Sesame oil ameliorates valproic acid-induced hepatotoxicity in mice: Integrated in vivo- in silico study

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Research Article

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Abstract

Sesame oil has been exhibited to have anti-inflammatory and antioxidant influences. The goal of this experiment was to look into sesame oil's hepato-protective properties and underlying processes in valproic acid-induced hepatotoxicity. Molecular docking was carried out to clarify the functional and structural underlying mechanism of sesame oil ameliorative effect. Mice were given 8 ml/kg/day of sesame oil (orally) and 100 mg/kg/day of valproic acid (i.p.) for 21 days. The results revealed that valproic acid caused a considerable increase in hepatic malondialdehyde (MDA) levels while decreasing the activity of glutathione peroxidase (GPx) enzyme. There was also a significant rise in serum levels of interleukines 1β & 6 (IL-1β & IL-6) and a significant decrease in hepatic (PXR) gene expression level. Sesame oil co-administration with valproic acid significantly normalized the antioxidant and anti-inflammatory status and upregulated the gene expression level of PXR. In silico docking analysis results confirmed these results. This study concluded that supplementation of sesame oil attenuated valproic acid induced oxidative stress and inflammation. Hence, it was recommended as a dietary supplement for protection against valproic acid induced hepatotoxicity.

Introduction

Valproic acid (VPA) is commonly prescribed for treatment of seizure disorders, bipolar disorder, and migraine prophylaxis. Despite its medical efficacy, hepatotoxicity has been reported as a serious complication after VPA administration. Pediatric patients are more susceptible to VPA-induced hepatotoxicity than adults [1].

Valproic acid is broadly metabolized in the liver. Biotransformation of VPA produced several metabolites such as hydroxylated, unsaturated and conjugated metabolites which may be responsible for its toxicity [2]. PXR has been well-established as a main regulator of xenobiotic clearance. It is chiefly expressed in liver and small intestine. PXR expression in other tissues such as stomach, lung, breast, uterus, ovary and bone marrow is minor. Activated PXR up regulates genes encoding enzymes that metabolize drugs and drug transporters [3].

Liver injury may be directly by the drug or its metabolites, or injury may result from the subsequent inflammatory reaction [4]. Many research focused on the pathophysiology of drug-induced toxicity mediated by reactive metabolites. Oxidative stress may perform a role in the etiology of drug produced hepatotoxicity via oxidative alteration of biological macromolecules such as nucleic acids, lipids and proteins [5]. Several factors are contributing to hepatotoxicity caused by VPA, including development of oxidative stress and VPA metabolites generate reactive metabolites, which then covalently bond to cellular proteins. During drug induced hepatotoxicity, inflammation of the liver is a common occurrence [6]. Inflammatory cytokines were found to be higher in rats with liver damage. The production of inflammatory mediators by activated hepatic macrophages has been shown to aggravate drug-induced hepatic damage [7].
For balancing the extreme production of free radicals, mechanisms of enhancing redox homeostasis should be maintained. Many natural compounds have been tested as antioxidants as part of these mechanisms [8]. Sesame oil (SO), derived from *Sesamum indicum L.*, is effective against numerous diseases and it possess anti-aging effects. The lignans contained in SO, in addition to sesaminol which is the primary antioxidant component, contribute to its antioxidant and antimutagenic capabilities. SO has a high concentration of phenol, sesamin, sesamol, and sesamolin, which contributes to its better oxidative stability [9]. As its antioxidant activity, SO has long been used as a daily dietary supplement to boost cell resistance to lipid peroxidation. Moreover, previous studies suggested that SO enhances the ability of liver to detoxify chemicals and xenobiotics [10]. Nevertheless, there is no previous experimental proof reporting the ameliorative influence of sesame oil against valproic acid-induced hepatotoxicity.

Thus, the purpose of this research is to look at the hepato-protective impact of sesame oil as an antioxidant and anti-inflammatory agent on valproic acid-induced hepatic cell damage in mice. In addition, a docking study was carried out to gain functional and structural insight into the binding mode of the valproic acid and some sesame oil constituents (sesamin, sesaminol, sesamol and sesamolin), and GPx, IL-1β, IL-6 and PXR.

**Materials And Methods**

**1-Chemicals**

El-Borg pharmacy in Beni-Suef, Egypt, sold Depakine® (Sodium valproate, Sanofi Synthelabo, France), each containing 200 mg of sodium valproate. Harraz for food industry and natural products Co., Cairo, Egypt, provided the sesame oil. Malondialdehyde (MDA) and glutathione peroxidase (GPx) commercial kits were purchased from Biodiagnostic company, Cairo, Egypt. Elabscience® in the United States provided ELISA kits for determining IL-1β and IL-6 serum levels.

**2- Treatments for animals**

Thirty ten-week-old male mice were obtained from a laboratory animal farm in Egypt's Beni-Suef governorate. Mice were given a balanced commercial meal and free access to water, and were housed at a chamber temperature of 25ºC, 45% moisture. All research procedures were carried out consistent with the guide for the care and use of laboratory animals as well as the Research Ethical Committee of Beni-Suef University's Faculty of Veterinary Medicine.

The mice were haphazardly assigned into three groups of ten mice each one week following acclimatization:

**Control group (C group):** Mice served as control and were intraperitoneal injected with equal volumes of 0.9% saline.

**Valproic acid group (VPA group):** VPA at dosage of 100 mg/kg body weight (dissolved in distilled water) was selected according to Abdella et al. [8]. Mice were intraperitoneal injected with VPA (100 mg/kg/day)
for 21 days.

**Sesame oil group (SO group):** Mice were given 8 ml/kg of sesame oil orally every day [10] concurrent with i.p. injection of VPA all over the period.

### 3-Sampling and Biochemical analysis

Blood samples were taken through retro-orbital hemorrhage 24 hours after the last dosage. Blood samples were left for 30 minutes at 37°C to clot. For serum separation, clotted blood samples were centrifuged at 3000 rpm for 15 minutes. The sera were maintained at −20°C till they were used.

#### 3.1-Sample collection

Cervical dislocation was used to kill the mice. The liver tissue was removed and cleaned in physiological saline after that. Two sections of the liver samples were taken. For homogenization, the first part from liver (0.5g) was put in 5 mL phosphate buffered saline (Teflon Homogenizer, India). Using a high-speed cooling centrifuge, the tissue homogenate was centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatants were kept at −20°C until the oxidative/antioxidant indices were determined. The other part was preserved at −80°C to determine the level of PXR gene expression.

### 3.2-Estimation of biochemical parameters

#### 3.2.1- Parameters of oxidative and antioxidant status

The MDA level and GPx activity in liver tissue homogenate were used to assess the oxidant-antioxidant status of the tissue. The concentration of MDA was determined using the technique of Wills [11]. GPx activity measurement was based on Paglia and Valentine [12].

#### 3.2.2-Determination of serum levels of IL-1β and IL-6

IL-1β and IL-6 serum levels were determined using ELISA kits in line with the manufacturer's commands.

#### 3.2.3- Real time-polymerase chain reaction (RT-PCR) for detection of PXR gene expression:

Total RNA was extracted from liver according to manufacturer's instructions using Ribozol™ RNA Extraction Reagents with the code N580. A UV spectrophotometer "Hitachi spectrophotometer, Model U-2000, Tokyo, Japan" was used to quantify the concentration of RNA.

cDNA synthesis: Five μg of RNA were reverse transcribed with oligonucleotide (dT)18 primer and denatured for two minutes at 70°C. On ice, denatured RNA was added to a reverse transcription mixture. The reaction tube was kept at 42°C for 1 hour before being heated to 92°C to end the reaction.

For quantitative RT-PCR, first-strand cDNA (5 μL) were used in a whole volume of 25 μL, which included 12.5 μL 2x SYBR Green PCR Master Mix and 200 ng of each primer as given in table (1). On the step one
plus RT-PCR system, PCR reactions comprising 95°C for 10 minutes (1 cycle), 94°C for 15 seconds, and 60°C for 1 minute (40 cycles), were performed (Applied Biosystems). The ABI Prism 7500 sequence detection system software was used to assess the data, and PE Biosystems’ v1.7 Sequence Detection Software was used to quantify it (Foster City, CA). The comparative threshold cycle approach was used to calculate the relative expression of the genes examined. All of these data were normalized to the beta-actin genes, all of these processes were performed in accordance with the procedure outlined by Livac and Schmittgen [13].

4-In silico molecular docking

Molecular docking was performed for Gpx, IL-1β, IL6, and PXR against sodium valproate and sesamin, sesaminol, sesamol, and sesamolin as main constituents of sesame oil. Initially, the biological data were collected from both NCBI (ID: EC 1.11.1.9, EC 16176, 16193, 10090) and UniProtKB (ID: P11352, Q3TI17, A2RTD1, O54915) databases for Gpx, IL-1β, IL6, and PXR respectively. Modelling of the 3D structure for each was performed by I-TASSER server. PubChem database was used for obtaining each structure. Modification for all structures were done by Swiss PDB Viewer (spdbv) software for energy minimization process. Open Babel (Version 2.3.1) software was used to convert the format from pdb to pdbqt. Before the molecular docking process, each protein and ligand was altered by the addition of hydrogen atoms, and metals were handled with the Discovery Studio software (version 2019). Eventually, Auto Dock Vina (Version 2.0) was used define the grid box with 1.00 Å spacing and a grid map of 42 54 42 XYZ Å points for Gpx, 46 46 48 XYZ Å points for IL-1β, 54 54 48 XYZ Å points for IL6, and 90 82 64 XYZ Å points for PXR enzyme. Van der Waals interactions, binding energy, and inhibition constant were ranked by Auto Dock [14].

5-Statistical analysis

All results were statistically evaluated using one-way analysis of variance (ANOVA) using SPSS software (Chicago, USA) then comparisons by using the Tukey post hoc test. The data were exhibited as a mean with standard error (SE). At p < 0.05, the differences were judged statistically significant.

Results

Our experimental results revealed that the administration of sesame oil modulated the valproic acid-induced changes in oxidant/antioxidant indices and pro-inflammatory mediators and upregulated PXR gene expression level as a result of its anti-inflammatory and antioxidant properties “Fig. 1,2 &3”.

From the docking analysis results, the binding energy ranged from -3.9: -7.1 through Gpx complexes with the five ligands, -4.4: -7.9 through IL-1β complexes, -5.1: -8.6 through IL6, and -5.5: -8.4 through PXR complexes (table 2). Figure 4, 5, 6, and 7 illustrated the 2D chemical interactions for each docked protein-ligand complex. Many interactions were identified such as conventional hydrogen bonds, carbon hydrogen bond, Alkyl, Van der Waals, pi-alkyl, pi-sigma, pi-anion, and pi-sulfur. Hydrophobicity areas were shown in Fig. 8, 9, 10, and 11 in all docked complexes with the brown color. Also, H-bond areas were
shown in Fig. 12, 13, 14, and 15. The solvent accessible surface (SAS) areas were shown in Fig. 16, 17, 18, and 19.

Discussion

Valproic acid is one of the histone deacetylase inhibitors used for management of epilepsy, bipolar disorder as well as migraine. Although the common use of VPA, its clinical use has been linked to hepatotoxicity which has been widely established [1]. One of the most essential features of VPA therapy is the development of innovative techniques to avoid or reduce hepatotoxicity. Subsequently in the current study, the preventive influence of sesame oil versus VPA- provoked hepatotoxicity was examined. It was reported that oxidative stress causes excessive formation of free radicals and exhaustion of antioxidants which are linked to liver dysfunction caused by VPA [15]. Our results revealed that VPA administration increased considerably hepatic MDA level and decreased Gpx enzyme activity as compared to control group. These results confirm previous work stated that VPA induced liver lipid peroxidation and decreased hepatic antioxidant enzymes activities [7]. The critical role of free radicals in cell injury is well understood, and it has been postulated that the covalent attachment of free radicals to macromolecules, in addition to reactive intermediates, may play a role in the severe adverse drug reactions. Several investigations have suggested that hepatotoxic medications produce reactive metabolites and free radicals [16].

Pre-treatment with SO improved the VPA-induced variations in oxidative stress indices where it significantly reduced hepatic MDA level and increased Gpx activity indicating the hepato-protective effect of SO. These findings were consistent with Hsu et al. [10] who explained that SO potently inhibited lipid peroxidation by preventing the production of hydroxyl radicals, peroxynitrite, and nitric oxide. It is strongly believed that the antioxidant properties of SO is owing to the existence of vitamin E and lignans which are non-fat constituents, such as sesamin, sesamol, sesaminol and sesamolin. Lignans as antioxidant have been observed in numerous studies and vitamin E has also been shown to boost glutathione tissue content [17]. Additionally, natural products provide protection against free radicals mediated damage because of their antioxidant properties, which eliminate free radicals from the biological environment [18].

The inflammatory reactions associated with VPA administration was clearly obvious during our study via the elevating serum level of IL-1β and IL-6 in mice of VPA group in line with Jin et al. [7] who reported that VPA induced a rise in nuclear factor-kappa B (NF-κB) level as well as stimulation of IL-1β and IL-6 expression. It appears that oxidative stress associated with VPA triggers transcription factors, as NF-κB, leading to oxidative damage and inflammation [19]. Sesame oil showed a protective effect against these inflammatory reactions via lowering the serum levels of both IL-1β and IL-6 in accordance with Hsu et al. [20] who found that SO diminished the levels of some inflammatory mediators (tumor necrosis factor-α, IL-1β and IL-6) via suppression of inducible nitric oxide synthase (iNOS) production and inhibition of neutrophil infiltration. Sesaminol, one of SO active constituent, decreased the inflammatory cytokine expression in mice's brains [21]. Previously, it was reported that SO inhibited NF-κB which had an anti-
inflammatory impact in cultured rat astrocytes. SO inhibited translocation of NF-κB from cytoplasm in to the nucleus thus, it is clear that SO has components that inhibit inflammation through blocking NF-κB activation [22]. These data suggested that suppression of inflammatory cytokine production could be part of the mechanism behind sesame oil's hepato-protective impact against VPA-induced liver damage.

PXR is a well-known drug and xenobiotic metabolism regulator. It's engaged in medication-drug interactions, which impact drug metabolism and excretion, lowering efficacy or raising toxicity [23]. In our study, VPA significantly decreased the expression level of PXR gene in liver tissue as a result of inflammatory reactions associated with its administration in line with Pascussi et al. [24] who reported that the levels of hepatic PXR mRNA have been downregulated in response to inflammatory signals. He found that PXR mRNA is downregulated by the pro-inflammatory cytokine IL-6 in primary human hepatocytes. PXR regulates the expression of several drug metabolizing enzymes. It defends the body against dangerous outside toxins as well as endogenous toxins [3].

The daily administration of SO significantly increased the gene expression level of PXR in agreement with accumulating data which revealed that PXR is a biological target of a variety of herbal extracts or active substances found in herbal medicine [25]. Natural products have been discovered to interact with PXR in some way, causing enzymes and transporters to up-regulate in order to speed up their own metabolism and, as a result, the metabolism of concomitant medications that are substrates for the same enzymes and transporters. PXR is participating in the adjustment of immunological and inflammatory reactions [3]. Therefore, PXR activators can be used for the remedy of liver inflammatory disorders. The benefits of SO and its components, such as sesaminol, can modify the amount of gene transcription in diverse targets, such as hepatocytes, endothelial cells, and macrophages, according to the majority of the research discussed [26].

We suggest that the protective effect of SO might be a result of its anti-inflammatory activity in harmony with Pavek [27] who reported that pro-inflammatory stimuli decrease the liver drug-metabolizing activities by inhibiting PXR activation. Sesame lignans or antioxidants such as sesamin, episesamin, sesaminol and sesamolin are responsible for many of sesame oil's distinctive chemical and physiological qualities. Sesamin improves liver detoxication, lowers the risk of chemically generated cancers, protects neuronal cells from oxidative stress, and has anti-inflammatory and anti-allergic properties. Additionally, the interaction of natural compounds with distinct cytochrome isoforms contributes to their protective potential [28].

After the in vivo experiment's procedure and analysis, it was essential to predict the changes during the inhibition process in different measured parameters. This prediction was carried out by the in silico docking [29]. Beginning with the binding energy: in the Gpx docked complexes, sesamin, and sesaminol showed the best results of -7.1, in the IL-1β docked complexes, sesaminol showed the best results of -7.9, in the IL-6 docked complexes, sesamin, and sesaminol showed the best results of -8.6; in the PXR, sesamin showed the best results of -8.4. The sesamin component generally offered the best results among all the docked complexes against most targeted proteins. Secondly, the 2D chemical interaction
analysis, which indicates the strongest of the binding interactions among each complex, showed many powerful interactions. In Gpx docked complexes with: sodium valproate, there were two conventional hydrogen bonds in the LEU A:22 and THR A: 23 residues, with sesamin there was one conventional hydrogen bond in the ARG A: 34, with sesaminol, there was one conventional hydrogen link in the ASP A:169 and one carbon-hydrogen link in the GLY A: 31, with sesamol there were three conventional hydrogen bonds in the LEU A: 22, THR A: 23, and PRO A: 104 residues and there was one pi-donor hydrogen bond in the LEU A: 108 residue, with sesamolin there were three conventional hydrogen bonds in the MET A: 1, CYS A: 2, and ARG A: 174 residues and there was one carbon hydrogen bond in the ASN A: 199 residue. Also, in IL-1β docked complexes with sodium valproate, there was one carbon hydrogen bond in the SER A:65, with, with sesamol there were two conventional hydrogen bonds in the ALA A: 2 and ASN A: 8 residues, and with sesamolin, there was one conventional hydrogen bond in the ASN A: 8 residue. In IL-6 docked complexes with: sodium valproate, there was one conventional hydrogen bond in the GLN A: 94, with sesaminol, there was one carbon hydrogen bond in the MET A: 127 residue, with sesamolin there was one carbon hydrogen bond in the SER A: 115 residue. And finally, in the 2D chemical interactions of PXR complexes with: sodium valproate there were two conventional hydrogen links in the GLN A: 92 and ASN A: 69 residues, with sesamin, there were two conventional hydrogen links in the LEU A: 332 and TYR A: 337 and there was one carbon hydrogen bonds in the LEU A: 327 residue, with sesaminol there were two conventional hydrogen bonds in the LEU A: 327 residue, with sesamolin there were one carbon hydrogen bonds in the LEU A: 332 and there were four carbon hydrogen bonds in the GLN A: 331, THR A: 287, TYR A: 337 and LEU A: 190 residues. In the drug design strategies of inhibitors, hydrophobicity is one of the most critical parameters of the success of the inhibition procedure. Especially examining how inhibitors interact with the hydrophobic and hydrophilic regions of the enzyme active site [30]. During the analysis of the most hydrophobic areas: Gpx-sesaminol, IL1β-sesamin, IL6-sesaminol, and PXR-sesamol docked complexes were the most in the brown color (hydrophobicity). On the other hand, during the changes in the binding site interactions, water may hinder the formation of solute intermolecular interactions by forming hydrogen bonds with proton donors and acceptors in the solute. So, H-bond as acceptor and doners is an essential parameter in the inhibition process. Among the areas of H-bonds as donors (pink color) and acceptors (green color) in the all docked complexes: Gpx-sesamin, IL1β-sesamol, IL6-sesamin, and PXR-sesamin were the most. Solvent accessible surface (SAS) is defined as the location of the solvent molecule's center as it rolls over the protein's van der Waals surface. The SAS interactions areas analysis (blue color) among the all docked complexes showed that Gpx-sesamin, IL1β-sodium valproate, IL6-sesaminol, and PXR-sesamin were the most.

**Conclusion**

Based on our results, we concluded that sesame oil has the potential capacity to alleviate valproic acid-induced hepatotoxicity due to its antioxidant and anti-inflammatory properties which are attributed to
radical scavenging activities of it and its components. Therefore, sesame oil can be considered as a promising compound to prevent liver toxicity induced by valproic acid.

**Abbreviations**

MDA: malondialdehyde  
GPx: glutathione peroxidase  
IL-1β: interleukine 1β  
IL-6: interleukine 6  
PXR: pregnane xenobiotic receptor  
VPA: valproic acid  
SO: sesame oil  
NF-κB: nuclear factor-kappa B  
iNOS: inducible nitric oxide synthase

**Declarations**

**Availability of data and materials**

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

**Competing interests**

The authors declare no competing financial interests.

**Funding**

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

**Authors’ contributions**

DSM, NSS & OS conceived of the idea. NSS & OS performed the experiment & laboratory works. MML performing the molecular docking study. DSM & OS analyzed the results. DSM the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.
References


temporal relationship with onset of toxicity. Toxicol Appl Pharmacol 252:318–324. doi: https://doi.org/10.1016/j.taap.2011.03.004


Tables

Table (1): Sequences of primers utilized in quantitative Real Time-PCR amplification of mRNAs expressing PXR.

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<th>mRNA</th>
<th>Sequences (5 → 3)</th>
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<td>PXR</td>
<td>Forward primer: GGTGTGGTCCAGCGCAGCGT</td>
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<td></td>
<td>Reverse primer: ACTGCTGGGTTTGCTGGGCGT</td>
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<tr>
<td>β-actin</td>
<td>Forward primer: ATGAGCCCCAGCCTTCTCCAT</td>
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<tr>
<td></td>
<td>Reverse primer: CCAGCCGAGCCACATCGCTC</td>
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Table (2): Docking score of binding energy for each docked protein-ligand complex.
<table>
<thead>
<tr>
<th>Name of ligand</th>
<th>Binding energy</th>
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<tr>
<td>Glutathione peroxidase (Gpx)</td>
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</tr>
<tr>
<td>Sodium Valproate</td>
<td>-3.9</td>
</tr>
<tr>
<td>Sesamin</td>
<td>-7.1</td>
</tr>
<tr>
<td>Sesaminol</td>
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<td>-4.4</td>
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<tr>
<td>Sesamolin</td>
<td>-7</td>
</tr>
<tr>
<td>Interleukin-1β (IL-1β)</td>
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<td>-8.4</td>
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</table>

**Figures**
Figure 1

**Hepatic MDA levels and Gpx activity in C, VPA and SO groups.**

Values as mean ± SE of mean. The significant difference at (P > 0.05) is represented as different superscript letters between the studied groups.

Figure 3

**Relative expression levels of PXR gene in C, VPA and SO groups.**
Values as mean ± SE of mean. The significant difference at (P > 0.05) is represented as different superscript letters between the studied groups.

**Figure 6**

Figure 7

Figure 9

Figure 10

Figure 11

Figure 13

**Figure 14**


**Figure 15**


**Figure 17**

Figure 18