.1361.1

Article Type:
Research Paper

Article info:
Received: 25 Aug 2023
Revised: 15 Sep 2023
Accepted: 27 Dec 2023

Keywords:
Diazinon, Malathion, Ginger, Organophosphorus, Toxicity

ABSTRACT

Background: Diazinon and Malathion are organophosphorus toxins. Ginger has been widely recognized for its antioxidant properties, and its ethanolic extract has shown potential therapeutic effects. This study aimed to evaluate the antioxidant effect of ethanolic ginger extract on toxicity caused by diazinon and malathion in human lung epithelial cells. Organophosphorus toxins are known to induce oxidative stress and cellular damage.

Materials and Methods: The ginger roots were crushed and solved in 70% ethanol. The ethanol solution was then removed by evaporation using a rotary evaporator to obtain a concentrated extract. To assess cell growth inhibition, the MTT assay was employed. Lipid peroxidation levels were determined using TBA reagents. Additionally, the glutathione content in human lung epithelial cells was measured using the DTNB reagent.

Results: The ginger extract in combination with malathion and diazinon led to a significant increase in cell viability, particularly at higher doses, compared to the control group ($P<0.05$). The ethanol extract of ginger exhibited a dose-dependent reduction in the MDA level in the treated groups, which was significantly lower than that of the control group ($P<0.05$). Moreover, the treatment groups receiving doses of 100, 500, and 1000 µg/ml of ginger extract showed a significant increase in glutathione levels ($P<0.05$). The protective effects were particularly pronounced at doses of 500 and 1000 µg/ml of ginger extract.

Conclusion: The ethanolic extract of ginger possesses significant antioxidant properties, which can mitigate the toxicity caused by diazinon and malathion in human lung epithelial cells.

*** Corresponding Author:**
Alireza Shahriary, Associate Professor.
Address: *Chemical Injuries Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.*
Phone: *+98 (939) 1357042*
E-mail: *shahriary961@yahoo.com*

Introduction

Organophosphorus pesticides, such as diazinon (DZ) and malathion, are widely used in agriculture and residential settings [1]. Initially, the application of pesticides was introduced as a means to prevent, control, and eradicate undesirable insects, pests, and the diseases they carry [2]. However, the escalated usage of these chemical compounds has raised significant concerns both for the environment and public health [1]. Malathion, an organophosphate insecticide, is applied in agriculture, commercial extermination, fumigation, veterinary practices, as well as domestic and public health initiatives [3]. Due to its low toxicity to mammals, malathion has become one of the most commonly employed organophosphate compounds in the United States, consequently serving as a primary source of occupational pesticide exposure [4]. DZ, chemically known as O,O-diethyl-O-[2-isopropyl-6-methyl-4 pyrimidinyl] phosphorothioate, is an organophosphorus compound extensively utilized as a soil pesticide in agricultural practices [5]. The presence of DZ in fruits and vegetables has emerged as a global concern. DZ demonstrates various toxic effects in experimental animals. It induces significant histopathological lesions in the liver, kidney, and brain, resulting in damage to several organ systems [6]. Apart from DZ and malathion's primary mechanism of action as acetylcholinesterase inhibitors, evidence suggests that organophosphorus pesticides can induce oxidative stress and cellular damage in various organs, including the lungs [7, 8]. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. Excessive ROS production leads to oxidative damage, including lipid peroxidation, protein oxidation, and DNA damage. The lungs, being constantly exposed to environmental pollutants and airborne toxins, are particularly susceptible to oxidative stress-induced injuries [9].

Ginger (*Zingiber officinale*) is a medicinal plant known for its various therapeutic properties. It contains bioactive compounds, such as gingerols, shogaols, and paradols, which possess potent antioxidant and anti-inflammatory activities [10, 11]. In the context of organophosphorus pesticide toxicity, it is crucial to explore potential natural remedies that can mitigate oxidative stress and protect lung health [12]. Understanding the protective effects of ginger in the context of organophosphorus pesticide-induced toxicity could provide valuable insights for developing preventive and therapeutic strategies. The aim of this study was to evaluate the antioxidant effect of ethanolic ginger extract on human lung epithelial cells exposed to DZ and malathion.

Materials & Methods

Preparation of the botanical sample and extraction process

To obtain the ginger extract, 10 g of ginger root was subjected to maceration in 70% ethanol at a ratio of 1:5 (w/v) for 72 hours. The ethanol was then evaporated using a rotary evaporator (Heidolph, Schwabach, Germany) until 99% of the solvent was removed. To obtain a pure extract, a fresh solvent was added three times. The concentrated extract was subsequently stored at 4 °C until it was ready for use.

Cell culture

In this study, the BEAS-2B cell line was utilized, which was obtained from the Pasteur Institute of Iran. To initiate cell culture, a flask containing culture medium, composed of DMEM, 1% Pen Strep, and 10% FBS, was prepared and cells were cultured in 5% CO_2 at 37 ˚C.

MTT assay

The cell suspensions were then incubated until reaching the log phase of growth, approximately 10^9 cells. When the primary culture approached confluence, the flask was incubated with trypsin-EDTA for 5 minutes to detach the cells. Subsequently, 100 µL of the cell suspension was added to 96-well plates and allowed to incubate for 4 hours. Following a 24-hour incubation period, the cell line was subjected to a single dose of malathion and DZ. Additionally, ginger was administered at concentrations of 0.1, 1, 10, 100, 500, and 1000 g/mL. The cells were then incubated for 48 hours. Subsequently, each well was treated with 50 µL of MTT solution and incubated for 4 hours. After removing the contents of each well and washing them with phosphate-buffered saline, 50 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan dye. Finally, the absorbance of the wells was measured at 490 nm and 630 nm using an ELISA reader.

Glutathione (GSH) concentration measurement

The GSH level in the cells was measured using DTNB (5,5′-dithiobis [2-nitrobenzoic acid]) as the indicator, and a spectrophotometer (UV-1601 PC, Shimadzu, Japan) was employed to determine the GSH content. In brief, a 0.5 ml sample or standard solution was mixed with 0.25 ml of 1 M sodium phosphate buffer (pH 6.8) and 0.5 ml of DTNB. After 5 minutes, the absorbance of the mixture was measured at 412 nm using the spectrophotometer. The GSH

concentration was calculated based on a standard curve generated using known GSH concentrations, and the results were expressed as micromoles (μM). This method provided a quantitative assessment of the GSH levels in the cells, allowing for the evaluation of the impact of the experimental conditions on the cellular antioxidant status.

Lipid peroxidation assessment

For lipid peroxidation assessment, the level of malondialdehyde (MDA) was measured using the thiobarbituric acid (TBA) test. A cell suspension was mixed with TBA reagent (0.1 mL) and phosphoric acid (0.1 mL). The mixture was then subjected to a warm water bath at 100°C for 30 minutes, followed by 5 minutes of cooling on ice. After cooling, n-butanol (0.2 mL) was added to the mixture, thoroughly shaken, and then centrifuged for 10 minutes at 3500 rpm. The supernatant was collected, and the absorbance of the resulting solution was measured at 532 nm using a BioTek ELx800 microplate reader.

Statistical analysis

The results were presented as Mean±SD, which was calculated based on three repetitions of the experiment. All statistical analyses were performed using Prism statistical software, version 9. To compare the data, a one-way analysis of variance (ANOVA) was conducted, followed by the Tukey test as the post-test for pairwise comparisons. A $P<0.05$ was considered to indicate a significant difference between the groups.

Results

The effect of ginger along with DZ and malathion

Malathion and DZ, both individually and in combination with ginger at all doses, exhibited a significant impact on cell viability compared to the control group, as well as naringin at a dose of 500 and ginger at a dose of 1000 ($P<0.05$). Furthermore, when compared to the naringin group, the combination of DZ with ginger at all doses displayed a significant effect ($P<0.05$). Notably, the combination of DZ with ginger at all doses resulted in a significantly higher cell viability compared to DZ alone ($P<0.05$). The results indicated that as the ginger dose increased, there was a significant enhancement in cell viability. These findings suggest a potential beneficial effect of ginger in mitigating the toxic effects induced by DZ and enhancing cell viability (Figures 1 and 2). Combining malathion with ginger at concentrations of 500 and 1000 μg/mL resulted in a significant improvement in cell viability compared to using malathion and DZ alone ($P<0.05$) (Figures 1 and 2).

The effect of ginger on oxidative stress markers

As shown in Table 1, treatment led to a decrease in GSH levels, which ginger treatment, in a dose-dependent manner, can suppress ($P<0.05$). Moreover, malathion and DZ treatment led to an increase in LPO levels, which can be suppressed with ginger treatment in a dose-dependent manner ($P<0.05$).

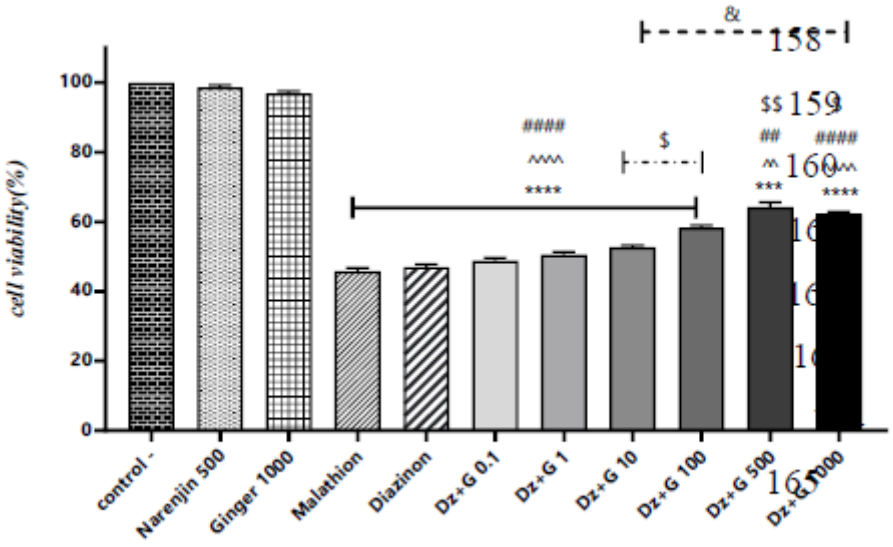

Figure 1. The effect of ginger in combination with diazinon on cell viability
*P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

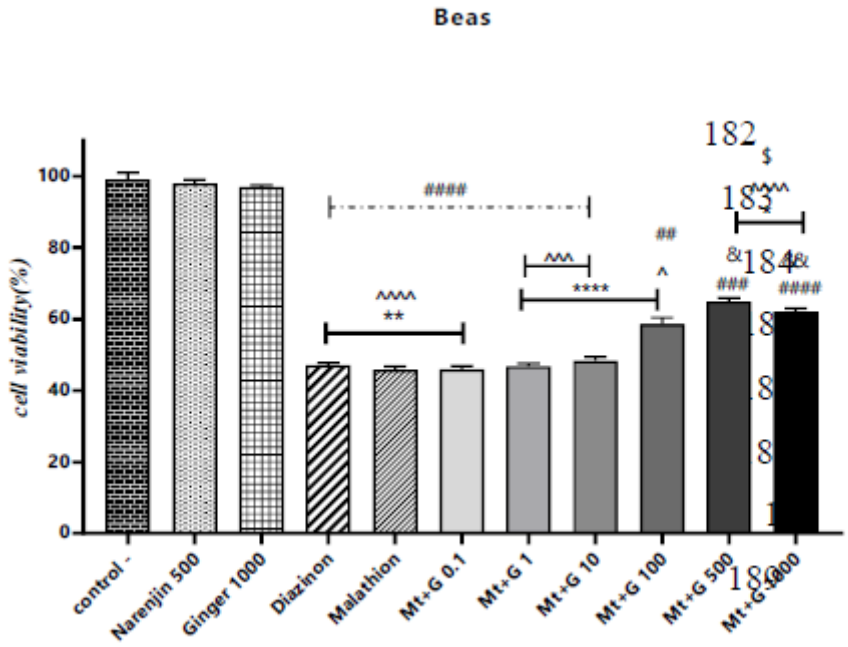

Figure 2. The effect of ginger in combination with malathion on cell viability
Mt: Malathion.
*P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

Table 1. The effect of ginger on oxidative stress markers in study groups

Variables / Groups	GSH		LPO	
	Diazinon	Malathion	Diazinon	Malathion
Control	98.67(0.58)	97(1)	2.24(0.83)	2.24(0.93)
Narenjin 500	98.33(0.578)	98.33(0.58)	1.38(0.68)	1.38(68)
Ginger 1000	96.67(0.58)	96.67(0.58)	3.53(0.61)	3.53(0.61)
Malathion	58.67(1.07)	58.67(1.07)	71.04(0.96)	71.24(0.70)
Diazinon	62.16(1.92)	62.16(1.92)	67.56(1.45)	67.59(0.76)
G 0.1	65(0.99)^{a}	66.06(0.94)^{a}	66.01(0.95)^{a}	64.11(1.01)^{a}
G 1	67.16(0.92)^{b}	68.69(1.14)^{ab}	63.1(0.92)^{b}	61.66(0.57)^{b}
G 10	69.23(.62)^{c}	71.47(1.47)^{ac}	59.7(1.11)^{bc}	58.59(1.24)^{abc}
G 100	78.3(1.47)^{ab}	76.23(0.43)^{ab}	55.6(0.88)^{bcd}	54.36(0.89)^{abcd}
G 500	82(1)^{abc}	79.63(1.11)^{abc}	49.97(0.92)^{abcd}	48.76(0.67)^{abcde}
G 1000	79(1)^{abc}	76.93(0.90)^{ab}	51.65(0.59)^{ab}	53.4(1.39)^{abce}

LPO: Lipid peroxidation, GSH: Glutathione.
Significant statistical differences between groups in each index are indicated by the different superscript letter (P<0.05).

Common letters in each column indicate a significant difference between two groups of the same column (for example, in column 1, the letter 'a' indicates a significant difference between the G 0.1 group and the G 100, 500, and 1000 groups).

Discussion

The present study aimed to assess the anti-oxidative properties of ginger extract on DZ and malathion toxicity in human lung epithelial cells. The findings revealed significant effects of ginger extract on cell viability, lipid peroxidation, and GSH levels, indicating its potential as a protective agent against organophosphorus toxin-induced toxicity. Firstly, the combination of malathion and DZ with ginger extract resulted in a notable increase in cell viability, especially at higher doses. This suggests that ginger extract may have a positive impact on cell survival and protection against the cytotoxic effects of these toxins. The observed increase in cell viability could be attributed to the antioxidant properties of ginger extract, which may help mitigate the oxidative stress induced by the toxins. The antioxidant effects of ginger have been confirmed previously. In this regard, Masuda et al. reported that the antioxidant activity might be due to not only the radical scavenging activity of antioxidants but also the affinity of the antioxidants for the substrates [13]. Moreover, Tohma et al. demonstrated the antioxidant properties of ginger extracts, suggesting that the consumption of the extract may reduce or delay the development of diseases associated with oxidative stress due to an imbalance in antioxidant supplementation [14].

Furthermore, the ethanol extract of ginger demonstrated a dose-dependent reduction in MDA levels compared to the control group. MDA is a marker of lipid peroxidation, and elevated MDA levels indicate cellular damage caused by oxidative stress. The significant decrease in MDA levels suggests that ginger extract has the ability to inhibit lipid peroxidation induced by DZ and malathion. This protective effect can be attributed to the presence of bioactive compounds in ginger extract, such as ginger and other phenolic compounds, which possess strong antioxidant properties [15]. In addition, the assessment of GSH levels in the treatment groups revealed a significant increase in cells treated with various doses of ginger extract. GSH is a key antioxidant molecule involved in cellular defense against oxidative stress [16]. The elevation of GSH levels indicates the enhanced antioxidant capacity of the cells, which may contribute to the reduction of toxicity induced by DZ and malathion [17]. Ginger extract may stimulate the synthesis of GSH or prevent its depletion, further strengthening the cellular antioxidant defense mechanism [18]. Previous studies have demonstrated the ability of ginger to effectively scavenge ROS and prevent oxidative damage induced by malathion. Additionally, ginger has been found to reduce lipid peroxidation by acting as a scavenger for free radicals. Moreover, ginger extract has been shown to lower lipid peroxidation levels and increase GSH levels [19, 20]. In the liver and kidney, ginger extract can decrease Malathion toxicity through liver enzyme modulation and antioxidant effects [21]. The anti-oxidative properties of ginger may be associated with its protective mechanisms, as GSH plays a crucial role in regulating ROS.

Moreover, earlier research has indicated that ginger possesses antioxidant properties, as it has been found to decrease oxidative stress induced by cisplatin [19]. This suggests that ginger has the ability to mitigate oxidative damage through its anti-oxidative effects. Notably, the protective effects of ginger extract were more pronounced at doses of 500 and 1000 µg/ml. This suggests a dose-dependent response, where higher concentrations of ginger extract lead to greater protection against toxin-induced toxicity. However, it is important to consider the potential toxicity or adverse effects of higher doses of ginger extract, which should be further investigated to ensure safety and optimal therapeutic efficacy. A different study demonstrated the hepatotoxic and nephrotoxic effects of malathion, emphasizing the importance of avoiding its exposure and providing protection through the supplementation of a mixture containing ginger and zinc [22]. The findings of this study provide valuable insights into the anti-oxidative properties of ginger extract against DZ and malathion toxicity in human lung epithelial cells. Ginger extract demonstrated significant protective effects, including increased cell viability, reduced lipid peroxidation, and enhanced GSH levels. These results support the potential use of ginger extract as a natural antioxidant in combating organophosphorus toxin-induced lung toxicity. Further studies are warranted to elucidate the underlying molecular mechanisms and to evaluate the effectiveness of ginger extract in vivo and in clinical settings.

Conclusion

The ethanolic extract of ginger possesses significant antioxidant properties, which can mitigate the toxicity caused by DZ and malathion toxins in human lung epithelial cells.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Baqiyatallah University of Medical Sciences, Tehran, Iran (Code: IR.BMSU.REC.1399.449). Besides, ethical issues (including plagiarism, data fabrication, and double publication) have been completely observed by the authors.

Funding

This article was extracted from a research project approved by Baqiyatallah University of Medical Sciences (Code: 99000280) and financially supported by the Baqiyatallah University of Medical Sciences, Tehran, Iran.

Authors contribution's

Conceptualization, methodology and funding acquisition: Alireza Shahriary and Hossein Rahmani; Validation: Alireza Shahriary, Hossein Rahmani and Mohammad Shokrzadeh; Formal analysis: Alireza Shahriary and Mohammad Shokrzadeh; Investigation: Alireza Shahriary, Hossein Rahmani, Saeid Yaghubii and Mohammad Shokrzadeh; Resources: Alireza Shahriary; Data collection: Saeid Yaghubii; Writing of the original draft: Hossein Rahmani; Review, and editing: Hossein Rahmani, Yazdan Hasani Nourian and Hadi Esmaeili Gouvarchin Ghaleh; Visualization and supervision: Hossein Rahmani, Alireza Shahriary and Yazdan Hasani Nourian; Project administration: Hossein Rahmani, Hadi Esmaeili Gouvarchin Ghaleh and Mohammad Shokrzadeh .

Conflict of interest

The authors declared no conflicts of interests.

Acknowledgements

The researchers gratefully thank the scientific colleagues who participated in this study.

References

[1] Sobolev VE, Sokolova MO, Jenkins RO, Goncharov NV. Molecular mechanisms of acute organophosphate nephrotoxicity. Int J Mol Sci. 2022; 23(16):8855. [DOI:10.3390/ijms23168855] [PMID]

[2] Flint ML, Van den Bosch R. Introduction to integrated pest management. Berlin: Springer Science & Business Media; 2012. [Link]

[3] Roberts JR, Karr CJ; Council On Environmental Health. Pesticide exposure in children. Pediatrics. 2012; 130(6):e1765-88. [DOI:10.1542/peds.2012-2758] [PMID]

[4] Abdo W, Elmadawy MA, Abdelhiee EY, Abdel-Kareem MA, Farag A, Aboubakr M, et al. Protective effect of thymoquinone against lung intoxication induced by malathion inhalation. Sci Rep. 2021; 11(1):2498. [DOI:10.1038/s41598-021-82083-w] [PMID]

[5] Briceño G, Schalchli H, Rubilar O, Tortella GR, Mutis A, Benimeli CS, et al. Increased diazinon hydrolysis to 2-isopropyl-6-methyl-4-pyrimidinol in liquid medium by a specific Streptomyces mixed culture. Chemosphere. 2016; 156:195-203. [DOI:10.1016/j.chemosphere.2016.04.118] [PMID]

[6] Slotkin TA, Ryde IT, Levin ED, Seidler FJ. Developmental neurotoxicity of low dose diazinon exposure of neonatal rats: Effects on serotonin systems in adolescence and adulthood. Brain Res Bull. 2008; 75(5):640-7. [DOI:10.1016/j.brainresbull.2007.10.008] [PMID]

[7] Agrawal AN, Sharma B. Pesticides induced oxidative stress in mammalian systems. Int J Biol Med Res. 2010; 1(3):90-104. [Link]

[8] Possamai FP, Fortunato JJ, Feier G, Agostinho FR, Quevedo J, Wilhelm Filho D, et al. Oxidative stress after acute and sub-chronic malathion intoxication in Wistar rats. Environ Toxicol Pharmacol. 2007; 23(2):198-204. [DOI:10.1016/j.etap.2006.09.003] [PMID]

[9] Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E. The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. Int J Mol Sci. 2021; 22(9):4642. [DOI:10.3390/ijms22094642] [PMID]

[10] Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, et al. Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). Foods. 2019; 8(6):185. [DOI:10.3390/foods8060185] [PMID]

[11] Arcusa R, Villaño D, Marhuenda J, Cano M, Cerdà B, Zafrilla P. Potential role of Ginger (Zingiber officinale Roscoe) in the prevention of neurodegenerative diseases. Front Nutr. 2022; 9:809621. [DOI:10.3389/fnut.2022.809621] [PMID]

[12] Zeng X, Du Z, Ding X, Jiang W. Protective effects of dietary flavonoids against pesticide-induced toxicity: A review. Trends Food Sci Technol. 2021; 109:271-9. [DOI:10.1016/j.tifs.2021.01.046]

[13] Masuda Y, Kikuzaki H, Hisamoto M, Nakatani N. Antioxidant properties of gingerol related compounds from ginger. Biofactors. 2004; 21(1-4):293-6. [DOI:10.1002/biof.552210157] [PMID]

[14] Tohma H, Gülçin İ, Bursal E, Gören AC, Alwasel SH, Köksal E. Antioxidant activity and phenolic compounds of ginger (Zingiber officinale Rosc.) determined by HPLC-MS/MS. J Food Meas Charact. 2017; 11:556-66. [DOI:10.1007/s11694-016-9423-z]

[15] Soror E, El Asely AM, Gawad EA, Radwan HA, Abbass AA. Recuperative effects effects of honey bee pollen, ginger (Zingiber officinale), and Moringa oleifera in Nile tilapia (Oreochromis niloticus L.) after sub-lethal exposure to dimethoate. Aquaculture. 2021; 530:735886. [DOI:10.1016/j.aquaculture.2020.735886]

[16] Surai PF, Kochish II, Fisinin VI, Kidd MT. Antioxidant defence systems and oxidative stress in poultry biology: An update. Antioxidants. 2019; 8(7):235. [DOI:10.3390/antiox8070235] [PMID]

[17] Ince S, Arslan-Acaroz D, Demirel HH, Varol N, Ozyurek HA, Zemheri F, et al. Taurine alleviates malathion induced lipid peroxidation, oxidative stress, and proinflammatory cytokine gene expressions in rats. Biomed Pharmacother. 2017; 96:263-8. [DOI:10.1016/j.biopha.2017.09.141] [PMID]

[18] Baliga MS, Haniadka R, Pereira MM, Thilakchand KR, Rao S, Arora R. Radioprotective effects of Zingiber officinale Roscoe (ginger): Past, present and future. Food Funct. 2012; 3(7):714-23. [DOI:10.1039/c2fo10225k] [PMID]

[19] Badr AM. Organophosphate toxicity: Updates of malathion potential toxic effects in mammals and potential treatments. Environ Sci Pollut Res Int. 2020; 27(21):26036-57. [DOI:10.1007/s11356-020-08937-4] [PMID]

[20] Shanmugam KR, Mallikarjuna K, Nishanth K, Kuo CH, Reddy KS. Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues. Food Chem. 2011; 124(4):1436-42. [DOI:10.1016/j.foodchem.2010.07.104]

[21] Abdelbaky MS, Hamad MS, El-Soauly RE. Effect of ginger on hepatotoxicity and nephrotoxicity induced by malathion in rats. J Appl Nutr Sci. 2022; 1(2):63-76. [Link]

[22] Baiomy AA, Attia HF, Soliman MM, Makrum O. Protective effect of ginger and zinc chloride mixture on the liver and kidney alterations induced by malathion toxicity. Int J Immunopathol Pharmacol. 2015; 28(1):122-8. [DOI:10.1177/0394632015572083] [PMID]

This Page Intentionally Left Blank