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Original article

Anti-inflammatory and ameliorative effects of gallic acid on fluoxetine-induced oxidative stress and liver damage in rats

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ABSTRACT

Background: Fluoxetine-induced liver damage is a cause of chronic liver disease. In the present study the hepatoprotective effects of gallic acid against fluoxetine-induced liver damage were examined.
Methods: Forty-eight male rats were divided into six groups as follow: group 1, the control group; group 2, rats receiving fluoxetine (24 mg/kg bw daily, *po*) without treatment; group 3, rats receiving 24 mg/kg bw fluoxetine, treated with 50 mg/kg bw silymarin and groups 4, 5, and 6 in which gallic acid (50, 100, and 200 mg/kg bw, *po*, respectively) was prescribed after the consumption of fluoxetine. The histopathological changes of hepatic tissues were checked out.
Results: Fluoxetine caused a significant increase in the levels of serum glutamate oxaloacetate transaminase (GOT), serum glutamate pyruvate transaminase (GPT), lipid profiles, urea, fasting blood sugar (FBS), creatinine (Cr), protein carbonyl (PC) content, malondialdehyde (MDA), and liver TNF-α as an inflammatory element. Also, the obtained results of group 2 revealed a significant decline in ferric reducing ability of plasma (FRAP), liver catalase (CAT), superoxide dismutase (SOD), and vitamin C levels. The treatment with gallic acid showed significant ameliorations in abnormalities of fluoxetine-induced liver injury as represented by the improvement of hepatic CAT, SOD activities, vitamin C levels, serum biochemical parameters, and histopathological changes, in addition to the recovery of antioxidant defense system status.
Conclusions: Gallic acid has inhibitory effects on fluoxetine-induced liver damage. The effect of gallic acid is derived from free radical scavenging properties and the anti-inflammatory effect related to TNF-α.

Introduction

Major depressive disorder (MDD), regarded as a genuine general health issue, is accompanied with enduring, incapacity, morbidity and morality [1–3], predicted to be the second largest burden in International Community of Health by 2020 [4]. Fluoxetine, an anti-depressive disorder drug, is normally used to treat disorders such as MDD, panic disorder, bulimia nervosa, and premenstrual dysphoric disorder [5–7]. Fluoxetine has inevitable side effects such as weight gain, dyslipidemia, type 2 diabetes, high blood pressure, and the risk of coronary heart disease [8,9]. It has been reported that fluoxetine-induced oxidative damage caused by the oxidant-antioxidant system, results in changes in liver tissue and serum markers of the liver [10]. Other studies reveled that reactive oxygen species (ROS) has a critical position in the start and development of oxidative stress and also stated that hepatic damage is caused by fluoxetine [11].

Nowadays, medicinal plants are attracting attention due to their being inexpensive, higher safety of use, and their few side effects. On the other hand, consumption of plant-derived compounds and fruit in diet can reduce the risk of disease incidence [12–14]. Besides, a large number of scientific reports show that polyphenolic compounds can have different pharmacological roles which might be related to antioxidant system [15]. Gallic acid (3,4,5-trihydroxybenzoic acid) found in tea leaves, sumac, oak bark, gallnuts, witch hazel, and other kinds of plants [16]. Some researches show that gallic acid has hepatoprotective activity against hepatotoxicity due to its hydroxyl groups [17]. Thus, the objective of this study was to evaluate the effects of gallic acid on liver injury caused by fluoxetine-administrated in Wistar rats.

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Materials and methods

Chemicals

Fluoxetine capsules (20 mg fluoxetine-hydrochloride) were purchased from Pars Daru Co. (Tehran, Iran). Triglyceride (TG), total cholesterol (TC), serum glutamate oxaloacetate transaminase (GOT), serum glutamate pyruvate transaminase (GPT), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), urea, fasting blood sugar (FBS), and creatinine (Cr) kits were purchased from Pars Azmoon Co. (Tehran, Iran). All other chemicals used, were of analytical grade.

Experimental animals

Forty-eight male Wistar rats (10–12 weeks old, 180–220 g) were purchased from Pasteur Institute of Iran (Tehran, Iran). They were kept under normal laboratory conditions ($22 \pm 2\,^{\circ}C$, $60 \pm 5\%$ humidity, and 12:12 light dark cycle). Animals had access to standard rat pellet diet and water. They were allowed to get accustomed to these conditions for two weeks before being used for the experiments. All procedures were approved by the Ethics Committee of University (Ethic number IR. SKUMS. REC. 1394. 203).

Experimental design

The rats were divided into six groups (n = 8). The fluoxetine solution and gallic acid solution were prepared daily and all groups were treated orally by stomach tube. Group 1, the control group, received 1 ml pure corn oil as a solvent of fluoxetine and 1 ml solvent of gallic acid (1:10 ethanol-distilled water) with an interval of one hour. Group 2 received 24 mg/kg body weight (bw) fluoxetine [18] and 1 ml solvent of gallic acid. Group 3, served as positive control and received 24 mg/kg bw fluoxetine and 50 mg/kg bw silymarin [19]. Groups 4, 5, and 6 received 24 mg/kg bw fluoxetine and were treated with 50, 100, and 200 mg/kg bw doses of gallic acid, respectively, every day for one month [20].

After one month of treatment animals were anesthetized with chloroform and then were sacrificed. Blood specimens were collected by cardiac puncture and centrifuged at 3000 rpm for 15 min. Plasma and serum were prepared from the blood samples for different biochemical analyses. Each liver sample was divided to determine liver catalase (CAT), superoxide dismutase (SOD), tumor necrosis factor-α (*TNF-α*) gene expression, and to conduct histopathological examinations.

Biochemical analysis

Determination of serum biochemical parameters

GOT, GPT, FBS, urea, Cr, and profile of serum lipids were determined by an auto-analyzer (BT3000, Rome, Italy) using Pars Azmoon kits (Tehran, Iran). The Friedewald equation was used to calculate VLDL-C [21]. Serum TNF-α was measured by enzyme-linked immune sorbent assay (ELISA) according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanghai, China).

Ferric reducing ability of plasma (FRAP)

FRAP was measured as described previously [22].

Determination of protein content

Bradford method was used to measure protein content of the samples [23]. Bovine serum albumin (BSA) was used as the standard.

Determination of the serum protein carbonyl (PC)

Reznick and Parker's spectrophotometric method was applied to measure protein carbonyl content. The absorbance of product was read at 366 nm [24].

Determination of lipid peroxidation product

The concentration of MDA, as a product of lipid peroxidation, was measured in serum and liver tissue through HPLC system (Agilent, USA) and Agarwal method [25].

Determination of hepatic vitamin C level

Omaye method was used to measure vitamin C level in hepatic tissues [26].

Determination of superoxide dismutase (SOD) activity

Flohe and Gunzler method was used to determine SOD activity of hepatic tissues [27].

Determination of liver catalase (CAT) activity

The liver CAT activity of experimental groups was estimated as it was already explained [13].

Determination of TNF-α gene expression

Analysis of *TNF- α* gene expression was carried out through Real-Time quantitative PCR (RT-qPCR) and the ΔΔCT method as described previously [28]. The primers used for *TNF-α* and *β-actin* were as follow: *TNF-α* forward: 5′-CTGGCGTGTTCATCCGTTC-3′,

Table 1
Effect of gallic acid on serum biochemical parameters in fluoxetine induced hepatic damage in rats.

parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
GOT (U/L)	141.12 ± 7.6	331.75 ± 31.39^{a}	169.25 ± 12.54^{b}	166.75 ± 11.87^{b}	154.12 ± 16.34^{b}	$241.87 \pm 23.3^{a,b,c,d,e}$
GPT (U/L)	75.5 ± 7.96	242 ± 12.77^{a}	$125.5 \pm 12.03^{a,b}$	$124.75 \pm 16.55^{a,b}$	$116.87 \pm 11.11^{a,b}$	$149.87 \pm 14.25^{a,b,c,d,e}$
FBS (mg/dL)	132.87 ± 7.19	177.25 ± 13.09^{a}	124.62 ± 6.25^{b}	122.25 ± 11.75^{b}	123.5 ± 7.7^{b}	133.25 ± 6.45^{b}
TC (mg/dL)	71.75 ± 7.97	149.37 ± 15.68^{a}	81.12 ± 6.83^{b}	89.62 ± 8.78^{b}	82 ± 6.92^{b}	$144.5 \pm 10.75^{a,c,d,e}$
TG (mg/dL)	65.12 ± 7.71	153.37 ± 13.55^{a}	$86.75 \pm 12.72^{a,b}$	$91.87 \pm 13.2^{a,b}$	76.75 ± 8.08^{b}	$147.75 \pm 9.11^{a,c,d,e}$
LDL-C (mg/dL)	8.75 ± 0.7	14.62 ± 0.95^{a}	$11.5 \pm 1.6^{a,b}$	$12 \pm 2.07^{a,b}$	$11.62 \pm 1.18^{a,b}$	$13.77 \pm 1.09^{a,c,d,e}$
VLDL-C (mg/dL)	13.41 ± 1.27	30.72 ± 2.7^{a}	$17.5 \pm 2.35^{a,b}$	$18.02 \pm 2.71^{a,b}$	15.35 ± 1.61^{b}	$29.1 \pm 2.13^{a,c,d,e}$
HDL-C (mg/dL)	72.18 ± 4.71	44.36 ± 5.72^{a}	$60.81 \pm 3.64^{a,b}$	$55.37 \pm 3.75^{a,b}$	$65.06 \pm 6.85^{b,d}$	$57.02 \pm 7.05^{a,b}$
Creatinine (mg/dL)	0.43 ± 0.05	0.43 ± 0.07	0.45 ± 0.05	0.43 ± 0.05	0.51 ± 0.09	$0.57 \pm 0.07^{a,b,c,d}$
Urea (mg/dL)	36.87 ± 2.9	51 ± 4.95^{a}	48.37 ± 3.66^{a}	47.37 ± 5.15^{a}	46.87 ± 2.47^{a}	47.37 ± 3.15^{a}

The data were expressed in mean ± SD and n = 8 in each group. Normal control (1); rats treated with fluoxetine-only (2); rats supplemented with fluoxetine and silymarin (3); rats supplemented with 50 mg/kg bw gallic acid (4); rats supplemented with 100 mg/kg bw gallic acid (5); and rats supplemented with 200 mg/kg bw gallic acid (6).
[a] $p < 0.05$ *vs.* group 1. [b] $p < 0.05$ *vs.* group 2. [c] $p < 0.05$ *vs.* group 3. [d] $p < 0.05$ *vs.* group 4. [e] $p < 0.05$ *vs.* group 5.