Evaluation of the Protective Effects of Ellagic Acid and Taurine on Lipid Profile and Gene Expressions of NF-κB, IL-1β, and TNF-α against Fluoxetine Induced Hepatotoxicity in Male Wistar Rats

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Research Article

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Abstract

Purpose

Fluoxetine by increasing free radicals can cause hepatotoxicity. Ellagic acid and taurine have antioxidant properties. In this study, the protective effects of ellagic acid and taurine against fluoxetine-induced liver damage were examined

Methods

The animals were divided into five groups as follows: group 1: controls receiving corn oil; group 2: receiving fluoxetine 15 mg/kg body weight (bw); group 3: receiving fluoxetine 15mg/kg bw and ellagic acid 50 mg/kg bw; group 4: receiving fluoxetine 15 mg/kg bw and taurine 100 mg/kg bw; and group 5: receiving 15mg/kg bw, ellagic acid 50 mg/kg bw, and taurine 100 mg/kg bw.

Results

Fluoxetine significantly raised serum uric acid, malondialdehyde (MDA), protein carbonyl (PC), lipids profile, serum glutamate pyruvate transaminase (GPT), serum glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP), total bilirubin, and serum interleukin-1 beta; and gene expressions of interleukin-1beta (IL-1B), nuclear factor kappa B (NF-κB), and tumor necrosis factor-alpha (TNF-α). Moreover, it significantly decreased ($p < 0.05$) high-density lipoprotein cholesterol (HDL-C), ferric reducing/antioxidant power (FRAP), catalase (CAT), vitamin C, and superoxide dismutase (SOD) in the liver compared to group 1. Treatment with ellagic acid and taurine significantly elevated antioxidant capacity and decreased hepatotoxic biochemical parameters and cells' inflammation compared to group 2. Also, the results confirm that treatment with ellagic acid and taurine improved tissue change and liver function.

Conclusion

Our study has concluded the beneficial effect of ellagic acid and taurine against fluoxetine-induced hepatotoxicity. This effect is derived from free radical scavenging properties and the anti-inflammatory effect related to IL-1B, NF-κB, TNF-α.

Introduction

Antidepressant medications like Fluoxetine are used under some conditions such as major depression, panic disorder, and obsessive-compulsive disorder (Gibbons et al., 2012, Baytunca et al., 2016). This drug is metabolized in the liver by cytochrome (CYP) CYP2D6 to the norfluoxetine. Fluoxetine and norfluoxetine inhibit hepatic cytochromes such as CYP2B6, CYP2C19, CYP2D6, and CYP2C (Mandrioli et

al., 2006, Sager et al., 2014). Accordingly, these cytochromes are components of electron transfer chain oxidases. The inhibition of hepatic cytochrome by norfluoxetine consequently leads to the production of reactive oxygen species)ROS) (Feng and He, 2013). Overproduction of ROS and depletion of antioxidant capacity cause oxidative stress (Lukaszewicz-Hussain, 2010). Under oxidative stress conditions, ROS binds to macromolecules such as proteins and lipids and then leads to their oxidation (Chapple, 1997). Side effects of fluoxetine include chronic liver failure, dyslipidemia, diabetes mellitus, and heart disease (Mastronardi et al., 2011). Mechanisms of hepatic damage include the increased hepatic enzymes such as aminotransferase, gluconeogenesis, change in the metabolism of glucose, and oxidative stress (Ranjbar et al., 2005). Of note, Hepatotoxicity has been reported in oxidative stress (Cai et al., 2015). besides, fluoxetine by the development of oxidative stress leads to hepatic damage (Mastronardi et al., 2011). Antioxidants are a class of substances that inhibit oxidation. These compounds prevent cell damage by collecting ROS (Halliwell, 2011). Antioxidants have been reported to treat some diseases such as drug-induced hepatotoxicity, cancer, and cardiovascular diseases (Zafarullah et al., 2003). Polyphenolic compounds like ellagic acid are found in green tea, grapes, apples, and pomegranates, which have strong antioxidant properties. Additionally, taurine is produced from decarboxylation and oxidation of sulfur amino acids that has antioxidant properties (Balkan et al., 2001, Rehman et al., 2012). Therefore, in this study, our aim was to evaluate the protective effects of ellagic acid and taurine on hepatotoxic biochemical parameters, lipid profile, antioxidant capacity, and gene expressions of interleukin-1beta (IL-1B), nuclear factor kappa B (NF-κB), and tumor necrosis factor-alpha (TNF-α) against fluoxetine induced hepatotoxicity in male Wistar rats.

Materials And Methods

Chemicals

Sodium acetate, 2-thiobarbituric acid, Ferric chloride, and H2O2 were obtained from Merck (Darmstadt, Germany). Fluoxetine capsules (20 mg fluoxetine-hydrochloride), ellagic acid, and taurine powder were also obtained from pars Daru Company (Tehran, Iran). In addition, nitro blue tetrazolium, 2, 4, 6-tripyridyl-s-triazine (TPTZ), and 2,4-Dinitrophenylhydrazine (2,4-DNPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). SYBR® Green PCR Master Mix was obtained from Qiagen Company (Düsseldorf, Germany). Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), Triglyceride (TG), total cholesterol (TC), serum glutamate pyruvate transaminase (GPT), serum glutamate oxaloacetate transaminase (GOT), alkaline phosphatase (ALP), and total bilirubin were bought from Pars Azmoon Company (Tehran, Iran).

Animals

In this study, 40 male Wistar rats were used. The rats were obtained from Tehran Pasteur Institute (Tehran, Iran). All of them were kept under laboratory conditions (at 25 °C temperature, 50% humidity, free access to standard food and water, and a 12hr light/dark cycle). We randomly divided all the animals into five groups. The first group (control group) received only 1 ml corn oil by stomach tube for three weeks

(Karimi-Khouzani et al., 2017). The second group (group 2) received fluoxetine 15 mg/kg body weight (bw) by stomach tube for three weeks (Zlatković et al., 2014). The third group (positive control) received fluoxetine 15 mg/kg body weight and ellagic acid 50 mg/kg body weight by stomach tube for three weeks (Pari and Sivasankari, 2008). The fourth group received fluoxetine 15 mg/kg body weight and taurine 100mg/kg body weight by stomach tube for three weeks (Kim et al., 2017). Finally, the fifth group received fluoxetine 15 mg/kg body weight, ellagic acid 50 mg/kg body weight, and taurine 100 mg/kg body weight by stomach tube for three weeks. Three weeks later, the rats were anesthetized with chloroform and blood of rats were collected by cardiac puncture. Next, bloods were centrifuged and serum and plasma were separated for biochemical analysis. Also, a piece of liver tissue was removed to determine histological studies, superoxide dismutase (SOD), catalase (CAT), TNF-α, IL-1β, and NF-κB gene expressions.

Biochemical analysis

Serum total bilirubin, ALP, GOT, GPT, HDL-C, LDL-C, TG, and TG levels were measured using an auto-analyzer (BT 3000, France). Additionally, we measured serum interleukin-1 beta (IL-1β) by ELISA kit (BT-Laboratory, China) in terms of the kits' protocol. VLDL-C was calculated using TG/5 by Friedewald et al (Friedewald et al., 1972)

Determination of Ferric Reducing/Antioxidant Power (FRAP)

Antioxidant capacity was measured using tripyridyl-s-triazine (TPTZ). Accordingly, this method was firstly described by Benzie and Strain (Benzie and Strain, 1996).

Determination of lipid peroxidation

Serum and liver malondialdehyde (MDA) was measured using Thiobarbituric acid described in a previous study by Heidarian & Soofiniya (Heidarian and Soofiniya, 2011).

Determination CAT and SOD in the liver tissue

Heidarian et al. measured CAT activity in a previous study (Heidarian et al., 2014). Additionally, Beauchamp and Fridivich measured SOD activity (Beauchamp and Fridovich, 1971).

Determination of protein oxidation

Protein carbonyl was measured using 6 M guanidine hydrochloride. Correspondingly, this method was described by Reznick & Packer (Reznick and Packer, 1994).

Determination of vitamin C in the liver tissue

Vitamin C was measured in liver tissue by the Omaye method (Omaye et al., 1979).

Determination of TNF-α IL-1β and NF-κB gene expression

Using RNX-plus kit, total RNA was extracted (GMbiolab, China) according to the kits' protocol. Quality and quantity total RNA was also measured with absorbance at 260/280 nm by spectrophotometer (Nanodrop2000, Thermo, USA). CDNA measurement was accomplished using PrimeScriptTM reagent kit (Takara Bio Inc. Japan) in terms of the kits' protocol. The amplification of cDNA was done by Real-time quantitative PCR (RT-qPCR) using specific primers for TNF-α, IL-1β and NF-κB and SYBR® Green PCR Master Mix (Qiagen, Germany). Accordingly, these primers were as follows: Forward for IL-1β: 5´-CAACAAAAATGCCTCGTGCTG-3´ reverse: 5´-TCGTTGCTTGTCTCTCCTTGTA-3´ , forward for TNF-α: 5´-CTGGCGTGTTCATCCGTTC-3´ reverse: 5´-GGCTCTGAGGAGTAGACGATAA-3´ , forward for NF-κβ: 5´-CTGGCCATGGACGATCTGTT-3´ reverse: 5´-TGATCTTGATGGTGGGGTGC-3´, and forward for β-actin: 5´-AGGAGTACGATGAGTCCGGC-3´ reverse: 5´-CGCAGCTCAGTAACAGTCCG-3´. Using primer 3.0 and oligo 7.0 software, these primers were designed and then confirmed by BLAST Nucleotide (NCBI). The amplification of cDNA was performed for 10 min at 95 °C. RT-qPCR was done in three steps and 40 cycles. These steps were as follows: for 15 s at 95 °C for secondary denaturation, for 20 s at 60 °C for annealing, and 25 s at 72 °C for extension). In addition, β-actin was used to control gene and normalize gene expression

Histopathological study

After sacrificing the rats, liver tissues of each experimental group were fixed in 20% formaldehyde. All these tissues were dissected in 5-μm pieces by a microtome. These pieces were then stained with hematoxylin-eosin (H&E). After performing this stage, the tissues were observed with a light microscope, up to the histopathological observation at the end, we photographed from the tissues using a digital camera (Nikon camera).

Statistical analysis

Results were shown as mean ± SD. Statistical analyses were done by one-way analysis of variance (ANOVA) and Tukey post hoc test using SPSS software (Statistical Package for the Social Sciences, version 20.0, SPSS Inc, Chicago, IL, USA). The statistical significance level was considered as $p < 0.05$

Results

Biochemical data

Table 1 shows the effects of ellagic acid and taurine on biochemical data. In the second group that received only fluoxetine, we observed a significant elevation ($p < 0.05$) in parameters such as GOT, ALP, GPT, total bilirubin, LDL-C, TG, VLDL-C, TC, and uric acid and a significant reduction ($p < 0.05$) in HDL-C compared to the group 1 (Table 1). Moreover, in the groups that received ellagic acid and taurine and group 5, we observed a significant reduction ($p < 0.05$) in serum GOT, ALP, GPT, TG, VLDL-C, TC, and uric acid as well as a significant elevation ($p < 0.05$) in HDL-C compared to the group 2 (only treated with fluoxetine). Also, was observed a significant reduction ($p < 0.05$) in total serum bilirubin and LDL-C in group 5 compared to group 2.

The effects of ellagic acid and taurine on FRAP and MDA levels

Table 2 shows the effects of ellagic acid and taurine on FRAP and MDA levels. In the second group receiving fluoxetine for three weeks, we observed a significant elevation ($p < 0.05$) in serum and tissue MDA content and a significant reduction ($p < 0.05$) in FRAP levels compared to the control group (Table 2). We found that in groups 3,4, and 5 that received ellagic acid and taurine FRAP levels significantly raised and ($p < 0.05$) serum and tissue MDA significantly decreased ($p < 0.05$) compared to group 2. Also, we observed a significant elevation ($p < 0.05$) in FRAP levels and a significant reduction ($p < 0.05$) in serum MDA in group 5 compared to groups 3 and 4.

Effects of ellagic acid and taurine on PC and vitamin C

Table 3 shows the effects of ellagic acid and taurine on PC and vitamin C. A significant increase was observed in serum PC in the second group ($p < 0.05$) compared to group 1. Moreover, in this group, a significant depletion was observed ($p < 0.05$) in liver vitamin C compared to group 1 (Table 3). The groups that received ellagic acid and taurine alone and group 5 was observed a significant elevation ($p < 0.05$) in liver vitamin C compared to group 2. In addition, a significant reduction ($p < 0.05$) was observed in serum PC compared to group 2 in groups 3, 4, and 5. However, in group 5 compared to groups 3 and 4, a significant depletion was observed ($p < 0.05$) in PC.

Effects of ellagic acid and taurine on SOD and CAT activity in the liver tissue

Fig. 1 shows the effects of ellagic acid and taurine on SOD and CAT activities in the liver tissue. In the second group that received fluoxetine for three weeks compared to group 1, we observed a significant reduction ($p < 0.05$) in SOD and CAT activities in the liver tissue (Fig. 1). In the groups that received ellagic acid and taurine and group 5 a significant elevation was observed in the SOD and CAT activities in the liver tissue ($p < 0.05$) compared to group 2. In the fifth group, a significant elevation ($p < 0.05$) was found in the SOD and CAT activities in the liver compared to the third and fourth groups.

The effects of ellagic acid and taurine on TNF-α, IL-1β, and NF-κB gene expressions and serum IL-1β

Fig. 2 shows the effects of ellagic acid and taurine on serum IL-1β, TNF-α, IL-1β, and NF-kB gene expressions. In the second group that only received fluoxetine, we observed a significant elevation ($p < 0.05$) in serum IL-1β, TNF-α, IL-1β, and NF-kB gene expressions compared to group 1 (Fig. 2). Additionally, in the groups that received ellagic acid and taurine and group 5 we observed a significant reduction ($p < 0.05$) in serum IL-1β, TNF-α, IL-1β, and NF-kB gene expressions compared to group 2. However, in group 5 compared to the groups 3 and 4, a significant depletion ($p < 0.05$) was observed in serum IL-1β, TNF-α, IL-1β, and NF-kB gene expressions.

Histopathological findings

In the first group, a normal morphology was observed (Fig. 3 (A)), but infiltration of lymphocyte cells was observed in the second group (just received fluoxetine) compared to group 1 (Fig. 3 (B)). Additionally, in

groups 3 and 4 that received ellagic acid and taurine alone, a depletion was observed in cells' inflammation compared to group 2 (Fig. 3 (C, D)). In group 5, a significant depletion was observed in cells' inflammation and infiltration of lymphocyte cells compared to group 2 (Fig. 3 (E)).

Discussion

Fluoxetine is one of the antidepressants considered for the treatment of depressive disorders, but it faces limitations due to causing liver complications. Fluoxetine leads to the release of free radicals and liver damage by impairing organs' function like mitochondria (Lyoo and Renshaw, 2002, Özden et al., 2005). Increasing liver markers such as GOT, GPT, total bilirubin, and ALP in the blood is known as one of the signs of liver damage that are measured as important markers in liver damage (Adeyemi and Olayaki, 2018). Of note, GOT, GPT, total bilirubin, and ALP in the second group significantly increased compared to group 1 (Table 1). Previous studies confirm the results of the present study (Inkielewicz-Stępniak, 2011, Karimi-Khouzani et al., 2017). The ellagic acid and taurine-treated groups showed a significant reduction in serum levels of these enzymes (Table 1). Previous researches have shown that antioxidants can maintain cell membrane and reduce enzyme release through free radical scavenging activity. Therefore, it can be said that antioxidant compounds prevent liver necrosis and liver dysfunction by protecting liver cells against free radicals (Feng et al., 2014, Karimi-Khouzani et al., 2017). Therefore it seems reducing the level of GOT, GPT, total bilirubin, and ALP were related to antioxidant properties of ellagic acid and taurine.

Changes in the serum lipid profile such as VLDL-C, LDL-C, TC, TG, and HDL-C usually occur in liver injury (Panneerselvam et al., 2013). The results of our study showed that fluoxetine consumption significantly changes the level of lipid profile. As well, Fluoxetine has been shown to elevate TC, TG, and LDL-C levels significantly. Also, a significant depletion was observed in HDL-C levels in group 2 compared to group 1 (Table 1). Previous works have reported hyperlipidemia is related to the effect of fluoxetine on gene expression of fatty acid synthesis and acetyl-CoA carboxylase 1. Previous studies confirm the results of the present study (Cheng et al., 2007, Karimi-Khouzani et al., 2017). Evaluation of lipid profile in different groups showed that ellagic acid and taurine were effective in reducing TC, TG, and LDL-C levels as well as increasing HDL-C levels in the treatment groups (Table 1). Previous research has shown that antioxidants can play an effective role in preventing the elevation of lipid profile levels in fluoxetine-induced liver damage (Tung et al., 2009, Heidarian et al., 2017, Sharifi-Rigi et al., 2019). It seems that the roles of ellagic acid and taurine in reducing the level of lipid profile were related to their antioxidant properties. Ellagic acid and taurine both exert their hypolipidemic activity by reducing de novo lipogenesis and TG esterification, while enhancing FA oxidation. Previous studies have shown that ellagic acid and taurine are able to enhance the phosphorylation of AMP-activated protein kinase (AMPK). AMPK activation has been shown to regulate energy homeostasis by inhibiting adipogenesis and de novo TG synthesis, and by augmenting FA oxidation (Kang et al., 2016, Han et al., 2020).

Some parameters such as PC and MDA are the other oxidative stress markers (Beal, 2002). In this report, a significant elevation we observed in PC and MDA in the fluoxetine receiving group as well as a

significant depletion in FRAP compared to group 1 (Tables 2 and 3). Previous studies confirm the results of the present study that fluoxetine by increasing free radicals leads to the production of PC and MDA (Zlatković et al., 2014, Karimi-Khouzani et al., 2017). In the other group that received ellagic acid and taurine, a significant elevation was found in FRAP levels as well as a significant depletion in contents of serum PC and MDA in serum and liver tissues compared to group 2 (Tables 2 and 3). Previous works have reported antioxidants by increasing antioxidant capacity lead to a reduction of free radicals (Mudnic et al., 2010, Heidarian et al., 2017, Esmaeilzadeh et al., 2020). It seems the role of ellagic acid and taurine to be relative to their antioxidant properties in the prevention of cell necrosis and inhibition of lipid peroxidation.

Natural antioxidants such as SOD and CAT are two kinds of important antioxidants to neutralize ROS. SOD and CAT neutralize ROS to compounds such as water and oxygen. Also, another antioxidant such as vitamin C is obtained by food, which eliminates ROS. Following the elevation of ROS production, the level of enzymatic antioxidants decreased (Sen et al., 2010, Pai et al., 2012). In the fluoxetine receiving group compared to group 1, contents of vitamin C, CAT and SOD significantly decreased in the liver tissue (Table 3 and Fig. 1). Previous studies confirm the results of the present study that fluoxetine leads to oxidative stress and under oxidative stress conditions antioxidants are reduced (Jayavelu et al., 2013, Zlatković et al., 2014, Karimi-Khouzani et al., 2017). In the ellagic acid and taurine receiving group compared to group 2 contents of vitamin, CAT, and SOD significantly increased in the liver tissue (Table 3 and Fig. 1). Moreover, in histopathological examination of liver tissue, we found a significantly decreased inflammation and tissue damage in the group receiving ellagic acid and taurine compared to group 2 (Fig. 3). Previous works have reported antioxidants by inhibiting oxidative stress lead to elevation of antioxidant capacity (Nouri et al., 2017, Nouri and Heidarian, 2019). These effects may be mediated by the antioxidant properties of the two compounds.

Previous research has shown that fluoxetine induced liver damage that is relative to inflammation in liver tissue. These changes may be due to the elevation of ROS production in the liver tissue. NF-κβ, TNF-α, and IL-1β are the main mediators initiating the cascade path of inflammation. The production of these cytokines in the liver is responsible for causing inflammation in the liver tissue, which ultimately leads to apoptosis and necrosis of hepatocytes (Dhami et al., 2013, Karimi-Khouzani et al., 2017). In the fluoxetine receiving group compared to group 1, significantly increased 1 TNF-α, IL-1β, and NF-κB gene expressions and serum IL-1β were observed (Fig. 2). Previous studies confirm the results of the present study (Karimi-Khouzani et al., 2017, Esmaeilzadeh et al., 2020). In the ellagic acid and taurine receiving group compared to the group 2, a significant decrease was observed in serum IL-1β, TNF-α, IL-1β, and NF-κB gene expressions (Fig. 2). Previous works have reported antioxidants to have anti-inflammatory activity by reducing inflammatory factors (Nouri et al., 2017, Nouri et al., 2021). It seems the roles of ellagic acid and taurine to be relative to their antioxidant properties.

This study evaluated the effects of ellagic acid and taurine on fluoxetine-induced liver damage. Since oxidative stress plays an important role in the development of fluoxetine-induced liver damage, it seems that antioxidant compounds can effectively prevent the structural and functional damages caused by

this drug. Our results show that the combined use of alginic acid and taurine further reduces oxidative stress and tissue damage and increases antioxidant reserves compared to the consumption of one of them alone.

Declarations

Conflict of interest

The authors have declared that there is no conflict of interest.

Compliance with ethical standards

All experiments were performed in accordance with the guidelines set by the ethical committee under ethics code IR.FUMS.REC.1398.174.

Consent to Participate

Not applicable

Consent to Publish

Not applicable

Author contributions

MM, RA, and TB conceived and designed research. MM and RA supervised the research. MM and RA contributed new reagents or analytical tools. MM, RA, TB, MS, and AS conducted experiments. MS, TB, and AS analyzed data and wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

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Availability of data and materials

Not applicable

References

Adeyemi WJ, Olayaki LA (2018) Diclofenac - induced hepatotoxicity: Low dose of omega-3 fatty acids have more protective effects. Toxicol Rep 5: 90-95

Balkan J, Dogğru-Abbasoğlul S, Kanbaglil ö, Çevikbas U, Aykaç-Toker G, Uysal M (2001) Taurine has a protective effect against thioacetamide-induced liver cirrhosis by decreasing oxidative stress. Hum Exp Toxicol 20: 251-254

Baytunca MB, Donuk T, Erermis S (2016) Evaluation of a neuropsychiatric disorder: from PANDAS to PANS and CANS. Turk Psikiyatri Dergisi 27

Beal MF (2002) Oxidatively modified proteins in aging and disease. Free Radic Biol Med 32: 797-803

Beauchamp C, Fridovich I (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal Biochem 44: 276-287

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239: 70-76

Cai Z, Lou Q, Wang F, Li E, Sun J, Fang H, Xi J, Ju L (2015) N-acetylcysteine protects against liver injure induced by carbon tetrachloride via activation of the Nrf2/HO-1 pathway. Int J Clin Exp Pathol 8: 8655-8662

Chapple IL (1997) Reactive oxygen species and antioxidants in inflammatory diseases. J Clin Periodontol 24: 287-296

Cheng D, Chu CH, Chen L, Feder JN, Mintier GA, Wu Y, Cook JW, Harpel MR, Locke GA, An Y, Tamura JK (2007) Expression, purification, and characterization of human and rat acetyl coenzyme A carboxylase (ACC) isozymes. Protein Expr Purif 51: 11-21

Dhami KS, Churchward MA, Baker GB, Todd KG (2013) Fluoxetine and citalopram decrease microglial release of glutamate and D-serine to promote cortical neuronal viability following ischemic insult. Mol Cell Neurosci 56: 365-374

Esmaeilzadeh M, Heidarian E, Shaghaghi M, Roshanmehr H, Najafı M, Moradi A, Nouri A (2020) Gallic acid mitigates diclofenac-induced liver toxicity by modulating oxidative stress and suppressing IL-1β gene expression in male rats. Pharm Biol 58: 590-596

Feng L, Mao W, Zhang J, Liu X, Jiao Y, Zhao X, Wang X, Zhang D, Cai D, Wang Y (2014) Pharmacokinetic variations of tetramethylpyrazine phosphate after oral administration in hepatic precancerous mice and its hepatoprotective effects. Drug Dev Ind Pharm 40: 1-8

Feng S, He X (2013) Mechanism-based inhibition of CYP450: an indicator of drug-induced hepatotoxicity. Curr Drug Metab 14: 921-945

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502

Gibbons RD, Hur K, Brown CH, Davis JM, Mann JJ (2012) Benefits from antidepressants: synthesis of 6-week patient-level outcomes from double-blind placebo-controlled randomized trials of fluoxetine and venlafaxine. Arch Gen Psychiatry 69: 572-579

Halliwell B (2011) Free radicals and antioxidants - quo vadis? Trends Pharmacol Sci 32: 125-130

Han HL, Zhang JF, Yan EF, Shen MM, Wu JM, Gan ZD, Wei CH, Zhang LL, Wang T (2020) Effects of taurine on growth performance, antioxidant capacity, and lipid metabolism in broiler chickens. Poult Sci 99: 5707-5717

Heidarian E, Jafari-Dehkordi E, Valipour P, Ghatreh-Samani K, Ashrafi-Eshkaftaki L (2017) Nephroprotective and Anti-Inflammatory Effects of Pistacia atlantica Leaf Hydroethanolic Extract Against Gentamicin-Induced Nephrotoxicity in Rats. J Diet Suppl 14: 489-502

Heidarian E, Saffari J, Jafari-Dehkordi E (2014) Hepatoprotective action of Echinophora platyloba DC leaves against acute toxicity of acetaminophen in rats. J Diet Suppl 11: 53-63

Heidarian E, Soofiniya Y (2011) Hypolipidemic and hypoglycemic effects of aerial part of Cynara scolymus in streptozotocin-induced diabetic rats. J MED PLANTS RES Journal 5: 2717-2723

Inkielewicz-Stępniak I (2011) Impact of fluoxetine on liver damage in rats. Pharmacol Rep 63: 441-447

Jayavelu A, Natarajan A, Sundaresan S, Devi K, Senthilkumar B (2013) Hepatoprotective activity of Boerhavia diffusa L.(Nyctaginaceae) against ibuprofen induced hepatotoxicity in wistar albino rats. Int J Pharm Sci Rev Res 2: 1-8

Kang I, Buckner T, Shay NF, Gu L, Chung S (2016) Improvements in Metabolic Health with Consumption of Ellagic Acid and Subsequent Conversion into Urolithins: Evidence and Mechanisms. Adv Nutr 7: 961-972

Karimi-Khouzani O, Heidarian E, Amini SA (2017) Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats. Pharmacol Rep 69: 830-835

Kim YS, Sung SH, Tang Y, Choi EJ, Choi YJ, Hwang YJ, Park PJ, Kim EK (2017) Protective Effect of Taurine on Mice with Doxorubicin-induced Acute Kidney Injury. Adv Exp Med Biol 975 Pt 2: 1191-1201

Lukaszewicz-Hussain A (2010) Role of oxidative stress in organophosphate insecticide toxicity–Short review. Pestic Biochem Physiol 98: 145-150

Lyoo IK, Renshaw PF (2002) Magnetic resonance spectroscopy: current and future applications in psychiatric research. Biol Psychiatry 51: 195-207

Mandrioli R, Forti GC, Raggi MA (2006) Fluoxetine metabolism and pharmacological interactions: the role of cytochrome p450. Curr Drug Metab 7: 127-133

Mastronardi C, Paz-Filho GJ, Valdez E, Maestre-Mesa J, Licinio J, Wong ML (2011) Long-term body weight outcomes of antidepressant–environment interactions. Mol Psychiatry 16: 265-272

Mudnic I, Modun D, Rastija V, Vukovic J, Brizic I, Katalinic V, Kozina B, Medic-Saric M, Boban M (2010) Antioxidative and vasodilatory effects of phenolic acids in wine. Food Chem 119: 1205-1210

Nouri A, Heibati F, Heidarian E (2021) Gallic acid exerts anti-inflammatory, anti-oxidative stress, and nephroprotective effects against paraquat-induced renal injury in male rats. Naunyn Schmiedebergs Arch Pharmacol 394: 1-9

Nouri A, Heidarian E (2019) Ameliorative effects of N-acetyl cysteine on diclofenac-induced renal injury in male rats based on serum biochemical parameters, oxidative biomarkers, and histopathological study. J Food Biochem 43: e12950

Nouri A, Heidarian E, Nikoukar M (2017) Effects of N-acetyl cysteine on oxidative stress and TNF-α gene expression in diclofenac-induced hepatotoxicity in rats. Toxicology mechanisms and methods 27: 561-567

Omaye ST, Turnbull JD, Sauberlich HE (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol 62: 3-11

Özden H, Bildirici K, Üstüner D, Üstüner C, Cengiz BP, Tülay A, YILMAZ V (2005) Histopathologic examination of rat liver after experimental application of fluoxetine. Tr Ekopatol Derg 11: 9-15

Pai PG, Chamari Nawarathna S, Kulkarni A, Habeeba U, Reddy C S, Teerthanath S, Shenoy JP (2012) Nephroprotective effect of ursolic Acid in a murine model of gentamicin-induced renal damage. ISRN Pharmacol 2012: 410902-410902

Panneerselvam L, Subbiah K, Arumugam A, Senapathy JG (2013) Ferulic acid modulates fluoride-induced oxidative hepatotoxicity in male Wistar rats. Biol Trace Elem Res 151: 85-91

Pari L, Sivasankari R (2008) Effect of ellagic acid on cyclosporine A-induced oxidative damage in the liver of rats. Fundam Clin Pharmacol 22: 395-401

Ranjbar A, Solhi H, Mashayekhi FJ, Susanabdi A, Rezaie A, Abdollahi M (2005) Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. Environ Toxicol Pharmacol 20: 88-91

Rehman MU, Tahir M, Ali F, Qamar W, Lateef A, Khan R, Quaiyoom A, Oday OH, Sultana S (2012) Cyclophosphamide-induced nephrotoxicity, genotoxicity, and damage in kidney genomic DNA of Swiss albino mice: the protective effect of Ellagic acid. Mol Cell Biochem 365: 119-127

Reznick AZ, Packer L (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 233: 357-363

Sager JE, Lutz JD, Foti RS, Davis C, Kunze KL, Isoherranen N (2014) Fluoxetine- and norfluoxetine-mediated complex drug-drug interactions: in vitro to in vivo correlation of effects on CYP2D6, CYP2C19, and CYP3A4. Clin Pharmacol Ther 95: 653-662

Sen S, Chakraborty R, Sridhar C, Reddy Y, De B (2010) Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. Int J Pharm Sci Rev Res 3: 91-100

Sharifi-Rigi A, Heidarian E, Amini SA (2019) Protective and anti-inflammatory effects of hydroalcoholic leaf extract of Origanum vulgare on oxidative stress, TNF-α gene expression and liver histological changes in paraquat-induced hepatotoxicity in rats. Arch Physiol Biochem 125: 56-63

Tung YT, Wu JH, Huang CC, Peng HC, Chen YL, Yang SC, Chang ST (2009) Protective effect of Acacia confusa bark extract and its active compound gallic acid against carbon tetrachloride-induced chronic liver injury in rats. Food Chem Toxicol 47: 1385-1392

Zafarullah M, Li WQ, Sylvester J, Ahmad M (2003) Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci 60: 6-20

Zlatković J, Todorović N, Tomanović N, Bošković M, Djordjević S, Lazarević-Pašti T, Bernardi RE, Djurdjević A, Filipović D (2014) Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. Eur J Pharm Sci 59: 20-30

Tables

Table 1. Effects of ellagic acid and taurine on biochemical tests

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
ALP (u/L)	185.87±17.46	449.75±47.87[a]	250.62±14.48 [ab]	250.38±47.02 [ab]	191.62±21.20 [bcd]
GPT (u/L)	63.62±6.16	135.88±10.74 [a]	94.87±7.05 [ab]	98.37±8.73 [ab]	64.37±12.80 [bcd]
GOT (u/L)	159.50±18.73	290.88±16.47 [a]	188.12±9.06 [ab]	192.00±13.03 [ab]	148.50±20.75 [bcd]
Total bilirubin (mg/dl)	0.85±0.06	2.27±0.70 [a]	1.88±0.131 [a]	1.79±0.29 [a]	0.84±0.10 [bcd]
LDL-C (mg/dl)	23.05±4.15	61.78±11.91 [a]	45.62±6.27 [ab]	44.63±13.10 [ab]	21.99±6.07 [bcd]
TG (mg/dl)	66.12±4.67	143.38±10.43 [a]	82.87±9.89 [ab]	82.00±7.83 [ab]	66.12±10.96 [bcd]
VLDL-C (mg/dl)	13.22±0.93	28.67±2.08 [a]	16.57±1.97 [ab]	16.40±1.56 [ab]	13.22±2.19 [bcd]
HDL-C (mg/dl)	37.62±2.76	14.67±3.43 [a]	28.70±3.66 [ab]	26.65±2.66 [ab]	36.35±2.92 [bcd]
TC (mg/dl)	77.50±5.50	126.12±7.03 [a]	95.50±7.15 [ab]	92.87±11.40 [ab]	76.62±8.31 [bcd]

The obtained data were expressed as mean ± SD (n = 8). Group 1: control, Group 2: received fluoxetine just, Group 3: received fluoxetine and ellagic acid 50 mg/kg, Group 4: received fluoxetine and taurine 100 mg/kg, and Group 5: received fluoxetine, ellagic acid50 mg/kg, and taurine 100 mg/kg. Data were analyzed by ANOVA followed by Tukey post hoc test for pairwise comparison. [a] significant difference in comparison with the control group (group 1) ($p < 0.05$), [b] significant difference in comparison with only fluoxetine group (group 2) ($p < 0.05$), [c] significant difference in comparison with the group receiving ellagic acid (50 mg/kg) (group 3) ($p < 0.05$), [d] significant difference in comparison with the group receiving taurine (100 mg/kg) (group 4) ($p < 0.05$).

Table 2. Effects of ellagic acid and taurine on FRAP and MDA contents

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Serum MDA (nmol/l)	8.52±0.56	19.58±1.30 [a]	12.99±0.82 [ab]	13.72±1.77 [ab]	8.92±1.42 [bcd]
Liver MDA (nmol/mg protein)	1.56±0.19	5.76±0.83 [a]	2.80±0.28 [ab]	2.99±0.58 [ab]	1.76±0.52 [bcd]
Plasma FRAP (µM)	616.13±52.41	369.25±38.76 [a]	513.12±40.08 [ab]	501.25±39.90 [ab]	634.12±44.34 [bcd]

The obtained data were expressed as mean ± SD (n = 8). Group 1: control, Group 2: received fluoxetine just, Group 3: received fluoxetine and ellagic acid 50 mg/kg, Group 4: received fluoxetine and taurine 100 mg/kg, and Group 5: received fluoxetine, ellagic acid50 mg/kg, and taurine 100 mg/kg. Data were analyzed by ANOVA followed by Tukey post hoc test for pairwise comparison. a significant difference in comparison with the control group (group 1) ($p < 0.05$), b significant difference in comparison with only fluoxetine group (group 2) ($p < 0.05$), c significant difference in comparison with the group receiving ellagic acid (50 mg/kg) (group 3) ($p < 0.05$), d significant difference in comparison with the group receiving taurine (100 mg/kg) (group 4) ($p < 0.05$).

Table 3. Effects of ellagic acid and taurine on PC and vitamin C

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Liver vitamin C (mg/g tissue)	14.30±1.30	8.88±1.12 [a]	12.10±0.61 [ab]	12.40±0.49 [ab]	14.23±1.15 [bcd]
Protein carbonyl (nmol NADPH/mg protein)	4.40±0.55	11.18±1.01 [a]	7.47±0.69 [ab]	7.27±1.30 [ab]	4.55±0.62 [bcd]

The obtained data were expressed as mean ± SD (n = 8). Group 1: control, Group 2: received fluoxetine just, Group 3: received fluoxetine and ellagic acid 50 mg/kg, Group 4: received fluoxetine and taurine 100 mg/kg, and Group 5: received fluoxetine, ellagic acid50 mg/kg, and taurine 100 mg/kg. Data were analyzed by ANOVA followed by Tukey post hoc test for pairwise comparison. a significant difference in comparison with the control group (group 1) ($p < 0.05$), b significant difference in comparison with only fluoxetine group (group 2) ($p < 0.05$), c significant difference in comparison with the group receiving ellagic acid (50 mg/kg) (group 3) ($p < 0.05$), d significant difference in comparison with the group receiving taurine (100 mg/kg) (group 4) ($p < 0.05$).

Figures

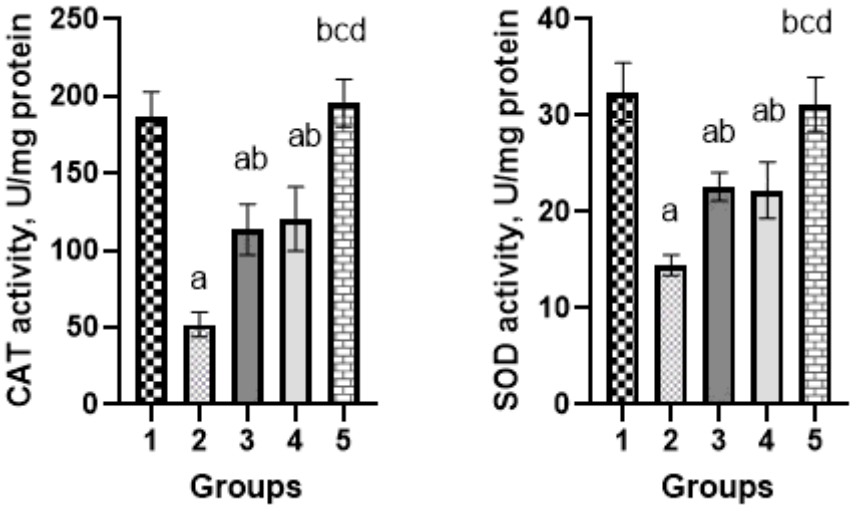

Figure 1

shows the effects of ellagic acid and taurine on SOD and CAT activities in the liver tissue. The obtained data were expressed as mean ± SD (n = 8). Group 1: control, Group 2: received fluoxetine just, Group 3: received fluoxetine and ellagic acid 50 mg/kg, Group 4: received fluoxetine and taurine 100 mg/kg, and

Group 5: received fluoxetine, ellagic acid50 mg/kg, and taurine 100 mg/kg. Data were analyzed by ANOVA followed by Tukey post hoc test for pairwise comparison. a significant difference in comparison with the control group (group 1) ($p < 0.05$), b significant difference in comparison with only fluoxetine group (group 2) ($p < 0.05$), c significant difference in comparison with the group receiving ellagic acid (50 mg/kg) (group 3) ($p < 0.05$), d significant difference in comparison with the group receiving taurine (100 mg/kg) (group 4).

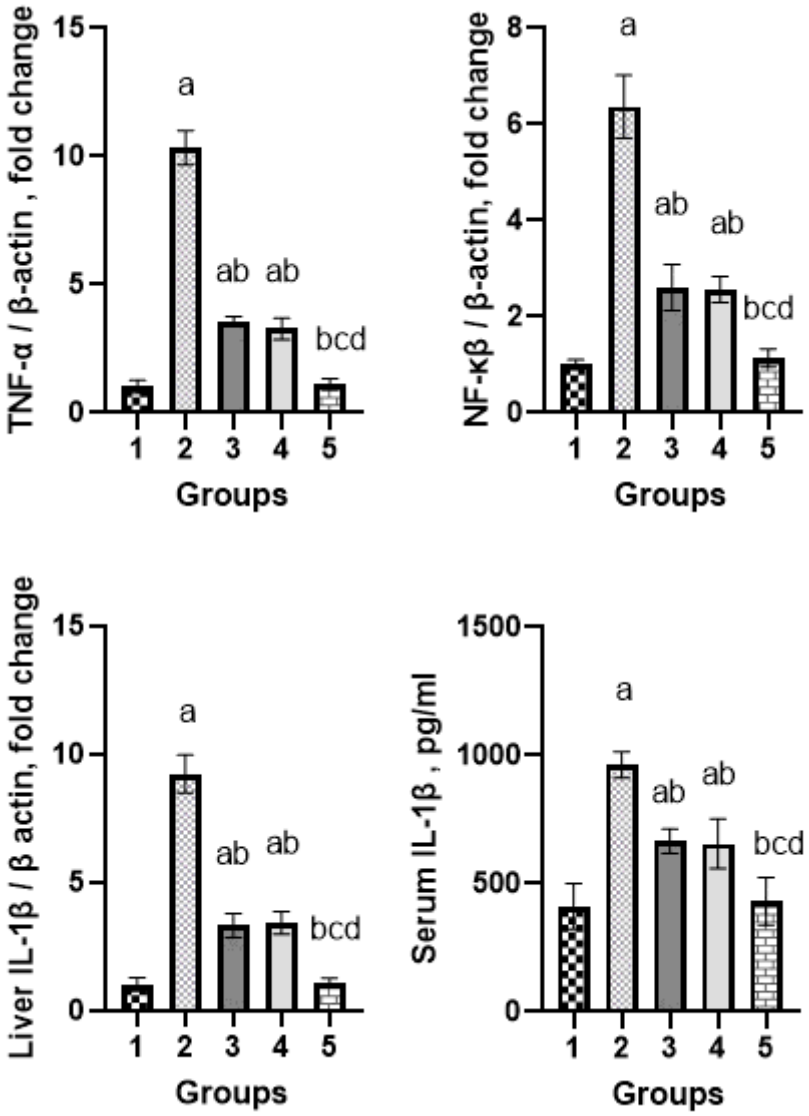

Figure 2

shows the effects of ellagic acid and taurine on serum IL-1β, TNF-α, IL-1β, and NF-κB gene expressions. The obtained data were expressed as mean ± SD (n = 8). Group 1: control, Group 2: received fluoxetine just, Group 3: received fluoxetine and ellagic acid 50 mg/kg, Group 4: received fluoxetine and taurine 100 mg/kg, and Group 5: received fluoxetine, ellagic acid50 mg/kg, and taurine 100 mg/kg. Data were analyzed by ANOVA followed by Tukey post hoc test for pairwise comparison. a significant difference in comparison with the control group (group 1) ($p < 0.05$), b significant difference in comparison with only fluoxetine group (group 2) ($p < 0.05$), c significant difference in comparison with the group receiving

ellagic acid (50 mg/kg) (group 3) ($p < 0.05$), d significant difference in comparison with the group receiving taurine (100 mg/kg) (group 4).

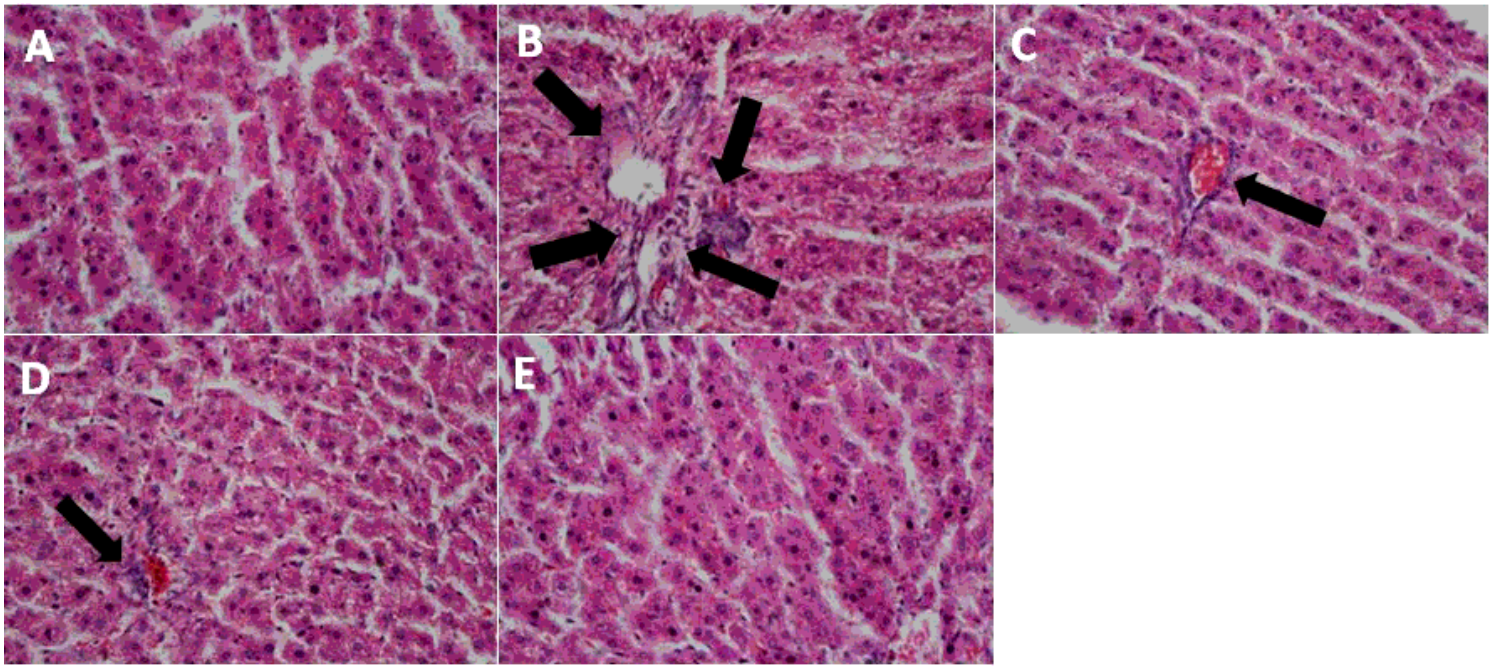

Figure 3

the effects of ellagic acid and taurine on hepatocyte cells. Group 1: control, Group 2: just received fluoxetine, Group 3: received fluoxetine and ellagic acid 50 mg/kg, Group 4: received fluoxetine and taurine 100 mg/kg, and Group 5: received fluoxetine, ellagic acid 50 mg/kg, and taurine 100 mg/kg. The black arrows show the infiltration of lymphocyte cells.

Supplementary Files

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- Control.jpg
- FLUEATUR.jpg
- FLUEA.jpg
- FLUTUR.jpg
- FLU.jpg
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- RealTimeIL1B.xlsx
- RealTimeNFkB.xlsx
- RealTimeTNFa.xlsx