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Some biological properties of ethanol extract prepared from the aerial parts of *Scutellaria albida* L. subsp. *condensata* (Rech.f.) J.R. Edm.

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ABSTRACT

Moderate effects of some plants with proven biological properties in the treatment of various diseases have increased the importance of them and the interest in alternative medicine. The members of the Lamiaceae family are one of the widely used in alternative medicine and agriculture due to their metabolite content. In order to gain valuable biological data for alternative medicine and new studies, *Scutellaria albida* subsp. *condensata* is a member of the Lamiaceae family and this plant was collected from a height of 1500 meters in Bitlis province in Turkey. Ethanol (EtOH) extract was prepared by using the aerial parts of the plant and used in all stages of the study. Firstly, the phenolic content of the extract was determined by HPLC. Myricetin and 4-Hydroxybenzoic acid at the highest concentrations were detected, but ascorbic acid, gallic acid, quercetin, and 3,4-Dihydroxybenzoic acid could not be determined in the extract. In order to test the antioxidant properties based on phenolic content, several *in vitro* antioxidant tests were performed and DNA protective properties were investigated. In the biological activity results, the extract was determined to have a similar antioxidant effect to standards or lower than them and exhibited relatively DNA protective activity at high concentration. Finally, the effects of the extract on some types of bacteria and fungi were investigated by the hollow agar method and 150 µL volume of the extract was shown to have better activity than ampicillin and Amikacin. Due to the limited studies on *Scutellaria albida* subsp. *condensata*, it is thought that this study will contribute to the literature.

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1. Introduction

Plants are important medical factors for our health because of their healing effects or positive effects on the some of diseases. Secondary metabolites (SMs), also known as phytochemicals, natural products, or plant constituents are important molecules that play a role in the cell's interaction with its environment [1]. The main classification system for SMs includes three major groups: terpenoids, alkaloids, and phenolics and each of them contain subclasses with complexity in structure [2]. Phenolics, one of the most important, have high antioxidant value and are known to be protective against neurodegenerative diseases, diabetes, and cancer associated with reactive oxygen species such as hydrogen peroxide (H₂O₂), superoxide (O₂⁻), and hydroxyl (OH[•]) [3, 4].

The members of the Lamiaceae family are widely used in traditional medicine and agriculture and they include plant species with economic value [5]. The use of this family in pharmaceutical production and agricultural fields increased the importance of family members and attracted the attention of

scientific world. They are spread almost all over the world and have 46 different genera and 758 taxa in our country, and the rate of endemism in our country is 45% [6]. The members of *Scutellaria* L. genus generally grow on stony and rocky slopes and have medical importance due to their SMs and other contents [7]. Previous studies have reported that the members of this family has many biological effects such as antimicrobial, antifungal, anti-inflammatory, anticancer, and antioxidant effects [8-12].

Based on the data suggested about the Lamiaceae genus in previous studies, it is aimed to gain a new plant species and its valuable biological properties for the literature and to provide a starting point for advanced studies carried out on similar studies and especially pharmacological research. For this purpose, ethanol (EtOH) extract was prepared from the aerial parts of *Scutellaria albida* subsp. *condensata*

collected in Bitlis province in Turkey and various biological properties of the extracts were investigated. Within the scope of the study, the concentrations of 17 phenolics were tried to determine by HPLC. Moreover, Various *in vitro* antioxidant properties and DNA protective properties on pBR322 plasmid

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DNA were studied using different concentrations of the extract to validate HPLC results. Finally, by investigating the antimicrobial effects of the extract, their compatibility with the effects of the antioxidant and DNA protective properties were tested.

2. Material and Method

2.1. Collection of the plants and extract preparation

Scutellaria albida subsp. *condensata* was collected during the vegetation period between 2014-2015 years in Bitlis province in Turkey. EtOH extract was prepared with the 50 g of aerial parts of the using the soxhlet extraction apparatus and stored at -18 °C, in dark bottles. The Plant was also converted into herbarium material and stored in Bitlis Eren University Science and Technology Application and Research Center with the code M.KURŞAT: 6049.

2.2. Determination of phenolic concentrations by HPLC

HPLC chromatograms using the 10mg/mL standard concentrations and calibration curves using their different concentrations were created. The chromatogram of phenolics is shown in **Figure 1**. Five different concentrations of standards were used to generate the calibration curve and prepared standard solutions were filtered through 0.45µm membrane filter. HPLC conditions were performed according to the method used in our previous article [13].

2.3. Microorganisms and antimicrobial activity

The microorganisms used in this study are as follows; *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Bacillus megaterium* DSM 32, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027, *Klebsiella pneumonia* ATCC 13883, *Yarrowia lipolytica*, *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 834. Standard antibiotics known to be effective on microorganisms were purchased from OXOID company and their antimicrobial effects were determined using the hollow agar method and the results were compared with the effects of the extract. The antibiotics used in this study are Erythromycin; E-15, Ampicillin/Sulbactam; SAM-20, Rifampicin; RD-5, Amikacin; AK-30 and Fluconazole; FCA-25. Test microorganisms were obtained from Muş Alparslan University Central research and application laboratory, and bacterial production was performed using the method of Hindler [14]. The blur of microorganisms was adjusted according to the 0.5 standard of Mc Farland. The different concentrations (75 µL, 100 µL, and 150 µL) of extract were prepared and antimicrobial effects of the extract and antibiotics were determined using the method of Sagdic, Karahan [15].

2.4. Antioxidant properties using in vitro studies

2.4.1. Total antioxidant activity by ferric thiocyanate method

The effect of the extract on lipid peroxidation was determined using the ferric thiocyanate method proposed by Mitsuda [16]. This method is based on the spectrophotometric measurement

of the peroxide formed as a result of linoleic acid oxidation at 500 nm at 10 hours intervals. The test was terminated when the control absorbance reached the highest value. Absorbance percentages in the inhibition of linoleic acid emulsion were calculated according to the following equation 1. In the equation, A sample; the value of the sample absorbance when the control absorbance is the highest and A control; the value of control absorbance when the control absorbance is the highest.

$$\text{Lipid Peroxidation Inhibition (\%)} = 100 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \times 100 \right) \quad (1)$$

2.4.2. Determination of total reduction

Total reduction power was performed according to the method proposed by Oyaizu [17]. According to this, the extract was prepared at different concentrations (25µg/mL, 50µg/mL, and 100µg/mL) and absorbance values were spectrophotometrically recorded at 700 nm.

2.4.3. DPPH radical scavenging activity

The effect of DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging was performed by Blois [18] method. This method is based on the reduction of DPPH radicals dissolved in alcohol in the presence of antioxidants having hydrogen donating groups and absorbance values are measured at 517 nm.

2.4.4. Cupric (Cu²⁺) reduction by the CUPRAC method

Cupric ions (Cu²⁺) reduction activities were performed using the method proposed by Apak, Güçlü [19]. According to the method, the absorbance values of different concentrations of the samples were recorded at 450 nm. The increase in the absorbance of the reaction mixture indicates the cupric ion reduction capacity.

2.5. Determination of DNA protective effect

In this study, pBR3222 plasmid DNA was used as model nucleic acid and DNA damage was created using H₂O₂. The protective effect of the extract on pBR322 plasmid DNA was demonstrated by agarose gel electrophoresis [20]. According to this, sample mixtures were prepared as shown in **Table 1** and 5µl of prepared extract and 5µl of loading buffer were mixed with each other and loaded to electrophoresis with the other electrophoresis components (**Figure 3**). Electrophoresis was performed at 40V for 2 hours and stained with ethidium bromide and agarose gel was then visualized using Londershausen 1996 imaging system.

3. Results

3.1. Phenolic Concentrations of the Extract

The phenolic concentration results are shown in **Table 1**. The study showed the myricetin concentration as the highest (28.795µg/mL) and the vanillin concentration as the lowest (0.728µg/mL). However, ascorbic acid, gallic acid, 3,4-dihydroxybenzoic acid, and quercetin could not be determined

in the extract.

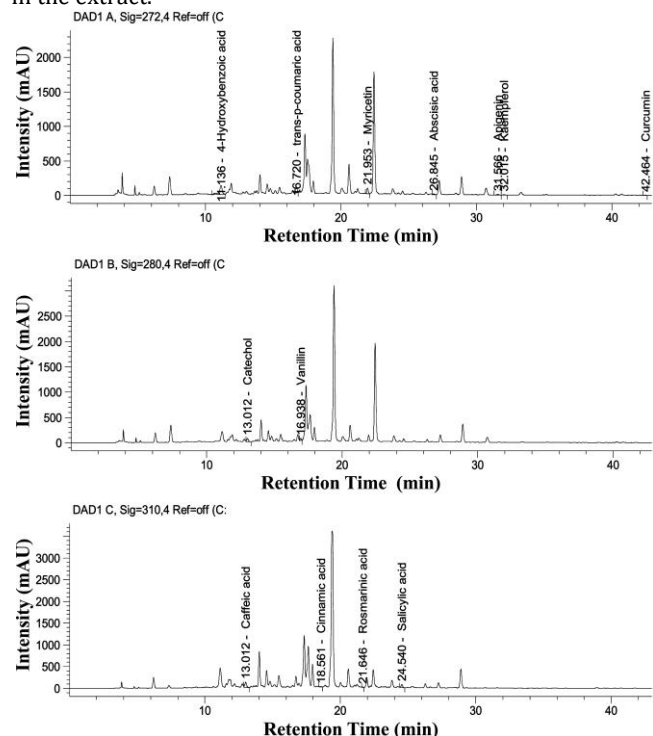


Figure 1. HPLC chromatogram showing retention times of phenolic

content in the extract.

Table 1. Phenolic concentrations of EtOH extract

Phenolic content	Concentration (µg/mL)
Ascorbic acid	N/A
Gallic acid	N/A
3,4-Dihydroxybenzoic acid	N/A
4-Hydroxybenzoic acid	24.823
Trans-p-coumaric acid	10.808
Myricetin	28.795
Abacetic acid	6.773
Quercetin	N/A
Apigenin	18.462
Kaempferol	2.799
Curcumin	4.111
Catechol	17.951
Vanillin	0.728
Caffeic acid	8.360
Cinnamic acid	1.428
Rosmarinic acid	0.780
Salicylic acid	14.913

3.2. Antimicrobial effects of the extract

Scutellaria albida subsp. *condensata* extract was tested on 3 Gram positive (*B. subtilis*, *S. aureus* ve *B. megaterium*), 4 gram negative (*E. aerogenes*, *E. coli*, *P. aeruginosa* ve *K. pneumonia*), and 3 Fungus (*Y. lipolytica*, *C. albicans* ve *S. cerevisiae*) and the results of the antimicrobial effects were calculated as mm (Table 2). When the antimicrobial effect results are evaluated, it is clear that primarily the antimicrobial effect increases depending on

the extract concentration and it can be said that although the extract has both antibacterial and antifungal effects, fluconazole has only antifungal effect and other antibiotics have only antibacterial effect. In addition to this, extract showed the lowest antimicrobial effect on *B. subtilis*, *E. aerogenes* and *C. albicans* (14±0,00 mm) and the highest antimicrobial effect on *B. megaterium* (20±1,00 mm).

Table 2. A: Diameters of the antimicrobial effect depending on the EtOH extract concentration (mm), B: Diameters of the antimicrobial effect depending on the type of antibiotics (mm)

A					B				
		EtOH extract			Antibiotics				
Microorganisms		75µl	100µl	150µl	Erythromycin	Ampicillin / Sulbactam	Amikacin	Rifampicin	Fluconazole
Gram (+)	<i>B. subtilis</i>	n/a	n/a	14±0,00	20±0,00	14±1,15	11±1,00	21±0,00	n/a
	<i>S. aureus</i>	n/a	n/a	n/a	21±1,00	10±0,00	9±0,00	18±1,15	n/a
	<i>B. megaterium</i>	15±0,00	16±0,00	20±1,00	25±0,00	n/a	10±1,00	16±0,00	n/a
Gram (-)	<i>E. aerogenes</i>	n/a	n/a	14±0,00	27±1,00	10±1,00	9±0,00	16±1,00	n/a
	<i>E. coli</i>	12±0,00	14±0,57	16±1,15	19±1,52	13±0,00	13±0,00	18±0,00	n/a
	<i>P. aeruginosa</i>	12±0,00	14±0,00	17±1,00	19±0,00	-	14±1,15	8±0,00	n/a
	<i>K. pneumonia</i>	n/a	n/a	n/a	19±1,73	16±0,57	10±0,00	19±1,73	n/a
Fungus	<i>Y. lipolytica</i>	14±0,00	15±0,57	15±0,57	n/a	n/a	n/a	n/a	21±0,00
	<i>C. albicans</i>	n/a	n/a	14±0,00	n/a	n/a	n/a	n/a	23±1,52
	<i>S. cerevisiae</i>	n/a	n/a	17±0,57	n/a	n/a	n/a	n/a	n/a

n/a: not available

3.3. Antioxidant study results

3.3.1. Total antioxidant activity results

Total antioxidant activity was investigated at different

concentrations (25µg/mL, 50µg/mL, and 100µg/mL) and generally increased depending on extract concentration. Inhibition effects of the extract and standards on linoleic acid emulsion were calculated based on the 30th hour when the control absorbance value reached the maximum. When the result of total antioxidant activity was evaluated, the highest

effect was observed at 100 µg/mL concentration (**Figure 2A**) and total antioxidant activity percentages were calculated for this test. According to this, the order of total antioxidant activities for the highest concentration is as follows; EtOH extract (73.19%) > BHT (72.76%) > BHA 72.34(%) > α-Tocopherol (57.87%)

3.3.2. Total reduction activity results

The reduction capacities of the samples were determined by measuring spectrophotometrically the absorbance values at 700 nm and their reduction capacities increased in parallel with the concentration and the order of the results obtained is as follows; BHA > BHT > α-Tocopherol > EtOH extract. (**Figure 2B**)

3.3.3. DPPH radical scavenging results

DPPH radical scavenging activities were evaluated for 25 µg/mL, 50 µg/mL and 100 µg/mL extract concentrations at 517 nm and the strongest effect was seen at 100 µg/mL (**Figure 2C**). Then, DPPH radical scavenging activity

percentages for 100 µg/mL were calculated. When looking at 100 µg/mL concentration results, besides the strong effect in the extract, it is clear that the extract has a lower radical scavenging effect than the standards and DPPH radical scavenging sequence is as follows; BHA(91,74%) ≥ α-Tocopherol (91,23%) ≥ BHT (90,50%) > EtOH extract (69,94%).

3.3.4. Cupric ion (Cu²⁺) reduction results

Cupric ions (Cu²⁺) reduction activities were performed using the method proposed by Apak, Güçlü [19]. The absorbances in different concentrations of the extract and standards were recorded at 450 nm. The cupric ion reduction capacity of the extract increased in parallel with extract concentration (**Figure 2D**). The order of cupric ion reduction capacities is as follows; BHA > BHT > α-Tocopherol > EtOH extract.

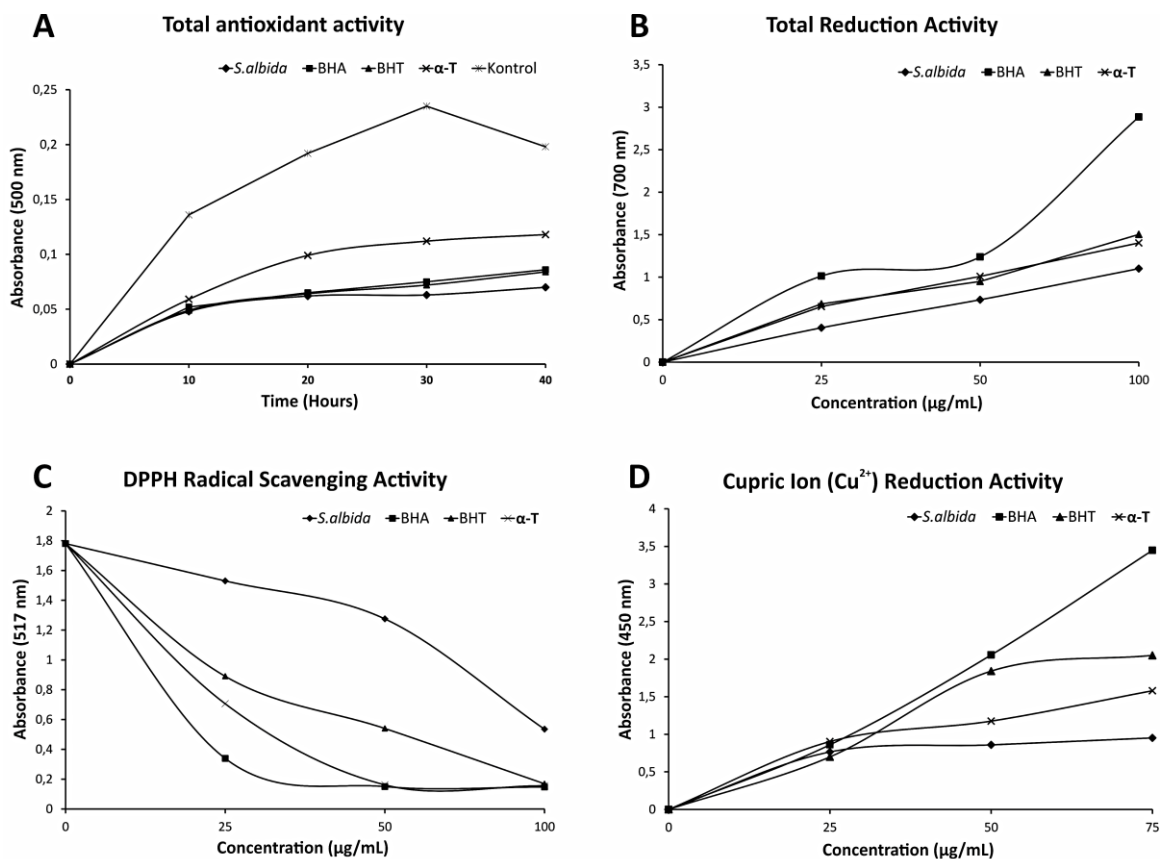


Figure 2. *In vitro* antioxidant activity results of EtOH extract prepared from *S. albida* subsp. *condensata* (*S. albida*) and standards. **A**; Total antioxidant activity depending on hours, **B**; Total reduction activity on concentrations **C**; DPPH radical scavenging activity on concentrations and, **D**; Cupric ion (Cu²⁺) reduction activity on concentrations.

3.4. DNA protective activity results

Whether the extract has a protective effect on plasmid DNA was determined by observing the form I, form II, and form III structures in pBR322 plasmid DNA (**Figure 3**). In the results of

agarose gel electrophoresis, it is understood that H₂O₂ disrupted the form I structure (**Line 2 in Figure 3**) and the mixture of H₂O₂ and DMSO destroyed completely the plasmid DNA (**Line 3 in Figure 3**). It was also observed that the high extract of *Scutellaria albida* subsp. *condensata* relatively

decreased the scavenging effects of the mixture of H_2O_2 and DMSO (Line 5 in Figure 3). In addition, the extract did not have a serious adverse effect on plasmid DNA when applied alone (Line 8, 9, 10 in Figure 3) however, when applied together with H_2O_2 , it had a seriously positive effect on the stability of the form II structure, especially at 100 $\mu\text{g}/\text{mL}$ concentration (Line 5 in Figure 3).

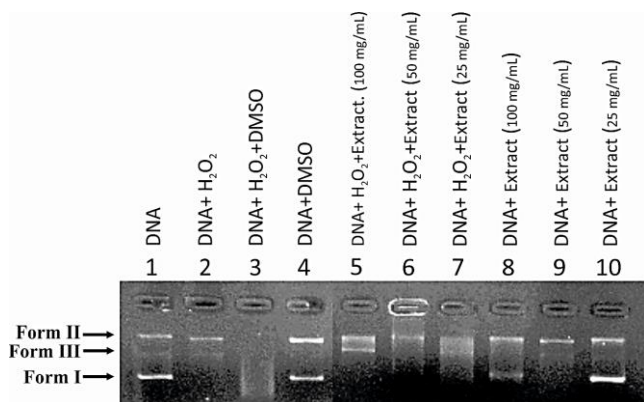


Figure 3. Agarose gel image showing the protective effect of EtOH extract on pBR322 plasmid DNA.

4. Discussion

It is known that plants have a protective effect against some diseases by supporting the immune system. In particular, plants whose healing effects have become the focus of researchers and new plant and biological property discoveries have gained momentum. As a result, it was understood that some of them have direct healing effects and the others have an indirect healing effect, and the reason for this is attributed to the types and concentrations of molecules in plant content. Lamiaceae family is widely researched and known to have strong biological properties. Previous studies have mentioned the high levels of rosmarinic acid in this family [21-23]. Gallic acid, myricetin, quercetin, apigenin, kaempferol, caffeic acid, cinnamic acid, and rosmarinic acid were reported to find in different species of this family [24]. Another study reports that this family has high phenolic concentrations known as antioxidants such as rosmarinic acid, coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, luteolin, apigenin, quercetin, rutin, epicatechin, and catechin [25-27]. In the HPLC results of our study, it was detected that the extract contains the highest level of myricetin (28.795 $\mu\text{g}/\text{mL}$) and was found to have very low curcumin concentration which is used extensively as food additive (4.111 $\mu\text{g}/\text{mL}$).

Extracts prepared from plants known to have antimicrobial effects were used in the treatment of bacterial infections in ancient times. Studies have reported that ethanol and methanol extracts prepared from the members of the Lamiaceae family have a strong effect on *S. aureus* and *B. subtilis*. The essential oils of the *Saturja hortensis* which is a member of the Lamiaceae family have been reported to show high antimicrobial activity [28] and acetone extract of the *Scutellaria* genus show the highest antibacterial activity against *Streptococcus mutans* [29]. 150 μL volume of extract in our study exhibited the highest antimicrobial activity on *B. megaterium* and generally showed a higher effect than antibiotics other than erythromycin and rifampicin. A study conducted with the Lamiaceae family emphasized that the extracts exhibited lower activity than

ampicillin and kanamycin [30]. It is clear that in this study, the antimicrobial effect at 150 μL extract volume was similar to ampicillin/sulbactam and amikacin or higher than them.

Some factors such as adverse environmental conditions and the misuse of medications cause oxidative stress and various metabolic diseases by increasing cellular radical concentration [31, 32]. Moreover, the radicals can transform the form I structure of DNA into form II and form III by disrupting the double helix of DNA [33]. Contrary to this, antioxidant molecules inhibit the radicals that are the product of adverse conditions and hereby prevent DNA structure against oxidative stress and DNA damage [34]. In the results of antioxidant studies, it was clear that the extract has high antioxidant properties, but still exhibited lower activity than standard antioxidants. It was also determined that total antioxidant activity was higher than standards but, the effects of the extract were weaker in the other *in vitro* tests. This result is evidence that *Scutellaria albida* subsp. *condensata* is a potential antioxidant but not kinetically active. A study emphasizes that the Lamiaceae family have a protective role on plasmid DNA damaged by H_2O_2 and UV [35] and reported that *Leucas aspera* is a member of the Lamiaceae family and it has a positive effect on DNA stability [36]. Similar to previous studies, it was shown in this study that EtOH extract reduced the DNA damage caused by only H_2O_2 or DMSO and H_2O_2 together by allowing the regeneration of the form II structure depending on extract concentration. It can also be said that the lowest volume of extract does not have a significant effect on pBR322 plasmid DNA.

5. Conclusions

In conclusion, *Scutellaria albida* subsp. *condensata* extract was found to contain optimum concentrations of phenolic antioxidants. In parallel with this result, they were also found to have quite power antioxidant properties despite being weak by standards and exhibited the valuable antibiotic effect by providing the desired effect at high concentrations. The extract did not show a serious negative effect on pBR322 plasmid DNA when applied alone at low concentration, but when applied together with H_2O_2 , the 100 $\mu\text{g}/\text{mL}$ concentration of the extract reduced relatively the scavenging effect of H_2O_2 .

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Orbital period behaviour of three semi-detached binaries: AI Cru, V1898 Cyg and Z Vul

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ABSTRACT

In this research, orbital period variation of three semi-detached binaries AI Cru, V1898 Cyg and Z Vul investigated using all published eclipse times. O-C analysis method has preferred as the method and the orbital periods of all systems are determined to increase. The changing rate of their period has been determined to be 0.8, 4.1 and 0.8 s/century for AI Cru, V1898 Cyg and Z Vul, respectively. Mass transfer between components has proposed as the cause of orbital period increase. For AI Cru, V1898 Cyg and Z Vul, mass transfer from less massive component to more massive and mass transfer rate was found to be 2.9×10^{-7} , 1.5×10^{-7} and $5.3 \times 10^{-8} M_{\odot}/\text{yr}$, respectively. Cyclic change has also seen in AI Cru and V1898 Cyg with increasing parabolic change. Cyclic periodic changes can be explained as being the result of a light-travel time effect via a tertiary body around the eclipsing pair. The minimum mass of probable tertiary components around AI Cru and V1898 Cyg were found to be $0.38 M_{\odot}$ and $0.26 M_{\odot}$, respectively.

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1. Introduction

One of the most important classifications of binary stars was made by Kopal (1955) according to Roche lobes, and this classification also provides information about the evolutionary states of stars. The radii of stars change during their evolution, but the expansion of binary stars it is limited to their Roche lobes. Semi-detached binaries (also known as classical Algols) is a classification made according to Roche classification, and one of the components filled the Roche lobe and the other did not. Classical Algols are semi-detached star systems and the primary component usually B or early A (rarely spectral type F) composed of spectral type, the second component is of the G, K, M spectral type of giant or sub-giant that filled the Roche lobe. Except for eclipsing systems, they do not show any significant change in their light curves. This the most important feature of the systems is that both eclipse depths are quite different from each other. The orbital periods of the vast majority are between one day and several days.

Orbital period analysis provides information about the evolution of such stars. So in this paper, the orbital period changes of three semi-detached systems (AI Cru, V1898 Cyg and Z Vul) are examined. Some basic physical parameters and data information of target systems collected in the literature

have given in Tables 1 and 2.

1.1. AI Cru

The eclipsing binary AI Crucis (GSC 08974-00877, TYC 8974-877-1, Gaia DR2 6058510765049380096, $V=9.69$ mag) was discovered by Oosterhoff (1933) and determined the orbital period as $P = 1.4177073$ day. Then the photometric solutions of the system were made by several authors (Ollongren 1956, Giuricin et al. 1980, Russo 1981). It was reported by Russo (1981) that the second component is a semi-detached system filling the Roche lobe. Both photometric and spectroscopic observations of AI Cru were carried out by Bell et al. (1987). The spectral type of the primary component was determined as B 1.5 by Bell et al. (1987) and the masses of the components in the same study were reported as $9.8 \pm 0.5 M_{\odot}$ and $5.8 \pm 0.3 M_{\odot}$. The orbital period analysis study of the system was performed by Zhao et al. (2010) and Zhao & Qian (2012). In both studies, it was determined that the orbital period of the system is increased. The last orbital period analysis study of the system was made by Zhao & Qian (2012) and the period increase rate was determined as $dP/dt = +1.00 (\pm 0.04) \times 10^{-7}$ days/yr.

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Table 1. Basic physical parameters of systems used in analysis.

Parameters	AI Cru	V1898 Cyg	Z Vul
Spectral type	B2IV ³	B2IV+GIII ⁴	B3-5V+A2-3III ⁵
Orbital period (day)	1.41771076 ¹	1.51312433 ¹	2.45492774 ¹
T_0 (HJD+ 2400000)	33466.3382 ¹	45960.6717 ¹	25456.118 ¹
M_1 - M_2 (M_\odot)	9.8-5.8 ²	6.05-1.16 ⁴	5.29-2.33 ⁵
R_1 - R_2 (R_\odot)	4.9-4.4 ²	3.53-2.64 ⁴	4.93-4.67 ⁵

References: 1: Kreiner et al. (2001); 2: Bell et al. (1987); 3: Wesselink (1969); 4: Dervişoğlu et al. (2011); 5: Lazaro et al. (2009).

1.2. V1898 Cyg

V1898 Cyg (GSC 03588-04247, TYC 3588-4247-1, Gaia DR2 2163574661395444864, $V=7.82$ mag) was discovered by Abt et al. (1972). Photometric observations on B and V filters were made by Halbedel (1985) and, contrary to spectroscopic observations, it was found that both eclipses were similar and the orbital period was determined as $P = 3.0239$ days. The new light curve was made by Caton & Smith (2005) and the orbital period of the system was determined as $P = 1.5131273$ days and it is half the value found by Halbedel (1985). Both photometric and spectroscopic observations of the V1898 Cyg system was carried out by Dervişoğlu et al. (2011) and in the same study, basic physical parameters such as mass, radius of the system were determined. The orbital period analysis study of the system was also carried out by Dervişoğlu et al. (2011) and it has been determined that the period has increased. They are the orbital period increase rate $P'/P = 6.68 (\pm 0.63) \times 10^{-7} \text{ yr}^{-1}$.

1.3. Z Vul

The eclipsing binary system Z Vul (GSC 02128-00966, TYC 2128-966-1, Gaia DR2 2023954311223665536, $V=7.33$ mag) was discovered by Herschel (it has been reported by Astbury, 1909). The radial velocity study was made by Popper (1957) and the mass ratio of the Z Vul system was determined as $q = 0.42$. The spectral type of the system was determined as B5 by Levato (1975) and the rotational velocity in the same study was determined as $v \sin i = 195 \text{ km/s}$. Light curve analysis simultaneously with radial velocity was carried out by Ghoreyshi et al. (2008) and in the same study, basic astrophysical parameters such as mass and radius have determined. Light curve and spectroscopic observations of Z Vul were made by Lazaro et al. (2009). Light and radial velocity curve analysis were also performed in the same study. The orbital period analysis study of the system was conducted by Kreiner & Ziolkowski (1978) and it has been stated that the orbital period has increased.

2. Material and Method

In this study, collected all visual, photographic, photoelectric and CCD data from the literature. The following equations have applied to the minimum times collected (most of which are taken from the O-C Gateway (Paschke and Brat; 2006)). Some information about the O-C data has given in Table 2.

Table 2. Data information used in the orbital period analysis of systems.

System	Data Range (year)	The total number of data	Type Min.I/Min.II
AI Cru	~90	29	26/3
V1898 Cyg	~30	13	13/-
Z Vul	~120	466	444/22

The orbital periods of binary star systems can change over time. These changes are it may be in the form of an increase or decrease. In the literature, the change in the periods of binary stars is explained by four different mechanisms. These are conserved mass transfer between components or nonconservative mass loss, magnetic activity, the effect of a third body in the system and axis rotation. From these four effects, mass transfer/mass loss and magnetic activity are it literally changes its period. The axis rotation and the third body do not literally change the orbital period. To determine the cause of the period change of a system (O: observed and C: calculated minima times) the change of the difference of O-C with respect to time it is based on interpretation. O-C analyzes of binary stars are often complex. These often involve one or more cyclical changes superimposed on an increasing or decreasing parabolic change. Parabolic changes are generally explained by mass transfer between components. However, decreased parabolic changes can also be explained by possible angular momentum loss from the system if at least one of the components is behind the F5 spectral type. Although sinus-like changes are generally accepted to be caused by possible components revolving around the binary system, in addition, the source of this change. There may also be magnetic cycles of cold and magnetic active components in the system (Applegate, 1992). In O-C graphs, interstellar mass and energy transfer or mass loss of the system appear as parabolic change. In this case, equation 1 is used for O-C data.

$$MinI = T_0 + E.P + Q.E^2 \quad (1)$$

where T_0 and P are the starting minimum time and orbital period, respectively, E is the number of cycles and Q is the coefficient of the parabolic term. If the period of the system is increasing, Q is positive, if it is decreasing, it is negative. Equation 2 is used to express the change in unit time, period.

$$\frac{\Delta P}{P} = \frac{2.Q}{P} \quad (2)$$

In order to model the sine-like changes seen in O-C graphs using the light-time effect (LITE) caused by the possible third object, the most basic expression was first given by Irwin (1959) with equation 3.

$$\Delta t = \frac{a_{12} \sin i'}{c} \left\{ \frac{1 - e'^2}{1 + e' \cos v'} \cdot \sin(v' + w') + e' \cos w' \right\} \quad (3)$$

The value given in the equation is the time delay caused by the possible third object. In the equation 3; a_{12} , i' , e' , v' and w' are the semi-major axis, inclination, eccentricity, true anomaly of the position of the binary system's mass center of orbit and the longitude of the periastron of the orbit of the eclipsing binary around the third component, respectively.

O-C changes due to magnetic cycle are cyclical and can be represented by equation 4.

$$MinI = T_0 + E.P + A_{mod} \sin \left[\frac{2\pi}{P_{mod}} (E - T_s) \right] \quad (4)$$

where A_{mod} , P_{mod} , and T_s represent the amplitude, period and minimum moment of the cyclic change, respectively. Conservative mass transfer between components can be recommended as the cause of orbital period increase. The following equation is used for mass transfer between components (Kwee, 1958).

$$\frac{\Delta P}{P} = \frac{3\dot{m}_1(m_1 - m_2)}{m_1 m_2} \quad (5)$$

where it is represented by the period change (ΔP) and the transferred mass amount (\dot{m}_1).

3. Results and Conclusion

In this study, the orbital period analysis study of three semi-detached systems has been done in detail and O-C method has used in the analysis. Semi-detached systems generally the second component filled the Roche lobe while the first component did not fill the Roche lobe. Therefore, the orbital period change of the systems is expected to increase since there will be a mass transfer from the second component filling the Roche lobe to the first component from Lagrange 1 point. It has been observed that the orbital periods of all systems increase.

O-C analysis of AI Cru has performed with a total of 29 minima times collected from the literature (an update of about 8 years has been made). Both cyclical change and increasing parabolic change has seen in AI Cru's O-C analysis (see Figure 1 and Figure 2). So equation 1 showing parabolic change and light time effect has used. Equation 2 has used to calculate the period change rate of AI Cru and the orbital period increase rate of the system has determined as $dP/dt = 9.3 \times 10^{-8}$ days/yr. In the last period analysis study conducted by Zhao & Qian (2012) for the AI Cru system, the rate of increase of the period has calculated as $dP/dt = 1.00(0.04) \times 10^{-7}$ days/yr. It was also noted in the same study that the conservative mass transfer between components would not be sufficient as the reason for the period increase of the system and it has been suggested that the loss of mass from the first component by the stellar wind may be the reason for the period increase. Therefore, in this study, both the conservative mass transfer rate and the mass loss ratio are calculated and given in Table 3. With the increase of the AI Cru system period, cyclical change was also observed and it has suggested that a possible third object could be the cause of cyclical change. The mass of the possible third component has calculated to be $0.38 M_\odot$.

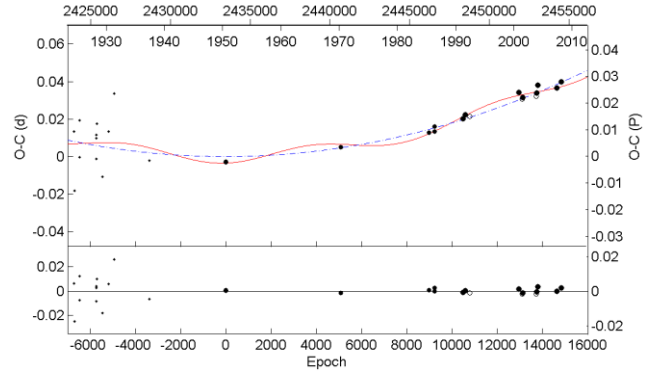


Figure 1. Representation of AI Cru system with O-C graph and theoretical curves. On the top panel; dashed line represents parabola, continuous line represents parabola + cyclical fit, bottom panel shows differences from theoretical curve.

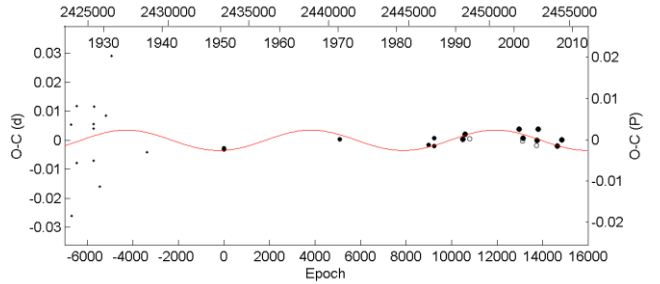


Figure 2. Cyclical O-C change and theoretical representation of AI Cru system.

Approximately 10 years of update has been made for the V1898 Cyg system and an orbital period analysis study has been performed with 13 minima times collected in the literature. As a result of O-C analysis of V1898 Cyg system, cyclic change has observed with parabolic change (see Figure 3 and Figure 4). Using the equations in section 2, the orbital period rate of change has calculated as $dP/dt = 4.73 \times 10^{-7}$ days/yr ($3.13 \times 10^{-7} \text{ yr}^{-1}$). The last orbital period analysis study of the system was made by Dervişoğlu (2011) and the period increase rate was calculated as $dP/P = 6.68 \times 10^{-7}$ days/yr. In this study, it has proposed that the orbital period change for V1898 Cyg can be a mass transfer between components with the assumption of conservative mass. Mass transfer between components has calculated as $dM/dt = 1.5 \times 10^{-7} M_\odot/\text{yr}$ from second component to primary component. In the analysis, it was seen that there was a cyclical change with the increase of the period. It has been suggested that there may be a third object as the cause of cyclical change and the minimum mass of this object has been calculated as $0.38 M_\odot$.

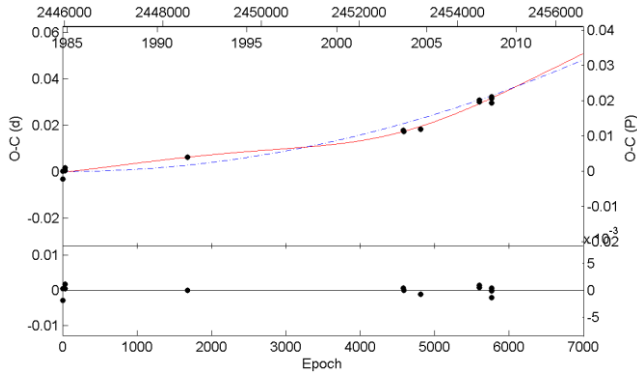


Figure 3. O-C values of V1898 Cyg (description is the same as fig. 1).

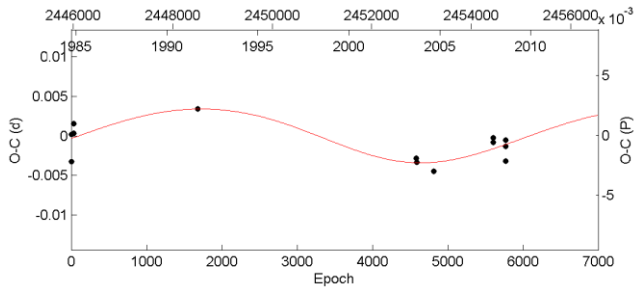


Figure 4. Sinusoidal fit on the O-C diagram of V1898 Cyg.

A detailed orbital period analysis study has been conducted for the Z Vul semi-detached system. The orbital period analysis of the system has made by Kreiner & Ziolkowski (1978) and it has reported that the period increased. Analysis has conducted with more sensitive data of about 40 years (with minima times 466 collected in the literature). It is seen that the period of the Z Vul system has increased and this rate of increase has been determined as $dP/P = 9.37 \times 10^{-8}$ days/yr. Mass transfer from the first component to the second component has been proposed as the cause of orbital period increase. The mass transfer ratio has calculated as $dM/dt = 5.3 \times 10^{-8} M_{\odot}/\text{yr}$ with the conservative mass assumption.

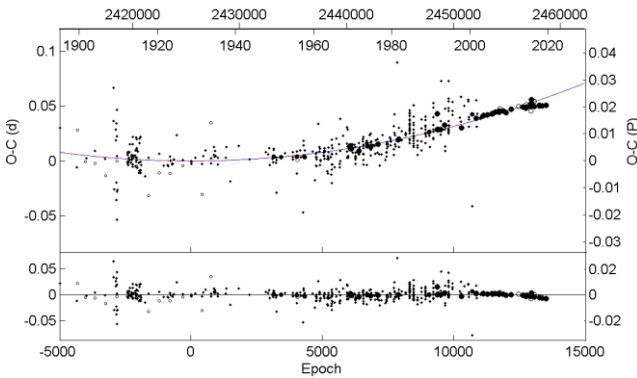


Figure 5. O-C values of the Z Vul system and the theoretical curve used to represent it and its differences.

Table 3. Parameters derived from O-C analysis of three systems in this study.

Parameters	AI Cru	V1898 Cyg	Z Vul
T_0 (HJD+2400000)	33466.3389 (37)	45960.6755 (13)	25456.1147 (24)
P_{orb} (day)	1.4177092 (4)	1.5131199 (18)	2.4549259 (7)
Q (day)($\times 10^{-10}$)	1.8(1)	9.8(2)	3.1 (5)
dP/dt (s/century)	0.8	4.1	0.8
dM/dt (M_{\odot}/yr)	2.9×10^{-7}	1.5×10^{-7}	5.3×10^{-8}
(conservative)			
dM/dt (M_{\odot}/yr)	1.3×10^{-7}	1.7×10^{-7}	5.9×10^{-8}
(estimated mass loss)			
A (day)	0.0035 (3)	0.0034 (9)	---
$a_{12} \sin i$ (AB)	0.61 (18)	0.59 (16)	---
e	0.12 (1)	0.10 (5)	---
ω (deg)	80 (19)	246 (48)	---
T' (HJD+2400000)	37010(626)	51226(533)	---
P_{12} (yr)	31 (4)	24 (4)	---
$f(m_3)$ (M_{\odot})	0.00023 (1)	0.00033 (8)	---
m_3 (M_{\odot}) for $i=90$	0.38	0.26	---
deg.			

A second way of explanation for cyclical variations is if one or both of the components are cold and the outer parts have a convective envelope, the magnetic cycle of the active components can also cause the cyclical period change. Therefore, with the parabolic term, the cyclic changes Applegate (1992), equation 4 was applied to the O-C data to explain the model and the Applegate parameters found were listed in table 4.

Table 4. Some parameters related to Applegate model for AI Cru and V1898 Cyg.

Parameters	AI Cru	V1898 Cyg
P_{mod} (yr)	31 (4)	24 (4)
$\Delta P/P$	3.88×10^{-6}	4.87×10^{-6}
ΔJ (erg s $^{-1}$)	-1.2×10^{49}	-1.2×10^{49}
$\Delta \Omega/\Omega$	0.016	0.032
ΔE (erg)	9.9×10^{42}	1.8×10^{43}
I_s (g cm 2)	1.42×10^{55}	7.95×10^{54}
ΔL_{rms} (L_{\odot})	8	20
B (kG)	8	2

As a result; the orbital period analysis study of three semi-detached systems has been done in detail. It has been observed that the orbital periods of all systems increase. This is what is expected of such systems. The changing rate of their period have been determined to be 0.8, 4.1 and 0.8 s/century for AI Cru, V1898 Cyg and Z Vul, respectively. Conservative mass between components have assumed as the orbital period reason. For AI Cru, V1898 Cyg and Z Vul, mass transfer from less massive component to more massive and mass transfer rate was found to be 2.9×10^{-7} , 1.5×10^{-7} and $5.3 \times 10^{-8} M_{\odot}/\text{yr}$, respectively. Cyclical change has also seen in AI Cru and V1898 Cyg with increasing parabolic change. Cyclical periodic changes can be explained as being the result of a light-travel time effect via a tertiary body around the eclipsing pair. The minimum mass of probable tertiary components around AI Cru and

V1898 Cyg were found to be $0.38 M_{\odot}$ and $0.26 M_{\odot}$, respectively.

In order to better understand the nature of such systems, more minima times and data accumulated over longer years are needed.

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Determination of hepatoprotective and antioxidant role of thyme (*Origanum onites* L.) infusion against ethyl alcohol induced oxidative stress in rats

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ABSTRACT

In this study, we aimed to determine the hepatoprotective and antioxidants role of thyme (*O. onites*) against ethyl alcohol (EtOH) induced oxidative stress in rats. The experiment conducted as control, 20% EtOH, 3% *O. onites* infusion and 3% *O. onites* + 20% EtOH groups. The antioxidant and hepatoprotective role of *O. onites* infusion was evaluated by histopathological change and serum damage biomarkers of liver (LDB), antioxidant constituents (ACs) and malondialdehyde (MDA). According to results, Liver damage serum enzymes activities were significantly higher in 20% EtOH group compared to normal control (NC) whereas the parameters were significantly lower in the infusion supplemented groups compared to 20% EtOH group. Also, MDA content of 20% EtOH group increased significantly in all tissues compared to NC group but decreased as comparison with 20% EtOH. The results suggest that the infusion restored the most of the parameters towards the NC with fluctuations in the ACs.

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1. Introduction

Today, humankind have been using the plants, herbs, and ethnobotanicals from the early stages and are still used everywhere around the world for the treatment of diseases and health improvement. Today's modern medicine is established on plant and natural sources and they take a large part in today's commercial drug development and manufacture. Nearly 25% of the prescribed medications around the world are obtained from plants. Even so herbs are commonly used in health care instead of medications. In addition, herbs are used in the treatment of acute and chronic diseases and problems like depression, prostate problems, heart diseases, inflammation and improving the immune system, and a lot of other conditions [1].

There is a copious amount of various compounds in plants a lot of these compounds are secondary metabolites including aromatic compounds and majority of them are phenols and oxygen-substituted derivatives like tannins and a lot of these compounds have antioxidant capacity [2, 3]. In drug development and pharmacological research ethnobotanicals are significant not just when plant components are directly used as therapeutic agents but also during drug synthesis as a starting material or as models for pharmacologically active compounds [4]. Also, plants and herbs can be handled and taken in various form and ways and these comprise teas, essential oil, whole herb, syrup, salves, ointment, rubs, capsules and tablets having powdered or ground form of the raw herb or its dried extract [1]. Natural antioxidants are thought of to be helpful agents for the hindrance of diseases. Many investigations have stated

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that the phenolic constituents in the essential oil of plants have antioxidant activity as a result of their ability to scavenge free radicals. In addition, it has been shown that there is a high amount of phenolic constituents in the essential oil of various species of *Origanum* [5].

In the last two decades years, many *Origanum* species are characterized by different main components. The contents of essential oil of oregano include carvacrol and/or thymol as the major constituents [6-17] and other minor components such as p-cymene, c-terpinene, terpinen-4-ol, linalool, sabinene hydrate, and terpinen-4-ol [15]. Also, in a current study, b-bisabolene, eucalyptol, borneol and other three of the presently unknown constituents from minor compounds are pointed out as aroma impact factors of *O. onites*. Carvacrol and thymol are the main factors of the biological activity of oregano and carvacrol has a lot of pharmacological activities [5]. On the other hands, it have reported that carvacrol has a lot of biological effects containing lipid peroxidase inhibition, antifungal, white blood cell macrophage stimulant, antibacterial, acetylcholine esterase inhibition, cardiac depressant activity and radical scavenging effect and the preservation of food due to their antioxidant properties [18].

In this study, we determined the healing effects of *O. onites* infusion against EtOH-induced hepatotoxicity and oxidative stress in rats evaluated by histopathological change, LDB, ACs and MDA. With this aim, *O. onites* infusion was given orally as beverage because the plants are well characterized in the healing effects.

2. Materials and Methods

2.1. Animals

Twenty eight *Wistar albino* female rats aged 3-4 months and weighing between 150-250 gr were provided by the Van Yüzüncü Yıl University Experimental Animal Research Centre. The rats were divided into 4 groups containing 7 rats. The authors decelerate that they have followed EU standards regulations for the protection of animals during experiment and approved by The Local Ethics Committee of Experimental Animal with the protocol number 2018/4.

2.2. Preparation of Foods

The plant material was provided from medical and aromatic plants garden of Van Yüzüncü Yıl University, Faculty of Agriculture Field Crops Department. Briefly for the *O. onites* infusion, we weighted 3 grams of *O. onites* for each 100 mL of water and then we poured the boiling tap water to the plant and let it brew for 15-20 minutes. After that, the mixture was filtered and putted aside to cool. For the 20% Ethyl alcohol mixture, we measured 20 mL of Ethyl alcohol and added 80 mL of tap water to it and then we shook it gently and putted it

aside. For the *O. onites* + EtOH mixture, we poured 80 mL of boiling tap water to 3 grams of *O. onites* and let it brew for 15-20 minutes. After that, the mixture was filtered and 20 mL of EtOH was added to it and putted aside to cool. All of these mixtures were prepared as brewed daily and freshly and the bottle sterilized as daily against contamination.

2.3. Experimental Design

28 rats used in this study were divided into 4 groups of randomly selected 7 rats in each group:

Group I (Control): Nothing was applied to this group; it was fed only with standard rat feed and water as *ad libitum*.

Group II (EtOH): The group rats were received 20% ethanol and fed with standard rat feed. The ethanol dose was chosen based on the fact that 20% concentration caused oxidative stress orally as *ad libitum* [19,20].

*Group III (%3 *O. onites* infusion)*: This group was given standard rat feed and freshly brewed and chilled 3% *O. onites* infusion as *ad libitum*.

*Group IV (%3 *O. onites* + 20% EtOH)*: This group was given standard feed and a mixture of 3% *O. onites* and 20% EtOH as *ad libitum*.

2.4. Preparation of tissues supernatant and erythrocyte pellets

The rats were anesthetized intra-peritoneally by ketamine (5 mg/ 100 g body weight) at the end of the designated experimental period. The intracardiac blood was taken from their hearts with the help of injectors for the determination of serum biomarkers and biochemical analysis. For tis purpose, a part of the taken blood in the biochemistry tubes and was centrifugated at 4000xg for 15 min at 4 °C. The remaining part used for the preparation erythrocyte pellets put into EDTA tubes. Then, the blood samples were centrifuged at 4000xg for 15 min at 4 °C and then these pellets were washed three times with physiological saline.

After the dissection of the liver and kidney tissues, the tissues were transferred to petri dishes and then the tissues were washed with 0.9% NaCl solution and then the samples were preserved at -78 °C until the time of analysis. After the addition of 50 mM ice-cold KH_2PO_4 (1:5 w/v) solution the tissues were homogenised for 5 min using stainless steel probe homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) Then, the obtained mixture was centrifuged at 7000xg for 15 min. All of these processes were carried out at 4 °C. The obtained erythrocyte pellets and Supernatants were used for the determination of MDA contents and ADS constituents [21-23].

2.5. Biochemical analysis

This is based on TBA reactivity, the tissue and erythrocyte

MDA concentration was determined using the technique reported by Jain et al [24]. The measurement of tissue and erythrocyte reduced glutathione (GSH) concentration was made according to the technique reported by Beutler et al [25]. Glutathione S-transferase (GST) assay was carried on as stated by Mannervik and Guthenberg [26] method based on the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm. Glutathione reductase (GR) assay was carried on as stated by Carlberg and Mannervik [27] method as based on the diminishing in the NADPH absorbance at 340 nm. Glutathione peroxidase (GPx) assay was carried on as stated by Paglia and Valentine [28] method based on that of GPx catalyses the oxidation of GSH. Superoxide dismutase (SOD) assay was made according to McCord and Fridovich method [29] based on the calculation of inhibition percentage of formazan dye formation at 505 nm. Catalase (CAT) assay was made according to Aebi [30] method based on the consumption of H_2O_2 at 37 °C and 240 nm.

2.6. Measurement of serum biomarkers

Serum biomarkers related to liver damage such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) enzyme activities and total protein (TP) and total cholesterol (TC) levels were via commercial kits (DPC; Diagnostic Products Corporation, USA) using autoanalyzer (INTEGRA-800 ROCHE).

2.8. Histopathological examination

Liver tissue samples were taken and fixated in 10% buffered formalin solution. The routine tissue follow-up and the samples were embedded into paraffin blocks. Then, 4 µm-sections were taken using microtome (Leica RM 2135) and stained with hematoxylin-eosin [31]. After, the preparation sections examined under light microscope (Nikon 80i-DS-R12) equipped with a video camera (DXM1200F, Nikon, Tokyo, Japan).

2.9. Analysis of data

Minitab 13 for windows packet program was used for the statistical analysis and all the data were expressed as mean ± standard deviation (SD). For the determination of the differences between means of the experimental groups one-way analysis of variance (ANOVA) statistical test were used and accepting the significance level at $p \leq 0.05$.

3. Results

3.1. Effect of the *O. onites* infusion on food, liquid intake and liver serum biomarkers

At the end of 30 day experiment, liver damage biomarkers in

serum such as AST, ALT activities, TP and TC levels were determined as an indicator of hepatoprotective effects of *O. onites* infusion against EtOH induced oxidative stress in rats. According to the results liver damage serum enzymes activities were significantly higher in EtOH group compared to normal control (NC) whereas the parameters were significantly lower in the infusion supplemented groups compared to EtOH group. As shown in Table 1, the food intake is not change, whereas the fluids consumption of EtOH and alcohol-thyme groups decreased during experimental period compared to that of the control group.

Table 1. Determination of hepatoprotective capacity of *Origanum onites* L. infusion against ethanol exposed rats

Parameters	Groups			
	Group I	Group II	Group III	Group IV
Food intake (g/week)	281±11	310±16	280±24	316±3
Water intake (mL/week)	236±19	121.4±15 ^a	247.6±115	101.9±3 ^a
AST (U/L)	129±7.5	140±12 ^a	108±13 ^a	113±9 ^{a,b}
ALT (U/L)	36±6	45±8.2 ^a	39.6±5	47±6.0 ^a
TP (g/dL)	72±3	64±3.4 ^a	69.9±3	68±3.8 ^a
TC (mg/dL)	69±6	45±5 ^a	48.9±6 ^a	49±5.6 ^a

Each value represents as Mean±SD.

^a: Significantly different from control

^b: Significantly different from EtOH

3.2. Effect of the FE extract on lipid peroxidation and antioxidant defence systems

Antioxidant enzymes that can be considered as an indicator of antioxidant activity of *O. onites* infusion in liver, kidney and erythrocyte tissue samples include CAT, SOD, GPx, GST, GR enzyme activities and GSH and MDA levels were determined. According to the results obtained at the end of the experiment the supplementation of rats with the EtOH and alcohol-thyme mixture infusion cause a shift in the antioxidant defence system constituent, MDA content and liver damage biomarker levels in comparison with the rats of control group (Table 2).

Table 2 Determination of Antioxidant capacity of *Origanum onites* L. infusion against ethanol exposed rats

	Parameters	Groups			
		Group I	Group II	Group III	Group IV
Liver	GSH (mg/g)	7.6±1.2	9.5±0.6 ^a	7.40±0.92	5.6±1.1 ^{a,b}
	MDA (nmol/g)	118.6±2	144.1±9 ^a	85.7±10 ^a	104.4±6 ^{a,b}
	GST (U/g)	0.76±0.1	0.80±0.06	0.722±0.03	0.45±0.07 ^{a,b}
	GPx (U/g)	1.4±0.03	1.51±0.15	1.54±0.14 ^a	1.62±0.14 ^a
	GR (U/g)	0.04±0.001	0.04±0.001	0.03±0.001 ^a	0.02±0.001 ^{a,b}
	SOD (U/g)	2229±28	2233±33	2169±62 ^a	2182±32 ^{a,b}
	CAT (U/g)	118.8±3.6	123.7±7	112.2±10.8	94.2±9 ^{a,b}
Kidney	GSH (mg/g)	5.3±1.02	6.3±0.9	4.6±0.8	3.85±0.7 ^{a,b}
	MDA (nmol/g)	99.5±9.1	124.1±6 ^a	112.4±4.4 ^a	114.7±7.8 ^{a,b}
	GST (U/g)	0.07±0.01	0.07±0.01	0.06±0.001 ^a	0.06±0.001 ^{a,b}
	GPx (U/g)	8.5±0.7	12.4±0.9 ^a	7.98±0.6	10.5±1.7 ^{a,b}
	GR (U/g)	0.1±0.01	0.1±0.001	0.03±0.001 ^a	0.04±0.001 ^{a,b}
	SOD (U/g)	2144±8	2217±25 ^a	2244±7 ^a	2242±8 ^{a,b}
	CAT U/g	62.8±5.9	69.15±8.5	52.76±7.9 ^a	46.4±4.6 ^{a,b}
Erythrocyte	GSH (mg/ml)	0.6±0.04	0.7±0.10 ^a	0.9±0.1 ^a	0.9±0.06 ^{a,b}
	MDA(nmol/ml)	15.±3.7	18.9±2.8	6.8±1.4 ^a	5.5±1 ^{a,b}
	GST (U/ml)	0.05±0.01	0.06±0.01	0.05±0.01	0.05±0.01 ^b
	GPx (U/ml)	0.85±0.09	0.94±0.21	0.68±0.08 ^a	0.89±0.15
	GR (U/ml)	0.01±0.001	0.01±0.001	0.01±0.001 ^a	0.01±0.001 ^{a,b}
	SOD (U/ml)	2294±4.3	2286±6 ^a	2286±4.3 ^a	2287±5.3 ^a
	CAT (U/ml)	105.9±6.2	109.9±8.7	92.01±6.98 ^a	89.61±8.04 ^{a,b}

Each value represents as Mean±SD.

^a: Significantly different from control

^b: Significantly different from EtOH

3. Histopathological Findings

In histopathological examination of the groups rats; microscopically, normal histological appearance of the liver was seen in the NC. Namely, in the group of NC rats, the structures of hepatocytes and portal areas were in normal appearance, and sinusoids in which the hepatocytes formed regular remark cords around the vena centralis and the remark cords were normal (Figure 1). Also, histological appearance of the *O. onites* group rats was similar to the control group. That whereas, almost similar morphological changes were observed in the livers of all rats in the EtOH group. These changes were localized especially in hepatocytes around the portal vein (periportal) of the pins. It was noted that degenerative changes (hydropic degeneration) occurred in the cytoplasm of hepatocytes in these regions. As a result of the degeneration in hepatocytes, the regular remark cord structure of hepatocytes was disrupted and sinusoids were narrowed. It was noted that there was no significant degeneration in hepatocytes around the vena centralist, remark cords and sinusoids had normal appearance (Figure 2). On the other hands, it was observed that morphological changes were very minimal in the alcohol-thyme group, and a view almost similar to the control group was observed (Figure 3).

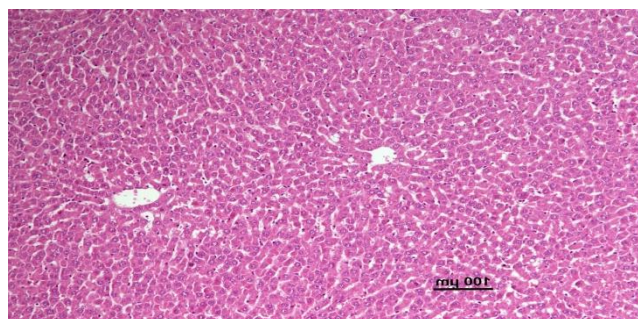


Figure 1 (Control and *O. Onites*) : The normal histological appearance of the liver is observed in the control and *O. onites* group.

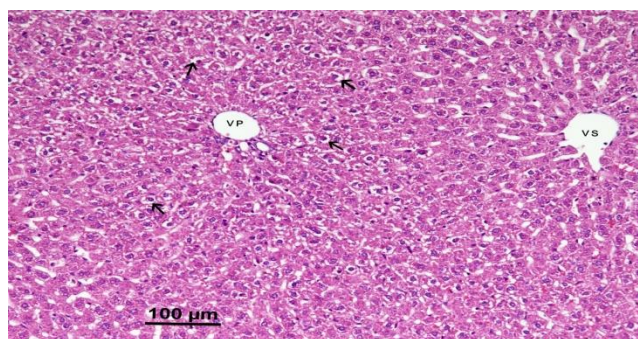


Figure 2 (20% EtOH): Hydropic degeneration is observed in hepatocytes around the portal vein (VP), and the normal appearance of remark cords and sinusoids around the vena centralist (VS).

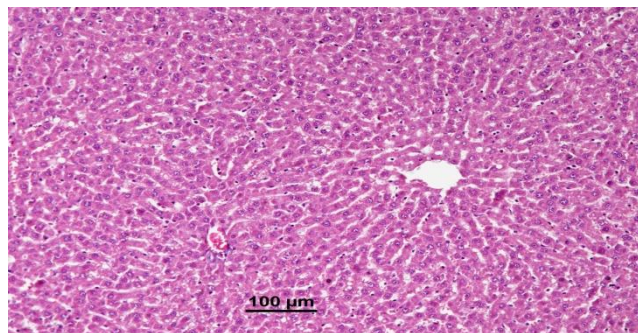


Figure 3 (3% *O. onites* + 20% EtOH): It was observed that morphological changes were very minimal in this group, and a view almost similar to the control group was observed.

4. Discussion

It known that one of the sources of free radicals is EtOH and it is reported that excessive EtOH consumption caused damage to liver tissue and many negative metabolic changes, and these adverse effects varied depending on dose and duration, individual strength, diet, and other factors [32]. In addition the rate of ethanol disposed of through the lungs and kidneys is only 2-10% of the EtOH ingested. The remaining alcohol should be oxidized primarily inside of the organism body liver because it contains the majority of the body's

enzymes capable of EtOH oxidation. The organ specificity to ethanol explain why ethanol metabolism causes such as big imbalances in the liver. So, there has to be attempts to provide protection against these harmful agents and experimental studies have indicated the influence of the functional plant, *O. onites* in this regard. The aim of this study was to investigate whether or not *O. onites* infusion can protect the liver from the toxicity of EtOH, by determination of histopathological changes of liver, serum liver biomarkers level, MDA content and its effectiveness on the antioxidant defence system in rats.

For the first time the results of the present investigation revealed that the treatment of rats with *O. onites* infusion protected the rats from EtOH-induced hepatotoxicity, as is clearly shown by the changes in the levels of serum biomarkers caused by degenerative changes occurred in hepatocytes. In the current study, the rats were feed with EtOH as drinking water for 30 days to induce experimental alcoholises. Hence, the toxicity of ethanol in rats was proved another time. Many studies have revealed the hepatotoxicity of ethanol in a variety of animal species. In addition, EtOH is a peroxidant which is commonly used to produce oxidative stress and experimental liver damage in living organisms [19,20,22,23,33]

As summarized in the table 1 and histopathological image examinations (figure 1-3), the current study, while food intake of the all of groups is not change, the fluids consumption of EtOH and alcohol-thyme groups decreased during experimental period compared to that of the control group. The reason for such an effect is not certain but, it can be speculated that the reason behind a significant the decreased consumptions of the rats to EtOH and the alcohol-thyme mixtures might have altered their drinking behaviour during experiment. Also, there was a significant increase in the AST and ALT levels of EtOH group in comparison with the control group and a significant decrease in the TP and TC content of EtOH group in comparison to control group. The alcohol-thyme mixture group caused a significant decrease in the AST levels in comparison with the EtOH group whereas, a significant increase in the TP and TC content of alcohol-thyme group in comparison with the EtOH group. The reason behind such an effect of EtOH and *O. onites* infusion *in vivo* is not clear to this day. However, the increase in the levels of these enzymes in our study may be the result of changes in the metabolism and detoxification activity of liver as a result of the toxic effects of EtOH. Because, some researchers stated that the liver-derived serum enzyme concentration such as, AST and ALT are both often multiplied in patients with alcoholism [34,35]. It is known that there is a high amount of ALT and AST in hepatocytes and these parameters are sensitive to hepatotoxins and the liver cells damage will discharge their contents such as AST and ALT enzymes into the extracellular space [36]. There is a large amounts of these

enzymes in the liver tissues and it is thought that rather than consumption of EtOH they indicate hepatotoxic damage per se. 39-47% of EtOH-dependent humans has shown elevated AST degrees [37,38]. Moreover, the AST to ALT proportion is seen as a valuable parameter for supporting alcoholic hepatitis diagnosis [39-41]. On the other hand, conflicting results in protein synthesis was seen *in vivo* after chronic administration of ethanol. After a few weeks of administration of a blend of fluid and solid diets containing ethanol a diminished incorporation of labelled amino acids into liver proteins was found in rats nourished the ethanol-containing diets described by researchers [19,42,43]. Furthermore, Morland [19] detected a decrease in the ability of EtOH fed rat liver to incorporate amino acids into protein after stimulation with dexamethasone, and reduced activity of enzymes (tyrosine aminotransferase, tryptophan oxygenase) involved in protein metabolism. All of these results and possibilities may clarify the valuable reduction in the total protein level of EtOH group in contrast to the control group in this study. In addition, one of the vital components that control cellular homeostasis and function are lipids. The liver takes a fundamental part in several stages of lipid synthesis, transportation and lipid digestion system. So, it is sensible that those who have extreme liver dysfunction to have an unusual lipid profile (LP). It is known that patients with hepatic failure and serious hepatitis have a noticeable reduction in plasma triglyceride (TG) and cholesterol levels because of a decrease in lipoprotein biosynthesis. In chronic liver diseases, commonly low levels of cholesterol and TG are observed as a result of diminished liver biosynthesis capacity [44]. In our study, a valuable reduction was shown in the total cholesterol level of EtOH group in contrast to the control group. This may have resulted from the changes in lipid homeostasis and liver degeneration induced by ethanol abuse.

EtOH administration in alcoholises studies may elicit severe injury of hepatic cells and architecture as histopathological findings including necrosis in hepatocytes, inflammatory cell infiltration, extensive vacuolization with disappearance of nuclei and impairment in portal area [45]. In present study, similar findings were detected in the EtOH group rats. There were noteworthy reduced of histopathological damages in alcohol-thyme group. According to these results, it was noted that degenerative changes occurred in hepatocytes in the periportal regions of the alcohol group, whereas in the alcohol-thyme group, a normal appearance was observed in the liver. It is understood that this hepatoprotective effect is related to the effect of antioxidant components such as carvacrol and thymol, which are the main factors of the biological activity of oregano that can be found in thyme infusion. The possible cause of degenerative changes in the alcohol group, especially in hepatocytes in the periportal regions, due to the histological structure of the liver, it is believed that hepatocytes in these

areas are the first to be exposed to toxic components from the digestive tract with vena porta.

As shown in table 2, the current study established that *O. onites* infusion has antioxidant role in rats. This was evident from the changes in the MDA content of alcohol-thyme group in comparison to the EtOH group. According to the obtained results, EtOH significantly increased MDA contents in the liver, kidney and erythrocyte tissues in comparison to those of control group. On the other hand, the *O. onites* infusion supplemented group's significantly decreased MDA contents in all tissues in comparison to those of EtOH group. The reason behind such an effect of EtOH and *O. onites* infusion in vivo is not clear to this day. However, the high MDA content is a critical marker of LP and MDA is one of the major oxidation outcomes of peroxidized polyunsaturated fatty acids [46]. Circulating MDA is one of the foremost commonly and broadly utilized oxidative stress biomarkers [47,48]. In the current study, the reason for the significant increase in MDA content levels in the EtOH group in comparison to other groups is likely because EtOH administration essentially diminished the antioxidant activities of biological systems and resulted in the aggregation of free radicals which along these lines started lipid peroxidation [49] which in turn increased MDA levels. And the higher levels of hepatic lipid peroxidation may be connected to the fact that the larger part of ethanol metabolism happens within the liver and a vital marker of lipid peroxidation is increased MDA content [50]. Moreover, the bunch by Marnett detailed in 2003 that MDA is mutagenic in human cells [51].

Meanwhile, there were significant fluctuations in the GPx, GR, SOD, GST and CAT activities and GSH levels in the EtOH-treated rats. Nevertheless, the effectiveness of *O. onites* infusion against these fluctuations wasn't determined and to this day the reason behind such effect of the supplemented functional plant is not understood. However, the protective activities of enzymatic antioxidants can be affected by oxidative stress resulted from the exposure of organisms to EtOH. The observed increase in the GSH, SOD and GPx activates may be the result of an adaptive response to ethanol-induced lipid peroxide toxicity [19-21, 23]. Also, the increase in GST enzyme may be was due to the induction of an antioxidant adaptation system. An adaptive change against ethanol-induced lipid peroxide toxicity may be the reason for this increased GST activity [20,22,36]. On the other hands, the reasons for such effect of *O. onites* may be due to the contents of essential oil of oregano, carvacrol and/or thymol as the major constituent(s) and other minor components such as p-cymene, c-terpinene, terpinen-4-ol, linalool, sabinene hydrate, and terpinen-4-ol [15]. Since the major constituent of the essential oil of *O. onites* is the phenolic compound carvacrol, although linalool and thymol chemotypes are observed rarely in wild populations, they may have hepatoprotective and antioxidant effect. Furthermore, it have been repoted that the

main component carvacrol has many effect such as cardiac depressant activity, antibacterial, antifungal, lipid peroxidase inhibition, radical scavenging effect, white blood cell macrophage stimulant and acetylcholine esterase inhibition. Carvacrol-rich oils are also recommended for the food preservation due to their antioxidant properties [18].

5. Conclusion

The observations made at the end of our research led us to summarize that the subchronic EtOH administration not only leads to a rise in MDA concentration and causes also a shift fluctuated in the antioxidative defence systems, injuries of hepatic cells and increases liver damage biomarkers. However, the supplementation of thyme infusion miht has been provided protective against EtOH induced liver toxicity and oxidative stress.

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The relationship between nitric oxide and cadmium toxicity in wheat (*Triticum aestivum* L.) seedlings

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ABSTRACT

In this study, biochemical responds against different cadmium concentrations (25 μ M, 50 μ M and 75 μ M) in seedlings belonging to three wheat (*Triticum aestivum* L.) varieties applied to different SNP (25 μ M and 50 μ M) concentrations. As the material of the study, fifteen days old seedlings of wheat (*Triticum aestivum* L.) were used. In all applications carried out to the seedlings, hydroponic method was preferred. The seedlings were divided into three groups in which pretreatment of SNP (sodium nitroprusside) for 48 hours were done. After that, different concentrations of cadmium were applied to these three groups to except controls (pure water and SNPs). In addition, reduced glutathione (GSH) / oxidized glutathione (GSSG) ratio, catalase (CAT) with superoxide glutathione (SOD) activities were detected in the leaves. According to the obtained results, (GSH) / (GSSG) ratio reduced in all three varieties; CAT activity was reduced in Bayraktar and Ikizce, but it was increased in Tosunbey. SOD activity was increased all three varieties. The most prominent responses of SOD enzyme activity in the leaves of wheat seedlings were determined in Tosunbey wheats. When the results are evaluated, generally, 50 μ M of SNP pre-application was found as more successful than 25 μ M of SNP application in terms of attenuating Cd toxicity. SNP was found to have a mitigating effect against Cd depending on the dose.

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1. Introduction

Heavy metal uptake negatively affects the quality of the biological activities of plants by creating stress in the plant (Khairy et al., 2016). Intake amounts of heavy metals vary depending on plant species and age. All plants are capable of collecting mineral elements necessary for growth and development from soil and water. Many plants have the ability to accumulate some heavy metals, although their biological functions are not yet known. These heavy metals may be Cd, Cr, Pb, Co, Ag, Se and Hg depending on the plant species. Both tolerable and accumulative upper limits of heavy metals differ in different plant species (Okçu et al., 2009). Plants have

various defense mechanisms that can tolerate the damages of heavy metals such as increasing the amount of antioxidant enzyme activities and antioxidant molecules and repairing cell membranes (Verma and Dubey, 2003; Zacchini et al., 2003; Benavides et al., 2005). Cadmium is a long half-life heavy metal and is commonly found in the environment (Gill et al., 2013). Cadmium is one of the most destructive heavy metals causing stress in plants (Hegedus et al., 2001). They are much more toxic (2-20 times) than other heavy metals. Cadmium is a moving element in soil and can be easily taken by plants (Oktüren Asri et al., 2007). It is known that nitric oxide acts as an antioxidant and antistress agent in plant responses to various biotic and abiotic stresses such as injury, infection, drought, low and high temperature, ultraviolet (UV), ozone in

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plants (Neill et al., 2003). In some plants, NO promotes tissue expansion at low concentrations in leaves and roots, while they exhibit inhibitory effects at high concentrations (Hufton et al., 1996; Leshem and Haramaty, 1996). When NO reacts with active oxygen species (H_2O_2 , OH^\bullet , O_2^\bullet , $ONOO^\bullet$ etc.), it shows either a toxic or protective effect. There are also indirect protective signal transduction pathways between NO and intracellular antioxidant systems such as glutathione, ascorbate, carotenoids and antioxidant enzymes (Beligni and Lamattina, 1999a, b). It has been reported that the application of Cd in citrus rootstocks has increased the amount of superoxide radical and hydrogen peroxide, and it has increased activities of lipoxygenase, superoxide dismutase and catalase (Jouili and El Ferjani, 2004). Nahar et al. (2016), reported that SOD and CAT activities of mung bean seedlings increased with Cd treatment compared to control, and also SNP (Sodium nitroprusside, it is nitric oxide donor) application caused a sustained increase in SOD activities and decrease in CAT activities (Nahar, et al., 2016). It was found that NO plays a protective role against cadmium stress in sunflower leaves (Laspina et al., 2005). It has been found that increased Cd amount caused loss plant weight and decreased of the activity of superoxide dismutase, catalase and peroxidase enzymes in *Oryza sativa* (Hassan et al., 2005).

In this study, the effects of SNP pretreatment on the physiological and biochemical parameters of different Cd concentrations on 3 different varieties of bread wheat (*Triticum aestivum* L. cv Tosunbey, Bayraktar cv. and Ikizce cv.) was investigated.

2. Test Material and Methods

To grow the seedlings of wheat (*Triticum aestivum* L. cv Tosunbey, cv Bayraktar and cv Ikizce that they were obtained from Ministry Of Agriculture And Forest Field Plants Central Research Institute Directorate, Turkey), homogenous seeds were soaked with pure water and kept in the dark for 6 hours at 23-25°C. At the end of this period, wheat seeds were arranged in germination plates and germinated in darkness for 23 days at 23-25 °C. Then, seedlings were added at regular intervals and equal amounts of soil solution in the long day period (16/8) until 15 days in normal daylight. 15 days old seedlings were selected as homogeneous and used as experimental material. 15 days old wheat seedlings were divided into 3 groups containing an equal number of seedlings and SNP was applied from the roots of the seedlings at different concentrations (25 and 50 mM) for 48 hours using the hydroponic method. Each group was hydroponically exposed to different concentrations of CdCl₂ (25, 50 and 75 µM) prepared for 2 days. Reduced glutathione (GSH) / oxidized glutathione (GSSG) ratio, catalase (CAT) and superoxide dismutase (SOD) enzyme analyzes were performed in the seedlings of the different applications groups.

Reduced Glutathione (GSH) and Oxidized Glutathione (GSSG) analyses were performed according to Yilmaz et al., (2009). Briefly, tissues were homogenized with 10mM EDTA and 50 mM NaClO₄, 0.1% H₃PO₄ mixture. For catalase enzyme activity, phosphate buffer with hydrogen peroxide

obtained by adding 30 mM H₂O₂ to the pH 7 phosphate buffer was used to determine the catalase activity. Analysis of catalase enzyme activity was measured based on the method of Aebi, (1984). Superoxide dismutase activity was determined according to the method developed by Mourente et al., (1999). Briefly, containing 0.5 ml of 100 mM potassium phosphate buffer, 0.1 mM EDTA, 200 µl adrenaline, 200 µl xanthine and 50 µl distilled water and 50 µl sample were prepared and the reaction initiated by the addition of 10 µl xanthine oxidase.

All parameters in our study were analyzed 3 times. Accuracy values of the data were tested by means of SPSS 15 software and one-way ANOVA. Differences between the groups were also differentiated at $p \leq 0.05$ significance level.

3. Results

3.1. Glutathione (GSH)/ Oxide Glutathione (GSSG) Levels

Compared to the control group of Reduced Glutathione (GSH) / Oxidized Glutathione (GSSG) ratio in the leaves of Tosunbey seedlings; 25 µM Cd, 25 µM SNP, 25 µM SNP + 25 µM Cd, 25 µM SNP + 75 µM Cd and 50 µM SNP + 75 µM Cd groups decreased by 52.38%, 56.06%, 67.74%, 56.44% and 65.80% respectively. ($P \leq 0.05$). It was determined that Reduced Glutathione (GSH) / Oxidized Glutathione (GSSG) ratio decreased 50 µM Cd, 75 µM Cd, 25 µM SNP + 50 µM Cd, 50 µM SNP, 50 SNP + 25 µM Cd and 50 µM SNP + 50 µM Cd groups compared to Control (Table 1). Although it was not statistically significant ($p > 0.05$) compared to the control group in the leaves of Bayraktar seedlings; in the 25 µM SNP + 25 µM Cd and 50 µM SNP + 50 µM Cd groups, a decrease of 24.72% and 7.79% was detected, respectively ($P \leq 0.05$). Compared to control group, The Reduced Glutathione (GSH) / Oxidized Glutathione (GSSG) ratio was decreased by 25 µM Cd, 50 µM Cd, 75 µM Cd, 25 µM SNP, 25 µM SNP + 50 µM Cd, 25 µM SNP + 75 µM Cd, 50 µM SNP, 50 SNP + 25 µM Cd and 50 µM SNP + 75 µM treatments. These datas were also found to be statistically insignificant ($P > 0.05$).

Table 1. Glutathione and oxide glutathione amounts in application groups

GROUPS	GSH / GSSG		
	TOSUNBEY	BAYRAKTAR	IKIZCE
CONTROL	13,86 ± 1,7	8.33 ± 0,7	13,59 ± 1,6
25 µM SNP	6,09 ± 1,9*	7.05 ± 0,5	9,72 ± 2,1
50 µM SNP	11,54 ± 5,6	7.77 ± 0,3	8,75 ± 1,4*
25 µM Cd	6,61 ± 3*	7.12 ± 0,2	10,78 ± 0,4
50 µM Cd	9,12 ± 1,8	8.08 ± 0,2	10,42 ± 2,5
75 µM Cd	7,11 ± 2,4	8.35 ± 1,5	11,63 ± 0,9
25 µM SNP- 25 µM	4,47 ± 0,6*	6.27 ± 0,9*	7,45 ± 1,4*
25 µM SNP-50 µM	9,28 ± 2,3	7.11 ± 0,9	7.79 ± 1,5*
25 µM SNP- 75 µM	6,03 ± 0,9*	7.01 ± 0,3	9,06 ± 1,9*
50 µM SNP-25 µM	12,25 ± 0,9	7.59 ± 0,5	8,88 ± 0,7*
50 µM SNP-50 µM	10,71 ± 1,3	7.45 ± 0,5 [□]	8,48 ± 0,9*
50 µM SNP-75 µM	4,74 ± 0,7*	7.11 ± 0,2	7,93 ± 1,2*

*: Compared to control □: Intergroup; Important at $p \leq 0.05$ levels. Average of data ± SE (n: 3)

3.2. Catalase (CAT) Activities

It was determined that, compared to the control group, the CAT activity of the leaves of Tosunbey seedlings; 25 μ M Cd, 50 μ M Cd, 75 μ M Cd, 25 μ M SNP, 25 μ M SNP + 25 μ M Cd, 25 μ M SNP + 50 μ M Cd, 50 μ M SNP + 25 μ M Cd, 50 μ M SNP + 75 μ M Cd (respectively 74.02%, 30.19%, 21.76%, 42.63%, 51.17%, 54.48%, 34.05% and 20.44%) caused decrease while 50 μ M SNP + 50 μ M Cd group caused an increase (26.21%) ($P \leq 0.05$). It was revealed that, compared to the control group, CAT activity in the leaves of Bayraktar seedlings, the CAT activity decreased by 25 μ M Cd, 50 μ M Cd, 75 μ M Cd, 25 μ M SNP+25 μ M Cd, 25 μ M SNP+50 μ M Cd, 25 μ M SNP+75 μ M Cd, 50 μ M SNP+50 μ M Cd ve 50 μ M SNP+75 μ M Cd groups (respectively % 14.16, % 26.64, % 29.79, % 10.23, % 23.96, % 29.75, % 26.84 and % 43.28) while 50 μ M SNP+25 μ M Cd group caused an increase (7.75%) ($P > 0.05$). It was found that CAT activity of the leaves of *T. aestivum* L. cv

İkizce seedlings decreased by 25 μ M Cd, 50 μ M Cd, 75 μ M Cd, 25 μ M SNP+25 μ M Cd, 25 μ M SNP+50 μ M Cd, 25 μ M SNP+75 μ M Cd, 50 μ M SNP+50 μ M Cd ve 50 μ M SNP+75 μ M Cd groups (respectively 11.99%, 17.21%, 21.74%, 22.88%, 22.35%, 21.96%, 25.10% and 39.86%) also 50 μ M SNP and it increased by 50 μ M SNP + 25 μ M Cd groups (respectively 7.04% and 28.07%) when compared to the control ($P \leq 0.05$).

Table 2. Catalase activities in application groups

GROUPS	CAT Activities (μ g/g)		
	TOSUNBEY	BAYRAKTAR	İKİZCE
CONTROL	357.63 \pm 1.2	420.03 \pm 5.0	629.73 \pm 5.1
25 μ M SNP	510.09 \pm 0.5*	421.84 \pm 5.9	628.51 \pm 10.4
50 μ M SNP	365.26 \pm 20.9	449.01 \pm 15.6	674.07 \pm 24.9*
25 μ M Cd	622.37 \pm 3.8*	360.51 \pm 15.2*	554.18 \pm 20.9*
50 μ M Cd	465.60 \pm 6.1*	308.08 \pm 3.1*	521.35 \pm 9.3*
75 μ M Cd	435.47 \pm 15.5*	294.87 \pm 6.3*	492.81 \pm 15.1*
25 μ M SNP- 25 μ M Cd	540.63 \pm 0.8*□	377.01 \pm 5.4*	485.61 \pm 4.3*□
25 μ M SNP-50 μ M	552.48 \pm 1.1*□	319.34 \pm 6.1*	488.96 \pm 13.0*
25 μ M SNP- 75 μ M	365.16 \pm 7.3 □	295.03 \pm 7.2*	491.38 \pm 9.2*
50 μ M SNP-25 μ M	479.43 \pm 7.8*□	452.59 \pm 2.1*□	452.94 \pm 16.4*□
50 μ M SNP-50 μ M	451.38 \pm 29.3*	307.26 \pm 0.8*	471.66 \pm 20.1*□
50 μ M SNP-75 μ M	284.50 \pm 4.7*□	238.22 \pm 26.7*□	378.67 \pm 3.2*□

*: Compared to control □: Intergroup; Important at $p \leq 0.05$ levels. Average of data \pm SE (n: 3)

3.3. Superoxide Dismutase (SOD) Activities

It was observed that SOD activity in the leaves of Tosunbey seedlings compared to control; 50 μ M Cd, 75 μ M Cd, 25 μ M SNP + 25 μ M Cd, 25 μ M SNP + 50 μ M Cd, 25 μ M SNP + 75 μ M Cd, 50 μ M SNP + 50 μ M Cd and 50 μ M SNP + 75 μ M Cd respectively ; 20.94%, 32.93%, 29.90%, 15.01%, 22.39%, 20.94% and 19.49% increase; A decrease of 17.52% was detected in the 50 μ M SNP treatment group ($P \leq 0.05$).

Compared to the control group, SOD activities in the leaves of Bayraktar seedlings; it was observed an increase in the 25 μ M Cd, 50 μ M Cd, 75 μ M Cd, 25 μ M SNP, 25 μ M SNP + 25 μ M Cd, 25 μ M SNP + 50 μ M Cd, 25 μ M SNP + 75 μ M Cd, 50 μ M SNP, 50 μ M

SNP + 25 μ M Cd, 50 μ M SNP + 50 μ M Cd and 50 μ M SNP + 75 μ M Cd treatments, but these increases were not found statistically significant ($p > 0.05$).

SOD activity in the leaves of İkizce seedlings when compared to control group it was determined that, increased ratio of 7.07% in 50 μ M SNP + 50 μ M Cd group ($P \leq 0.05$). Also, an increase was found in the 25 μ M Cd, 50 μ M Cd, 75 μ M Cd, 25 μ M SNP, 25 μ M SNP + 25 μ M Cd, 25 μ M SNP + 50 μ M Cd, 25 μ M SNP + 75 μ M Cd, 50 μ M SNP, 50 μ M SNP + 25 μ M Cd and 50 μ M SNP + 75 μ M Cd groups, but it was not found statistically significant ($P > 0.05$).

Table 3. Superoxide Dismutase activities in application groups

GROUPS	CAT Activities (μ g/g)		
	TOSUNBEY	BAYRAKTAR	İKİZCE
CONTROL	7.59 \pm 0.7	12.01 \pm 0.1	14.71 \pm 0.3
25 μ M SNP	8.2 \pm 0.2	12.27 \pm 1.1	14.64 \pm 0.5
50 μ M SNP	6.268 \pm 0.3*	12.24 \pm 0.2	14.92 \pm 0.3
25 μ M Cd	7.71 \pm 0.1	12.19 \pm 0.1	15.19 \pm 0.2
50 μ M Cd	9.18 \pm 0.3*	12.15 \pm 0.1*	15.08 \pm 0.3
75 μ M Cd	10.09 \pm 1.3*	12.39 \pm 0.2	14.71 \pm 0.2
25 μ M SNP- 25 μ M	9.86 \pm 0.3*□	11.98 \pm 0.1	15.43 \pm 0.3□
25 μ M SNP-50 μ M	8.73 \pm 0.2*	12.25 \pm 0.1	15.16 \pm 0.9
25 μ M SNP- 75 μ M	9.29 \pm 0.4*	12.35 \pm 0.3	15.05 \pm 0.3
50 μ M SNP-25 μ M	8.62 \pm 0.2	12.25 \pm 0.1	14.88 \pm 0.3
50 μ M SNP-50 μ M	9.18 \pm 0.1*	12.43 \pm 0.3	15.73 \pm 0.3*
50 μ M SNP-75 μ M	9.07 \pm 0.7*	12.05 \pm 0.1	15.15 \pm 0.1

*: Compared to control □: Intergroup; Important at $p \leq 0.05$ levels. Average of data \pm SE (n: 3)

4. Discussion

It has been determined that the most obvious reactions of the wheat seedlings in terms of GSH / GSSG ratios are given by İkizce wheat. There is information in the literature that GSH / GSSG ratios are significantly decreased in seedlings without SNP pre-treatment (Romero-Puertas et al., 2007) and SNP pre-applied (Nahar et al., 2016). In this regard, the effort of SNP pretreatment to alleviate the destructive effect of CD was found to be significant at 50 μ M compared to 25 μ M. GR, a glutathione reductase enzyme, converts oxidized glutathione (GSSG) to reduced glutathione (GSH) through a reaction related to NADPH⁺. Reduced glutathione is an important non-enzymatic antioxidant that plays a role in defending against oxidative stress. GSH and GR form the compounds of ascorbate-glutathione metabolism, which plays a role in responding to stress in plants (Kaya and Doğanlar, 2016).

It has been determined that the most prominent responses in the leaves of wheat seedlings in terms of CAT enzyme activity are given by İkizce wheat. Compared to control seedlings, seedlings without SNP pre-application (Hassan et al., 2005; Kotapati et al., 2016; Nahar et al., 2016; Liang et al., 2018) and SNP pre-applied (Kaya and Ashraf, 2015) caused decreased CAT enzyme activity. The effort of SNP pretreatment to alleviate the destructive effect of CD was found significant at 50

μM compared to 25 μM . Similar results were seen in Bayraktar seedlings. It was determined that without SNP pre-application (Chaudhary and Sharma, 2009; Jouili and El Ferjani, 2004), or SNP pre-applied (Nahar et al., 2016; Ali et al., 2017; Amooaghaie et al., 2017) in Tosunbey seedlings an increase CAT activity compared to the control group, and also in the Tosunbey seedlings an increase CAT activity in 25 μM SNP, and 50 μM SNP in İlkizce seedlings were observed. Catalase is found in organelles called peroxidase in all cells of plants and plays a protective role by keeping H_2O_2 level at a certain level for the cell. Catalase enzyme detoxifies H_2O_2 in high concentrations and provides the plant to get rid of stress with minimum damage. Increased activity of antioxidant enzymes such as CAT is as a result of the detoxification mechanism that enables lipid peroxidation to be reduced (Santos and Silva, 2015). It was determined that the most prominent responses of the wheat seedlings in terms of SOD enzyme activities were given by Tosunbey wheat. SOD enzyme activities increased in both seedlings, without SNP pre-application (Jouili and El Ferjani, 2004) and SNP pre-applied (Nahar et al., 2016; Liang et al., 2018) compared to control seedlings. Similar results were observed in Bayraktar and İlkizce wheat. The effort of SNP pretreatment in Tosunbey seedlings to alleviate the destructive effect of Cd was found significant at 25 μM compared to 50 μM . 50 μM SNP application was found to be significant in Tosunbey seedlings compared to control. The antioxidant system plays an important role in protecting cell compounds from the damage of reactive oxygen species produced under stress. The increase in the production and accumulation of reactive oxygen species in plant cells under optimal growth conditions brings disruption of cellular homeostasis (Wang et al., 2015). Increasing SOD activity under stress conditions shows that superoxide radical reactive oxygen species are produced more. Because SOD plays a role in removing the superoxide radical from chloroplasts and converting it into H_2O_2 (Santos and Silva, 2015).

5. Conclusion

In this study, we observed that Cd, which is not a necessary element for plants, is toxic for wheat plants even at very low concentrations. It was found that SNP had a mitigating effect against Cd depending on the dose. Results showed that; SNP can regulate the oxidative stress caused by Cd on certain parameters to a limited extent. This state is depending on the chosen concentrations, planned application and duration. However, we believe that the different studies to be carried out will contribute to the fully understanding of the subject since the studies on this subject are limited and inadequate.

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Augmented reality practices in health services: Literature review

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ABSTRACT

Augmented reality (AR) is a version of virtual reality (VR) technology, and digital three-dimensional interfaces and virtual objects appear before us as a dynamic and innovative technology allowing simultaneous interaction with the real world. Today, we encounter wearable technologies as the widespread use of augmented reality technologies. Along with the rapid proliferation of mobile devices and wireless network technologies, innovative technologies such as augmented reality (AR) and virtual reality (VR) have also become useable in each domain. The development of health technologies also in the same pace brings along the opportunities for using such technologies in the domain of health. In the present study, literature review on AR practices used in the domain of health was made, and the data was analyzed.

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1. Introduction

Milgram and Kishino (1994) had defined augmented reality as “the environment of reality in which the products of digital media are being used instead of the objects of the real world”. Augmented reality (AR) is a version of virtual reality (VR) technology, and digital three-dimensional interfaces and virtual objects appear before us as a dynamic and innovative technology allowing simultaneous interaction with the real world (Azuma, 1997; Sommerauer and Müller, 2014). When the definitions in literature are reviewed, augmented reality may be defined as real worlds that are enriched with the use of virtual objects. According to this, augmented reality is an environment that consists of real and virtual objects formed by virtual objects placed in the real-world environment with

experiential purposes. Today, we encounter wearable technologies as the widespread use of augmented reality technologies. In this manner, the co-occurrence of virtual objects and real beings ensures the increase of sense of reality in the users of augmented reality (Bokyung, 2008). Along with the rapid proliferation of mobile devices and wireless network technologies, innovative technologies such as augmented reality (AR) and virtual reality (VR) have also become useable in each domain. The development of health technologies also in the same pace brings along the opportunities for using such technologies in the domain of health.

When AR practices’ usage area in the domain of health is considered, they are being used in order to easily determine the point of interference in surgical operations, and in order to minimize the risk of infection by making smaller cuts on the patient. In the domain of neurosurgery, it was observed that it

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is being used for accessing the area of tumor, decreasing the size of cut, or displaying three dimensionally the construct of veins in brain in cases of aneurysm. And in the domain of physiotherapy and rehabilitation, it is encountered that it is being used both on adults and children with the purpose of having the patients perform the given movements in a correct manner along with the creation of the perception of reality. Lastly, the domain that is frequently encountered is education. In medical education, especially in the domain of anatomy, it is observed that AR practices are being used, and thus that it is very useful in cases where training on cadaver cannot be made, or where the students want to study a subject again. In addition, we are also observing that it started to be used on the education of health habits such as getting the children adopting the habit of brushing their teeth.

1.1. The purpose of the research

The purpose of the present study is to determine AR practices' usage area in the domain of health. For this purpose, an extensive literature review was performed. Moreover, it is being considered to also reveal information on the states of the researchers performing such researches, the change of researches as per years, and the magazines where they were published. Along with the determination of the subjects on which it was concentrated the most in this field, it was intended to reveal the results that will shed light on the future researches.

2. Method

For an extensive literature review, EDS search engine, enabling access to all the subscribed databases from a single access point by the provider service of "EBSCO Discovery Services", was directly accessed through the website of the Library and Documentation Directorate of University of Bezmialem Foundation. The key words of "augmented reality" and "healthcare" were written in the search section, and in the content providers section, Complementary Index, Scopus®, IEEE Xplore Digital Library, Newspaper Source Plus, Academic Search Complete, Science Citation Index, Regional Business News, Business Source Complete, MasterFILE Complete, Networked Digital Library of Theses & Dissertations, MEDLINE, MEDLINE Complete, General OneFile, Supplemental Index, Directory of Open Access Journals, ScienceDirect, Social Sciences Citation Index, Newswires, CINAHL Complete, arXiv, ERIC, Library & Information Science Source, Library, Information Science & Technology Abstracts, Food Science Source, Journals@OVID, Arts & Humanities Citation Index, Teacher Reference Center, DergiPark, China Science & Technology Journal Database, Emerald Insight, National Database of ULAKBİM (Turkish Academic Network and Information Center), Dentistry & Oral Sciences Source, eBook Academic Collection Trial, JSTOR Journals, GreenFILE, Research Starters, Books at JSTOR, Dynamed, Bibliotheksverbund Bayern, eBook Index, Google Scholar, and ProQuest Dissertation and Thesis database were selected.

The conformity to key words of the listed articles was examined again, and 46 publications in total were obtained. Each of the reviewed publications were listed with the used of MS Excel program in the direction of the intended objectives, and the obtained data was analyzed.

3. Findings

The distribution of the authors as per the states is listed as seen in Graph 1. According to the analysis performed, it is being observed that most of the researches on AR practices used in health services are originating from USA [16,19,22,42,44,45,]. And then Canada is following USA in terms of researches performed on the same [18,20,24,43].

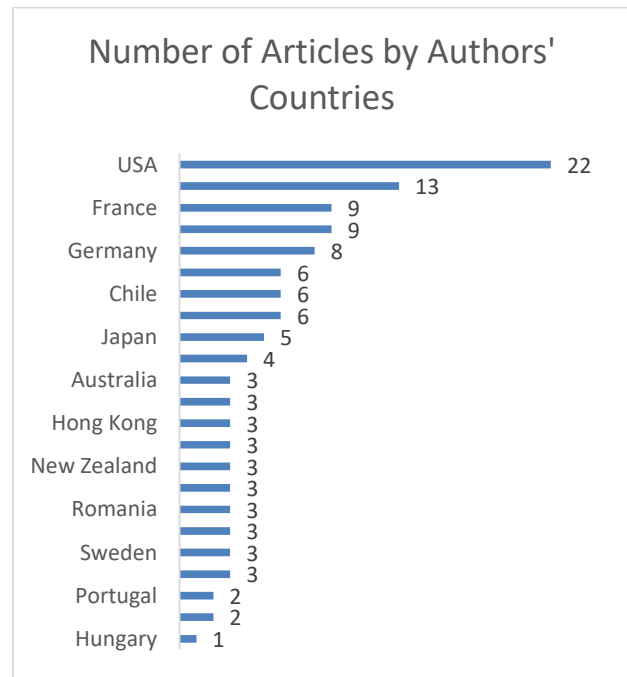


Figure 1. Distribution as per states of AR studies performed in the domain of health

And when the research methods used in the studies were examined, it was observed that literature review [8,9,10,13,14,19,33,34,36,40], compilation and AR practices applied on surgical operations were published at a high degree [17,18,20,24,26,35,44]. (Graph 2)



Figure 2. Methods used in the publications on AR practices used in the domain of health

When the fields of application of AR, used in trainings provided in the domain of health, were examined, it is being observed by Graph 3 that it was used the most in the medical education [5,7,8,11,14,22,36,40,43].

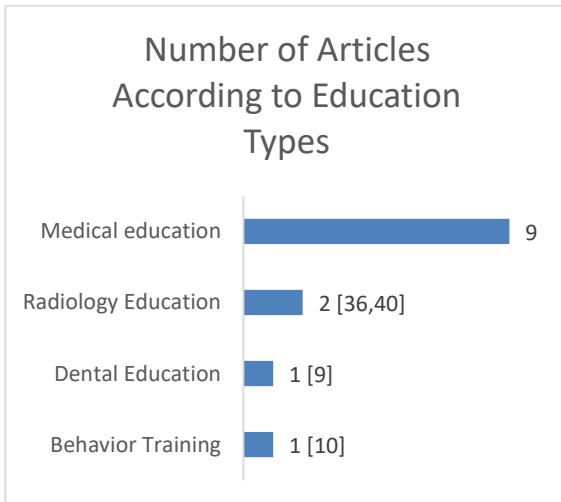


Figure 3. Types of health education provided by AR practices

When articles on AR practices used in surgical sciences were examined, it was observed that the highest number of publications was on the domain of General Surgery as clinical domain [13,17,20,44].

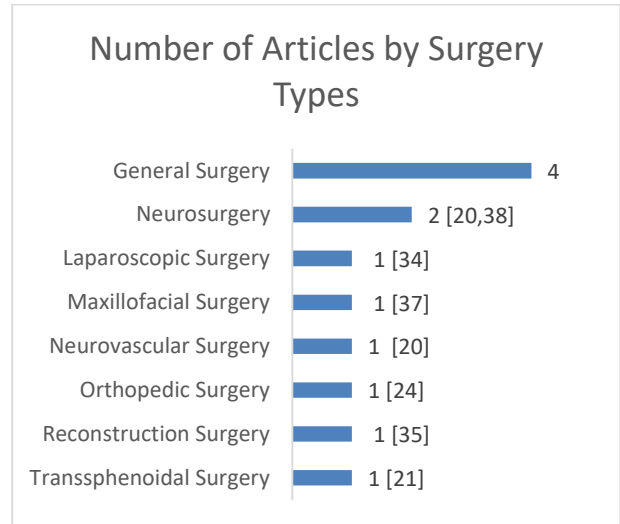


Figure 4. Use of AR practices in surgical branches

And when the key words of the publications were analyzed, it is being observed by Graph 5 that the most frequently used key word was “Augmented Reality” [5,7,8,9,11,13,14,15,16,17,19,20,21,22,23,26,27,29,31,33,34,35,37,38,39,41,42,44,45,46], and the second one was “Virtual Reality” [8,9,13,14,16,25,27,37,41].

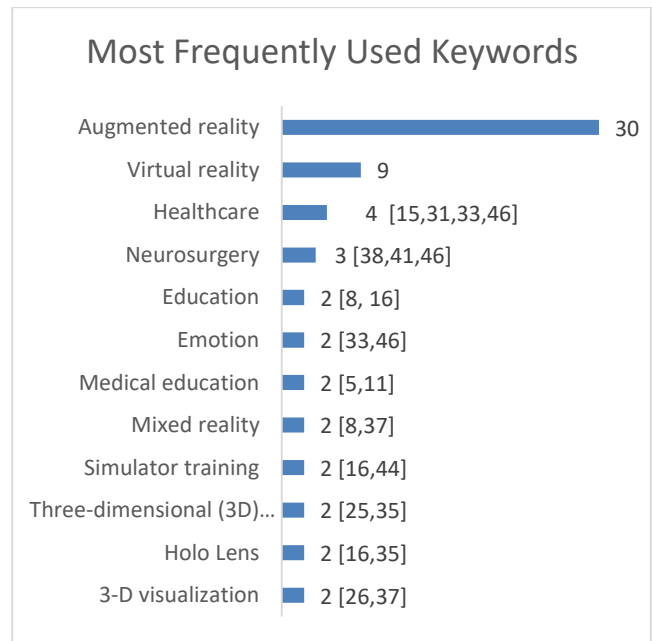


Figure 5. The most frequently used key words

And when number of articles was examined as per years, the highest leap had occurred in 2018 by 20 articles as seen on Graph 6.

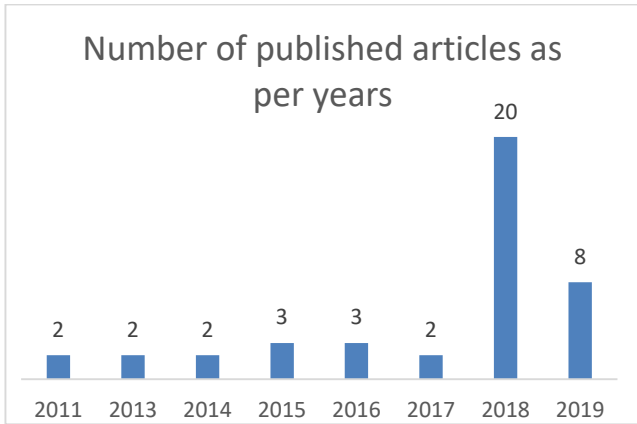


Figure 6. Number of published articles as per years

The following results were obtained by the analysis of journals on which the articles were published

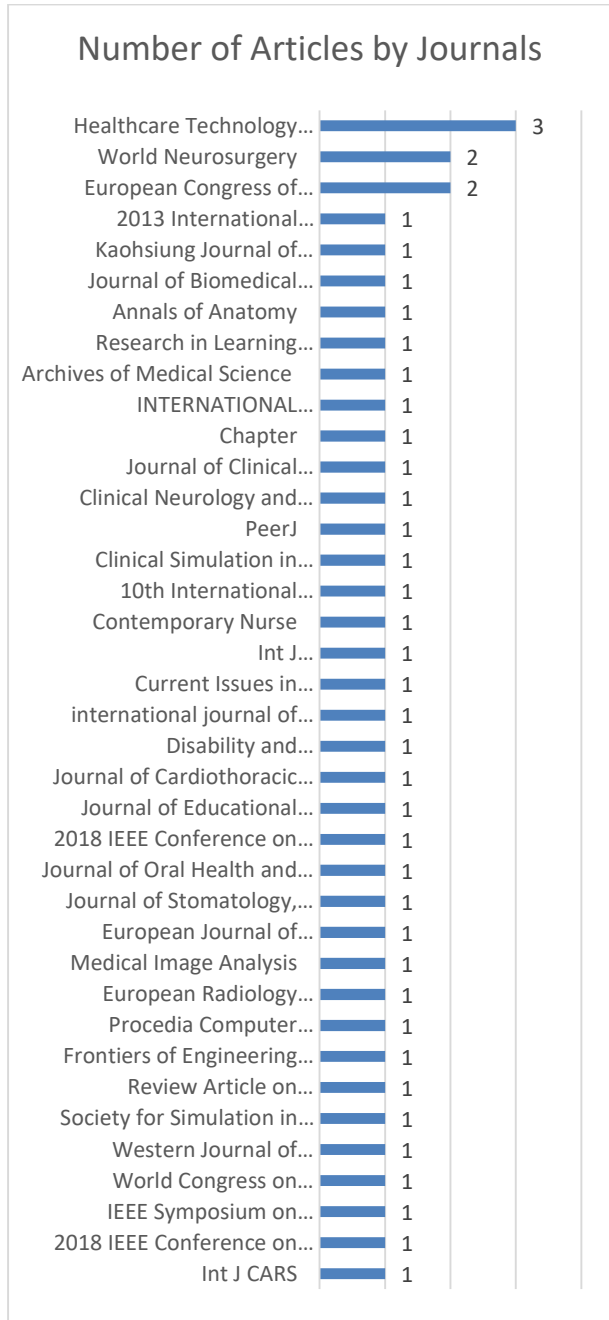


Figure 7. Number of articles by journals

4. Conclusions

According to the obtained results, we observe that AG practices had significantly increased as of year 2018. It will not be incorrect to say that ease of access to the referred services along with the development of AR systems had caused that increase. And along with the increase of malpractice actions in the whole world, it is a reality that the use of practices, that will minimize human error, will increase. It is being anticipated

that this technology will be increasingly used both in the domain of clinics and in the domain of education.

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The effect of different negative parameters on the performance of steel structures

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ABSTRACT

In this study, we present the effect of various negativity parameters on steel structures that cause post-earthquake damage based on performance-based assessment. In accordance with this purpose, eigenvalue and pushover analysis are carried out for different negativity parameters such as number of story, soft story, short column, hill-slope effect and irregularity in plan for a sample steel structure in this study. Structural models were created over the reference building to cover each negativity parameter within the scope of this study. Natural vibration period, base shear force, target displacements for damage estimation and stiffness values are obtained for each structural model separately. The comparisons with the reference building model results are made. The effect to behavior of structures is determined and a reduction coefficient is proposed, for each negativity parameter, respectively. The proposed coefficients can be used to determine the risk priority in steel structures. It was concluded that each negativity parameter considered in this study reduces the behavior of the building under the effect of earthquakes.

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1. Introduction

The failure of the structures due to their insufficient vulnerability is the one of the main reason of significant loss of life and properties after the earthquakes (Hadzima-Nyarko et al., 2017; Pavić et al., 2020; Harirchian et al., 2020). Vulnerability can be defined simply as sensitivity of exposure to earthquake hazard (Coburn and Spence, 2003). Building stocks vulnerability can be expressed in terms damage potential respect of similar structures subjected to a seismic hazard (s) (Işık et al., 2018; Harirchian and Lahmer, 2020). The destructive power of seismic hazard reveals some weaknesses in urban environments (Inel and Meral, 2016; Işık et al., 2017). It is important to anticipate and take appropriate measures to reduce vulnerability and expected losses in order to prepare for management in the event of a potential earthquake (Ademović et al., 2020; Arslan et al., 2015). There are many

structural parameters that can adversely affect the vulnerability of structures under earthquake impact (Işık 2016; Šipoš and Hadzima-Nyarko, 2017). These negative structural parameters are caused by most structural damage and increased the damage amount after devastating earthquake (Yakut, 2004; Sucuoğlu and Yazgan, 2003; Chever, 2012). The main negative building characteristics that increase the amount of damage after earthquakes are included in both seismic design codes and rapid seismic assessment methods such as number of story, soft story, short column, hill-slope effect and irregularity in plan as stated in previous studies. (Harirchian, 2020; Sucuoğlu et al., 2007; Yakut et al., 2007; Ozmen et al., 2014; Tesfamariam and Liu, 2010; Alam and Alam, 2012; Jain et al., 2010; Ozcebe et al., 2003). In these studies, include rapid evaluation methods of structures and suggested a coefficient for the negativities generally found in reinforced-concrete (RC) structures.

Within the scope of this study, number of stories, soft story,

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short column, hill-slope effect and irregularity in plan, which are among the main parameters that cause vulnerability of buildings, are examined in terms of steel structures. Pushover and eigenvalue analyses were performed to determine how these parameters affect the earthquake behavior of the structures considering the structural models created for each negativity parameter. Natural vibration periods were obtained based on eigenvalue analysis. The base shear force, target displacements for damage estimation and stiffness values were obtained by using pushover analysis. A reduction coefficient is proposed for each negativity parameter considered in this study by using the values obtained from these two analyses. These reduction coefficients that obtained from this study can be used for the rapid assessment method for steel structures. Information is given about analysis and main parameters that taken into consideration in this study. After giving detailed information about the structural characteristics for sample steel structure, the analysis results are stated.

2. Analysis Types

In this study, Seismostruct, one of the software related to structures, was used (SeismoSoft, 2018). Two different analyses were carried out such as eigenvalue and pushover analysis.

2.1. Eigenvalue Analysis

Eigenvalue analyzes were performed for the building models created for each variable used in this study. Mode shapes and natural frequency for any structure can be obtained by eigenvalue analysis. Material properties always remain constant during the calculation (Antoniou and Pinho 2003; Kutanis et al., 2017; Aksoylu and Arslan, 2019). Briefly, it can be evaluated as pure elastic structural analysis. The cross section can be expressed by material cross-sectional properties such as torsion constant, moment of inertia, modulus of elasticity and stiffness modules (Lou et al., 2017; Nikoo et al., 2017; Zuo and Zha, 2018). Structure related modal period, frequency, modal participation factors, effective modal masses and their percentages can be calculated by eigenvalue analysis.

2.2. Pushover Analysis

Pushover analysis is one of the practical and effective methods to determine seismic capacities of structures in the design and evaluation process (Hsiao et al., 2015; Estêvão and Oliveira 2015; Krawinkler and Seneviratna, 1998). This analysis method was developed to understand the nonlinear behaviour characteristics and performance of structures under horizontal loads (Chopra and Goel, 2002; Antoniou and Pinho, 2004; Elnashai, 2001). Many data can be obtained such as the base shear capacity, displacement ductility of the structure, the damages in the structural system elements and the distribution of forces in the structure after damage by pushover analysis (Aydinolglu, 2003; Gupta and Kunnath, 2000; Papanikolaou

and Elnashai, 2005; Bracci et al., 1997). The capacity curve representing the relationship between the base shear force and peak displacement is also obtained from the pushover analysis. To obtain this curve, the lateral forces are increased monolithically until the displacement of the top of the building reaches a predetermined displacement value (Inel and Ozmen, 2006; Antoniou and Pinho, 2004; Işık and Kutanis, 2015; Jalayer et al., 2015). Typical pushover curve was given in Figure 1.

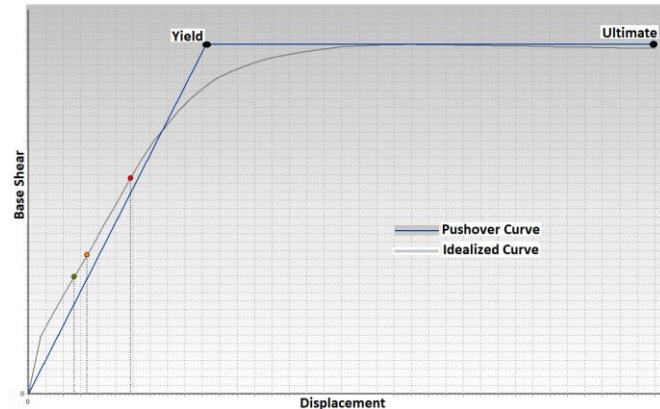


Figure 1. Typical pushover and idealized capacity curves

3. Description of Sample Steel Structure and Modelling

In this study, ZA local ground type that given in Eurocode-8 (Eurocode-8, 2005) was chosen as the local soil class for all structural models. The characteristics of this soil type (ZA) are given in Table 1.

Table 1. Local ground type ZA (Eurocode-8 2005)

Ground Type	Description of stratigraphic profile	Parameters		
		(VS) ₃₀ [m/s]	NSPT [blows /30cm]	(cu) ₃₀ [kPa]
ZA	Rock or other rock-like geological like geological formation, including at most 5 m of weaker material at the surface.	>800	----	----

Material models have an important place in seismic analysis of the buildings (Işık and Özdemir, 2017). Calculations were made for steel model selected as Menegotto-Pinto steel model (stl_mp) (Menegotto, 1973) in this study. The stress-strain relationship of the material model is demonstrated in Figure 2.

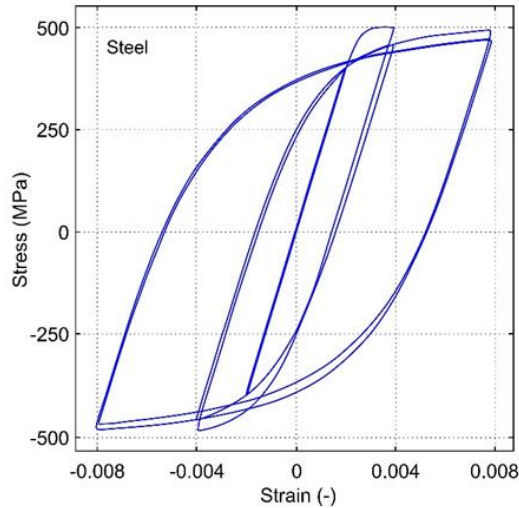


Figure 2. Material models for concrete and steel considered in the study

The cross-sectional representation and the dimensions of the selected profile are shown in Figure 3.

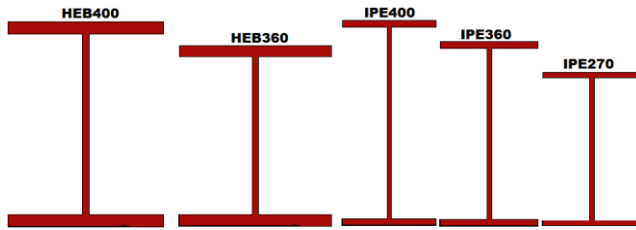


Figure 3. Cross sections of the steel profiles

The cross-section dimensions of profiles that used in this study are given in Table 2.

Table 2. Cross section dimensions of the profiles

Profile	HEB 360	HEB 400	IPE 270	IPE 360	IPE 400
Bottom Flange Width (m)	0.3000	0.3000	0.1350	0.1700	0.1800
Bottom Flange Thickness (m)	0.0225	0.0240	0.0102	0.0127	0.0135
Top Flange Width (m)	0.3000	0.3000	0.1350	0.1700	0.1800
Top Flange Thickness (m)	0.0225	0.0240	0.0102	0.0127	0.0135
Web height (m)	0.3150	0.3520	0.2496	0.3346	0.3730
Web Thickness (m)	0.0125	0.0135	0.0066	0.008	0.0086

The floor plan of the steel structure considered in the study is shown in Figure 4.

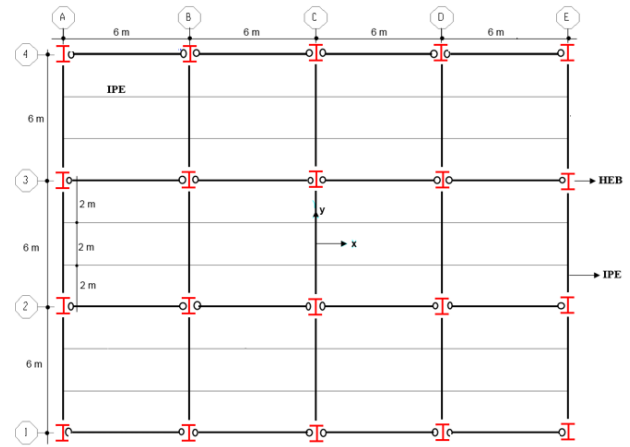


Figure 4. The plan of the sample steel structure

The 2D model of an exemplary axis of the steel structure considered in the study is given in Figure 5.

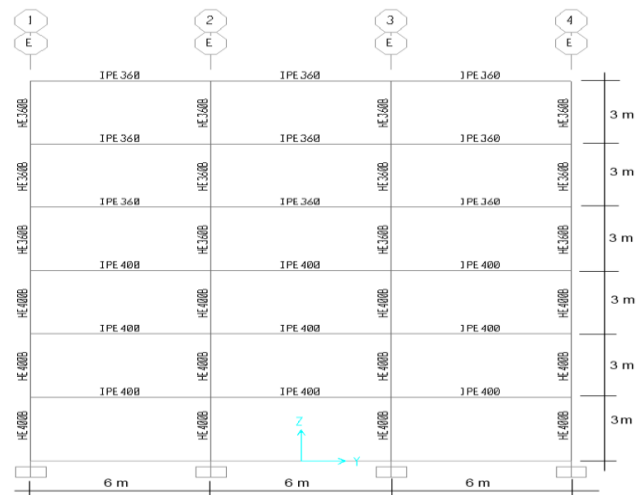


Figure 5. 2D model of the sample steel structure

The three-dimensional model obtained in the software for the structure and the loads that were applied are given in Figure 6. The loading condition is taken as a constant for all building models.

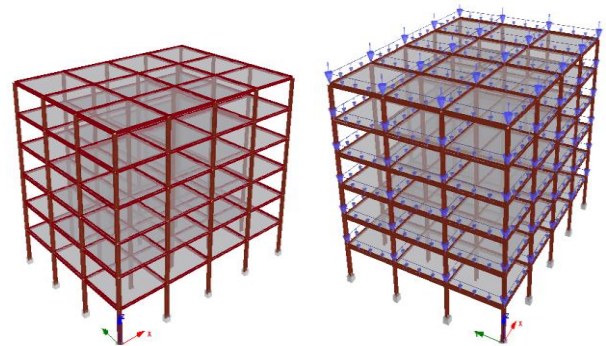


Figure 6. The 3D model and applied loads of the sample steel structure

In performance-based Earthquake Engineering, determining target displacements is essential for damage estimation when definite performance limits of structural elements are reached. The limit states are given in Eurocode-8 (Part 3) (Eurocode, 2005; Pinto et al, 2011) were taken into account for damage assessment used universal in the structural analysis. The damage assessment of limit states are presented in Table 3, along with Eurocode-8. These values were computed for all building models.

Table 3. Limit states in Eurocode-8 (Part 3)

Limit State	Description	Return Period (year)	Probability of exceedance (in 50 years)
Limit state of damage limitation (DL)	Only lightly damaged, damage to non-structural components economically repairable	225	0.20
Limit state of significant damage (SD)	Significantly damaged, some residual strength and stiffness, non-structural components damaged, uneconomic to repair	475	0.10
Limit state of near collapse (NC)	Heavily damaged, very low residual strength & stiffness, large permanent drift but still standing	2475	0.02

Base shear forces for each structural model were calculated separately for both directions. The displacement at the moment of yield (d_y), elastic stiffness (K_{elas}) and effective stiffness (K_{eff}) values were also calculated separately for all structural models.

4. Main Negative Structural Parameters and Analysis Results

Some properties of the structures closely affect the earthquake damages that occur. In this study, five main vulnerabilities that cause earthquake damages have been taken into consideration. The number of stories, soft story, short column, hill- slope effect and irregularity in the plan were chosen as variables in structural analysis in this study. The specified analyzes for each variable were made separately. The comparisons are made between reference model and building model with negativity.

4.1. Number of Stories

One of the factors causing structural damage is the total number of stories of the building. It is revealed that there is an almost linear relationship between the number of studies and building damage in buildings in previous observations and studies (Şengezer, 1999) Analyzes were carried out by selecting three different numbers of stories for this parameter. Story heights are taken as 3m and equal in all stories in all structural models. Only the number of stories is taken as variable. Two-dimensional building models are shown in Figure 7.

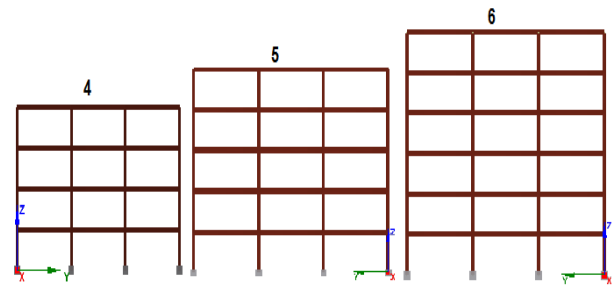


Figure 7. 2D models obtained for number of stories

Three-dimensional models obtained from the software of the selected structures, 4-story, 5-story and 6-story, are given in Figure 8.

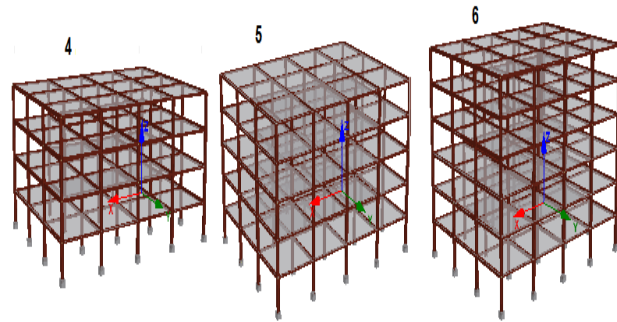


Figure 8. 3D models of the building models

Comparison of pushover analysis curves obtained for different story number in both direction is given in Figure 9.

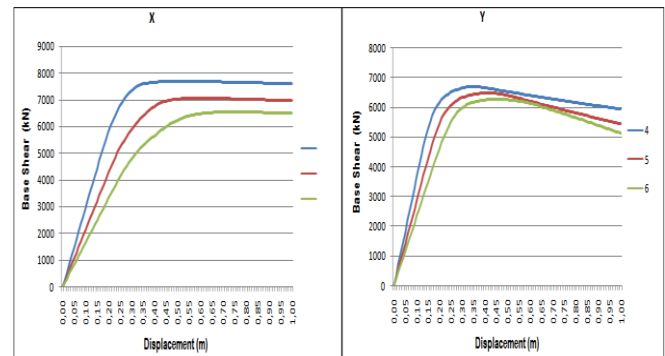


Figure 9. Comparison of pushover curves obtained for story change

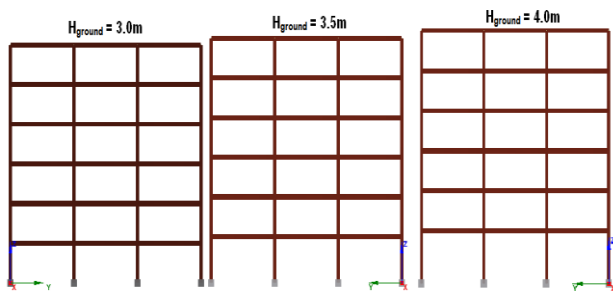
The comparisons of eigenvalue and pushover analysis results are given in Table 4.

Table 4. Comparison of the values obtained for story change

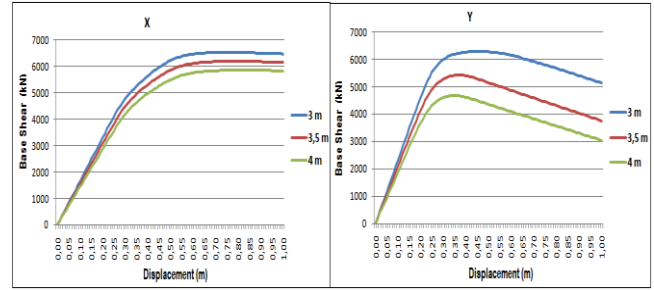
Story Number	Direction	Period (s)	Base Shear (kN)	K _{elas} (kN/m)	K-eff (kN/m)	d _y (m)	DL (m)	SD (m)	NC (m)
6	X	0.406	6538	16485	14992	0.436	0.022	0.029	0.045
5	X	0.326	7052	21586	18224	0.355	0.014	0.017	0.031
4	X	0.250	7683	33141	27913	0.275	0.00734	0.00942	0.01633
6	Y	0.40579	6272.83	23248.5	22247.5	0.282	0.01591	0.02041	0.03539
5	Y	0.32620	6489.65	28545.2	26906.8	0.241	0.01069	0.01371	0.02378
4	Y	0.24991	6695.2	36451.8	33815.2	0.198	0.00650	0.00834	0.01446

4.2. Soft Story

The stiffness and strength of any story in the building is significantly less than the other stories, creates the concept of soft story. Soft story can be occurring due to different characteristics between stories (such as the height of the stories), changing of the structural system, using different floors system or due to the change in the amount of partition walls that are not considered to belong to the structural system (Inel et al., 2011; Tezcan et al., 2007; Jara et al., 2020). Within the scope of this study, a soft story was created with the height of the ground story being higher than the other stories. The ground story height was chosen differently according to other story heights and analyzes were carried out according these structural models. The heights on the other stories remained constant in each model. Apart from this, all parameters that are essential for structural analysis are taken as the same in each model. In order to examine the ground story height change, three different building models have been created by taking the ground story height such as 3m, 3.5m and 4.0m. Two-dimensional models of these structures are given in Figure 10.


Figure 10. 2D models of the different ground story height

Comparison of the curves obtained as a result of pushover analysis in X and Y directions due to changing of ground story height are given in Figure 11.


Figure 11. Comparison of pushover curves obtained for different ground story change

Comparison of analysis results values for changing ground story height is given in Table 5.

Table 5. Comparison of the results for ground story height change

Ground Story Height (m)	Direction	Period (s)	Base Shear (kN)	K _{elas} (kN/m)	K-eff (kN/m)	d _y (m)	DL (m)	SD (m)	NC (m)
3	X	0.40579	6537.71	16485.2	14992.3	0.4361	0.02235	0.02867	0.04970
3.5	X	0.42106	6191.32	15366.2	13977.6	0.443	0.02490	0.03190	0.05529
4	X	0.43730	5870.31	14311.1	12984.5	0.452	0.02770	0.0356	0.06166
3	Y	0.40579	6272.83	23248.5	22247.5	0.282	0.01591	0.02041	0.03539
3.5	Y	0.42106	5421.24	21013.9	20222.7	0.268	0.01860	0.02380	0.04127
4	Y	0.43730	4669.74	20479	18080.3	0.258	0.02190	0.02810	0.04876

4.3. Short Column

Short columns may formed different ways. A column being shorter than the way it was originally designed in the building is defined with the concept of short column (Bal et al., 2008). Short columns may occur either because the original column length-to-depth ratio is small, or due to the presence of an obstacle along a certain height of the column (e.g. low brick masonry wall) in which case the effective length of the column is reduced and equals the column's unflanked length. (Moretti and Tassios, 2013; Chen et al., 2010; Çağatay et al., 2009). While creating the short column, the ground and 1st story height values in the building were chosen to be lower than the other stories. While all story heights are 3m, ground story height is 3.5 m and 2nd story height is 2.5m. The total height of the building has not been changed. Two-dimensional models of these structures are given in Figure 12.

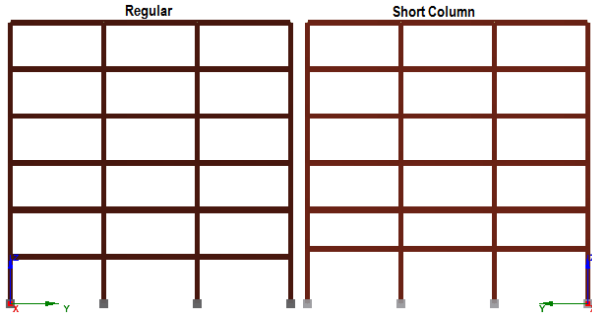


Figure 12 . 2D models obtained for short column effect

Comparison of curves obtained as a result of static pushover analysis in X and Y directions due to short column change are given in Figure 13.

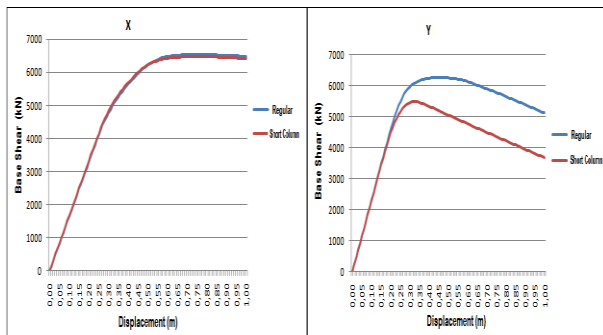


Figure 13. Pushover curves for short column

Comparison of analysis results values for short column is given in Table 6.

Table 6. Comparisons of results for short column

Model	Direction	Period (s)	Base Shear (kN)	K _{elas} (kN/m)	K _{eff} (kN/m)	dy (m)	DL(m)	SD (m)	NC (m)
Reference	X	0.405	6537.7	16485.2	14992.3	0.4361	0.0223	0.0287	0.0497
Short Column	X	0.402	6472.7	16668.3	15239.3	0.425	0.0221	0.0284	0.0493
Reference	Y	0.405	6272.8	23248.5	22247.5	0.282	0.0159	0.0204	0.0354
Short Column	Y	0.402	5489.5	22978.9	22224.6	0.247	0.0164	0.0210	0.0364

4.4. Hill-slope Effect

The fact that the building is prominently on a hill or on a high slope will slightly increase the earthquake effects it will be exposed to or exposed to. For geological and topographic reasons, buildings have to be built under the influence of hill-slope. As a result, level differences occur on the story where the building meets the ground. This situation creates height differences in the structural system elements and causes the formation of short and long columns (Mohammad et al., 2017; Tezcan et al., 2011). The structural models were created by reducing the height values of the columns on the ground story

of the building while considering the hill - slope effect. Two-dimensional building models obtained while considering the hill-slope effect are given in Figure 14.

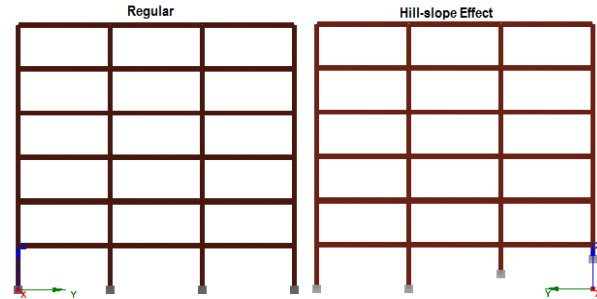


Figure 14. 2D models for hill-slope effect

Comparison of curves obtained as a result of pushover analysis in X and Y directions due to hill-slope effect are given in Figure 15.

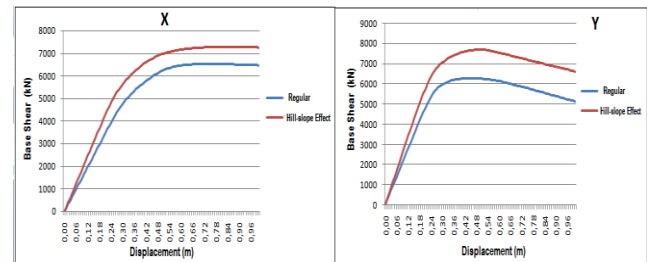


Figure 15. Comparisons of pushover curves for hill-slope effect

The comparison of analyses results for hill-slope effect is given in Table 7.

Table 7. Comparison of result values for the hill-slope effect

Model	Direction	Period (s)	Base Shear (kN)	K _{elas} (kN/m)	K _{eff} (kN/m)	dy (m)	DL (m)	SD(m)	NC (m)
Reference	X	0.405	6537.71	16485.2	14992.31	0.436	0.022	0.0286	0.0497
Hill-slope	X	0.384	7258.32	20476.6	17951.33	0.404	0.016	0.0214	0.0371
Reference	Y	0.405	6272.83	23248.5	22247.51	0.282	0.016	0.0204	0.03539
Hill-slope	Y	0.384	7697.63	28578.5	25952.23	0.297	0.012	0.0151	0.0262

4.5. Irregularity in Plan

Structures can be built with irregularities in the plan instead of simple geometry like a rectangular plan due to various reasons. Seismic design codes suggest that the plan should not have as large indentations and protrusions as possible (TBEC-2018; Herrera and Soberon, 2008). The floor form plan of the reference building has been changed for the irregularity of the plan. Floor views of regular and irregular in plan were given in Figure 16.

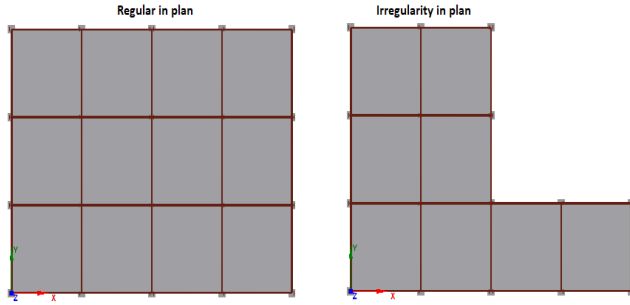


Figure 16. The plans for regular and irregular building model in plan
Three-dimensional models of both models were given in Figure 17.

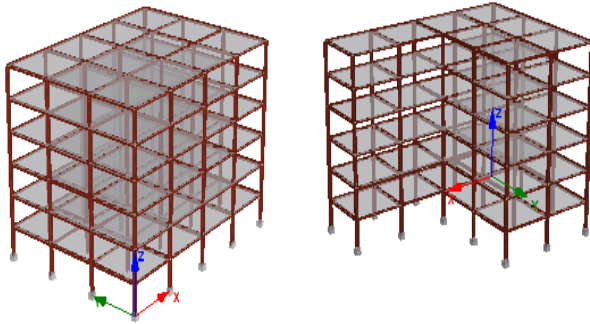


Figure 17. Three-dimensional models of structures with reference and irregular building models

Comparison of curves obtained as a result of pushover analysis in X and Y directions due to irregularity in plan are given in Figure 18.

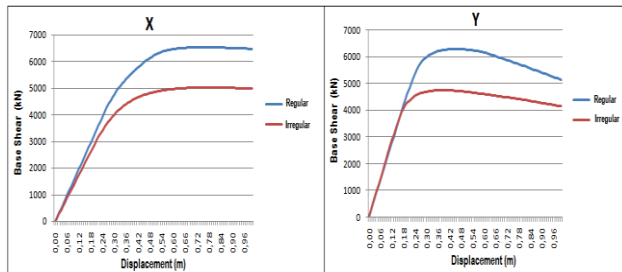


Figure 18. Comparisons of pushover curves for irregularity in plan

The comparisons of analyses result for irregularity in plan are given in Table 8.

Table 8. Comparison of result values for irregularity in plan

Model	Direction	Period (s)	Base Shear (kN)	K _{elas} (kN/m)	K _{eff} (kN/m)	dy (m)	DL (m)	SD (m)	NC (m)
Reference	X	0.406	6537.71	16485.21	14992.31	0.436	0.022	0.029	0.049
Irregularity	X	0.405	5024.33	14569.97	13005.89	0.436	0.019	0.025	0.043
Reference	Y	0.406	6272.83	23248.50	22247.51	0.282	0.016	0.021	0.035
Irregularity	Y	0.405	4733.24	23986.10	22437.87	0.282	0.0098	0.013	0.022

4.6. Local Soil Conditions

It is a known fact that local ground conditions directly affect the seismic behavior of structures. In the analysis results obtained, the differentiation of local soil conditions did not change the results significantly. Therefore, in order to obtain more meaningful results, Turkish Earthquake Hazard Map application, which used for Turkish Building Earthquake Code (2018), was used to determine the effect of local soil conditions (AFAD, 2020). Turkish Earthquake Hazard Map Interactive Web Application has become available for the computation of earthquake parameters for any geographic location since the beginning of 2019. For this purpose, results were obtained according to different soil classes for any geographic location where the building will be built by using this application. Comparison of the values obtained as a result of this application is shown in Table 9.

Table 9. Change of parameters obtained for different soil conditions

Parameter	ZA	ZB	ZC	ZD	ZE
F _s	0.800	0.900	1.250	1.301	1.502
F _i	0.800	0.800	1.500	2.246	3.507
S _{DS}	0.499	0.562	0.780	0.812	0.937
S _{D1}	0.142	0.142	0.266	0.398	0.621
T _A (s)	0.057	0.050	0.068	0.098	0.132
T _B (s)	0.284	0.252	0.340	0.490	0.662
T _{AD} (s)	0.019	0.017	0.023	0.033	0.044
T _{BD} (s)	0.095	0.084	0.113	0.163	0.221

Changes in the values obtained through this application related to the soil type given in TBEC-2018 are given in Table 10.

Table 10. Change of soil conditions parameters given (%)

Soil Type	F _S	F ₁	S _{DS}	S _{D1}	T _A	T _B	T _{AD}	T _{BD}
ZA	0	0	0	0	0	0	0	0
ZB	12.50	0.00	12.63	0.00	14.00	11.27	11.76	11.58
ZC	56.25	87.50	56.31	87.32	19.30	19.72	21.05	18.95
ZD	62.63	180.75	62.73	180.28	71.93	72.54	73.68	71.58
ZE	87.75	338.38	87.78	337.32	131.58	133.10	131.58	132.63
Mean	43.83	121.33	43.89	120.98	47.360	47.326	47.614	46.948

5. Conclusions and Discussions

The lack of sufficient earthquake vulnerability of the buildings is one of the main causes of large-scale loss of life and property. Depending on the power of the seismic hazard, the possibility of damage to buildings varies depending on the weaknesses of the structures during design and construction. Several negative parameters in the structures directly affect the amount of damage that occurs. In this study, six different negative parameters such as number of story, soft story, short column, hill-slope effect, local soil conditions and irregularity in plan, which are the main causes of earthquake damages, have been taken into consideration. The effects of these parameters on the earthquake performance of buildings have been investigated based on pushover and eigenvalue analysis in terms of steel structures. The reduction coefficient for each parameter is proposed by evaluating all the obtained analysis results together.

There are many studies in the literature about the effects of single or multiple negativity parameters on earthquake vulnerabilities in RC structures due to more damage in the earthquakes. Therefore, studies on the subject of steel structures are limited and only one parameter should be considered in general. This study will have an important place in determining the earthquake vulnerability of steel structures with the same building characteristics but various negative parameters. In addition, the reduction coefficients obtained as a result of this study can be a source for their usability in rapid evaluation methods to be developed for steel structures.

In the scope of the study, for the total number of stories, which is the first parameter considered, three different numbers of stories were taken into account such as 4-, 5- and 6-story. As the number of stories increased, natural fundamental periods increased due to the decrease in stiffness values in both X and Y directions. The seismic capacity of steel structure was increased due to the decrease in the number of stories. The target displacements for damage estimation expected from the structure have increased according to the amount of the movement of the structures will increased due to higher periods. Therefore, as the number of stories increases, the level of vulnerability expected from the building will also increase. An average effect of 39% was obtained for number stories for this study by considering all analysis results.

The soft story was created by choosing the ground story height of the building higher than the other stories. The natural fundamental period of the structure increased, accordingly its rigidity and seismic capacities decreased for the ground floor height increased. With the consideration of all these values and

the increase in the ground story height, the target displacements for damage estimation expected from the building have also increased. It showed an average of 51% change due to the soft story. The most unfavourable situation among all negativity parameters was obtained for soft story.

The column heights on the 1st story of the building were changed while creating the short column. In the case of a short column, although the period appears smaller and more rigid, the first damages were obtained under lower load conditions. The stiffness continuity expected from the building is interrupted by the change of column heights between stories. It showed an average of 5% change due to the short column.

The column heights on the ground story have different heights according to the selected ground-slope for the hill-slope effect. In this case, the structure also has a short column situation. While the natural fundamental period value of the structure decreased under hill-slope effect, the rigidity and seismic capacity increased. The target displacements for damage estimation were decreased according to hill-slope effect. However, in the columns with the least height, the damage occurred at lower load conditions. The damage status of the structure showed that the earthquake vulnerability of the building was weakened due to the hill-slope effect. The exchange value obtained for this parameter is 10%.

Only one case is taken into account for irregularity in the plan. In case of irregularity in the plan, the period of the structure increased, the stiffness value decreased and the seismic capacity decreased accordingly to these values. The target displacements for damage estimation were decreased for irregularity in the plan. Considering all the values obtained, the average change was calculated as 31% for irregularity in plan.

Local soil conditions directly affect the seismic behaviour of the structures. Close values were obtained for all soil conditions in the software. Therefore, the local ground coefficient values foreseen in TBEC-2018 were used. 47% value was calculated as the change.

Within the scope of this study, the recommended reduction coefficients for six different parameters based on the analysis results are shown in Table 11.

Table 11. Recommended values for each parameter

Parameter	Reduction Coefficient
Soft story	0.51
Short column	0.05
Irregularity in plan	0.31
Number of story	0.39
Local soil conditions	0.47
Hill-slope effect	0.10

In this study, each main parameter has been examined separately. It is useful to investigate how the earthquake performance of the structure will be in case of multiple parameters at the same time. In addition, only one different building model was taken into consideration in this study. Considering different types of building models in future studies will further support the results to be obtained.

In cases where the number of stories is higher than the number of stories considered in the study, these values can be calculated for high-rise steel buildings, in future studies. Only one case for soft story, short column, hill-slope effect and irregularity in plan was considered in this study by using one software only. Different situations for main parameters and software can be considered to examine these main parameters in more detail in future studies.

Conflicts of interest

The authors declare that there are no conflicts of interest in the study.

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Performance prediction of a single-stage refrigeration system using R134a as a refrigerant by artificial intelligence and machine learning method

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ABSTRACT

In this study, COP and heat capacities of evaporator and condenser were calculated by artificial intelligence and machine learning method in a vapor compression mechanical refrigeration cycle using well-known R134a as a refrigerant. Dataset was obtained with CoolPack software to train the model. Evaporating, condensing, superheating and subcooling temperatures are selected as input data. COP, heat capacities of evaporator and condenser are included in the dataset as target values. Artificial Neural Network (ANN) model was created with Matlab R2018b software and validated with target data. The output files obtained were compared with the target files and it was found that the mean square error value was quite close to one. The results of this study show that the ANN method can be used to obtain cycle parameters in one stage refrigeration cycle with high accuracy.

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1. Introduction

People spend 90 % of their time inside building [1]. Fresh air supply is very important in ventilated spaces to provide thermal comfort and sufficient air quality. These outputs are necessary for the occupants, to obtain and maintain specified thermal conditions. Efficiency of office staff and productivity are dependent on the thermal environment conditions [2][3]. If ventilation rates above minimum levels, productivity could be raised and It will pay for itself while decreasing the intensity of SBS (Sick Build Symptoms) symptoms and improving perceived air quality [4]. Ventilation-related energy has an important role in energy consumption. This energy need is increasing and may represent up to 50% of the building total energy usage, particularly for certain typologies such as office buildings [5].

Cooling units, especially split type air conditioners, are very

common in Turkey. Researches that will increase the efficiency of these systems or find the factors that cause them to work inefficiently are very important due to the above-mentioned effects. Artificial intelligence and machine learning is a method that is very popular today, which enables the performance of these and similar thermal systems to be predicted very accurately.

Kılıç [6], investigated estimation of the coefficient of performance (COP) in the transcritical CO₂ one-stage vapor compression cycles with ANN. He compared the ANN results with target data and (R²) value was obtained as 0.99907. He suggested that ANN model can be used to prediction of the coefficient of performance (COP) in the transcritical CO₂ one-stage vapor compression cycles.

Hosoz and Ertunc [7], investigated the applicability of ANN to predict various performance parameters like evaporating

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temperature, compressor power and coefficients of performance for the lower and higher circuits) of a cascade vapor compression refrigeration system. They compared ANN output data with experimental data and ANN output data is agreed well with the experimental target data. They compared ANN output data with experimental data and ANN output data is agreed well with the experimental target data.

Kızılkın et al. [8], investigated the thermodynamic properties such as saturation pressure, liquid and vapor enthalpies, superheated steam enthalpy and temperature of the R410a refrigerant, with Artificial Neural Networks (ANN) method. They used empirical and experimental data available in the literature. They developed new formulations with ANN model to predict the thermodynamic properties of R410a. They said that, these equations obtained from the model were much faster and solutions were simpler.

Yılmaz and Atik [9], performed some experiments about effects of changing cooling water flow rate on the cooling capacity of the mechanical cooling system. They calculated power consumption, thermal efficiency, coefficient of performance (COP) of the system for each scenarios and trained the ANN model. As a result of their work, they stated that artificial neural networks can be used in this field.

There are several studies in the academic literature about artificial intelligence and machine learning and vapor compression mechanical refrigeration cycle. However, all of them examined the subject from a different point of view, and within the knowledge of the author, there is no study similar to this study. R134a is used as refrigerant in this study and ANN model is used to obtain COP and evaporator and condenser capacities.

2. Methods

2.1. Vapor Compression Mechanical Refrigeration Cycle

The vapor compression mechanical refrigeration cycle shown in Figure 1 consists of four main elements: condenser, evaporator, throttle valve and compressor. It is based on the principle that the refrigerant is mechanically compressed by the compressor as a result of the work given. In this system, the refrigerant compressed to high pressure in the compressor is sent to the condenser as superheated steam. The refrigerant, which condenses by giving heat to the environment, enters the evaporator in wet-vapor state by throttling to low pressure in the throttle valve. The refrigerant, which has a temperature below the ambient temperature surrounding the evaporator, absorbs the heat of the environment, cools the internal environment and is absorbed by the compressor as saturated vapor at the

evaporator outlet. [10] The scheme of the vapor compression mechanical refrigeration cycle is given Figure 1.

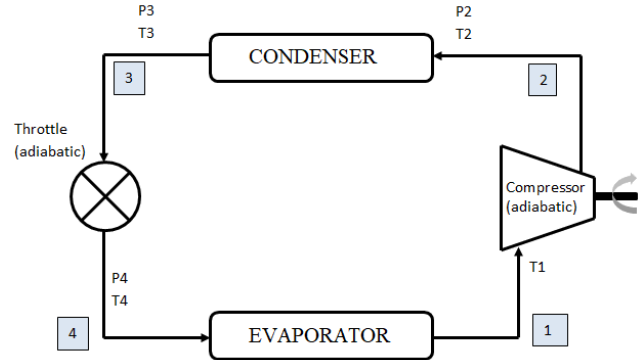


Figure 1. The scheme of the vapor compression mechanical refrigeration cycle

The compressor is adiabatic in this system, Compressor power can be calculates as below:

$$-w_k = h_2 - h_1 \left(\frac{kJ}{kg} \right) \quad (1)$$

Heat is extracted at constant pressure in the condenser and condenser capacity is ($P_2=P_1=\text{constant}$):

$$q_y = q_{23} = h_2 - h_3 \left(\frac{kJ}{kg} \right) \quad (2)$$

Throttle at constant enthalpy at the throttling valve:

$$h_3 = h_4 \left(\frac{kJ}{kg} \right) \quad (3)$$

Heat transfer to the refrigerant at constant pressure in the evaporator:

$$q_b = q_{41} = h_1 - h_4 \left(\frac{kJ}{kg} \right) \quad (4)$$

COP value of the vapor compression mechanical refrigeration cycle:

$$COP = \frac{h_1 - h_4}{h_2 - h_1} \quad (5)$$

56 different COP, q_b and q_c values were calculated with CoolPack software according to principles above. R134a was selected as refrigerant.

2.2. Artificial Intelligence and Machine Learning

Artificial neural networks are a system modeled with inspiration to the basis of the human neural [11]. It aims to solve the problems that cannot be solved by classical methods. ANN has important features such as learning, predicting, and making inferences between inputs even if there is missing information. Artificial neural networks, which can perform activities such as prediction, classification, and learning by using the relationship between cells that are linked to each other in the evaluation of complex relationships, are one of the most preferred optimization techniques recently [12].

The ANN model for the one-stage refrigeration cycle was developed using the available dataset obtained from CoolPack software. Dataset consists of 56 rows of input data which include evaporator (T_e), condenser (T_c), superheating and subcooling temperatures. Target data of ANN model is COP and heat capacities of evaporator and condenser. %70 of the data set is applied to ANN model to train it, %30 of data is used for testing and validating procedures. The scheme of the ANN model for the one-stage cooling cycle is shown in Figure 2. Levenberg–Marquardt algorithm was used in ANN model and weighting coefficients, which is a variant of the back-propagation algorithm, obtained from this algorithm. ANN model is set and computed in Matlab R2018b.

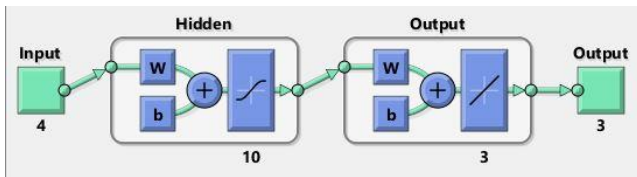


Figure 2. One-stage refrigeration cycle ANN scheme

3. Results

Matlab R2018b, Deep Learning Tool, is used to model vapor compression mechanical refrigeration according to ANN method. Wide range of data of Evaporator, condenser, superheat and subcooling temperatures are created in CoolPack and used to train ANN model.

It is important to get highly accurate predicted data in these systems to determine the performance of cooling. Performance statement is important not only mechanically but also economically. High performance systems decreases energy consumption. In addition, optimizing system performance will increase thermal comfort and, accordingly, employee productivity. Thus, the dataset obtained from theoretical calculations was used in the training of the ANN model and was used in the retesting of the predictions of this learned model.

The information obtained from this study will be a preliminary evaluation to repeat the study experimentally.

Dataset created in CoolPack imported to ANN model and ANN model was created with Matlab R2018b software. Input and target values was defined. Input, hidden layer, output layer and output can be seen from Figure 2. Evaporator, condenser, superheat and subcooling temperature is the data given to input layer. This layers gives COP and evaporator and condenser capacities. The evaporator capacity of cooling cycle in the dataset is in the range of 178.525 kJ/kg to 188.837 kJ/kg, the condenser capacity is in the range of 215.146 to 237.276 kJ/kg.

Figure 3 shows the comparison of output COP and target COP. Output values are in a good agreement with target values. R^2 is 0.998. COP is essential for predicting the performance of cooling cycle. ANN model can predict the COP value with high accuracy.

R^2 is one of the statistical performance evaluation criteria values and it is also in a good agreement with q_e target data. It can be seen from Figure 5. R^2 is 0.999, predicted q_e values are also in a good agreement with target data.

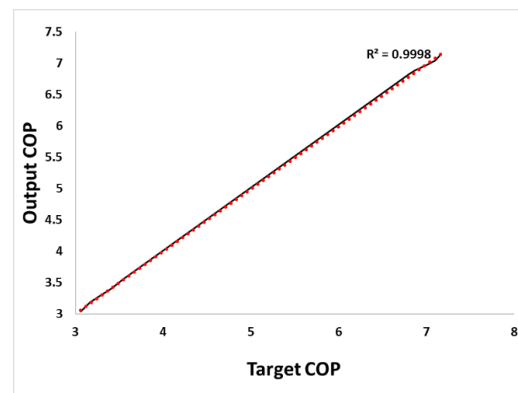


Figure 3. Comparison of output COP and target COP

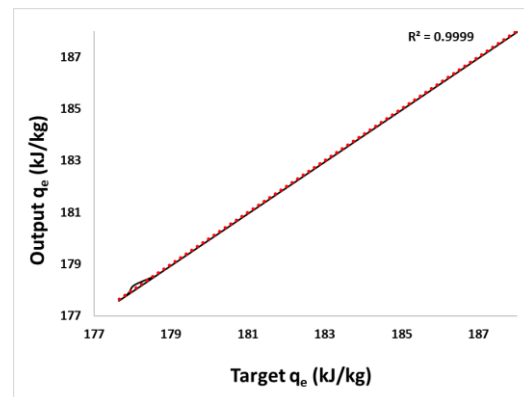


Figure 4. Comparison of output q_e and target q_e

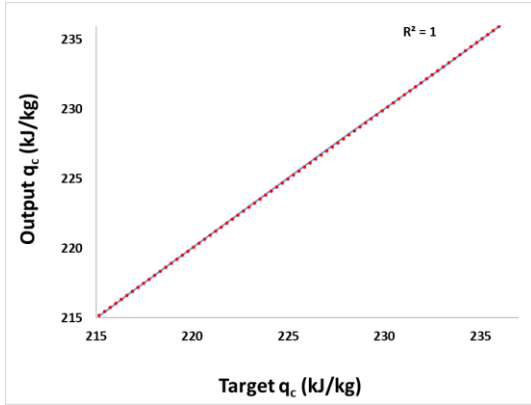


Figure 5. Comparison of output q_c and target q_c

4. Conclusions

In this paper, ANN approach is applied for thermal analysis of vapor compression mechanical refrigeration cycle. The ANN is applied for predicting the coefficient of performance (COP) and evaporator and condenser capacity. Predicted values and target values, which obtained from CoolPack, is compared and results are in a good agreement. It is suggested that ANN model can be applied all refrigerants and other cooling systems. Artificial intelligence and machine learning can be easily applied to thermodynamic cycles and can give fast and reliable results with experimental data.

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