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Fluoxetine and *Gundelia tournefortii* L. Plant Extract in Rats Exposed to Chronic Immobilization Stress; Determination of Effect on Anxiety, Motor Activity, Kidney and Liver Tissues*

Hamdullah YUKSEL[1], Bedia BATI[1], Gokhan OTO[2], Omer BINGOL[1]

[1]Van Yuzuncu Yil University, Faculty of Education, Department of Mathematics and Science Education, Van-TURKEY
[2]Van Yuzuncu Yil University, School of Medicine, Department of Medical Pharmacology, Van-TURKEY

Corresponding author: Bedia BATI; E-mail: bediabati@yyu.edu.tr; ORCID ID: 0000-0001-9501-7822
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Summary: This study was completed with the aim of investigating the effects of extracts obtained from *Gundelia tournefortii* L. and fluoxetine on anxiety, motor activity, biochemical and antioxidant parameters in rats exposed to chronic immobilization stress. The study included a total of 40 female Wistar albino rats with live weight of 200-220 g, randomly divided into groups of 8 rats with the study duration planned as 30 days. The groups created in the study were "control", "physiologic serum + chronic immobilization", "*Gundelia tournefortii* L. plant extract", "*Gundelia tournefortii* L. plant extract + chronic immobilization" and "fluoxetine + chronic immobilization". At the end of 30 days, all the groups had rotarod test (motor activity) and anxiety test (elevated plus maze) applied and after all the tests were completed the rats were sacrificed. At the end of the study, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid (UA), creatinine (CRE), total protein (TP) and albumin levels were determined. Additionally, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione *S*-transferase (GST), glutathione reductase (GR) activity and reduced glutathione (GSH) and malondialdehyde (MDA) levels were identified in liver and kidney tissue samples. In conclusion, administration of both *Gundelia tournefortii* L. plant extract and fluoxetine can be said to have positive effects on enzyme activities and MDA parameter value against experimentally-induced immobilization stress. Additionally, *Gundelia tournefortii* L. was not identified to have any effect on motor activity, but identified to have anxiolytic effect according to elevated plus maze test results.
Key words: Chronic immobilization stress, elevated plus maze, fluoxetine, *Gundelia tournefortii* L., rotarod

Kronik İmmobilizasyon Stresine Maruz Bırakılan Ratlarda Fluoksetin ve *Gundelia tournefortii* L. Bitki Ekstresinin; Anksiyete, Motor Aktivite, Böbrek ve Karaciğer Dokuları Üzerine Etkisinin Belirlenmesi

Özet: Bu çalışma, kronik immobilizasyon stresine maruz bırakılan ratlarda anksiyete, motor aktivite, biyokimyasal ve antioksidan parametre değerleri üzerine *Gundelia tournefortii* L. bitkisinden elde edilen ekstrelerin ve Fluoksetin'in etkilerinin incelenmesi amacıyla gerçekleştirilmiştir. Çalışmada canlı ağırlıkları 200-220 gr olan toplam 40 adet Wistar – albino ırkı dişi sıçan her grupta 8 sıçan olacak şekilde gruplara rastgele dağıtılmış ve çalışmanın süresi toplam 30 gün olarak planlanmıştır. Çalışmadaki gruplar; "Kontrol", "Serum fizyolojik su + Kronik İmmobilizasyon", "*Gundelia tournefortii* L. bitki ekstresi", "*Gundelia tournefortii* L. bitki ektresi + Kronik immobilizasyon" ve "Fluoksetin + Kronik immobilizasyon stresi" şeklinde oluşturulmuştur. 30'uncu gün sonunda tüm gruplara rotarod testi (motor aktivite testi) ve anksiyete testi (yükseltilmiş artı labirent testi) uygulananarak tüm testler tamamlandıktan sonra uygulama sonunda sıçanlar sakrifiye edilmiştir. Çalışma sonunda aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), alkalen fosfataz (ALP), ürik asit(UA), kreatinin (CRE), total protein (TP), albumin düzeyleri belirlenmiştir. Ayrıca, karaciğer ve böbrek dokusu örneklerinde katalaz (CAT), süperoksid dismutaz (SOD), glutatyon peroksidaz (GPx), glutatyon *S*-transferaz (G*S*T), glutatyon redüktaz (GR) aktiviteleri ve redükte glutatyon (GSH) ile malondialdehit (MDA) düzeyleri tespit edilmiştir. Sonuç olarak, deneysel olarak oluşturulan immobilizasyon stresine karşı, hem *Gundelia tournefortii* L. bitki ekstresinin hem de Fluoksetin uygulamasının enzim aktiviteleri üzerine ve MDA parametre değeri üzerine olumlu etkiler sergilediği söylenebilir. Bununla birlikte, *Gundelia tournefortii* L. bitkisinin; motor aktivite üzerine herhangi bir etkisi olmadığı tespit edilirken, yükseltilmiş artı labirent testi sonuçlarına göre, anksiyolitik etkisi olduğu belirlenmiştir.
Anahtar kelimeler: Fluoksetin, *Gundelia tournefortii* L., kronik immobilizasyon stresi, rotarod, yükseltilmiş artı labirent

Introduction

Anxiety is defined as worry, or even a feeling of fear, a person feels in response to a perceived internal or external threat caused by different situations. Different events or formations may cause anxiety (APPI). In recent years, anxiety is frequently observed around the world, causing an increase in clinical and if necessary preclinical studies related to treatment of this disease. Preclinical studies research the anxiolytic effects of new medications in animal studies. For

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these models, rats and mice are most commonly used amongst rodents (Aykac et al., 2015). These species are intensely used for research into underlying mechanisms of emotional and motivational functions on the ground. This increased behavior of rats in situations when they are placed in areas where they feel unprotected is closely related to anxiety. As the anxiety situation increases, research behavior of rats reduces (Barnett, 2007; Aykac et al., 2015). Some studies have led to the consideration that stress and anxiety may be the result of inflammation and oxidative stress processes (Salim et al., 2011; Yang et al., 2016).

Stress ensures the stimulation of many adaptive responses in the central and peripheral nervous system, but may lead to negative physiologic outcomes like insufficient growth metabolism, circulation, reproduction and immune responses and excessive or long-term stimulation (Barbieri et al., 2012). Long-term stress may cause a variety of discomforts like behavioral depression (Pedreanez et al., 2011), post-traumatic stress disorder (Kessler and Sonnega, 1995), and anxiety (Gulati et al., 2009). When metabolic activity increases, free radical production also increases, causing the production of reactive oxygen species (ROS) in the environment and imbalance in the antioxidant system (Lobo et al., 2010; Dias et al., 2014; Dong et al., 2016). ROS causes oxidative injury to different molecules in cells like proteins, lipids and nucleic acids (Ali et al., 2017; Turan and Celik 2016). Oxidative stress is thought to be the main factor in the development of many complications like neurodegenerative diseases, mainly, chronic kidney disease, hepatic inflammation, hepatic cirrhosis, hypercholesterolemia, diabetes, etc. (Gutteridge, 1995). As a result, morbidity rates related to a variety of diseases may increase as a result of exposure to long-term physiologic and psychological stress (Djordjevic et al., 2010).

Currently stress is a frequently encountered problem, with antidepressant medications used with the aim of treating depression caused by stress. One of the most commonly used of these antidepressant medications in fluoxetine. Fluoxetine is a prototype serotonin reuptake inhibitor. It is commonly used for treatment of panic and other anxiety disorders. Clinical observation of panic attack patients has confirmed its efficacy and reliability in reducing panic attack incidence, phobic symptoms, depressive and anxiety symptoms (Starevic et al., 2004). Additionally, fluoxetine has variable anxiety and depression effects in animal models (Borsini et al., 2002). There is a need for studies in this area to investigate the effects of this antidepressant medication.

In Turkey and the world in general, different plants are used with the aim of treating anxiety. One of these plants, with large area of use among the public, is the plant *Gundelia tournefortii* L. known as "Kenger" in Iran. *Gundelia tournefortii* L. is an edible member of the Asteraceae family specific to Iran, Turkey, Azerbaijan, Jordan, Cyprus and other regions (Coruh et al., 2007; Matthäus and Ozcan, 2011). Generally, the stem of the plant is used for medication aims in traditional medicine as hepatoprotective and blood cleaning and for diabetes, heart attacks, etc. in the Middle East (Hamdan and Afifi, 2004; Jamshidzadeh et al., 2005; Halabi et al., 2005). *Gundelia tournefortii* L. has high phenolic content, with caffeoylquinic acid derivatives, quercetin, and gallic acid and is very rich in other compounds like limonene, zingiberene and saponins responsible for the biological activity of the plant (Nakatani et al., 2000; Haghi et al., 2011; Asadi-samani et al., 2013). Polyphenols in this plant are potential antioxidants and may play an important role in preventing a variety of pathologic situations (Coruh et al., 2007; Asgary et al., 2009; Haghi et al., 2011). Some studies have evaluated the antiatherosclerotic properties of *Gundelia tournefortii* L. as being the result of hypolipidemic, anticoagulant and antioxidant properties (Hammadi and Salam, 2004; Asgary et al., 2009). Some animal studies have shown *Gundelia tournefortii* L. may regulate lipid profiles. The plant has also displayed antiinflammatory, analgesic and antioxidant effects during in vitro and in vivo studies (Coruh et al., 2007; Oryan et al., 2011).

The aim of this study was to determine the effect of *Gundelia tournefortii* L. on anxiety in rats with chronic immobilization stress and to determine the effect of this plant on various antioxidant enzyme levels and oxidative stress markers in liver and kidney tissues.

Materials and methods

Reagents

Thiobarbituric acid, butylated hydroxytoluene, trichloroacetic acid, ethylenediaminetetraacetic acid, reduced glutathione, metaphosphoric acid, DTNB, Tris, CDNB, GSSG, nicotinamide adenine dinucleotide phosphate, potassium dihydrogen phosphate and sodium chloride of technical grade used in this study were obtained from Sigma Chemical Company, St. Louis, MO, USA. Reagents for antioxidant enzyme analysis were purchased from Randox Laboratories Ltd.

Collection of plant material and preparation of Gundelia tournefortii L. *water extract*

The plant material used in this study of *Gundelia tournefortii* L. was collected from around the province of Diyarbakır in May. Plants placed in cloth bags were first washed with tap water and then with distilled water to remove dust and contamination. The plants were spread on drying paper in the laboratory environment and then air-dried. The drying paper under

the lower sections of the plants was changed each day. Plants were rotated daily to prevent humidity and mold. The *Gundelia tournefortii* L. plants were ground in an electric mill and passed through a 0.5 mm sieve. The plants were left in tightly-sealed colored glass jars until use. Hot water extraction of *Gundelia tournefortii* L. was completed with modifications of the decoction method applied by Eddouks et al. (2005). After 1 g of ground *Gundelia tournefortii* L. was boiled in 100 ml distilled water for 10 minutes, it was left in the open to cool for 15 minutes. The extract was filtered with Whatman filter paper and water was completely removed in a lyophilizer and the lyophilized extract stored at -80 °C was diluted daily and administered to rats orally in 300 mg/kg dose (Azeez and Kheder, 2012).

Experimental animals

The study used 40 female Wistar albino rats with live weight of 200-220 g as animal material. The study received written consent from Van Yuzuncu Yil University Experimental Animals Unit Ethics Committee dated 02.03.2017 and numbered "02". Rats were housed at room temperature (25±1 ^{o}C), with 12-hour light/12-hour dark period and fed *ad libitum*. A total of 5 groups were created in this study which lasted 30 days.

Control group: Rats with no additional intervention other than standard feed and water.

Physiologic serum + chronic immobilization group (Stress): Rats exposed to 30 minutes immobilization stress every day for 30 days, administered oral physiologic serum 30 minutes before being placed in immobilization cages.

Gundelia tournefortii L. water extract group (Gundelia**):** Rats administered 300 mg/kg oral *Gundelia tournefortii* L. water extract for 30 days.

Gundelia tournefortii L. water extract + chronic immobilization group (Gundelia + Stress) : Rats exposed to 30 minutes immobilization stress every day for 30 days, administered 300 mg/kg oral *Gundelia tournefortii* L. water extract 30 minutes before being placed in immobilization cages.

Fluoxetine + chronic immobilization group (Fluoxetine): Rats exposed to 30 minutes immobilization stress every day for 30 days, administered 10 mg/kg/day oral fluoxetine 30 minutes before being placed in immobilization cages.

Chronic immobilization stress (restrainer) was applied with the aim of inducing anxiety among rats in groups 2, 4 and 5 in the study. In order to perform biochemical analyses at the end of the study, rats were anesthetized with 10% ketamine and sacrificed.

Behavioral evaluations

There are many behavior test models used for evaluation of the cognitive and locomotor status of rats. These tests assess more than one status like anxiety, autonomic functions, learning, memory and locomotor activity in rats (Buyukdereli, 2008). With this aim, present study applied the elevated plus maze test and the rotarod test.

The elevated plus maze is a common technique used to evaluate anxiety. This technique is used to research the physiologic, behavioral and pharmacologic effects of medications or other materials by testing emotional activity. With different sizes prepared for mice and rats, this setup comprises a test pattern with two open arms and two closed arms in a plus shape (+) at a certain elevation (Walf and Frye, 2007).

One of the most commonly used tests to measure motor coordination is the rotarod performance scale. The rotarod test is a method used to assess performance, resistance power, balance and coordinated movements (Kaur and Ling, 2008).

Anxiety test: Rats had the elevated plus maze applied. The test has two open and two closed arms (width 12 cm, length 50 cm) and a central region where the arms meet, set up 50 cm above the floor. Each subject is left in the center of the setup looking at the open arms, and monitored for 5 minutes with a Noldus Ethno Vision Tracking camera system. After the experiments, statistical analysis is performed on the time the animal stays in the open arms of the setup and number of entries into the open arms.

Motor activity test: The rotarod test is a method used to assess performance, resistance power, balance and coordinated movements. The test basically measures the duration an experimental animal can stay on a rod rotating with fixed speed without falling. During the test, subjects have duration on the rotating rod recorded in seconds with an automatic counter (Kurt et al., 2002; Ferrante et al., 2002). In the first stage of the study, rats are taught to stay on the device rotating at 6 rpm for 3 minutes. Then each animal is given 3 60-second attempts at 16 rpm. In this way, the total score is the total from the three attempts (maximum value 180 s). The duration (s) the rats stay on the device without falling is accepted as rotarod performance.

Biochemical analysis

Preparation of tissue supernatant: At the end of the 30-day experiment, rats were anesthetized with 10% ketamine and sacrificed. Blood samples were taken from the heart with an injector for biochemical analysis. Liver and kidney tissues were washed in dilute physiologic serum (0.9% NaCl) and stored at -

78 °C until analysis. Tissues were homogenized for 5 minutes in 50 mM ice-cold KH_2PO_4 solvent (1:5 w/v) with an ultrasonic homogenator (20 KHz frequency ultrasound, Jencons Scientific Co.). Homogenate was centrifuged at 7000 g for 15 minutes at +4 °C. Liver and kidney tissue were prepared for analysis. Supernatants were used to determine antioxidant defense system and MDA content (Yurt and Celik, 2011).

Determination of antioxidant system in liver and kidney tissue: In liver and kidney tissue, SOD enzyme activity was measured in accordance with the Randox brand spectrophotometric kit protocol. CAT activity catalyzing the hydrolysis of oxygen and water in hydrogen peroxide used the Aebi (100) method (Aebi, 1974). The tissue was tested according to Randox's spectrophotometric kit protocol for GPx measurement. GR activity was tested with the Carlberg and Mannervik method (Carlberg and Mannervik, 1985). GST activity was tested with the Mannervik and Guthenberg method (Mannervik and Guthenberg, 1981). Tissue GSH activity was measured with the Beutler et al. method (Beutler et al., 1963). MDA concentration was determined using the method described by Jain et al. (Jain et al., 1989).

Measurement of biochemical parameters: AST, ALT, ALP, uric acid (UA), creatinine (CRE), total protein (TP) and albumin were measured by an auto analyzer (COBAS 8000/ROCHE/Germany/Serial No 1296-08) using the Roche kits.

Statistical analysis

Inter-group comparisons were performed with one-way analysis of variance (ANOVA) and chi-square analysis (for comparison of rates in independent groups), while multiple comparisons used the Tukey test in the R 3.5.1 (R Core Team, 2018) program. Significance level was taken as P<0.05.

Result

The anxiety test results obtained in this study are shown in Tables 1, 2 and 3, while motor activity test results are shown in Table 4.

According to Table 1, on the 15th day of the experiments, the *Gundelia tournefortii* L. groups and fluoxetine group had significant increases in the duration in the open area compared to the control group (P<0.05). Additionally, the groups administered *Gundelia tournefortii* L. and fluoxetine group had significant increases in time spent in the open area compared to the group only exposed to immobilization stress (P<0.05). On the 30th day experiments, the duration of time spent in the open area was significantly reduced for only the immobilization group compared to the control group at P<0.05 level. Groups administered *Gundelia tournefortii* L. had significant increases in time spent in the open area compared to the control group, while the fluoxetine group did not show statistical significance compared to the control group in terms of time spent in the open area (P>0.05). The *Gundelia tournefortii* L. groups and fluoxetine groups showed significant increases in time spent in the open area compared to the group only exposed to immobilization stress (P<0.05).

According to Table 2, on the 15th day experiments, the number of entries into the open area was identified to statistically significantly reduce in the group undergoing only immobilization stress compared to the control group (P<0.05). Only the number of entries into the open area of the group administered *Gundelia tournefortii* L. was not statistically significant compared to the control group (P>0.05), while there was a significant increase in the *Gundelia tournefortii* L. + stress group compared to the control group at P<0.05 level. The fluoxetine group had an increase in the number of entries into the open area, but was not determined to show statistical significance compared to the control group (P>0.05). Compared to the group only exposed to immobilization stress model, the *Gundelia tournefortii* L., *Gundelia tournefortii* L. + stress and fluoxetine groups had significant increases in number of entries into the open area (P<0.05). In experiments on the 30th day, the number of entries into the open area was reduced in the group only undergoing immobilization stress compared to the control group; however, this reduction was not determined to be statistically significant (P>0.05). Com-

Table 1. Time spent by the rats in the open area (sec)

	Groups					
Days	Control $\bar{X} \pm S_{\bar{x}}$	Stress $\bar{X} \pm S_{\bar{x}}$	*Gundelia* $\bar{X} \pm S_{\bar{x}}$	*Gundelia* + Stress $\bar{X} \pm S_{\bar{x}}$	Fluoxetine $\bar{X} \pm S_{\bar{x}}$	P Values
15th day	31.64 ± 1.50	17.84 ± 1.77	66.30 ± 7.65 [ab]	85.17 ± 6.71 [ab]	54.43 ± 4.49 [abd]	0.0008
30th day	25.37 ± 1.32	16.89 ± 1.28 [a]	42.57 ± 2.08 [ab]	52.68 ± 2.61 [abc]	33.47 ± 0.90 [bcd]	0.0007

a: Statistically significant difference compared to control group (P<0.05)
b: Statistically significant difference compared to the stress group (P<0.05)
c: Statistically significant difference compared to the Gundelia group (P<0.05)
d: Statistically significant difference compared to the Gundelia +stress group (P<0.05)
$\bar{X}$: Mean, $S_{\bar{x}}$: Standard error

Table 2. Number of entries into the open area.

Days	Groups					P Values
	Control $\bar{X} \pm S_{\bar{x}}$	Stress $\bar{X} \pm S_{\bar{x}}$	*Gundelia* $\bar{X} \pm S_{\bar{x}}$	*Gundelia* + Stress $\bar{X} \pm S_{\bar{x}}$	Fluoxetine $\bar{X} \pm S_{\bar{x}}$	
15th day	6.00 ± 0.38	4.25 ± 0.31 [a]	6.13 ± 0.55 [b]	7.88 ± 0.44 [abc]	7.38 ± 0.38 [b]	0.0003
30th day	1.25 ± 0.16	0.50 ± 0.19	2.88 ± 0.44 [ab]	3.38 ± 0.38 [ab]	2.38 ± 0.26 [b]	0.0007

a: Statistically significant difference compared to control group (P<0.05)
b: Statistically significant difference compared to the stress group (P<0.05)
c: Statistically significant difference compared to the Gundelia group (P<0.05)
$\bar{X}$: Mean, $S_{\bar{x}}$: Standard error

pared to the control group, the *Gundelia tournefortii* L. and *Gundelia tournefortii* L. + stress groups had significant increases in entries into the open area (P<0.05). The increase in entries into the open area in the fluoxetine group was not statistically significant compared to the control group (P>0.05). The *Gundelia tournefortii* L., *Gundelia tournefortii* L. + stress and fluoxetine groups had significant increases in the number of entries into the open area compared to the group only administered the immobilization stress model (P<0.05).

show statistical significance compared to the control group (P>0.05). Compared to the group only undergoing the immobilization stress model, the *Gundelia tournefortii* L., *Gundelia tournefortii* L. + stress and fluoxetine groups had significant increases in percentage entering the open area (P<0.05). In the 30th day experiments, the percentage entering the open area was reduced in the group only undergoing immobilization stress compared to the control group; however, this reduction was not determined to be statistically significant (P>0.05). Compared to the

Table 3. Total number of entrances to open area and percentage

Days		Groups					P Values
		Control	Stress	*Gundelia*	*Gundelia* + Stress	Fluoxetine	
15th day	N	48	34	49	63	59	0.0002
	%	31	34 [a]	43 [ab]	45 [bc]	44 [b]	
30th day	N	10	4	23	27	19	0.0009
	%	17	16	37 [ab]	31 [ab]	28 [b]	

a: Statistically significant difference compared to control group (P<0.05)
b: Statistically significant difference compared to the stress group (P<0.05)
c: Statistically significant difference compared to the Gundelia group (P<0.05)
N: Total number of entries to the open area.

According to Table 3, the percentage entering the open area on 15th day experiments were significantly reduced in the group with only immobilization stress compared to the control group (P<0.05). Only the *Gundelia tournefortii* L. group did not show statistical significance compared to the control group in terms of percentage entering the open area (P>0.05), while the *Gundelia tournefortii* L. + stress group had P<0.05 significant increase compared to the control group. The increase in percentage entering the open area in the fluoxetine group was not determined to

control group, the *Gundelia tournefortii* L. and *Gundelia tournefortii* L. + stress groups had significant increases in terms of percentages entering the open area (P<0.05). The increase in percentage entering the open area in the fluoxetine group did not reach statistical significance compared to the control group (P>0.05). The *Gundelia tournefortii* L., *Gundelia tournefortii* L. + stress and fluoxetine groups had increases in percentages entering the open area compared to the group only administered the immobilization stress model (P<0.05).

Table 4. Motor activity test results (s)

Days	Groups					P Values
	Control $\bar{X} \pm S_{\bar{x}}$	Stress $\bar{X} \pm S_{\bar{x}}$	*Gundelia* $\bar{X} \pm S_{\bar{x}}$	*Gundelia* + Stress $\bar{X} \pm S_{\bar{x}}$	Fluoxetine $\bar{X} \pm S_{\bar{x}}$	
0th day	60.00 ± 0.10	57.00 ± 2.10	57.25 ± 2.13	59.00 ± 1.00	59.75 ± 0.25	0.345
15th day	56.63 ± 1.83	58.75 ± 1.25	60.00 ± 0.10	55.75 ± 2.11	57.63 ± 1.24	0.468
30th day	60.00 ± 0.10	56.50 ± 2.41	58.63 ± 1.02	57.63 ± 1.63	60.00 ± 0.10	0.211

$\bar{X}$: Mean, $S_{\bar{x}}$: Standard error

According to Table 4, the rotarod test results for the 0, 15th and 30th days determined exposure to chronic immobilization stress or *Gundelia tournefortii* L. extracts did not affect motor activity (P>0.05).

Table 5 gives the effect of chronic immobilization stress on liver and kidney tissue biomarkers.

According to Table 5, there was no statistical change between Gundelia and Gundelia + stress groups compared to the control and stress groups in terms of AST levels, with a statistical increase in the fluoxetine group compared to the control and stress groups (P<0.05). ALT levels were statistically significantly increased in the fluoxetine group compared to the stress group, while TP levels were statistically significantly reduced in the Gundelia, Gundelia + stress and fluoxetine groups compared to the control group (P<0.05). Additionally, there was a significant reduction in the Gundelia groups compared to the control group in terms of albumin levels (P<0.05). Additionally, ALP, UA and CRE levels in the experimental

Table 5. Effect of chronic immobilization stress on liver and kidney tissue biomarkers

Parameters	GROUPS Control $\bar{X} \pm S_{\bar{X}}$	Stress $\bar{X} \pm S_{\bar{X}}$	Gundelia $\bar{X} \pm S_{\bar{X}}$	Gundelia + Stress $\bar{X} \pm S_{\bar{X}}$	Fluoxetine $\bar{X} \pm S_{\bar{X}}$	P Values
AST (U/L)	96.50 ± 5.79	125.25 ± 11.67	149.75 ± 14.57	140.75 ± 11.51	227.43 ± 34.62[ab]	0.001
ALT (U/L)	33.00 ± 2.03	33.63 ± 1.01	29.50 ± 0.98	37.00 ± 2.21	41.57 ± 4.77[b]	0.024
ALP (U/L)	223.00 ± 12.53	252.30 ± 5.98	266.3 ± 9.43	261.50 ± 12.95	259.30 ± 32.90	0.474
CREAC (mg/dL)	0.53 ± 0.01	0.56 ± 0.02	0.53 ± 0.02	0.56 ± 0.01	0.54 ± 0.01	0.269
UA (mg/dL)	2.78 ± 0.36	3.75 ± 0.78	3.04 ± 0.65	3.21 ± 0.45	2.96 ± 0.90	0.874
TP (g/dL)	72.00 ± 1.65	68.38 ± 1.31	67.13 ± 0.81[a]	66.88 ± 0.35[a]	64.86 ± 0.63[a]	0.001
Albumin (g/dL)	35.33 ± 1.33	33.88 ± 0.93	31.88 ± 0.48[a]	32.50 ± 0.33	32.43 ± 0.78	0.038

a: Statistically significant difference compared to control group (P<0.05)
b: Statistically significant difference compared to the stress group (P<0.05)
$\bar{X}$: Mean, $S_{\bar{X}}$: Standard error

Table 6. Effect of chronic immobilization stress of lipid peroxidation and the antioxidant defense system in liver tissue

Parameters	GROUPS Control $\bar{X} \pm S_{\bar{X}}$	Stress $\bar{X} \pm S_{\bar{X}}$	Gundelia $\bar{X} \pm S_{\bar{X}}$	Gundelia + Stress $\bar{X} \pm S_{\bar{X}}$	Fluoxetine $\bar{X} \pm S_{\bar{X}}$	P Values
SOD (U/mgp)	2106.41 ± 50.58	1745.90 ± 150.91[a]	2012.82 ± 27.28	1952.59 ± 25.39	1965.41 ± 50.67	0.038
CAT (U/mgp)	163.98 ± 7.94	163.51 ± 10.65	131.03 ± 4.89	162.58 ± 6.52	154.02 ± 18.70	0.191
GST (U/mgp)	2.05 ± 0.15	1.51 ± 0.33	1.63 ± 0.18	1.56 ± 0.24	1.42 ± 0.23	0.265
GPX (U/mgp)	18.75 ± 3.37	21.99 ± 3.74	20.82 ± 3.79	27.43 ± 4.62[a]	21.3 ± 3.87	0.007
GR (U/mgp)	0.61 ± 0.04	0.52 ± 0.08	0.37 ± 0.07[a]	0.39 ± 0.03[a]	0.22 ± 0.02[ab]	0.001
GSH (µmol/mgp)	24.41 ± 0.63	19.29 ± 0.82	21.31 ± 1.08	19.69 ± 2.05	20.48 ± 1.69	0.094
MDA (nmol/mgp)	12.20 ± 0.42	24.50 ± 7.79[a]	17.49 ± 1.67	21.8 ± 4.06	13.92 ± 0.91	0.039

a: Statistically significant difference compared to control group (P<0.05)
b: Statistically significant difference compared to the stress group (P<0.05)
$\bar{X}$: Mean, $S_{\bar{X}}$: Standard error

Table 7. Effect of chronic immobilization stress on lipid peroxidation and antioxidant defense system in kidney tissue

Parameters	GROUPS Control $\bar{X} \pm S_{\bar{X}}$	Stress $\bar{X} \pm S_{\bar{X}}$	Gundelia $\bar{X} \pm S_{\bar{X}}$	Gundelia + Stress $\bar{X} \pm S_{\bar{X}}$	Fluoxetine $\bar{X} \pm S_{\bar{X}}$	P Values
SOD (U/ mgp)	1947.78 ± 58.45	1966.74 ± 48.03	2033.39 ± 18.68	1956.70 ± 20.63	2026.65 ± 8.87	0.315
CAT (U/mgp)	33.69 ± 5.00	48.39 ± 8.05	23.16 ± 2.12[b]	26.34 ± 2.12	34.71 ± 7.19	0.031
GST (U/mgp)	0.15 ± 0.02	0.18 ± 0.02	0.19 ± 0.05	0.12 ± 0.01	0.20 ± 0.03	0.297
GPX (U/mgp)	33.92 ± 5.73	30.63 ± 5.16	29.41 ± 4.96	29.98 ± 5.05	27.23 ± 1.76[a]	0.015
GR (U/mgp)	0.38 ± 0.03	0.33 ± 0.03	0.27 ± 0.02	0.46 ± 0.04[b]	0.26 ± 0.02[a]	0.001
GSH (µmol/ mgp)	12.44 ± 0.62	12.75 ± 1.01	16.29 ± 1.02[ab]	14.14 ± 1.34	6.20 ± 0.26[ab]	0.001
MDA (nmol/ mgp)	65.46 ± 2.23	83.66 ± 1.90[a]	69.62 ± 6.18	66.06 ± 4.24[b]	75.02 ± 3.96	0.021

a: Statistically significant difference compared to control group (P<0.05)
b: Statistically significant difference compared to the stress group (P<0.05)
$\bar{X}$: Mean, $S_{\bar{X}}$: Standard error

groups were not found to have any significant variation when compared with the control and stress groups (P>0.05).

Tables 6 and 7 give the lipid peroxidation and antioxidant defense system analysis results for liver and kidney tissue, respectively.

According to Table 6, in liver tissue SOD activity levels were significantly reduced in the stress group compared to the control group, while GPx activity levels were observed to have a statistical increase in the Gundelia + stress group compared to the control group (P<0.05). Additionally, GR activity levels in the fluoxetine, Gundelia and Gundelia + stress groups had a statistical reduction observed compared to the control group, while the fluoxetine groups also had a statistical decrease compared to the stress group (P<0.05). The MDA levels in the stress group were determined to be statistically significantly increased compared to the control group (P<0.05).

According to Table 7, in kidney tissue CAT activity levels were statistically low in the Gundelia group compared to the stress group, while GPx activity levels were statistically low in the fluoxetine group compared to the control group (P<0.05). GR activity levels were low in the fluoxetine group compared to the control group, with a statistical increase in the gundelia + stress group compared to the stress group (P<0.05). GSH levels was statistically low in the fluoxetine group compared to both the control and stress groups, while there was a statistically significant increase identified in the Gundelia group compared to both the control and stress groups (P<0.05).

Discussion and Conclusion

In the elevated plus maze test, the open areas of the platform increase anxiogenic behavior. It is thought that agents with anxiolytic effect will increase the duration spent in these areas (Crawley and Goodwin, 1980; Belzung et al., 2001). For example, diazepam, an anxiolytic medication used clinically, increased duration spent in the open area and increased the number of entries into the open area (Crawley and Goodwin, 1980). The results of anxiety tests performed on the 15th and 30th days of the study show that *Gundelia tournefortii* L. caused significant anxiolytic effects in rats exposed to chronic stress. When compared with fluoxetine, used clinically with anxiolytic aims, *Gundelia tournefortii* L. appeared to show better anxiolytic effects than this medication. Different studies have reported that anxiolytic effects of plants are due to the combined effect of active materials, with flavonoids, especially, leading the list of these active materials (Coleta et al., 2006; Doukkali et al., 2016). The anxiolytic effects of flavonoids found in plants are reported to be due to modulation of the GABAergic system (Marder and Paladini, 2002). It is thought that the anxiolytic effect of *Gundelia tournefortii* L. is due to flavonoids contained in its structure.

In a study, the researchers stated that chronic restriction stress causes oxidative damage to tissues such as brain, kidney, liver. In addition, it was reported that various stress models decreased antioxidant enzyme activities and increased ROS production (Samarghandian et al., 2017). Similarly, according to the results of this study, experimentally-induced stress is understood to cause oxidative stress in liver and kidney tissue (Tables 6 and 7). Additionally, the

stress model developing due to immobilization stress applications was shown to cause increases and reductions in antioxidant levels and increased free radical production. Stress is considered to trigger ROS formation in mitochondria, peroxisomes, lysosomes, cytosol and plasma membranes in the body (Djordevic et al., 2010).

Lipid peroxidation is known to be one of the basic causes of injury occurring in hepatocytes (Turan and Celik, 2016) and is due to imbalance forming between the oxidant-antioxidant systems (Yuan et al., 2013). According to present study results, fluctuations occurred in both MDA and enzyme activities and this situation caused cytotoxicity in liver and kidney tissues, leading to excessive free radical production in metabolism resulting in insufficient antioxidant defense mechanisms.

Enzyme systems within the defense system are effective against free radicals formed in the organism. SOD, CAT and GPx are among the most important enzymatic antioxidants preventing free radical accumulation and initiation of lipid peroxidation in the environment (Gutteridge, 1995; Halliwell and Gutteridge 1999; Valko et al., 2007). In this study, the experimentally-induced chronic stress caused an increase in lipid peroxidation and appeared to cause fluctuations in SOD, CAT and GPx activities and enzyme activities in both liver and kidney tissue to deal with this situation.

Davydov et al. (2004) stated that immobilization stress triggered free radical formation in the liver of elderly and adult rats. In parallel with this, in present study, the low SOD level in liver tissue showed superoxide radical formation rates were induced by immobilization stress. In this study, the decrease in GSH levels in the liver is in parallel with studies in this field. Studies by Zaidi et al. (2005) and Sahin and Gumuslu (2007) observed a decrease in GSH levels in liver tissue of rats exposed to immobilization stress.

The study observed increases and decreases in SOD, CAT, GR, GST and GPx activities. CAT may be said to detoxify based on H_2O_2 in kidneys. CAT shows antioxidant enzyme activity at high concentrations of H_2O_2, while GPx shows activity at lower concentrations of H_2O_2 (Jones and Kennedy, 1983), with CAT considered to show more activity than GPx in this tissue. According to present study results, CAT in kidney tissue showed more activity compared to GPx, with this result appearing to support previous studies (Jones and Kennedy, 1983).

The TP levels in Gundelia, Gundelia + stress and fluoxetine groups were statistically significantly reduced compared to the control group, which may be said to be caused by some metabolic disorders in the liver damage. According to Bandyopadhyay et al. (1999), the majority of dissolved solid material in blood plasma is due to proteins. TP level in blood plasma or serum, 3.5-5 g/dL is serum albumin while 2.5-3.2 g/dL comprise globulins. The liver is an important tissue for the synthesis of serum proteins, and the level of serum proteins can be reduced in some liver and kidney diseases. For these reasons, some amino acids appear to be the main cause of metabolic dysfunction in oxidative injury in liver and kidney tissues.

Fluctuation in AST, ALT and ALP levels in the groups in present study change the transport and membrane permeability functions of these cells due to hepatocyte injury and as a result cause leakage of these enzymes. Enzymes like AST, ALT and ALP are released into blood after liver and kidney injury and this causes an increase in activity in serum samples (Gokcimen et al., 2007). Studies of ALT, AST and LDH enzymes reported that they are signs of cellular injury in situations where intracellular values are much higher than extracellular values or if there are increases in the amounts of these enzymes in plasma (El et al., 2002).

In this study, enzyme leakage occurring linked to injury cause an increase in uric acid and creatinine values in the stress, gundelia + stress and fluoxetine groups. Elevated uric acid levels are a common finding of chronic kidney disease. In the past, it was stated that elevated uric acid developed linked to reductions in excretion from the kidneys, while currently it was revealed that uric acid simultaneously has an active role in the formation and progression of kidney injury (Borges et al., 2010). As markers of kidney functions, urea, creatinine, BUN and uric acid levels are measured in blood. These measurements provide information about kidney functions (Soliman et al., 2007). Serum urea, creatinine and uric acid levels caused by the reduction in glomerular filtration are proposed to begin to elevate linked to increases in tubular reabsorption (Erdem et al., 2012).

According to the results of this study, *Gundelia tournefortii* L. showed strong anxiolytic effect. Serum biochemical analysis results showed that *Gundelia tournefortii* L. does not affect blood biochemistry when used alone or with chronic immobilization stress; however, fluoxetine used clinically for anxiolytic aims elevated serum enzymes and lowered total protein and albumin ratios. Additionally, fluoxetine appeared to lower lipid peroxidation levels. In both tissues, chronic immobilization stress appeared to cause both increases and decreases in enzyme activity levels. These results support the study by Zafir and Banu (2007). In this study fluoxetine was identified to lower lipid peroxidation and protein oxidation levels in depressive patients.

Many studies have shown that limited stress causes oxidative stress and thus causes imbalance in antioxidant status (Kovacheva-Ivanova et al., 1994; Oishi et al., 1999). Chronic immobilization stress is a good model to observe the formation of oxidative stress occurring in rat tissues. According to present results, administration of Gundelia plant extract and fluoxetine with immobilization stress affected antioxidant enzyme activities and was understood to cause lipid peroxidation in tissues and trigger free radical formation. Immobilization stress was observed to increase lipid peroxidation in both liver and kidney tissue, while *Gundelia tournefortii* L. and fluoxetine lowered lipid peroxidation.

In conclusion, chronic immobilization stress caused oxidative stress in liver and kidney tissue and administration of *Gundelia tournefortii* L. and fluoxetine was understood to have positive effects on enzyme activity and MDA levels during injury to these tissues. The study data shows that *Gundelia tournefortii* L. and fluoxetine used as antidepressant had beneficial effects against the oxidative injury caused by experimentally-induced stress. Additionally, *Gundelia tournefortii* L. was identified not to affect motor activity, but had anxiolytic effect according to the elevated plus maze test results.

Conflict of interest statement

The authors have no conflict of interest to declare within this article.

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