Research Article

Assessment of Protective Effects of Carvacrol on Haloperidol-Induced Oxidative Stress and Genotoxicity in Human Peripheral Blood Lymphocytes

Ehsan Zamani,1 Alireza Ahmadi Shad,2,3 Hediye Fatemi,1,2 Saba Mahboubi,2,3 Azadeh Motavallian,1,4 and Mehdi Evazalipour

1Department of Pharmacology and Toxicology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran
2Student Research Committee, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran
3Department of Pharmaceutical Biotechnology, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran
4Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, Netherlands

Correspondence should be addressed to Mehdi Evazalipour; evazalipour@yahoo.com

Received 2 August 2022; Accepted 14 October 2022; Published 25 October 2022

Academic Editor: Anurag Sharma

Copyright © 2022 Ehsan Zamani et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Haloperidol is a first-generation antipsychotic drug that has several indications in a wide range of mental conditions. The extensive prescription of haloperidol is correlated with some less-known adverse effects such as genotoxicity. Carvacrol is a monoterpenoid mainly found in oregano and thyme. It has the potential to scavenge free radicals in addition to increasing antioxidant defense enzyme activities and glutathione levels. In this study, we attempted to explore the possible potential of haloperidol in inducing genotoxicity in human peripheral lymphocytes as well as the protective role of carvacrol against this effect. The lymphocytes were divided into separate groups as follows: control group (cosolvent and NS); carvacrol group (5 μM); haloperidol group (25, 50, and 100 ng/ml); haloperidol (25, 50, and 100 ng/ml) + carvacrol (5 μM); positive control (0.8 μg/ml Cisplatin). After 24 hours of treatment, we conducted a cytokinesis-Block micronucleus test and an alkaline comet assay in order to determine genetic damage. Additionally, we measured glutathione and MDA levels as the biomarkers associated with oxidative stress. Significant increases in the levels of genotoxicity biomarkers (micronucleus frequency, DNA percentage in tail and tail moment) were observed in haloperidol-treated cells. The result of our oxidative stress tests also demonstrated that haloperidol had the potential to induce oxidative stress via reducing the levels of glutathione and increasing lipid peroxidation. Treatment with carvacrol significantly decreased the genotoxic events. It can be presumed that the induction of oxidative stress by haloperidol is the critical event associated with haloperidol-mediated genotoxicity. Therefore, using carvacrol as a natural antioxidant protected human lymphocytes against haloperidol genetic damage.

1. Introduction

Antipsychotic drugs are commonly prescribed to manage the symptoms of various psychiatric conditions, in particular schizophrenia and bipolar disorder [1]. Generally, they are classified into first generation and second-generation antipsychotics [2]. It is assumed that this class of drugs mainly acts by blocking dopaminergic signal transmission in the nigrostriatal pathway. Due to the aforementioned mechanism of action, it is of high probability that the side effects associated with the motor system, including extrapyramidal syndrome, may be observed [3, 4].

Haloperidol is classified as a typical antipsychotic drug and is extensively prescribed in order to manage various acute and chronic psychiatric conditions, such as schizophrenia, Tourette syndrome, different behavioral disorders in children, and acute mania. Also, haloperidol can be prescribed for chemotherapy-induced nausea and vomiting, and persistent hiccups [5–12]. Like any other first-generation antipsychotic, haloperidol mainly acts by blocking
dopaminergic receptors, in particular D2 receptors. The blockade of the dopaminergic pathway consequently leads to numerous side effects, such as tardive dyskinesia [13]. Chronic administration of haloperidol is assumed to be associated with an increase in the turnover rate of dopamine, which can possibly result in the production of reactive oxygen species (ROS) [14]. Moreover, it is proposed that haloperidol may cause a remarkable decline in the amount of glutathione [15].

The excessive production of free radicals, such as ROS, and the inability of the antioxidant defense system to stabilize them leads to a situation referred to as oxidative stress [16]. The probable role of free radicals in damaging macromolecules, like DNAs, proteins, and lipids, has been demonstrated in several studies [17–20]. More specifically, the results of some studies have illustrated the harmful effects of excessive ROS production on the genetic components of cells [21–25].

There have been promising reports on the major role of natural antioxidants in ameliorating oxidative stress [26, 27]. Carvacrol, (2-methyl-5-(1-methylethyl)-phenol), is a phe nolic monoterpenoid, which can be found predominantly in the essential oils of various plants, such as oregano and thyme [28]. It has numerous beneficial features, including antibacterial, antifungal, antiviral, and antiproliferative activities [29–32]. Additionally, potential antioxidant properties have been observed with carvacrol. It is assumed that one of the main mechanisms is the inhibition of lipid peroxidation [33, 34].

As regards the extensive use of haloperidol, it is of high significance to evaluate the toxic effects, which are more obscure. Furthermore, there are few studies on haloperidol’s probable genotoxicity. Therefore, the main objective of this study is to assess the possible genotoxic activity of haloperidol as well as the protective effects of carvacrol on human peripheral blood lymphocyte cells.

2. Materials and Methods

2.1. Chemicals. Haloperidol, carvacrol, phytohaemagglutinin (PHA), cytochalasin B, thiobarbituric acid, Tris-HCl, Tris ammonium, MgCl2, Disodium hydrogen phosphate, TCA, sucrose, EDTA, sodium acetate, Triton X-100, and Giemsa stain were from Sigma; DMEM cell culture and phosphate buffered saline (PBS) were from Gibco. Phosphoric acid, methanol, acetic acid glacial, potassium chloride, n-Butanol, sodium chloride, DTNB, Na2EDTA, sodium hydroxide, sodium lauryl sarcosinate, DMSO, and normal melting point agarose were from Merck. The low melting point (LMP) agarose was from Cleaver Scientific.

2.2. Blood Sampling and Treatment. The heparinized blood sample was obtained from the donor (a healthy, young, nonalcoholic, nonsmoking male). The donor had not been exposed to any chemicals or ionizing radiation that might have interfered with the results of the test throughout the six-month period of time prior to the blood sampling. An informed consent form was signed by the donor. The study was approved by the Guilan University of Medical Sciences’ Ethics Committee.

The blood sample was mixed with DMEM, which also contained 10% FBS, Glutamine, and Pen/Strep. The resulting mixture was divided into separate groups as follows:

- Control group (cosolvent and NS);
- Carvacrol group (5 μM) [35];
- Haloperidol group (25, 50, and 100 ng/ml) [36];
- Haloperidol (25, 50, and 100 ng/ml) + carvacrol (5 μM);
- Positive control (0.8 μg/ml Cisplatin).

After that, all groups were incubated for 24 hours (under the conditions of 37°C and 5% CO2 pressure).

2.3. Cytokinesis-Block Micronucleus Assay (CBMN Assay). The cytokinesis-block micronucleus assay (CBMN assay) was conducted as previously described with minor modifications [16]. Phytohaemagglutinin (PHA) was added to all the mentioned groups in order to stimulate mitosis in the culture. Subsequently, the cell mixtures were incubated for 72 hours. After 44 hours, cytochalasin-B (3 μg/ml) was added to the samples, leading to the inhibition of cytokinesis. After 28 hours, the culture was harvested, all groups were centrifuged at 200 × g and the supernatants were discarded. Subsequently, the samples were treated with hypotonic KCl followed by the addition of a prefixing solution (3 : 5, methanol: acetic acid) to the pellet. Right after that, the samples were centrifuged at 146 × g for 10 minutes, discarding the supernatant. Then, a fixative solution (3 : 1, methanol: acetic acid) was added. This stage was carried out two times. Next, a few drops of cell mixture were dropped on slides. Following that, the samples were air-dried. Eventually, the samples were stained using a 5% Giemsa solution. At least one thousand binuclear lymphocytes were analyzed under a microscope (Micros Austria daffodil MCX100, Vienna, Austria) at 400X magnification to determine the frequency of micronuclei (MN).

2.4. Single-Cell Gel Electrophoresis Assay (Alkaline Comet Assay). The alkaline comet assay was conducted as stated by Singh et al. with minor modifications [37]. The samples were centrifuged at 200 × g and the pellet pertaining to each sample group was mixed with 1% LMP agarose. The resultant mixtures were layered on fully frosted slides, which were already precoated with 1% normal melting point agarose. Immediately after that, the slides were covered and placed in a dark place for 10 minutes at 4°C in order for the LMP agarose to solidify. Subsequently, the samples were placed in a lysis solution at 4°C and in a dark chamber for 24 hours. Following lysis, the samples were placed horizontally in an alkaline electrophoresis buffer for 20 minutes at 4°C, and right after that, the electrophoresis was conducted for 20 minutes at 4°C, 1 V/cm, and 300 mA. Eventually, the slides were rinsed using Tris buffer as a neutralizing solution and then dehydrated with ethanol. This stage was repeated two more times. Next, the slides were stained using SYBR® Gold stain for 15 minutes in the dark and rinsed with deionized
water in order to remove excess stain. A total number of 100 nucleotides per slide were analyzed using a fluorescence invert microscope (Nikon Eclipse TS100, Tokyo, Japan) at 200X magnification. In order to quantify DNA damage, the head and tail intensities were measured using CASPLab® (CASP1.2.3 beta2) software, and the tail moment was calculated afterwards.

2.5. Measurement of Oxidative Stress Parameters

2.5.1. Measurement of Lipid Peroxidation. Thiobarbituric acid was used in order to measure malondialdehyde (MDA) levels as previously claimed with minor modifications [38]. After that, 0.05 M sulfuric acid was added to the cell homogenates, and after that, 0.2% TBA was added to the cell mixtures. Following that, for 30 minutes, the samples were put in a boiling water bath. Right after that, the samples were placed on ice, and n-butanol was added to all groups at the same time. Subsequently, the cell mixtures were centrifuged at \(3500 \times g\) for 10 minutes. Eventually, the supernatant of each group was analyzed with three replicates at 532 nm using an ELISA microplate reader. The malondialdehyde level was determined from a standard curve and the malondialdehyde level was expressed as \(\mu M\).

2.5.2. Measurement of Glutathione Content. Glutathione content was assessed as it was previously described, with minor modifications [38]. DTNB (5, 5-dithio-bis-(2-nitrobenzoic acid) was used as the indicator for the determination of glutathione concentration. Then, TCA was added to the cell suspensions in order to precipitate the proteins, and then the samples were centrifuged at 5000 \(\times g\) for 5 minutes at 4°C. Next, the supernatant pertaining to each group was mixed with DTNB and PBS. The samples were evaluated at 412 nm using a spectrophotometer. A standard curve was used to determine total glutathione. The total glutathione concentration was expressed as \(\mu M\) glutathione.

2.6. Statistical Analysis. All statistical analyses were performed using GraphPad Prism® software (version 6). The results were expressed as the mean ± SD. The assays were performed in at least triplicate. Comparison between groups was made using the one-way ANOVA test, followed by the post hoc Tukey’s test. \(P < 0.05\) was considered statistically significant.

3. Results

3.1. Cytokinesis-Block Micronucleus Assay (CBMN Assay). CBMN was conducted to examine the probable genotoxic effect of haloperidol on lymphocytes as well as the protective effect of carvacrol. In order to assess the cytogenetic damage, the frequency of MN was measured in binucleated lymphocytes (Figure 1). As shown in Figure 2, the frequencies of MN increased significantly in samples treated with haloperidol in comparison with the control group \((P < 0.001)\), whereas the frequency of MN significantly decreased in the groups treated with haloperidol and carvacrol \((P < 0.001)\).

3.2. Alkaline Comet (SCGE) Assay. The alkaline comet (SCGE) assay is a sensitive method, which was performed in this study to evaluate haloperidol-induced DNA strand breaks and the effects of carvacrol on it. Figure 3 shows the observed comets which were formed in each group. The results are demonstrated as the percentage of DNA in the tail (Figure 4) and the tail moment (Figure 5). As shown in both figures, the percentage of DNA in the tail and the tail moment increased significantly in the haloperidol group at...
all concentrations compared to the control group \( (P < 0.05) \). However, using carvacrol at haloperidol concentrations of 50 and 100 ng/ml decreased the percentage of DNA in the tail and tail moment \( (P < 0.05) \).

### 3.3. Measurement of Oxidative Stress Parameters

#### 3.3.1. Measurement of Lipid Peroxidation

The MDA level was assessed in this study as the final by-product of lipid peroxidation. The MDA levels significantly increased in the haloperidol treated groups at a concentration of 100 ng/ml, which is demonstrated in Figure 6 \( (P < 0.01) \). Whereas, the results indicated a statistically significant decrease when carvacrol was used with haloperidol at the concentration of 100 ng/ml \( (P < 0.05) \).

#### 3.3.2. Measurement of Glutathione Content

Glutathione concentration was measured in this study as the other indicator of oxidative stress. Figure 7 demonstrates significant decreases in the haloperidol group at all concentrations compared to the control group \( (P < 0.05) \), while the level of glutathione increased significantly when carvacrol was used with haloperidol at the concentration of 100 ng/ml \( (P < 0.05) \).
4. Discussion

Haloperidol is one of the most commonly prescribed antipsychotic drugs [39]. It has been reported in various studies that the induction of oxidative stress might be the principal mechanism correlated to haloperidol-induced adverse effects [40–42].

The main objective of this study was to provide further evidence on the probable potential of haloperidol in inducing genotoxicity through oxidative stress and the role of carvacrol in ameliorating it. In order to demonstrate the genotoxic potential of haloperidol, we conducted the CBMN test and the alkaline comet assay, both of which can detect various genotoxic events in various cell lines [43, 44].

According to the results reported in various studies, it is presumed that the optimal therapeutic window of haloperidol is between 5 and 25 ng/ml. However, it is possible that the plasma concentration of a drug increases to 100 ng/ml or more as a consequence of drug accumulation in the case of drug overdose or chronic administration. So, we chose haloperidol concentrations close to these ranges [45–47]. Based on the results of this study, exposing human lymphocytes to different concentrations of haloperidol (25, 50, and 100 ng/ml) resulted in significant increases in the frequency of MN, DNA percentage in tail, and tail moment, indicating that haloperidol might have the potential to induce genotoxicity. This is consistent with the results reported in previous studies [36, 47–51]. In a similar study, the CBMN test and alkaline comet assay were conducted on human lymphocytes at lower concentrations of haloperidol (5, 10 and 20 ng/ml) compared to our study. The study found out that all mentioned concentrations of haloperidol increased DNA tail length significantly after 24 and 48 hours of exposure, in addition to a significant increase in the frequency of MN at 10 ng/ml. Interestingly, the results of this study indicated that haloperidol might cause genotoxicity in relatively clinical concentrations [36]. In another study, therapeutic concentrations (5–25 ng/ml) and higher concentrations (100 and 500 ng/ml) of haloperidol were evaluated using a chromosome aberration test. The results of the test demonstrated significant increases at 25, 100, and 500 ng/ml. Additionally, exposing cells to haloperidol decreased the mitotic index significantly at all concentrations, which provided further evidence that haloperidol may have harmful effects on the rate of mitosis through the inhibition of DNA synthesis [47]. The possible role of antipsychotics in changing the epigenetic status of the brain has been proposed and assessed in several studies [48–50]. The results of a recent study also revealed that haloperidol may potentiate the induction of DNA methylation in neuroblastoma cells. It was hypothesized that inducing DNA methylation is associated with haloperidol’s strong blockade of dopamine D2 receptors [51]. The blockade of dopaminergic transmission by haloperidol leads to an increase in the turnover rate of dopamine, which will consequently result in the excessive production of reactive oxygen species and oxidative stress [52].
The key role of reactive oxygen species in the haloperidol-induced neurotoxicity was assessed in various studies. Based on their results, it was proposed that haloperidol may increase oxidative stress in cortical neurons of the brain, leading to a cascade of cellular events including an increase in the activity of the caspase-3 enzyme, which might result in cellular apoptosis, necrosis, and DNA fragmentation [42, 53, 54].

As regards oxidative stress, we found that haloperidol significantly reduced the level of cellular glutathione at all concentrations ($P < 0.05$), followed by a significant increase in lipid peroxidation at 100 ng/ml. This is in agreement with the findings of previous studies [41, 42, 55–63]. An early study on patients prescribed with haloperidol reported that a 2-week period of haloperidol administration significantly reduced the level of glutathione in the patients’ cerebrospinal fluid, in addition to a significant increase in MDA level [55]. Furthermore, in a study on male Sprague-Dawley rats that were chronically treated with haloperidol, it was revealed that haloperidol caused significant depletion in the levels of brain antioxidant defense enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) [42]. In another study, male Wistar rats were under chronic treatment of haloperidol for 21 days. It was reported that treatment with haloperidol led to significant decreases in the levels of glutathione and the activity of antioxidant enzymes SOD and catalase, along with a significant increase in lipid peroxidation in the forebrain [56]. Exposing human plasma to haloperidol demonstrated significant increases in TBARS, which are accounted for as biomarkers of lipid peroxidation [57–59]. Moreover, the results of some studies indicated that haloperidol might cause hepatotoxicity and nephrotoxicity through the depletion of reduced glutathione and antioxidant enzyme activity as well as generating ROS in the kidney and liver [41, 60–63]. Additionally, the induction of oxidative stress by haloperidol was observed in a cell study. It was reported that the exposure of rat primary cortical neurons and hippocampal HT-22 cell lines to haloperidol initiated sequences of cellular changes that resulted in cell death. According to the findings of the study, haloperidol increased the generation of ROS and decreased the glutathione level, followed by increasing the intracellular Ca$^{2+}$ level, which led to cell death [64].

The prominent role of antioxidants in reducing drug-induced cell death and cytotoxicity has been demonstrated in some studies [65–67]. In consonance with that, Behl et al. indicated that using vitamin E as a lipophilic antioxidant and free radical scavenger prevented DNA degradation and, therefore, necrotic cell death in random isolated cells treated with haloperidol. Based on the results of this study, it can be presumed that free radicals play the main role in haloperidol-induced necrotic cell death [68].

In recent years, the major role of natural antioxidants in preventing drug-induced side-effects correlated to oxidative stress has become a major area of focus [69]. Several studies have reported the alleviation of haloperidol-induced oxidative stress after treatment with natural antioxidant compounds [41, 42, 56, 59, 63, 70–72]. In our study, we also attempted to outline the potential of carvacrol as a natural antioxidant polyphenol compound. Carvacrol is a natural monoterpane, which has demonstrated anxiolytic and antidepressant properties in mice models [73, 74]. As a natural polyphenolic compound, carvacrol shares some common features with other compounds of the same family, including its ability to act as an antioxidant and scavenge free radicals, more specifically, reactive oxygen species. It is also assumed that monoterpenoids can have protective effects on the antioxidant defense system existing in the body [34, 75, 76]. Accordingly, the results of a TRAP/TAR assay (Total reactive antioxidant potential/Total antioxidant reactivity assay) revealed that carvacrol could scavenge peroxyl radicals [77]. The antioxidant properties of carvacrol are often attributed to the polyphenolic structure of this compound as well as its weak acid feature through which it can possibly react with free radicals and turn them into more stable radicals [78]. The results derived from various studies on drug-induced oxidative stress have also revealed that carvacrol improved glutathione levels significantly and restored the antioxidant defense enzymes [79–82]. Furthermore, carvacrol showed antilipid peroxidation ability by significantly decreasing markers such as MDA [83, 84]. We found that treating cells with carvacrol was effective in reducing haloperidol-induced oxidative stress and genotoxic events significantly. Our findings suggest that the concomitant administration of carvacrol as a supplement with haloperidol is a potential therapeutic strategy that might impose preventative impacts against haloperidol-induced adverse effects. However, further studies are required to be conducted.

5. Conclusion

In this study, we showed that haloperidol could mediate genotoxicity in human lymphocytes via the induction of oxidative stress. In addition, we observed that treating cells with carvacrol protected the cells against haloperidol-induced genotoxicity by reducing lipid peroxidation by-product MDA and increasing glutathione levels. Therefore, it can be presumed that oxidative stress plays an important role in haloperidol-mediated genotoxicity.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The data provided in our research were extracted from a part of Pharm D thesis and supported by the research council of the Guilan University of Medical Sciences, Rasht, Iran (Registration code: IR.GUMS.REC.1398.359).
References

[1] E. G. Katsioulis, M. Argyraki, M. T. Ekonomopoulou, C. Mourelatos, Z. Isakidou-Krifi, and D. Mourelatos, “Typical versus atypical antipsychotics: genotoxicity and cytostaticity testing,” Integrative Cancer Science and Therapeutics, vol. 7, pp. 1–6, 2020.

[2] M. De Hert, J. Detraux, R. Van Winkel, W. Yu, and C. U. Correll, “Metabolic and cardiovascular adverse effects associated with atypical antipsychotics,” Nature Reviews Endocrinology, vol. 8, no. 2, pp. 114–126, 2012.

[3] A. Galbraith, S. Bullock, E. Manias, and B. Hunt, Fundamentals of Pharmacology: An Applied Approach for Nursing and Health, Pearson Education, London, UK, 2007.

[4] L. Farde, A.-L. Nordström, F.-A. Wiesel, S. Pauli, C. Halldin, and G. Sédvall, “Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine: relation to extrapyramidal side effects,” Archives of General Psychiatry, vol. 49, no. 7, pp. 538–544, 1992.

[5] J. M. Kane and S. R. Marder, “Psychopharmacologic treatment of schizophrenia,” Schizophrenia Bulletin, vol. 19, no. 2, pp. 287–302, 1993.

[6] J. Quezada and K. A. Coffman, “Current approaches and new developments in the pharmacological management of tourette syndrome,” CNS Drugs, vol. 32, no. 1, pp. 33–45, 2018.

[7] T. Pringsheim, L. Hirsch, D. Gardner, and D. A. Gorman, “The pharmacological management of oppositional behaviour, conduct problems, and aggression in children and adolescents with attention-deficit hyperactivity disorder, oppositional defiant disorder, and conduct disorder: a systematic review and meta-analysis. Part 2: antipsychotics and traditional mood stabilizers,” The Canadian Journal of Psychiatry, vol. 60, no. 2, pp. 52–61, 2015.

[8] G. S. Sachs, F. Grossman, S. N. Ghaemi, A. Okamoto, and C. L. Bowden, “Combination of a mood stabilizer with risperidone or haloperidol for treatment of acute mania: a double-blind, placebo-controlled comparison of efficacy and safety,” American Journal of Psychiatry, vol. 159, no. 7, pp. 1146–1154, 2002.

[9] J. R. Hardy, A. O’Shea, C. White, K. Gilshenan, L. Welch, and C. Douglas, “The efficacy of haloperidol in the management of nausea and vomiting in patients with cancer,” Journal of Pain and Symptom Management, vol. 40, no. 1, pp. 111–116, 2010.

[10] C. J. Woelk, “Managing hiccups,” Canadian Family Physician, vol. 57, no. 6, pp. 672–675, 2011.

[11] J. Kornhuber, A. Schultz, J. Wiltfang et al., “Persistence of haloperidol in human brain tissue,” American Journal of Psychiatry, vol. 156, no. 6, pp. 885–890, 1999.

[12] E. J. Cobos, E. Pozo, and J. M. Baeyens, “Irreversible blockade of sigma-1 receptors by haloperidol and its metabolite in guinea pig brain and SH-SY5Y human neuroblastoma cells,” Journal of Neurochemistry, vol. 102, no. 3, pp. 812–825, 2007.

[13] R. Grohmann, R. Koch, and L. G. Schmidt, “Extrapyramidal symptoms in neuroleptic recipients,” Agents and Actions Supplements, vol. 29, pp. 71–82, 1990.

[14] V. Khot, M. Egan, T. Hyde, and R. Wyatt, “Neuroleptics and classic tardive dyskinesia,” in Drug Induced Movement Disorders, A. E. Lang and W. J. Weiner, Eds., Drug Induced Movement Disorders, Futura Publishing Company, New York, NY, USA, pp. 121–161, 1992.

[15] B. R. Shivakumar and V. Ravindranath, “Oxidative stress and thiol modification induced by chronic administration of haloperidol,” Journal of Pharmacology and Experimental Therapeutics, vol. 265, no. 3, pp. 1137–1141, 1993.

[16] E. Zamani, M. Shokrzadeh, M. Modanloo, and F. Shaki, “In vitro study towards role of acrylamide-induced genotoxicity in human lymphocytes and the protective effect of L-carnitine,” Brazilian Archives of Biology and Technology, vol. 61, no. 6, 2018.

[17] R. T. Dean, S. Fu, R. Stocker, and M. J. Davies, “Biochemistry and pathology of radical-mediated protein oxidation,” Biochemical Journal, vol. 324, no. 1, pp. 1–18, 1997.

[18] Q. Cai, L. Tian, and H. Wei, “Age-dependent increase of indigenous DNA adducts in rat brain is associated with a lipid peroxidation product,” Experimental Gerontology, vol. 31, no. 3, pp. 373–385, 1996.

[19] N. Ogawa, “Free radicals and neural cell damage,” Clinical Neurology, vol. 34, no. 12, pp. 1266–1268, 1994.

[20] A. D. Muthuswamy, K. Vedagiri, M. Ganesan, and P. Chinnakanu, “Oxidative stress-mediated macromolecular damage and dwindle in antioxidant status in aged rat brain regions: role of L-carnitine and DL-n-lipoic acid,” Clinica Chimica Acta, vol. 368, no. 1–2, pp. 84–92, 2006.

[21] M. Hanot-Roy, E. Tobeuf, A. Guilbert et al., “Oxidative stress pathways involved in cytotoxicity and genotoxicity of titanium dioxide (TiO2) nanoparticles on cells constitutive of alveolo-capillary barrier in vitro,” Toxicology in Vitro, vol. 33, pp. 125–135, 2016.

[22] U. S. Srinivas, B. W. Tan, B. A. Vellayappan, and A. D. Jeyasekharan, “ROS and the DNA damage response in cancer,” Redox Biology, vol. 25, Article ID 101084, 2019.

[23] C. Angélê-Martínez, K. V. T. Nguyen, F. S. Ameer, J. N. Anker, and J. L. Brumaghim, “Reactive oxygen species generation by copper (II) oxide nanoparticles determined by DNA damage assays and EPR spectroscopy,” Nanotoxicology, vol. 11, no. 2, pp. 278–288, 2017.

[24] S. Kawanishi, Y. Hiraku, M. Murata, and S. Oikawa, “The role of metals in site-specific DNA damage with reference to carcinogenesis,” Free Radical Biology and Medicine, vol. 32, no. 9, pp. 822–832, 2002.

[25] H. Shi, L. G. Hudson, and K. J. Liu, “Oxidative stress and apoptosis in metal ion-induced carcinogenesis,” Free Radical Biology and Medicine, vol. 37, no. 5, pp. 582–593, 2004.

[26] H. Zhang and R. Tsao, “Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects,” Current Opinion in Food Science, vol. 8, pp. 33–42, 2016.

[27] D. Ezhilarasan, E. Sokal, S. Karthikeyan, and M. Najim, “Plant derived antioxidants and antifibrotic drugs: past, present and future,” Journal of Coastal Life Medicine, vol. 2, no. 2, pp. 738–745, 2014.

[28] M. Sharifi-Rad, E. M. Varoni, M. Iriri et al., “Carvacrol and human health: a comprehensive review,” Phytotherapy Research, vol. 32, no. 9, pp. 1675–1687, 2018.

[29] C. Lioios, O. Gortzi, S. Lalas, J. Tsaknis, and I. Chinou, “Liposomal incorporation of carvacrol and thymol isolated from the essential oil of Origanum dictamnus L. and in vitro antimicrobial activity,” Brazilian Journal of Phytomedicine, vol. 11, no. 3, pp. 77–83, 2009.

[30] P. S. Chavan, and S. G. Tupe, “Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts,” Food Control, vol. 46, pp. 115–120, 2014.

[31] M. Sökmen, J. Serkedjieva, D. Daferera et al., “In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of Origanum dictamnus,” Phytotherapy Research, vol. 25, no. 6, pp. 865–871, 2011.
of Origanum acutidens,” Journal of Agricultural and Food Chemistry, vol. 52, no. 11, pp. 3309–3312, 2004.

[32] K. Arunasree, “Anti-proliferative effects of carvacrol on a human metastatic breast cancer cell line, MDA-MB 231,” Phytotherapy, vol. 17, no. 8–9, pp. 581–586, 2010.

[33] N. V. Yanishlieva, E. M. Marinova, M. H. Gordon, and V. G. Raneva, “Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems,” Food Chemistry, vol. 64, no. 1, pp. 59–66, 1999.

[34] V. Lagouri, G. Bekas, M. Tsimidou, S. Kokkini, and D. Boskou, “Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece,” Zeitschrift für Lebensmittel-Untersuchung und Forschung, vol. 197, no. 1, pp. 20–23, 1993.

[35] N. Kumar, A. Yadav, S. Gulati, N. Aggarwal, N. Aggarwal, and R. Gupta, “Antigenotoxic effect of curcumin and carvacrol against parathion induced DNA damage in cultured human peripheral blood lymphocytes and its relation to GSTM1 and GSTT1 polymorphism,” Journal of Toxicology, vol. 2014, pp. 1–7, 2014.

[36] G. GaJski, M. Gerić, and V. Garaj-Vrhovac, “Evaluation of the in vitro cytogenotoxicity profile of antipsychotic drug haloperidol using human peripheral blood lymphocytes,” Environmental Toxicology and Pharmacology, vol. 38, no. 1, pp. 316–324, 2014.

[37] N. P. Singh, M. T. McCoy, R. R. Tice, and E. L. Schneider, “A simple technique for quantification of low levels of DNA damage in individual cells,” Experimental Cell Research, vol. 175, no. 1, pp. 184–191, 1988.

[38] M. Evazalipour, P. Safarzadeh Kozani, P. Safarzadeh Kozani, S. Shabani, B. Rezaei Soufi, and E. Zamani, “Acrylamide induced oxidative cellular senescence in embryonic fibroblast cell line (NIH 3T3): a protection by carvacrol,” Jundishapur Journal of Natural Pharmaceutical Products, vol. 16, no. 4, 2021.

[39] J. Kame, “Tardive dyskinesia: epidemiological and clinical presentation,” in Psychopharmacology, F. E. Bloom and D. J. Kupfer, Eds., pp. 1485–1495, Psychopharmacology: The Fourth Generation of Progress. Raven Press Ltd., New York, NY, USA, 1995.

[40] I. Jeding, P. J. Evans, D. Akanmu et al., “Characterization of the potential antioxidant and pro-oxidant actions of some neuroleptic drugs,” Biochemical Pharmacology, vol. 49, no. 3, pp. 359–365, 1995.

[41] S. A. El-Awdan, G. A. A. Jaleel, and D. O. Saleh, “Alleviation of haloperidol induced oxidative stress in rats: effects of sucrose vs grape seed extract,” Biochemical Pharmacology, vol. 53, no. 1, pp. 29–35, 2015.

[42] J. Perera, J. H. Tan, S. Jeevathayapan, S. Chakravarthi, and N. Haleagrarahar, “Neuroprotective effects of alpha linoleic acid on haloperidol-induced oxidative stress in the rat brain,” Cell & Bioscience, vol. 1, no. 1, pp. 12–16, 2011.

[43] M. Fenech, M. Kirsch-Volders, A. T. Natarajan et al., “Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells,” Mutagenesis, vol. 26, no. 1, pp. 125–132, 2011.

[44] R. R. Tice, E. Agurell, D. Anderson et al., “Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing,” Environmental and Molecular Mutagenesis, vol. 35, no. 3, pp. 206–221, 2000.

[45] T. Van Putten, S. R. Marder, J. Mintz, and R. E. Poland, “Haloperidol plasma levels and clinical response: a therapeutic window relationship,” American Journal of Psychiatry, vol. 149, no. 4, pp. 500–505, 1992.

[46] J. Volavka, T. Cooper, P. Czobor, I. Bitter, M. Meisner, and E. Laska, “Haloperidol blood levels and clinical effects,” Arch Gen Psychiatry, vol. 49, no. 5, pp. 354–361, 1992.

[47] R. Saxena and Y. R. Ahuja, “Genotoxicity evaluation of haloperidol on human lymphocytes in vitro,” Journal of Human Ecology, vol. 10, no. 4, pp. 259–263, 1999.

[48] M. Kurita, T. Holloway, and J. Gonzalez-Maeso, “HDAC2 as a new target to improve schizophrenia treatment,” Expert Review of Neurotherapeutics, vol. 13, no. 1, pp. 1–3, 2013.

[49] M. de la Fuente Revenga, D. Ibi, T. Cuddy et al., “Chronic clozapine treatment restrains via HDAC2 the performance of mGlu2 receptor agonism in a rodent model of antipsychotic activity,” Neuropsychopharmacology, vol. 44, no. 2, pp. 443–454, 2019.

[50] B. Swathy and M. Banerjee, “Understanding epigenetics of schizophrenia in the backdrop of its antipsychotic drug therapy,” Epigenomics, vol. 9, no. 5, pp. 721–736, 2017.

[51] J. Du, Y. Nakachi, T. Kiyono et al., “Comprehensive DNA methylation analysis of human neuroblastoma cells treated with haloperidol and risperidone,” Frontiers in Molecular Neuroscience, vol. 14, Article ID 792874, 2021.

[52] M. Raudenska, J. Gumulec, P. Babula et al., “Haloperidol cytotoxicity and its relation to oxidative stress,” Mini Reviews in Medicinal Chemistry, vol. 13, no. 14, pp. 1993–1998, 2013.

[53] J. S. Koh, H. J. Kang, E. Y. Kim et al., “Haloperidol-induced neuronal apoptosis: role of p38 and c-Jun-NH2-terminal protein kinase,” Journal of Neurochemistry, vol. 75, no. 6, pp. 2327–2334, 2008.

[54] P. Gass, S. Mas, O. Molina, M. Bernardo, A. Lafuente, and E. Parellada, “Neurotoxic/neuroprotective activity of haloperidol, risperidone and paliperidone in neuroblastoma cells,” Progress in Neuro-Psychopharmacology and Biological Psychiatry, vol. 36, no. 1, pp. 71–77, 2012.

[55] B. Nagesh Pai, N. Janakiramaiah, B. Gangadhar, and V. Ravindranath, “Depletion of glutathione and enhanced lipid peroxidation in the CSF of acute psychotics following haloperidol administration,” Biological Psychiatry, vol. 36, no. 7, pp. 489–491, 1994.

[56] P. S. Naidu, A. Singh, and S. K. Kulkarni, “Effect of Withania somnifera root extract on haloperidol-induced orofacial dyskinesia: possible mechanisms of action,” Journal of Medicinal Food, vol. 6, no. 2, pp. 107–114, 2003.

[57] A. Dietrich-Muszalska, B. Kontek, and J. Rabe-Jabłońska, “Quetiapine, olanzapine and haloperidol affect human plasma lipid peroxidation in vitro,” Neuropsychobiology, vol. 63, no. 4, pp. 197–201, 2011.

[58] A. Dietrich-Muszalska, B. Olas, B. Kontek, and J. Rabe-Jabłońska, “Beta-glucan from Saccharomyces cerevisiae reduces plasma lipid peroxidation induced by haloperidol,” International Journal of Biological Macromolecules, vol. 49, no. 1, pp. 113–116, 2011.

[59] A. Dietrich-Muszalska, B. Kontek, B. Olas, and J. Rabe-Jabłońska, “Epicatechin inhibits human plasma lipid peroxidation caused by haloperidol in vitro,” Neurochemical Research, vol. 37, no. 3, pp. 557–562, 2012.

[60] J. K. Akintunde and O. K. Abubakar, “Novel therapeutic approaches of natural oil from black seeds and its underlying mechanisms against kidney dysfunctions in haloperidol-induced male rats,” Drug Metabolism and Personalized Therapy, vol. 32, no. 2, pp. 97–107, 2017.

[61] O. M. Abdel-Salam, E. R. Youness, Y. A. Khadrany, and A. A. Slem, “Brain and liver oxidative stress after sertraline and haloperidol treatment in mice,” Journal of Basic and
Clinical Physiology and Pharmacology, vol. 0, no. 0, pp. 1–9, 2013.

[62] A. C. Andreazza, V. E. Barakauskas, S. Fazeli et al., "Effects of haloperidol and clozapine administration on oxidative stress in rat brain, liver and serum," Neuroscience Letters, vol. 591, pp. 36–40, 2015.

[63] E. A. Abdel-Sattar, S. M. Mouneir, G. F. Asaad, and A. C. Andreazza, V. E. Barakauskas, S. Fazeli et al., "Induction of reactive oxygen species in neurons by haloperidol-induced oxidative stress in rat," Toxicology and Industrial Health, vol. 30, no. 2, pp. 147–153, 2014.

[64] Y. Sagara, "Induction of reactive oxygen species in neurons by haloperidol," Journal of Neurochemistry, vol. 71, no. 3, pp. 1002–1012, 2002.

[65] H. A. El-Beshbishy, O. M. Tork, M. F. El-Bab, and Y. Sagara, "Induction of reactive oxygen species in neurons by haloperidol-induced oxidative stress in rat," H. A. El-Beshbishy, O. M. Tork, M. F. El-Bab, and Y. Sagara, "Induction of reactive oxygen species in neurons by haloperidol-induced oxidative stress in rat," Pathophysiology, vol. 18, no. 2, pp. 125–135, 2011.

[66] S. Chaudhary, P. Ganjoo, S. Raisuddin, and S. Parvez, "Antioxidant and antiapoptotic effects of green tea polyphenols against azathioprine-induced liver injury in rats," Journal of Biochemical and Molecular Toxicology, vol. 11, no. 2, pp. 17–22, 2010.

[67] G. Nicolin, R. Rigolito, M. Miloso, A. A. Bertelli, and G. Tredici, "Anti-apoptotic effects of resveratrol on paclitaxel-induced apoptosis in the human neuroblastoma SH-SY5Y cell line," Neurochemistry Letters, vol. 302, no. 1, pp. 41–44, 2001.

[68] C. Behl, R. Rupprecht, T. Skutella, and F. Holsboer, "Haloperidol-induced cell death—mechanism and protection with vitamin E in vitro," NeuroReport, vol. 7, no. 1, pp. 360–364, 1995.

[69] A. Dietrich-Muszalska and B. Olas, "Inhibitory effects of haloperidol and clozapine administration on oxidative stress potential of the monoterpenes α-pinene and 1, 8-cineole against H2O2-induced oxidative stress in PC12 cells," Zeitschrift für Naturforschung C, vol. 71, no. 7–8, pp. 191–199, 2016.

[70] A. Pr, B. N. Rao, and B. S. Rao, "In vivo radioprotective potential of thymol, a monoterpane phenol derivative of cymene," Mutation Research/Genetic Toxicology and Environmental Mutagenesis, vol. 726, no. 2, pp. 136–145, 2011.

[71] B. Halliwell and J. M. Gutteridge, "Free Radicals in Biology and Medicine," Oxford University Press, Oxford, UK, 2015.

[72] R. Lima and M. Cardoso, "Família Lamiaceae: importantes óleos essenciais com ação biológica e antioxidante," Farm-Manguinhos - Revista Fitos, vol. 3, no. 3, pp. 14–24, 2013.

[73] M. Bozkurt, S. Em, P. Oktayoglu et al., "Carvacrol prevents methotrexate-induced renal oxidative injury and renal damage in rats," Clinical & Investigative Medicine, vol. 37, no. 1, pp. E19–E25, 2014.

[74] E. S. Em, A. A. Ar, M. Am, and E. A. Aa, "Thymol and carvacrol prevent cisplatin-induced nephrotoxicity by abrogation of oxidative stress, inflammation, and apoptosis in rats," Journal of Biochemical and Molecular Toxicology, vol. 29, no. 4, pp. 165–172, 2015.

[75] H. Ahmadvand, M. Tavafi, V. Asadollahi, L. Jafaripour, F. Hadipour-Moradi, and R. Mohammadrezaei-Khoramabadi, "Protective effect of carvacrol on renal functional and histopathological changes in gentamicin-induced nephrotoxicity in rats," vol. 18, no. 4, Article ID e6446, 2016.

[76] B. Aristatile, K. S. Al-Numair, A. H. Al-Assaf, C. Veeramani, and K. V. Pugalendi, "Protective effect of carvacrol on oxidative stress and cellular DNA damage induced by UBV irradiation in human peripheral lymphocytes," Journal of Biochemical and Molecular Toxicology, vol. 29, no. 11, pp. 497–507, 2015.

[77] S. Gunes, A. Ayhanci, V. Sahinturk, D. U. Altay, and R. Uyar, "Anti-apoptotic effect of trans-resveratrol on oxidative stress induced by valproic acid," Protoplasma, vol. 252, no. 1, pp. 209–217, 2015.

[78] M. Bozkurt, S. Em, P. Oktayoglu et al., "Carvacrol prevents cisplatin-induced nephrotoxicity by abrogation of oxidative stress, inflammation, and apoptosis in rats," vol. 18, no. 10, pp. 165–172, 2015.

[79] H. Ahmadvand, M. Tavafi, V. Asadollahi, L. Jafaripour, F. Hadipour-Moradi, and R. Mohammadrezaei-Khoramabadi, "Protective effect of carvacrol on renal functional and histopathological changes in gentamicin-induced nephrotoxicity in rats," vol. 18, no. 4, Article ID e6446, 2016.

[80] B. Aristatile, K. S. Al-Numair, A. H. Al-Assaf, C. Veeramani, and K. V. Pugalendi, "Protective effect of carvacrol on oxidative stress and cellular DNA damage induced by UBV irradiation in human peripheral lymphocytes," Journal of Biochemical and Molecular Toxicology, vol. 29, no. 11, pp. 497–507, 2015.

[81] S. Nafees, S. Ahmad, W. Arjumand, S. Rashid, N. Ali, and S. Sultana, "Carvacrol ameliorates thioacetamide-induced hepatotoxicity by abrogation of oxidative stress, inflammation, and apoptosis in liver of Wistar rats," Human & Experimental Toxicology, vol. 32, no. 12, pp. 1292–1304, 2013.