Protective Effect of Tert Butyl Hydroquinone on Diazinon-Induced Oxidative Stress in Brain and Heart of Male Rats

Saman Sargazi,1 Amir Moghadam-Jafari,2 and Mohammad Heidarpour3*

1Department of Biochemistry and Molecular Biology, Shahid Sadoughi University of Medical Sciences, Yazd, I.R. Iran
2Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, I.R. Iran
3Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, I.R. Iran

Corresponding author: Mohammad Heidarpour, Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, I.R. Iran. E-mail: heidarpour@um.ac.ir

Received 2015 January 08; Accepted 2015 May 22.

Abstract

Background: The present study was designed to investigate the chronic effects of diazinon on oxidative stress markers in brain and heart of rats and the possible protective effects of tert butyl hydroquinone (TBHQ), as an antioxidant.

Objectives: Therefore, the present study was undertaken to evaluate the protective role of TBHQ on oxidative stress induced by diazinon in brain and heart tissues of rats after 7 weeks exposure to sub-lethal dose of diazinon.

Materials and Methods: In this experimental study, 28 male Wistar rats which were randomly divided into four groups: diazinon group (10 mg/kg, once a day), TBHQ group (0.028 g/kg of diet, once a day), diazinon + TBHQ group (diazinon, 10 mg/kg, once a day + TBHQ, 0.028 g/kg of diet, once a day) and control group (corn oil, as vehicle of diazinon and TBHQ). The animals were treated with diazinon, TBHQ or corn oil orally using a stomach tube for 7 weeks.

Results: At the end of 7th week, total thiol groups, ferric reducing antioxidant power (FRAP) and malondialdehyde (MDA) levels in brain and heart tissues were investigated. A significant increase in MDA levels (P = 0.01) in heart tissue was evident in diazinon group, when compared to control group. Rats of the TBHQ and diazinon + TBHQ groups presented a significant increase in thiol groups (P = 0.01), when compared to control and diazinon groups. In addition, TBHQ administration significantly increased the FRAP level of brain tissue in TBHQ and TBHQ + diazinon groups, when compared with control and diazinon groups, respectively (P = 0.001).

Conclusions: The results of the present study showed that TBHQ treatment could improve antioxidant status in brain and heart tissues of rats with chronic toxicity of diazinon. However, it could not ameliorate the lipid peroxidation sufficiently.

Keywords: Diazinon, Tert Butyl Hydroquinone, Oxidative Stress, Brain, Heart

1. Background

Population growth and consequently the increase in food consumption, especially agricultural products, have prompted farmers to increase their yields. Increasing planting, will consequently lead to the increase in using pesticides. One of the main concerns of the world health organization (WHO) is the uncontrolled use of pesticides in the agricultural industry [1] and exposure to pesticides is considered as a public health problem in rural areas [2]. Among the pesticides, organophosphates are commonly used which is due to their non-biodegradability decomposition and discontinuous nature [3]. Organophosphate pesticides generally have high toxicity and have a high potential to negatively act on non-target organisms. As a significant matter, poisoning from organophosphates is a major reason for the prevalence of disease and death in third world countries [4]. A number of factors such as dosage, way of exposure, absorption rate, physicochemical properties and the level of neutralization of the toxin by the body, are involved in the intensity and duration of poisoning [5]. The main mechanism of pathogenicity in acute poisoning with organophosphate, is the irreversible inhibition of the enzyme acetylcholinesterase, which causes an accumulation of acetylcholine and acute muscarinic and nicotinic effects. However, in sub chronic or chronic poisoning by organophosphate, induction of oxidative stress is called as a central mechanism [6]. Oxidative stress represents an imbalance between the production of various free radicals of oxygen and the ability of the biological system for detoxification or repairing the damaging effects of their oxidative damages. Therefore, it will result in oxidative damages to the cell, texture, or organs of the body. Both increased production of reactive oxygen and decreased antioxidant capacity of the body may cause oxidative stress [7]. In several studies the role of oxidative stress in organophosphate-induced damage has been reported [8-13]. Diazinon (O, O-diethyl-o-[2-isopropyl-
6-methyl-4-pyrimidinyl[phosphorothioate] is one of the most commonly used organophosphates in the world [14]. Diazinon affects mitochondrial membrane transportation and cytochrome P450 system in hepatocytes [15, 16]. Administration of diazinon to rats resulted in depletion of glycogen from the brain and peripheral tissues [17]. It has also been shown that diazinon caused an increase in lipid peroxidation in rat erythrocytes [18]. Diazinon treatment in rats decreased renal antioxidants and enhanced lipid peroxidation with concomitant renal damage, which are involved in the diazinon-induced renal oxidative stress and toxicity [19]. Anti-oxidants are substances that destroy the effects of free radicals in the body and prevent their acts. Antioxidants exist in some specific nutrients and neutralize free radicals. Tert butyl hydroquinone (TBHQ) is an organic aromatic compound which is derived from the hydroquinone. TBHQ is a powerful antioxidant which is used as an additive for unsaturated vegetable oils and many edible animal fats. TBHQ is able to induce the nuclear translocation of transcription factor NF-E2-related factor 2 (Nrf2), which in turn regulates the expression of vitagenes codifying for cytoprotective phase 2 antioxidant proteins, such as glutathione-S-transferase, NAD(P)H quinone oxidoreductase and heme-oxygenase-1 [20]. The use of this antioxidant in case of proving its protective effect could be a useful solution for the prevention of chronic toxicity in individuals who are exposed to the organophosphates, particularly the farmers. Combined effects of diazinon and TBHQ have not been studied before.

2. Objectives

Therefore, the present study was undertaken to evaluate the protective role of TBHQ on oxidative stress induced by diazinon in brain and heart tissues of rats after 7 weeks exposure to sub-lethal dose of diazinon.

3. Materials and Methods

3.1. Chemicals, Animals and Protocol Design

In this experimental study, all the chemicals used in the present study were of technical grade and were supplied by Sigma-Aldrich (Germany) or Merk (Germany) companies.

Twenty eight adult male Wistar-rats, weighing in average 230 - 250 g, which were purchased from the Razi Vaccine and Serum Research institute, Mashhad, Iran. The animals were acclimatized for one week before the onset of experiment. During the experiment, all ethical principles of animal testing were fully considered. The animals were housed in plastic (polypropylene) cages using paddy husk bedding at room temperature (25 ± 1°C) in a 12-hour light/dark cycle with 50 ± 5% humidity. The animals had free access to commercial pellet diet (manufactured in Javaneh Khorasan, Mashhad, Iran) and water ad libitum. The experiment was approved by the Animal Welfare Committee of the School of the Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. The animals were randomly divided into 4 groups having 7 animals in each. The compounds were administrated in the morning (between 9:00 and 11:00 am) to non-fasted rats. All rats were treated for 7 weeks.

3.2. Group 1, Control group

That received corn oil (vehicle of diazinon and TBHQ) through gavages once a day.

3.3. Group 2. Diazinon-Treated Group

Diazinon at the dose of 10 mg/kg/day in corn oil was given through gavage to rats once a day. The selection of dose regimen was based on previously published studies which indicate substantial alterations in many of the biochemical parameters at this dose [14, 19].

3.4. Group 3. TBHQ Treated Group

TBHQ at a dose of 0.028 g/kg of diet was given through gavage to rats once a day. The selected dose of TBHQ was based on previously published studies which indicate antioxidative effects of TBHQ at this level [21]. The food intake of rats was measured during the acclimatization period. Pre-weighed food was provided in standard stainless steel hoppers. After 24 hours, the amount of food remaining, including any on the bottom of the cages or any that had spilled onto plastic sheets placed under each cage was recorded. Intake was calculated as the weight (in grams) of food provided less that recovered.

3.5. Group 4, TBHQ + Diazinon-Treated Group

TBHQ and diazinon (at above-mentioned doses) were administered orally via gavage needle.

3.6. Tissue Sample Preparation and Biochemical Analysis

The animals were euthanized by CO₂ 24 hours after the last oral administration. Brain and heart tissues of these animals were taken quickly, cleaned free of extraneous material and perfused immediately with sodium phosphate buffer (pH = 7.4). Tissue samples were minced, cut into small pieces and then dried on a filter paper and homogenized (10% w/v) in ice-cold 1.15% KCl-0.01 M sodium, potassium phosphate buffer (pH = 7.4) by Silent crusher M type homogenizer (Heidolph Instruments GmbH and Co. KG, Schwabach, Germany). The homogenate was centrifuged.
at 18,000 g for 20 minutes at 4°C, and the resultant super- 
natant was used for the determination of oxidative stress 
markers.

3.7. MDA Assay

The extent of lipid peroxidation was estimated as the 
concentration of thiobarbituric acid reactive product mal-
ondialdehyde (MDA) by using the method of Placer et al. 
[22]. The reaction mixture consisted of 0.2 mL of homog-
enized tissue, 1.3 mL of 0.2 M Tris-0.16 M KCl buffer (pH = 
7.4) and 1.5 mL of thiobarbituric acid reagent. The mix-
ture was heated in a boiling water bath for 10 min. After 
cooling, 3 mL of pyridine/n-butanol (3:1, v/v) and 1 mL of 1-
normal sodium hydroxide were added and mixed by vigor-
ous shaking. A blank was run simultaneously by incorpo-
rating 0.2 mL distilled water instead of the homogenized 
tissue. The absorbance of the test sample was read at 548 
nm. The nanomols of MDA per milliliter of homogenized 
tissue were calculated using $1.56 \times 10^5$ as extinction coeffi-
cient.

3.8. Total Thiol Group Assay

Total thiol groups of homogenized tissues were mea-
sured spectrophotometrically at 412 nm using DTNB [5, 5'-
dithiobis-(2-nitrobenzoic acid)] as the reagent [23]. After 
adding tris buffer to homogenized tissue, first absorbance 
was read at 412 nm (A1). Then DTNB was added and second 
absorbance at 412 nm was done (A2). The concentration of 
total thiol groups was calculated and expressed as mmol/L.

3.9. FRAP Assay

The total antioxidant capacity of the homogenized tis-
ues was measured using FRAP assay, which depends upon 
the reduction of ferric tripyridyltriazine [Fe(III)-TPTZ] com-
plex to the ferrous tripyridyltriazine [Fe(II)-TPTZ] by a re-
ductant at low pH. [Fe(II)-TPTZ] has an intensive blue color 
and can be monitored at 593 nm [24].

3.10. Statistical Analysis

Statistical analysis was conducted using SPSS for win-
dows (Release 16, SPSS Inc., Chicago, IL) with a value of P < 
0.05 as statistically significant. Data were expressed as 
mean ± standard deviation (SD). One way ANOVA was used 
to compare means among the different groups. Following 
analysis of variance, significant between-group differences 
were detected by the Bonferroni test.

4. Results

A significant increase in MDA levels (P = 0.01) in heart 
tissue was evident in the diazinon group, when compared 
to the control group. TBHQ alone or associated with diazi-
non, did not reduce lipid peroxidation, and no significant 
differences were observed for MDA level between TBHQ 
and control groups and between diazinon and diazinon + 
TBHQ groups (Table 1). No significant differences were 
found for total thiol groups between diazinon and control 
groups. However, rats of the TBHQ and diazinon + TBHQ 
groups presented a significant increase in thiol groups (P = 
0.01), when compared to control and diazinon groups 
(Table 1). A significant increase (P = 0.013) in FRAP levels 
in heart and brain tissues was observed in the diazinon 
group, when compared to the control group. TBHQ admin-
istration significantly increased (P = 0.001) the FRAP level 
of brain tissue in both TBHQ and TBHQ + diazinon groups, 
when compared with the control and diazinon groups, re-
spectively (Table 1).

5. Discussion

Extensive application of organophosphates is usually 
accompanied with serious problems of pollution and 
health hazards. Organophosphates act as pro-oxidants and 
elicit oxidative effects in multiple organs. Reactive oxygen 
spices (ROS) are produced as the result of the metabolism 
of OPs by cytochrome P450s. Glycated proteins activate 
specific membrane receptors and induce an intracellular 
oxidative stress. In recent years, some agents with an-
tioxidant effects (e.g. vitamins C and E, N-acetylcysteine 
and zinc) have been used to decrease cellular oxidative 
stress and thus cellular damage, in cases of organophos-
phate poisoning [18, 25]. Tert-butylhydroquinone (TBHQ) 
is one of the few antioxidants permitted for use in foods. It 
has furthermore been shown to protect the living animal 
and cell lines against acute toxicity and oxidative results 
[2]. The present study was designed to elucidate changes 
of oxidative stress markers in heart and brain of rats fol-
lowing chronic administration of diazinon and the poten-
tial antioxidant effects of TBHQ , as a food-additive antiox-
idant. It has been shown that organophosphate intoxica-
tion produces oxidative stress by generation of ROS and 
free radicals and induces tissue lipid peroxidation [6]. In 
the present study, administration of 10 mg/kg of diazinon 
for 7 weeks induced lipid peroxidation in heart tissue. In 
consistent with the present study, Ogutcu et al. reported 
that diazinon (10 mg/kg, for 7 weeks) caused increase of 
MDA level of rat heart [26]. Enhanced lipid peroxidation in-
duced by diazinon, has also been reported in other cells or 
tissues such as erythrocytes [27], liver [28] brain and spleen
Table 1. Effects of Diazinon and TBHQ on the MDA, Total Thiol Groups and FRAP levels in Heart and Brain of Rats

<table>
<thead>
<tr>
<th>Effects</th>
<th>Control (n = 7)</th>
<th>Diazinon (n = 7)</th>
<th>TBHQ (n = 7)</th>
<th>Diazinon-TBHQ (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>6.01 ± 1.02b</td>
<td>6.83 ± 0.99b</td>
<td>5.55 ± 0.88b</td>
<td>7.30 ± 0.68c</td>
</tr>
<tr>
<td>Heart</td>
<td>3.77 ± 0.82b</td>
<td>7.55 ± 1.04b</td>
<td>5.30 ± 0.32b</td>
<td>6.48 ± 1.12c</td>
</tr>
<tr>
<td>Total thiol groups, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.34 ± 0.09b</td>
<td>0.31 ± 0.06b</td>
<td>0.51 ± 0.06c</td>
<td>0.55 ± 0.12c</td>
</tr>
<tr>
<td>Heart</td>
<td>0.32 ± 0.04b</td>
<td>0.27 ± 0.05b</td>
<td>0.48 ± 0.11c</td>
<td>0.55 ± 0.08c</td>
</tr>
<tr>
<td>FRAP, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>1.18 ± 0.07b</td>
<td>1.34 ± 0.09c</td>
<td>1.35 ± 0.04c</td>
<td>1.50 ± 0.10d</td>
</tr>
<tr>
<td>Heart</td>
<td>1.23 ± 0.04b</td>
<td>1.37 ± 0.09cd</td>
<td>1.27 ± 0.09ce</td>
<td>1.45 ± 0.07d</td>
</tr>
</tbody>
</table>

Abbreviation: FRAP, ferric reducing antioxidant power; MDA, malondialdehyde; TBHQ, tert butyl hydroquinone.

aValues are expressed as mean ± SD.
bMeans within rows lacking a common lowercase letters (b, c, d) differ (P < 0.05).

The severity of diazinon-induced lipid peroxidation in different tissues depends on several factors such as oxygen consumption, metabolic activity rate and susceptibility to oxidants [29]. The use of antioxidants has been suggested as one of the methods of dealing with the toxicity of organophosphates in various studies [18, 25, 26, 31]. TBHQ is a derivative of hydroquinone, substituted with tert-butyl group. TBHQ is a highly effective antioxidant. In foods, it is used as a preservative for unsaturated vegetable oils and many edible animal fats. In the present study, TBHQ treatment improved antioxidant status in heart and brain tissues of rats. Rats of the TBHQ and diazinon + TBHQ groups presented a significant increase in total thiol groups and FRAP levels. TBHQ is a very potent activator of Nrf2 transcription factor, which has an important role in enzyme defending of the cells against the oxidative agents. However, it has been found that its antioxidant mechanism is mostly due to autophagy process to happen by AMP-activated protein kinase (AMPK) factor that through this mechanism, protects cells from lipotoxicity and is considered as a beneficial antioxidant [32, 33]. The dosage of TBHQ used in the present study was selected based on recommendations in the AIN-93G formula [34]. Several limitations of the present study especially the low amount of TBHQ administrated to rats and also the low number of animals in each group might be responsible for the observed results.

The present study is the first investigation in which the protective effects of TBHQ was evaluated against diazinon-induced oxidative stress in brain and heart of rats. TBHQ increased antioxidant levels in rats receiving diazinon. However, it could not attenuate diazinon-induced lipid peroxidation. The total antioxidant defense mechanism was not able to protect the tissues from lipid peroxidation caused by diazinon. Using higher amounts of TBHQ might be able to ameliorate diazinon-induced lipid peroxidation.

Acknowledgments

This study was supported by research fund of Ferdowsi University of Mashhad (project no. 3/29214). The authors wish to thank technicians who kindly helped us for sample collection of this study.

Footnotes

Authors’ Contribution: All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest: The authors declare there is no conflict of interests.

Funding/Support: Ferdowsi University of Mashhad.

References


