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AMAÇ VE KAPSAM

Caucasian Journal of Science Dergisi 2014 yılından bu yana aralıksız olarak yayınlanan uluslararası hakemli bir dergidir. Dergi, haziran ve aralık aylarında olmak üzere yılda iki kez yayınlanmaktadır. Caucasian Journal of Science dergisinin amacı; fen bilimleri, fen eğitimi, mühendislik ve sağlık alanlarında, araştırma makaleleri, kısa bildiriler, olgu sunumları, derleme yazıları ve editöre mektuplar yayınlanmak ve bu sayede akademik çalışmalara destek sağlamaktır. Fen bilimleri, fen eğitimi, mühendislik ve sağlık alanlarında, araştırma makaleleri, kısa bildiriler, olgu sunumları, derleme yazıları ve editöre mektuplar gibi akademik çalışmaların bağımsız ve ön yargısız olarak değerlendirilmesi en önemli ilkemizdir. Bu sebeple, çift kör hakemlik sistemi uygulanmakta ve gerekli durumlarda üçüncü hakeme müracaat edilmektedir. Yazıların değerlendirilmesinde ICMJE standartları gözetilir. Yayınlanan yazıların tam metinlerine erişim ücretsizdir. Dergimizin etik politikası gereği, intihal tolere edilemez. Dergimize yayımlanmak üzere gönderilen tüm makalelerin içeriği, intihal denetimi yazılımı ile kontrol edilmektedir.

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On Fuzzy Hypersoft Topological Spaces

Adem YOLCU[1], Taha Yasin ÖZTÜRK[2]

Makalenin Alanı: Matematik

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Anahtar Kelimeler
Fuzzy hypersoft küme
Fuzzy hypersoft topoloji
Fuzzy hypersoft taban
Fuzzy hypersoft alt uzay

Öz

Bu makale fuzzy hypersoft kümeler üzerinde bir topoloji kurmayı amaçlamaktadır. Bu çalışmada fuzzy hypersoft topoloji üzerinde fuzzy hypersoft açık(kapalı) kümeler, fuzzy hypersoft iç, fuzzy hypersoft kapanış, fuzzy hypersoft taban ve fuzzy hypersoft alt uzay topoloji kavramları tanıtılmış ve bazı önemli teoremler ispatlanmıştır.

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Abstract

This paper is aimed at constructing a topology on fuzzy hypersoft sets. The concepts of fuzzy hypersoft topology, fuzzy hypersoft open(closed) sets, fuzzy hypersoft interior, fuzzy hypersoft closure, fuzzy hypersoft base and fuzzy hypersoft subspace topology are also introduced here and some important theorems have been established.

1. INTRODUCTION

In sociology, economics, climate, engineering, etc., we can not use classical mathematical methods to solve any kind of problems because these sort of problems have their own difficulties. The Fuzzy set theory, first proposed in 1965 by researcher Zadeh (Zadeh, 1965), has become a very useful technique for solving these types of problems and provides an effective structure for representing ambiguous concepts by enabling partial membership. Both mathematicians and computer scientists have studied Fuzzy Set Theory and, over the

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years, fuzzy control systems, fuzzy logic, fuzzy topology, etc. Many implementations of fuzzy set theory have appeared as such. There is also a probability theory besides this theory, a rough set theory (Pawlak, 1982) that deals with solving these problems. Molodtsov (Molodtsov, 1999) introduced the idea of soft set theory, which is a completely new approach to modeling uncertainty, each of these theories has its inherent difficulties. The fundamental results of this new theory were established by Molodtsov and the soft set theory was successfully applied in many directions, such as smoothness of functions, operations analysis, Riemann integration, game theory, probability theory, etc. by Molodtsov. Maji (Maji et al., 2003) identified and studied several fundamental notions of soft set theory. The study of Maji et al. (Maji et al., 2003) was improved by Pei and Miao (Pei & Miao, 2005), Feng et al. (Feng et al., 2008), Chen et al. (Chen et al., 2005), Aktaş and Çağman (Aktaş & Çağman, 2007), Irfan Ali et al. (Ali et al., 2009) and Ozturk and Bayramov (Ozturk & Bayramov, 2014).

The research involving both fuzzy sets and soft sets was initiated by Maji et al. (Maji et al., 2001). The fuzzy soft set structure, which is a combination of soft set structure and fuzzy set structure, has been actively used by researchers and many studies have been added to the literature (Ahmad & Kharal, 2009; Roy & Maji, 2007; Yang et al., 2009). It is pointed out in (Pei & Miao, 2005) that several scientists from many fields have studied information systems and that certain compact ties exist between soft sets and information systems. It is also shown in (Pei & Miao, 2005) that soft sets are a class of special information systems , called fuzzy information systems, and that it is possible to unify research on soft sets and information systems. In addition , it is possible to predict some new outcomes and approaches from this unification. In (Kharal & Ahmad, 2009), in order to improve this fuzzy soft theory, The concept of mapping fuzzy soft sets was defined by Kharal and Ahmad and the properties of fuzzy soft images and fuzzy soft inverse images of fuzzy soft sets were studied.

Fuzzy soft topology on a fuzzy soft set over an initial universe was described by Tanay and Kandemir (Tanay & Kandemir, 2011). They defined some notions of fuzzy soft topological spaces and investigated some properties. Roy and Samanta (Roy & Samanta, 2012) defined a fuzzy soft topology over the initial universe, and they also introduced the base and subbase for this space , by giving some characterizations.

Smarandache (Smarandache, 2018) has implemented new uncertainty management methods. He extended the soft set to a hypersoft set by transforming the features into a multi-decision method. Hypersoft set structure is a more general form of soft set structure since it

consists of elements selected from different attributes. Due to the fact that this new structure is more useful, a lot of research have been done in a short time (Gayen et al., 2020), (Martin & Smarandache, 2020), (Rana et al., 2019), (Saeed et al., 2020), (Saqlain et al., 2020a, 2020b). Yolcu and Ozturk (Yolcu & Ozturk, 2021) defined the fuzzy hypersoft set structure by combining fuzzy and hypersoft set structures. In this paper, we are trying to extend this notion to topological spaces. Then we define a fuzzy hypersoft topology, fuzzy hypersoft open(closed) sets, fuzzy hypersoft interior, fuzzy hypersoft closure, fuzzy hypersoft base and fuzzy hypersoft subspace topology and also here we established some important theorems related to this spaces.

2. PRELIMINARIES

Definition 2.1 (Zadeh, 1965) Let U be a initial universe. A fuzzy set Λ in U, $\Lambda = \{(u, \mu_\Lambda(u)) : u \in U\}$, where $\mu_\Lambda : U \to [0,1]$ is the membership function of the fuzzy set Λ; $\mu_\Lambda(u) \in [0,1]$ is the membership $u \in U$ in Λ. The set of all fuzzy sets ove U will be denoted by $FP(U)$.

Definition 2.2 (Molodtsov, 1999) Let U be an initial universe and E be a set of parameters. A pair (F, E) is called a soft set over U, where F is a mapping $F : E \to \mathrm{P}(U)$. In other words, the soft set is a parameterized family of subsets of the set U.

Definition 2.3 (Maji et al., 2001) Let U be a initial universe, E be a set of parameters and $FP(U)$ be the set of all fuzzy sets in U. Then a pair (f, E) is called a fuzzy soft set over U, where $f : E \to FP(U)$ is a mapping.

Definition 2.4 (Smarandache, 2018) Let U be the universal set and $P(U)$ be the power set of U. Consider $e_1, e_2, e_3, ..., e_n$ for $n \geq 1$, be n well-defined attributes, whose corresponding attribute values are resspectively the sets $E_1, E_2, ..., E_n$ with $E_i \cap E_j = \varnothing$, for $i \neq j$ and $i, j \in \{1, 2, ..., n\}$, then the pair $(\Theta, E_1 \times E_2 \times ... \times E_n)$ is said to be Hypersoft set over U where $\Theta : E_1 \times E_2 \times ... \times E_n \to P(U)$.

Definition 2.5 (Yolcu & Ozturk, 2021) Let U be the universal set and $FP(U)$ be a family of all fuzzy set over U and $E_1, E_2, ..., E_n$ the pairwise disjoint sets of parameters. Let A_i be the

nonempty subset of E_i for each $i = 1, 2, ..., n.$ A fuzzy hypersoft set defined as the pair $(\Theta, A_1 \times A_2 \times ... \times A_n)$ where; $\Theta : A_1 \times A_2 \times ... \times A_n \to FP(U)$ and

$\Theta(A_1 \times A_2 \times ... \times A_n) = \{< u, \Theta(\alpha)(u) >: u \in U,$
$\alpha \in A_1 \times A_2 \times ... \times A_n \subseteq E_1 \times E_2 \times ... \times E_n\}$

For sake of simplicity, we write the symbols Σ for $E_1 \times E_2 \times ... \times E_n$, Γ for $A_1 \times A_2 \times ... \times A_n$ and α for an element of the set $\Gamma.$ The set of all fuzzy hypersoft sets over U will be denoted by $FHS(U, \Sigma).$ Here after, FHS will be used for short instead of fuzzy hypersoft sets.

Definition 2.6 (Yolcu & Ozturk, 2021)

i) A fuzzy hypersoft set (Θ, Σ) over the universe U is said to be null fuzzy hypersoft set and denoted by $0_{(U_{FH}, \Sigma)}$ if for all $u \in U$ and $\varepsilon \in \Sigma$, $\Theta(\varepsilon)(u) = 0$.

ii) A fuzzy hypersoft set (Θ, Σ) over the universe U is said to be absolute fuzzy hypersoft set and denoted by $1_{(U_{FH}, \Sigma)}$ if for all $u \in U$ and $\varepsilon \in \Sigma$, $\Theta(\varepsilon)(u) = 1$.

Definition 2.7 (Yolcu & Ozturk, 2021) Let U be an initial universe set $(\Theta_1, \Gamma_1), (\Theta_2, \Gamma_2)$ be two fuzzy hypersoft sets over the universe U. We say that (Θ_1, Γ_1) is a fuzzy hypersoft subset of (Θ_2, Γ_2) and denote $(\Theta_1, \Gamma_1) \subseteq (\Theta_2, \Gamma_2)$ if

i) $\Gamma_1 \subseteq \Gamma_2$

ii) For any $\varepsilon \in \Gamma_1$, $\Theta_1(\varepsilon) \subseteq \Theta_2(\varepsilon).$

Definition 2.8 (Yolcu & Ozturk, 2021) The complement of fuzzy hypersoft set (Θ, Γ) over the universe U is denoted by $(\Theta, \Gamma)^c$ and defined as $(\Theta, \Gamma)^c = (\Theta^c, \Gamma),$ where $\Theta^c(\varepsilon)$ is complement of the set $\Theta(\varepsilon),$ for $\varepsilon \in \Gamma.$

Definition 2.9 (Yolcu & Ozturk, 2021) Let U be an initial universe set and $(\Theta_1, \Gamma_1), (\Theta_2, \Gamma_2)$ be two fuzzy hypersoft sets over the universe U. The union of (Θ_1, Γ_1) and (Θ_2, Γ_2) is denoted by $(\Theta_1, \Gamma_1) \tilde{\cup} (\Theta_2, \Gamma_2) = (\Theta_3, \Gamma_3)$ where $\Gamma_3 = \Gamma_1 \cup \Gamma_2$ and

$$\Theta_3(\varepsilon) = \begin{cases} \Theta_1(\varepsilon) & \text{if } \varepsilon \in \Gamma_1 - \Gamma_2 \\ \Theta_2(\varepsilon) & \text{if } \varepsilon \in \Gamma_2 - \Gamma_1 \\ \max\{\Theta_1(\varepsilon), \Theta_2(\varepsilon)\} & \text{if } \varepsilon \in \Gamma_1 \cap \Gamma_2 \end{cases}$$

Definition 2.10 Let U be an initial universe set and $(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2)$ be fuzzy hypersoft sets over the universe U. The intersection of (Θ_1,Γ_1) and (Θ_2,Γ_2) is denoted by $(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)=(\Theta_3,\Gamma_3)$ where $\Gamma_3=\Gamma_1\cap\Gamma_2$ and each $\varepsilon\in\Gamma_3, \Theta_3(\varepsilon)(u)=\min\{\Theta_1(\varepsilon)(u),\Theta_2(\varepsilon)(u)\}$. (Yolcu & Ozturk, 2021)

3. FUZZY HYPERSOFT TOPOLOGICAL SPACES

Definition 3.1 Let $FHS(U,\Sigma)$ be the set of all fuzzy hypersoft subsets of (U,Σ) over the universe U and $\tilde{\tau}$ be a subfamily of $FHS(U,\Sigma)$. Then $\tilde{\tau}$ is called a fuzzy hypersoft topology on U if the following condition are satisfied.

1. $0_{(U_{FH},\Sigma)}$ and $1_{(U_{FH},\Sigma)}$ belongs to $\tilde{\tau}$,
2. The union of any number of fuzzy hypersoft sets in $\tilde{\tau}$ belongs to $\tilde{\tau}$,
3. The intersection of any two fuzzy hypersoft sets in $\tilde{\tau}$ belongs to $\tilde{\tau}$.

The triple $(U,\tilde{\tau},\Sigma)$ is called a fuzzy hypersoft topological space over U. Every member of $\tilde{\tau}$ is called a fuzzy hypersoft open set in U.

Definition 3.2 Let $FHS(U,\Sigma)$ be the set of all fuzzy hypersoftsets over the universe U. Then,

1. If $\tilde{\tau}=\{0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)}\}$, then $\tilde{\tau}$ is called to be fuzzy hypersoft indiscrete topology and $(U,\tilde{\tau},\Sigma)$ is called to be fuzzy hypersoft indiscrete topological space over the universe U.
2. If $\tilde{\tau}=FHS(U,\Sigma)$, then $\tilde{\tau}$ is called to be fuzzy hypersoft discrete topology and $(U,\tilde{\tau},\Sigma)$ is called to be fuzzy hypersoft discrete topological space over the universe U.

Example 3.1 Let $U=\{u_1,u_2,u_3\}$ be the set of mobile phones and also consider the set of attributes E_1 = Screen Size, E_2 = RAM, E_3 = Operating System, E_4 = Battery Power are given as;

E_1 = Screen Size = $\{6.55in(\alpha_1),6.78in(\alpha_2)\}$

E_2 = RAM = $\{12GB(\beta_1),8GB(\beta_2),4GB(\beta_3)\}$

E_3 = Operating System = $\{Android(\gamma_1),IOS(\gamma_2)\}$

E_4 = Battery Power = $\{4300mAH(\delta_1),4115mAH(\delta_2),5100mAH(\delta_3)\}$

Suppose that

$A_1 = \{\alpha_1\}, A_2 = \{\beta_1, \beta_2\}, A_3 = \{\gamma_2\}, A_4 = \{\delta_1, \delta_3\}$

$B_1 = \{\alpha_2\}, B_2 = \{\beta_2, \beta_3\}, B_3 = \{\gamma_1\}, B_4 = \{\delta_1, \delta_2\}$

Let

$$\tilde{\tau} = \{0_{(U_{FH},\Sigma)}, 1_{(U_{FH},\Sigma)}, (\Theta_1, \Gamma_1), (\Theta_2, \Gamma_2), (\Theta_3, \Gamma_3)\}$$

be a subfamily of $FHS(U, \Sigma)$ where $(\Theta_1, \Gamma_1), (\Theta_2, \Gamma_2), (\Theta_3, \Gamma_3)$ for $\forall \Gamma \subseteq \Sigma$, fuzzy hypersoft sets over U and defined as follows;

$$(\Theta_1, \Gamma_1) = \left\{ \begin{array}{l} < (\alpha_1, \beta_1, \gamma_2, \delta_1), \{\frac{u_1}{0,2}, \frac{u_2}{0,5}\} >, \\ < (\alpha_1, \beta_1, \gamma_2, \delta_3), \{\frac{u_1}{0,5}, \frac{u_2}{0,1}, \frac{u_3}{0,7}\} >, \\ < (\alpha_1, \beta_2, \gamma_2, \delta_1), \{\frac{u_1}{0,6}, \frac{u_3}{0,2}\} >, \\ < (\alpha_1, \beta_2, \gamma_2, \delta_3), \{\frac{u_2}{0,2}, \frac{u_3}{0,3}\} > \end{array} \right\},$$

$$(\Theta_2, \Gamma_2) = \left\{ \begin{array}{l} < (\alpha_2, \beta_2, \gamma_1, \delta_1), \{\frac{u_1}{0,4}, \frac{u_2}{0,7}, \frac{u_3}{0,2}\} >, \\ < (\alpha_2, \beta_2, \gamma_1, \delta_2), \{\frac{u_1}{0,8}, \frac{u_2}{0,2}, \frac{u_3}{0,4}\} >, \\ < (\alpha_2, \beta_3, \gamma_1, \delta_1), \{\frac{u_1}{0,3}, \frac{u_2}{0,4}\} >, \\ < (\alpha_2, \beta_3, \gamma_1, \delta_2), \{\frac{u_2}{0,7}, \frac{u_3}{0,8}\} > \end{array} \right\},$$

$$(\Theta_3,\Gamma_3)=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,2},\frac{u_2}{0,5}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,5},\frac{u_2}{0,1},\frac{u_3}{0,7}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,6},\frac{u_3}{0,2}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_2}{0,2},\frac{u_3}{0,3}\}>,\\ <(\alpha_2,\beta_2,\gamma_1,\delta_1),\{\frac{u_1}{0,4},\frac{u_2}{0,7},\frac{u_3}{0,2}\}>,\\ <(\alpha_2,\beta_2,\gamma_1,\delta_2),\{\frac{u_1}{0,8},\frac{u_2}{0,2},\frac{u_3}{0,4}\}>,\\ <(\alpha_2,\beta_3,\gamma_1,\delta_1),\{\frac{u_1}{0,3},\frac{u_2}{0,4}\}>,\\ <(\alpha_2,\beta_3,\gamma_1,\delta_2),\{\frac{u_2}{0,7},\frac{u_3}{0,8}\}>\end{array}\right\}.$$

Then $\tilde{\tau}$ is a fuzzy hypersoft topology on U and hence $(U,\tilde{\tau},\Sigma)$ is a fuzzy hypersoft topological space.

Remark 3.1 It is clear that each fuzzy hypersoft topology is also fuzzy soft topology. We consider that Example 3.1. If we select the parameters from a single attribute set such as E_2 while creating fuzzy hypersoft topology, then the resulting topology becomes fuzzy soft topology. Therefore fuzzy hypersoft topology is also fuzzy soft topology. But the reverse is not true since there are no different attributes in the fuzzy soft set structure, such as the fuzzy hypersoft set structure. From here, it is seen that the fuzzy hypersoft structure is a more general structure than the fuzzy soft structure.

Proposition 3.1 Let $(U,\tilde{\tau}_1,\Sigma)$ and $(U,\tilde{\tau}_2,\Sigma)$ be two fuzzy hypersoft topological space over U. Then $(U,\tilde{\tau}_1\tilde{\cap}\tilde{\tau}_2,\Sigma)$ is a fuzzy hypersoft topological space over the universe U.

Proof. 1. Since $0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)}\in\tilde{\tau}_1$ and $0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)}\in\tilde{\tau}_2$, then $0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)}\in\tilde{\tau}_1\tilde{\cap}\tilde{\tau}_2$.

2. Suppose that $\{(\Theta_i,\Gamma_i):i\in I\}$ be a family of fuzzy hypersoft sets in $\tilde{\tau}_1\tilde{\cap}\tilde{\tau}_2$. Then $(\Theta_i,\Gamma_i)\in\tilde{\tau}_1$ and $(\Theta_i,\Gamma_i)\in\tilde{\tau}_2$ for all $i\in I$, so $\underset{i\in I}{\cup}(\Theta_i,\Gamma_i)\in\tilde{\tau}_1$ and $\underset{i\in I}{\cup}(\Theta_i,\Gamma_i)\in\tilde{\tau}_2$. Thus $\underset{i\in I}{\cup}(\Theta_i,\Gamma_i)\in\tilde{\tau}_1\tilde{\cap}\tilde{\tau}_2$.

3. Suppose that $\left\{(\Theta_i,\Gamma_i): i=\overline{1,n}\right\}$ be a family of the finite number of fuzzy hypersoft sets in $\tilde{\tau}_1 \tilde{\cap} \tilde{\tau}_2$. Then $(\Theta_i,\Gamma_i)\in\tilde{\tau}_1$ and $(\Theta_i,\Gamma_i)\in\tilde{\tau}_2$ for all $i=\overline{1,n}$, so $\underset{i=1}{\overset{n}{\cap}}(\Theta_i,\Gamma_i)\in\tilde{\tau}_1$ and $\underset{i=1}{\overset{n}{\cap}}(\Theta_i,\Gamma_i)\in\tilde{\tau}_2$. Thus $\underset{i=1}{\overset{n}{\cap}}(\Theta_i,\Gamma_i)\in\tilde{\tau}_1 \tilde{\cap} \tilde{\tau}_2$.

Remark 3.2 The union of two fuzzy hypersoft topologies over U may not be a fuzzy soft topology on U. This condition showed the following example.

Example 3.2 We consider the Example 3.1. Let

$$\tilde{\tau}_1=\{0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)},(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2),(\Theta_3,\Gamma_3)\}$$

$$\tilde{\tau}_2=\{0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)},(\Theta_2,\Gamma_2),(\Theta_4,\Gamma_4)\}$$

where

$$(\Theta_4,\Gamma_4)=\left\{\begin{array}{l}<(\alpha_2,\beta_2,\gamma_1,\delta_1),\{\frac{u_1}{0,5},\frac{u_2}{0,7},\frac{u_3}{0,4}\}>,\\ <(\alpha_2,\beta_2,\gamma_1,\delta_2),\{\frac{u_1}{0,9},\frac{u_2}{0,4},\frac{u_3}{0,6}\}>,\\ <(\alpha_2,\beta_3,\gamma_1,\delta_1),\{\frac{u_1}{0,5},\frac{u_2}{0,7}\}>,\\ <(\alpha_2,\beta_3,\gamma_1,\delta_2),\{\frac{u_2}{0,8},\frac{u_3}{0,9}\}>\end{array}\right\}.$$

It is clear that $\tilde{\tau}_1 \tilde{\cap} \tilde{\tau}_2$ is a fuzzy hypersoft topology. But $(\Theta_1,\Gamma_1)\cup(\Theta_4,\Gamma_4)\notin\tilde{\tau}_1\cup\tilde{\tau}_2$ and hence $\tilde{\tau}_1\cup\tilde{\tau}_2$ is not fuzzy hypersoft topology.

Proposition 3.2 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U. Then the collection $\tilde{\tau}=\left\{(\Theta,\Gamma):(\Theta,\Gamma)\in\tilde{\tau}\right\}$ defines a fuzzy soft topology and $(U,\tilde{\tau},\Sigma)$ is a fuzzy soft topological space over U.

Proof. Suppose that $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U. Let us shoe that the collection $\tilde{\tau}=\left\{(\Theta,\Gamma):(\Theta,\Gamma)\in\tilde{\tau}\right\}$ provides the conditions of fuzzy soft topological spaces.

1. $0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)}\in\tilde{\tau}$,

2. Suppose that $\{(\Theta_i,\Gamma_i): i\in I\}$ be a family of fuzzy hypersoft sets in $\tilde{\tau}$. Since $\tilde{\tau}=\{(\Theta,\Gamma):(\Theta,\Gamma)\in\tilde{\tau}\}$, $\{(\Theta_i,\Gamma_i): i\in I\}$ is also a family of fuzzy soft sets in $\tilde{\tau}$. Then $\underset{i\in I}{\cup}(\Theta_i,\Gamma_i)\in\tilde{\tau}$ and hence $\underset{i\in I}{\cup}(\Theta_i,\Gamma_i)\in\tilde{\tau}$.

3. Suppose that $\{(\Theta_i,\Gamma_i): i=\overline{1,n}\}$ be a family of the finite number of fuzzy hypersoft sets in $\tilde{\tau}$. Then $\{(\Theta_i,\Gamma_i): i=\overline{1,n}\}$ is a family of fuzzy soft sets in $\tilde{\tau}$. Then $\overset{n}{\underset{i=1}{\cap}}(\Theta_i,\Gamma_i)\in\tilde{\tau}$ so $\overset{n}{\underset{i=1}{\cap}}(\Theta_i,\Gamma_i)\in\tilde{\tau}$.

Definition 3.3 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and (Θ,Γ) be a fuzzy hypersoft set over U. Then (Θ,Γ) is said to be a fuzzy hypersoft closed set if its complement $(\Theta,\Gamma)^c$ belongs to $\tilde{\tau}$.

Example 3.3 We consider the Example 3.1. It is clear that $\left(0_{(U_{FH},\Sigma)}\right)^c$, $\left(1_{(U_{FH},\Sigma)}\right)^c$, $((\Theta_1,\Gamma_1))^c$, $((\Theta_2,\Gamma_2))^c$, $((\Theta_3,\Gamma_3))^c$ are fuzzy hypersoft closed sets.

$$\left(0_{(U_{FH},\Sigma)}\right)^c=1_{(U_{FH},\Sigma)},\left(1_{(U_{FH},\Sigma)}\right)^c=0_{(U_{FH},\Sigma)}$$

$$((\Theta_1,\Gamma_1))^c=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,8},\frac{u_2}{0,5},\frac{u_3}{1}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,5},\frac{u_2}{0,9},\frac{u_3}{0,3}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,4},\frac{u_2}{1},\frac{u_3}{0,8}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_1}{1},\frac{u_2}{0,8},\frac{u_3}{0,7}\}>\end{array}\right\},$$

$$\left((\Theta_2,\Gamma_2)\right)^c=\left\{\begin{array}{l}<(\alpha_2,\beta_2,\gamma_1,\delta_1),\{\frac{u_1}{0,6},\frac{u_2}{0,3},\frac{u_3}{0,8}\}>,\\<(\alpha_2,\beta_2,\gamma_1,\delta_2),\{\frac{u_1}{0,2},\frac{u_2}{0,8},\frac{u_3}{0,6}\}>,\\<(\alpha_2,\beta_3,\gamma_1,\delta_1),\{\frac{u_1}{0,7},\frac{u_2}{0,6},\frac{u_3}{1}\}>,\\<(\alpha_2,\beta_3,\gamma_1,\delta_2),\{\frac{u_1}{1},\frac{u_2}{0,3},\frac{u_3}{0,2}\}>\end{array}\right\},$$

$$\left((\Theta_3,\Gamma_3)\right)^c=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,8},\frac{u_2}{0,5},\frac{u_3}{1}\}>,\\<(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,5},\frac{u_2}{0,9},\frac{u_3}{0,3}\}>,\\<(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,4},\frac{u_2}{1},\frac{u_3}{0,8}\}>,\\<(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_1}{1},\frac{u_2}{0,8},\frac{u_3}{0,7}\}>\\<(\alpha_2,\beta_2,\gamma_1,\delta_1),\{\frac{u_1}{0,6},\frac{u_2}{0,3},\frac{u_3}{0,8}\}>,\\<(\alpha_2,\beta_2,\gamma_1,\delta_2),\{\frac{u_1}{0,2},\frac{u_2}{0,8},\frac{u_3}{0,6}\}>,\\<(\alpha_2,\beta_3,\gamma_1,\delta_1),\{\frac{u_1}{0,7},\frac{u_2}{0,6},\frac{u_3}{1}\}>,\\<(\alpha_2,\beta_3,\gamma_1,\delta_2),\{\frac{u_1}{1},\frac{u_2}{0,3},\frac{u_3}{0,2}\}>\end{array}\right\}.$$

Proposition 3.3 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U . Then the following properties are provide.

1. $0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)}$ are fuzzy hypersoft closed sets over U.
2. The intersection of any number of fuzzy hypersoft closed set is a fuzzy hypersoft set over U.
3. The union of any two fuzzy hypersoft closed set is a fuzzy hypersoft closed set over U.

Proof. Straightforward.

Definition 3.4 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and (Θ,Γ) be a fuzzy hypersoft set over U. The fuzzy hypersoft closure of (Θ,Γ) denoted by $cl_{FH}(\Theta,\Gamma)$ is the intersection of all fuzzy hypersoft closed super sets of (Θ,Γ).

It is clear that $cl_{FH}(\Theta,\Gamma)$ is the smallest fuzzy hypersoft closed set over U which contain (Θ,Γ).

Theorem 3.1 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and $(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2)\in FHS(U,\Sigma)$. Then,

1. $cl_{FH}\left(0_{(U_{FH},\Sigma)}\right)=0_{(U_{FH},\Sigma)}$ and $cl_{FH}\left(1_{(U_{FH},\Sigma)}\right)=1_{(U_{FH},\Sigma)}$,
2. $(\Theta_1,\Gamma_1)\subseteq cl_{FH}(\Theta_1,\Gamma_1)$,
3. (Θ_1,Γ_1) is a fuzzy hypersoft closed set if and only if $(\Theta_1,\Gamma_1)=cl_{FH}(\Theta_1,\Gamma_1)$,
4. $cl_{FH}\left(cl_{FH}(\Theta_1,\Gamma_1)\right)=cl_{FH}(\Theta_1,\Gamma_1)$,
5. If $(\Theta_1,\Gamma_1)\subseteq(\Theta_2,\Gamma_2)$, then $cl_{FH}(\Theta_1,\Gamma_1)\subseteq cl_{FH}(\Theta_2,\Gamma_2)$,
6. $cl_{FH}\left[(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\right]=cl_{FH}(\Theta_1,\Gamma_1)\cup cl_{FH}(\Theta_2,\Gamma_2)$.

Proof. 1 and 2 are clear from the definition of closure.

3. Let (Θ_1,Γ_1) be a fuzzy hypersoft closed set. By (2), we have $(\Theta_1,\Gamma_1)\subseteq cl_{FH}(\Theta_1,\Gamma_1)$. Since $cl_{FH}(\Theta_1,\Gamma_1)$ is the smallest fuzzy hypersoft closed set over U which contain (Θ_1,Γ_1), then $cl_{FH}(\Theta_1,\Gamma_1)\,\tilde{\subset}\,(\Theta_1,\Gamma_1)$. Hence $(\Theta_1,\Gamma_1)=cl_{FH}(\Theta_1,\Gamma_1)$. Conversely, suppose that $(\Theta_1,\Gamma_1)=cl_{FH}(\Theta_1,\Gamma_1)$. Since $cl_{FH}(\Theta_1,\Gamma_1)$ is a fuzzy hypersoft closed set, then (Θ_1,Γ_1) is closed.

4. Let $(\Theta_2,\Gamma_2)=cl_{FH}(\Theta_1,\Gamma_1)$. Then, (Θ_2,Γ_2) is a fuzzy hypersoft closed set, $(\Theta_2,\Gamma_2)=cl_{FH}(\Theta_2,\Gamma_2)$. So, we have $cl_{FH}\left(cl_{FH}(\Theta_1,\Gamma_1)\right)=cl_{FH}(\Theta_1,\Gamma_1)$.

5. If $(\Theta_1,\Gamma_1)\subseteq(\Theta_2,\Gamma_2)$, then
$$(\Theta_2,\Gamma_2)=(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\Rightarrow$$
$$cl_{FH}(\Theta_2,\Gamma_2)=cl_{FH}\left[(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\right]=$$
$$cl_{FH}(\Theta_1,\Gamma_1)\cup cl_{FH}(\Theta_2,\Gamma_2)\Rightarrow$$
$$cl_{FH}(\Theta_1,\Gamma_1)\subseteq cl_{FH}(\Theta_2,\Gamma_2).$$

6. Since $(\Theta_1,\Gamma_1)\subseteq(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)$ and $(\Theta_2,\Gamma_2)\subseteq(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)$, from the (5), $cl_{FH}(\Theta_1,\Gamma_1)\subseteq cl_{FH}\left[(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\right]$ and $(\Theta_2,\Gamma_2)\subseteq cl_{FH}\left[(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\right]$. Therefore

$cl_{FH}(\Theta_1,\Gamma_1)\cup cl_{FH}(\Theta_2,\Gamma_2)\subseteq cl_{FH}\left[(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\right]$. Conversely, since $(\Theta_1,\Gamma_1)\subseteq cl_{FH}(\Theta,\Gamma)$ and $(\Theta_2,\Gamma_2)\subseteq cl_{FH}(\Theta_2,\Gamma_2)$ are fuzzy hypersoft closed sets, from the proposition 3, $cl_{FH}(\Theta_1,\Gamma_1)\cup cl_{FH}(\Theta_2,\Gamma_2)$ is a fuzzy hypersoft closed set over U being the union of two fuzzy hypersoft fuzzy soft closed sets. Then, $cl_{FH}\left[(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\right]\subseteq cl_{FH}(\Theta_1,\Gamma_1)\cup cl_{FH}(\Theta_2,\Gamma_2)$. Hence $cl_{FH}\left[(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)\right] = cl_{FH}(\Theta_1,\Gamma_1)\cup cl_{FH}(\Theta_2,\Gamma_2)$ is obtained.

Definition 3.5 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and (Θ,Γ) be a fuzzy hypersoft set over U. The fuzzy hypersoft interior of (Θ,Γ) denoted by $int_{FH}(\Theta,\Gamma)$ is the union of all fuzzy hypersoft open subsets of (Θ,Γ).

It is clear that $int_{FH}(\Theta,\Gamma)$ is the largest fuzzy hypersoft open set contained in (Θ,Γ).

Theorem 3.2 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and $(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2)\in FHS(U,\Sigma)$. Then,

1. $int_{FH}\left(0_{(U_{FH},\Sigma)}\right) = 0_{(U_{FH},\Sigma)}$ and $int_{FH}\left(1_{(U_{FH},\Sigma)}\right) = 1_{(U_{FH},\Sigma)}$,
2. $int_{FH}(\Theta_1,\Gamma_1)\subseteq(\Theta_1,\Gamma_1)$,
3. (Θ_1,Γ_1) is a fuzzy hypersoft open set if and only if $int_{FH}(\Theta_1,\Gamma_1) = (\Theta_1,\Gamma_1)$,
4. $int_{FH}\left(int_{FH}(\Theta_1,\Gamma_1)\right) = int_{FH}(\Theta_1,\Gamma_1)$,
5. If $(\Theta_1,\Gamma_1)\subseteq(\Theta_2,\Gamma_2)$, then $int_{FH}(\Theta_1,\Gamma_1)\subseteq int_{FH}(\Theta_2,\Gamma_2)$,
6. $int_{FH}\left[(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)\right] = int_{FH}(\Theta_1,\Gamma_1)\tilde{\cap} int_{FH}(\Theta_2,\Gamma_2)$.

Proof. 1 and 2 are clear from the definition of interior.

3. Let (Θ_1,Γ_1) be a fuzzy hypersoft open set. Since $int_{FH}(\Theta_1,\Gamma_1)$ is the largest fuzzy hypersoft open set contained in (Θ_1,Γ_1), $int_{FH}(\Theta_1,\Gamma_1) = (\Theta_1,\Gamma_1)$. Conversely, suppose that $int_{FH}(\Theta_1,\Gamma_1) = (\Theta_1,\Gamma_1)$. Since $int_{FH}(\Theta_1,\Gamma_1)$ is a fuzzy hypersoft open set, (Θ_1,Γ_1) is also fuzzy hypersoft open set.

4. Let $int_{FH}(\Theta_1,\Gamma_1) = (\Theta_2,\Gamma_2)$. Since (Θ_2,Γ_2) is a fuzzy hypersoft open set $int_{FH}(\Theta_2,\Gamma_2) = (\Theta_2,\Gamma_2)$, so $int_{FH}\left(int_{FH}(\Theta_1,\Gamma_1)\right) = int_{FH}(\Theta_1,\Gamma_1)$ is obtained.

5. Let $(\Theta_1,\Gamma_1)\tilde{\subseteq}(\Theta_2,\Gamma_2)$. $int_{FH}(\Theta_1,\Gamma_1)\tilde{\subseteq}(\Theta_1,\Gamma_1)$ and hence $int_{FH}(\Theta_1,\Gamma_1)\tilde{\subseteq}(\Theta_2,\Gamma_2)$ also $int_{FH}(\Theta_2,\Gamma_2)$ is the largest fuzzy hypersoft open set contained in (Θ_2,Γ_2) and $int_{FH}(\Theta_1,\Gamma_1)\tilde{\subseteq}int_{FH}(\Theta_2,\Gamma_2)$.

6. $int_{FH}(\Theta_1,\Gamma_1)\tilde{\subseteq}(\Theta_1,\Gamma_1)$ and $int_{FH}(\Theta_2,\Gamma_2)\tilde{\subseteq}(\Theta_2,\Gamma_2)$. Hence $int_{FH}(\Theta_1,\Gamma_1)\tilde{\cap}int_{FH}(\Theta_2,\Gamma_2)\tilde{\subseteq}(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)$. Since the largest fuzzy hypersoft open set contained in $(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)$ is $int_{FH}\left[(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)\right]$, $int_{FH}(\Theta_1,\Gamma_1)\tilde{\cap}int_{FH}(\Theta_2,\Gamma_2)\tilde{\subseteq}$ $int_{FH}\left[(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)\right]$.

Conversely $int_{FH}(\Theta_1,\Gamma_1)\tilde{\cap}int_{FH}(\Theta_2,\Gamma_2)\tilde{\subseteq}int_{FH}(\Theta_1,\Gamma_1)$ and $int_{FH}(\Theta_1,\Gamma_1)\tilde{\cap}int_{FH}(\Theta_2,\Gamma_2)\tilde{\subseteq}int_{FH}(\Theta_2,\Gamma_2)$. Hence $int_{FH}\left[(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)\right]\tilde{\subseteq}$ $int_{FH}(\Theta_1,\Gamma_1)\tilde{\cap}int_{FH}(\Theta_2,\Gamma_2)$.

Example 3.4 We consider the attributes in Example 3.1. Let

$$(\Theta_1,\Gamma_1)=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,2},\frac{u_2}{0,5}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,5},\frac{u_2}{0,1},\frac{u_3}{0,7}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,6},\frac{u_3}{0,2}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_2}{0,2},\frac{u_3}{0,3}\}>\end{array}\right\},$$

$$(\Theta_2,\Gamma_2)=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,3},\frac{u_2}{0,7},\frac{u_3}{0.3}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,6},\frac{u_2}{0,2},\frac{u_3}{0,7}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,7},\frac{u_3}{0,4}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_2}{0,3},\frac{u_3}{0,5}\}>\end{array}\right\},$$

Obviously, $\tilde{\tau}=\{0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)},(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2)\}$ is a fuzzy hypersoft topology on U.

1. Suppose that any $(\Theta_3,\Gamma_3)\in FHS(U,\Sigma)$ be defined as follow;

$$(\Theta_3,\Gamma_3)=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,4},\frac{u_2}{0,7},\frac{u_3}{0.6}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,8},\frac{u_2}{0,4},\frac{u_3}{0,9}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,8},\frac{u_3}{0,7}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_1}{0,4},\frac{u_2}{0,3},\frac{u_3}{0,5}\}>\end{array}\right\}$$

Then $0_{(U_{FH},\Sigma)},(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2)\subseteq(\Theta_3,\Gamma_3).$ Therefore

$int_{FH}(\Theta_3,\Gamma_3)=0_{(U_{FH},\Sigma)}\cup(\Theta_1,\Gamma_1)\cup(\Theta_2,\Gamma_2)=(\Theta_2,\Gamma_2).$

2. Suppose that any $(\Theta_4,\Gamma_4)\in FHS(U,\Sigma)$ be defined as follow;

$$(\Theta_4,\Gamma_4)=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,5},\frac{u_2}{0,2},\frac{u_3}{0,4}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,3},\frac{u_2}{0,4},\frac{u_3}{0,2}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,1},\frac{u_2}{0,2},\frac{u_3}{0,3}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_1}{0,4},\frac{u_2}{0,5},\frac{u_3}{0,1}\}>\end{array}\right\}$$

Now we find the complement of $(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2),$

$$(\Theta_1,\Gamma_1)^c=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,8},\frac{u_2}{0,5},\frac{u_3}{1}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,5},\frac{u_2}{0,9},\frac{u_3}{0,3}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,4},\frac{u_2}{1},\frac{u_3}{0,8}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_1}{1},\frac{u_2}{0,8},\frac{u_3}{0,7}\}>\end{array}\right\},$$

$$(\Theta_2,\Gamma_2)^c=\left\{\begin{array}{l}<(\alpha_1,\beta_1,\gamma_2,\delta_1),\{\frac{u_1}{0,7},\frac{u_2}{0,3},\frac{u_3}{0.7}\}>,\\ <(\alpha_1,\beta_1,\gamma_2,\delta_3),\{\frac{u_1}{0,4},\frac{u_2}{0,8},\frac{u_3}{0,3}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_1),\{\frac{u_1}{0,3},\frac{u_2}{1},\frac{u_3}{0,6}\}>,\\ <(\alpha_1,\beta_2,\gamma_2,\delta_3),\{\frac{u_1}{1},\frac{u_2}{0,7},\frac{u_3}{0,5}\}>\end{array}\right\},$$

$\left(0_{(U_{FH},\Sigma)}\right)^c=1_{(U_{FH},\Sigma)},\left(1_{(U_{FH},\Sigma)}\right)^c=0_{(U_{FH},\Sigma)}$

Obviously, $\left(0_{(U_{FH},\Sigma)}\right)^c,\left(1_{(U_{FH},\Sigma)}\right)^c,(\Theta_1,\Gamma_1)^c,(\Theta_2,\Gamma_2)^c$ are all fuzzy hypersoft closed sets over $(U,\tilde{\tau},\Sigma)$. Then $(\Theta_4,\Gamma_4)\subseteq\left(0_{(U_{FH},\Sigma)}\right)^c,(\Theta_1,\Gamma_1)^c,(\Theta_2,\Gamma_2)^c$. Therefore

$$cl_{FH}(\Theta_4,\Gamma_4)=\left(0_{(U_{FH},\Sigma)}\right)^c\tilde{\cap}(\Theta_1,\Gamma_1)^c\tilde{\cap}(\Theta_2,\Gamma_2)^c$$
$$=(\Theta_2,\Gamma_2)^c.$$

Theorem 3.3 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and $(\Theta,\Gamma)\in FHS(U,\Sigma)$. Then,

1. $\left(cl_{FH}(\Theta,\Gamma)\right)^c=int_{FH}\left((\Theta,\Gamma)^c\right)$,
2. $\left(int_{FH}(\Theta,\Gamma)\right)^c=cl_{FH}\left((\Theta,\Gamma)^c\right)$.

Proof. 1.

$$cl_{FH}(\Theta,\Gamma)=\tilde{\cap}\left\{(\Theta,\Gamma)\in\tilde{\tau}^c:(\Theta_2,\Gamma_2)\tilde{\subset}(\Theta,\Gamma)\right\}$$

$$\Rightarrow\left(cl_{FH}(\Theta,\Gamma)\right)^c=\left(\tilde{\cap}\left\{(\Theta,\Gamma)\in\tilde{\tau}^c:(\Theta_2,\Gamma_2)\tilde{\subset}(\Theta,\Gamma)\right\}\right)^c$$

$$=\cup\left\{(\Theta,\Gamma)\in\tilde{\tau}:(\Theta,\Gamma)^c\tilde{\subset}(\Theta_2,\Gamma_2)^c\right\}=int_{FH}\left((\Theta,\Gamma)^c\right)$$

2. $int_{FH}(\Theta,\Gamma)=\cup\left\{(\Theta,\Gamma)\in\tilde{\tau}:(\Theta,\Gamma)\tilde{\subset}(\Theta_2,\Gamma_2)\right\}$

$$\Rightarrow\left(int_{FH}(\Theta,\Gamma)\right)^c=\left(\cup\left\{(\Theta,\Gamma)\in\tilde{\tau}:(\Theta,\Gamma)\tilde{\subset}(\Theta_2,\Gamma_2)\right\}\right)^c$$

$$=\tilde{\cap}\left\{(\Theta,\Gamma)\in\tilde{\tau}^c:(\Theta_2,\Gamma_2)^c\tilde{\subset}(\Theta,\Gamma)^c\right\}=cl_{FH}\left((\Theta,\Gamma)^c\right).$$

Definition 3.6 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and $\tilde{B}\subseteq\tilde{\tau}$. $\tilde{B}$ is called a fuzzy hypersoft basis for the fuzzy hypersoft topology $\tilde{\tau}$ if every element of $\tilde{\tau}$ can be written as the fuzzy hypersoft union of elements of $\tilde{B}$.

Proposition 3.4 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and $\tilde{B}$ be fuzzy hypersoft basis for $\tilde{\tau}$. Then $\tilde{\tau}$ equals the collection of fuzzy hypersoft union of elements of $\tilde{B}$.

Proof. The proof is clear from definition of fuzzy hypersoft basis.

Example 3.5 We consider that the Example 3.1. Then $\tilde{B}=\{0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)},(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2)\}$ is a fuzzy hypersoft basis for the fuzzy hypersoft topology $\tilde{\tau}$.

Theorem 3.4 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and (Θ,Γ) be a fuzzy hypersoft set over U Then the collection $\tilde{\tau}_{(\Theta,\Gamma)}=\{(\Theta,\Gamma)\cap(\Xi,\Delta):(\Xi,\Delta)\in\tilde{\tau}\}$ is a fuzzy hypersoft topology on the fuzzy hypersoft subset (Θ,Γ) relative parameter set Γ.

Proof. 1. $0_{(U_{FH},\Sigma)},1_{(U_{FH},\Sigma)}\in\tilde{\tau}_{(\Theta,\Gamma)}$,

2. Suppose that $(\Theta_1,\Gamma_1),(\Theta_2,\Gamma_2)\in\tilde{\tau}_{(\Theta,\Gamma)}$. Then for each $i=1,2$, there exist $(\Delta_i,\Gamma_i)\in\tilde{\tau}$ such that $(\Theta_i,\Gamma_i)=(\Theta,\Gamma)\tilde{\cap}(\Delta_i,\Gamma_i)$. Then

$$(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)=\left[(\Theta,\Gamma)\tilde{\cap}(\Xi_1,\Delta_1)\right]\tilde{\cap}$$
$$\left[(\Theta,\Gamma)\tilde{\cap}(\Xi_2,\Delta_2)\right]=(\Theta,\Gamma)\tilde{\cap}$$
$$\left[(\Xi_1,\Delta_1)\tilde{\cap}(\Xi_2,\Delta_2)\right].$$

Since $(\Xi_1,\Delta_1)\tilde{\cap}(\Xi_2,\Delta_2)\in\tilde{\tau}$, we have $(\Theta_1,\Gamma_1)\tilde{\cap}(\Theta_2,\Gamma_2)\in\tilde{\tau}_{(\Theta,\Gamma)}$.

3. Let $\{(\Xi,\Delta)_k:k\in K\}$ be a subfamily of $\tilde{\tau}_{(\Theta,\Gamma)}$. Then for each $k\in K$, there is a fuzzy soft set $(\Xi_2,\Delta_2)_k$ of $\tilde{\tau}$ such that $(\Xi,\Delta)_k=(\Theta,\Gamma)\tilde{\cap}(\Xi_2,\Delta_2)_k$. Then we have,

$$\underset{k\in K}{\cup}(\Xi,\Delta)_k=\underset{k\in K}{\cup}\left[(\Theta,\Gamma)\tilde{\cap}(\Xi_2,\Delta_2)_k\right]$$

$$=(\Theta,\Gamma)\tilde{\cap}\left(\underset{k\in K}{\cup}(\Xi_2,\Delta_2)_k\right)$$

Since $\underset{k\in K}{\cup}(\Xi_2,\Delta_2)_k\in\tilde{\tau}$, we have $\underset{k\in K}{\cup}(\Xi,\Delta)_k\in\tilde{\tau}_{(\Theta,\Gamma)}$.

Definition 3.7 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and (Θ,Γ) be a fuzzy hypersoft set over U. Then the fuzzy hypersoft topology $\tilde{\tau}_{(\Theta,\Gamma)} = \{(\Theta,\Gamma)\cap(\Xi,\Delta) : (\Xi,\Delta) \in \tilde{\tau}\}$ is called fuzzy hypersoft subspace topology and $\left((\Theta,\Gamma),\tilde{\tau}_{(\Theta,\Gamma)},\Gamma\right)$ is called a fuzzy hypersoft subspace of $(U,\tilde{\tau},\Sigma)$.

Example 3.6 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U and $(\Theta,\Gamma) \in FHS(U,\Sigma)$. We consider the fuzzy hypersoft topology in Example 4 and (Θ,Γ) be defined as follow;

$$(\Theta,\Gamma) = \left\{ \begin{array}{l} < (\alpha_1,\beta_1,\gamma_2,\delta_1), \{\frac{u_1}{0,3},\frac{u_2}{0,3}\} >, \\ < (\alpha_1,\beta_1,\gamma_2,\delta_3), \{\frac{u_1}{0,6},\frac{u_2}{0,3},\frac{u_3}{0,8}\} >, \\ < (\alpha_1,\beta_2,\gamma_2,\delta_1), \{\frac{u_1}{0,2},\frac{u_3}{0,6}\} >, \\ < (\alpha_1,\beta_2,\gamma_2,\delta_3), \{\frac{u_2}{0,4},\frac{u_3}{0,5}\} > \end{array} \right\}$$

Then the collection

$$\tilde{\tau}_{(\Theta,\Gamma)} = \left\{ \begin{array}{l} 0_{(U_{FH},\Sigma)} \tilde{\cap} (\Theta,\Gamma), 1_{(U_{FH},\Sigma)} \tilde{\cap} (\Theta,\Gamma), \\ (\Theta_1,\Gamma_1) \tilde{\cap} (\Theta,\Gamma), (\Theta_2,\Gamma_2) \tilde{\cap} (\Theta,\Gamma) \end{array} \right\}$$

is a fuzzy hypersoft subspace topology and $\left((\Theta,\Gamma),\tilde{\tau}_{(\Theta,\Gamma)},\Gamma\right)$ is a fuzzy hypersoft topological subspace of $(U,\tilde{\tau},\Sigma)$.

Theorem 3.5 Let $(U,\tilde{\tau},\Sigma)$ be a fuzzy hypersoft topological space over U, $\tilde{B}$ be a fuzzy soft basis for $\tilde{\tau}$ and $(\Theta,\Gamma) \in FHS(U,\Sigma)$. Then the collection

$$\tilde{B}_{(\Theta,\Gamma)} = \{(\Theta,\Gamma) \tilde{\cap} (\Xi,\Delta) \mid (\Xi,\Delta) \in \tilde{B}\}$$

is a fuzzy hypersoft basis for the subspace topology $\tilde{\tau}_{(\Theta,\Gamma)}$.

Proof. Let (Ξ,Δ) be in $\tilde{\tau}_{(\Theta,\Gamma)}$. Then there is a fuzzy hypersoft set (Ξ_2,Δ_2) in $\tilde{\tau}$ such that $(\Xi,\Delta) = (\Theta,\Gamma) \tilde{\cap} (\Xi_2,\Delta_2)$. Since $\tilde{B}$ is a base for $\tilde{\tau}$, we can find a subcollection of $\tilde{B}$ such that $(\Xi_2,\Delta_2) = \underset{i\in I}{\cup}(k,\Gamma_3)_i$. Hence we have that

$$\begin{aligned} (\Xi,\Delta) &= (\Theta,\Gamma) \tilde{\cap} (\Xi_2,\Delta_2) = (\Theta,\Gamma) \tilde{\cap} \left(\underset{i\in I}{\cup}(k,\Gamma_3)_i\right) \\ &= \underset{i\in I}{\cup}\left[(\Theta,\Gamma) \tilde{\cap} (k,\Gamma_3)_i\right] \end{aligned}$$

which implies that $\tilde{B}_{(\Theta,\Gamma)}$ is a fuzzy hypersoft basis for the fuzzy hypersoft subspace topology $\tilde{\tau}_{(\Theta,\Gamma)}$.

4. CONCLUSION

Topology is an interesting and important field in mathematics and can provide many links between other fields of science and mathematical models. The soft set theory, initiated by Molodtsov (Molodtsov, 1999) and easily extended to many problems of social life uncertainties, has recently been studied and developed by many researchers. In the present paper, we first gave the concept of fuzzy hypersoft topology and presented its fundamental properties with some important examples. This paper also can be the initial step for studies on fuzzy hypersoft topology; for example, by using fuzzy hypersoft functions, one can study on fuzzy hypersoft continuity, separation axioms, compact spaces, connected spaces and since there are compact ties between hypersoft sets and information systems, the results deducted from the fuzzy hypersoft topological space studies can be used to strengthen these types of ties.

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Investigation of the Effects of Sodium Phenylpyruvate on Pulmonary Adenocarcinoma (A549) and Mammary Adenocarcinoma (MDA-MB-231) on Cell Lines

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Öz

Bu araştırma akciğer (A549) ve meme kanseri (MDA-MB-231) hücre hatlarında sodyum fenilpirüvatın antitümöral etkilerinin belirlenmesi amacıyla yapılmıştır. Araştırmada distile suyla sodyum fenilpirüvatın değişik yoğunluklarda çözeltileri hazırlandı. Bu çözeltilerden içerisinde $1x10^3$ adet kanser hücresi bulunan mikropleyt kuyucuklarına sırasıyla kontrol, 0.0375 mM, 0.075 mM, 0.15 mM, 0.3 mM, 0.6 mM, 1.2 mM ve 2.4 mM'lik konsantrasyonlarından 100 mikrolitre hacimlerde ilave edildi. Aynı doz sağlıklı hücrelere de uygulandı (HUVEC). Her kuyucuğa 24, 48 ve 72. saatlerde CVDK-8 cell viability test kitinden (Eco-Tech) 10'ar µL eklendi ve 1 saat sonra mikropleytlerin 450 nm dalga boyunda verdikleri absorbansları spektrofotometrede ölçülerek hücrelerin yoğunlukları belirlendi. Kontrol ve deney gruplarından elde edilen sonuçlar istatistiki yönden değerlendirildi. Deney gruplarda kontrole göre kanser hücre sayılarının azaldığı, hücre katlanma süreleriin ise arttığı tespit edildi. Kontrole göre en fazla azalma akciğer karsinom hücre hattında 2.4 mM dozda 24. saatte, meme adenokarsinom hücre hattında 2.4 mM dozda 72. saatte ve human umbilical vein endothelial cells (HUVEC) hattında 1.2 mM dozda 48. saatte olduğu tespit edildi. Sodyum fenilpirüvatın akciğer ve meme kanseri hücreleri üzerine *in vitro* şartlarda sitotoksik etki gösterdiği sonucuna varıldı.

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Abstract

This study was conducted to determine the antitumoral effects of sodium phenylpyruvate on cell lines in lung (A549) and breast cancer (MDA-MB-231). In the study, solutions of different concentrations of sodium phenylpyruvate with distilled water were prepared. In these solutions, 100 µL volumes of control, 0.0375 mM, 0.075 mM, 0.15 mM, 0.3 mM, 0.6 mM, 1.2 mM and 2.4 mM concentrations were added to the microplate wells containing 1x103 cancer cells, respectively. The same dose was applied to healthy cells (HUVEC). 10 µL of CVDK-8 cell viability test kit (Eco-Tech) was added to each well at 24, 48 and 72th hours, and after 1 hour, the absorbance of the microplates at 450 nm wavelength was measured through a spectrophotometer and the density of the cells was determined. The results obtained from the control and experimental groups were evaluated statistically. It was determined that the number of cancer cells decreased and the time of cell folding increased in the experimental groups compared to the control group. Compared to the control groups, the highest decrease was observed in the lung carcinoma cell line with a dose of 2.4 mM at the 24th hour, in the breast adenocarcinoma cell line with a dose of 2.4 mM at the 72nd hour, and in the human umbilical vein endothelial cells (HUVEC) line with a 1.2 mM dose at the 48th hour. It was concluded that sodium phenylpyruvate had an cytotoxic effect on lung and breast cancer cells *in vitro*.

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1. INTRODUCTION

Cancer is a disease that is characterized by the uncontrolled and abnormal reproduction of cells. 70-75% of cancer is caused by chemical carcinogens, and the remainder by irritation (physical) and viruses (Dogan, 2017; Dogan, 2016). Genetic predisposition is also important in the formation of cancer. In 2018, 2.09 million people worldwide were diagnosed with lung cancer. This number constitutes 11.6% of all the detected cancer cases. 1.76 million people died of lung cancer worldwide in 2018 (Herreros-Pomares et al., 2021). Breast cancer is the second deadliest type of cancer in women in the United States. Thanks to the treatments applied for breast cancer, the survival rate has increased from 75% to 90%. In 10-15% of diagnosed breast cancer cases, there are no estrogen and progesterone receptors and HER2 protein is negative. The 10-year recurrence-free rate in this type of breast cancer is 97% (Bussard et al., 2021). There is a close relationship between the survival rate of breast cancer and socioeconomic status in Singapore (Wong et al., 2021). Delayed diagnosis and development of resistance to drugs in cancer stem cells increase the death rate in cancer (Herreros-Pomares et al., 2021). Detection of mutated genes serves a function to understand the biology of cancer. However, the investigation of the cancer treatments in terms of genes alone may not solve the problem completely.

Phenylpyruvic acid is chemical 2-oxo-3-phenylpropanoic acid. It is chemically synthesized. Microorganisms such as *Proteus* that synthesize the deaminase enzyme, from alpha-keto acid from amino acids (phenylalanine is converted to phenylpyruvic acid). *P. vulgaris* strains have been shown to have high deaminase activity (Coban et al., 2014). Recombinant *Escherichia coli BL21* (DE3) strain was obtained by transferring the membrane bound L-amino acid deaminase (L-AAD) gene in *Proteus mirabilis KCTC 2566*. With the help of this recombinant *E. coli*, phenylpyruvic acid could be produced from L-phenylalanine in high yield. This method does not release toxic products for the environment (Hou et al., 2015). Phenylpyruvic acid could be produced with 98% yield by oxidizing D,L-phenylalanine with D-amino acid oxidase (DAAO)/catalase enzyme obtained from *Trigonopsis variabilis* (Ferandez-Lafuente et al., 1998). The presence of 13-D-glucopyranoside, which is an enolic derivative of phenylpyruvic acid, has been demonstrated in the water extract of *Aspalathus linearis*. Bacteria and plants can perform the biosynthesis of important aromatic amino acids L-phenylalanine and L-tyrosine by using phenylpyruvic acid in the shikimic acid pathway (Marais et al., 1996). Phenylpyruvic acid is found in the urine of patients with phenylketonuria (Marais

et al., 1996; Blau et al., 2010). Phenylketonuria, an autosomal recessive disease, has not the enzyme phenylalanine hydroxylase. Since phenylalanine cannot be converted to tyrosine, the level of tyrosine in the body decreases (Blau et al., 2010; Rosa et al., 2012). The levels of phenylalanine and its metabolites such as phenylpyruvic acid, phenyllactic acid and phenylacetic acid increase in blood and tissues. These metabolites are excreted in the urine (Blau et al., 2010; Rosa et al., 2012).

Alpha-keto acids (R-COCOOH) are compounds used in the production of amino acids, food additives, medicine and pesticides. Phenylpyruvic acid is an alpha-keto acid (Coban et al., 2014). Today, more than 10.000 tons of phenylpyruvic acid is produced in the world. Phenylpyruvic acid is used in the pharmaceutical, food and chemical industries (phenylalanine and phenyllactic acid synthesis, etc.). Aspartame, a sweetener, is obtained from phenylalanine (Hou et al., 2015; Li et al., 2017). Phenylpyruvic acid and other alpha-keto acids are added to foods as flavor enhancers. Phenylpyruvic acid is used to develop specific odor and taste in cheese and wine production. It is included in the diets of the patients with renal failure to reduce the accumulation of urea in the body. It is also recommended to be added to poultry feeds to prevent excessive nitrogen excretion with fertilizers. Phenylpyruvic acid is searched in the urine for the diagnosis of phenylketonuria (Coban et al., 2014). High oral doses of phenylpyruvic acid can cause irritation and inflammation in the digestive tract.

Phenylalanine is transaminated into phenylpyruvic acid (with pyruvic acid). Phenylpyruvic acid is reduced to phenyllactic acid by forming a hydroxy group with the enzyme lactate dehydrogenase. Phenylpyruvic acid synthesis could be increased by transamination by adding phenylalanine to the growth medium of *L. plantarum*. Transamination is an important step in the conversion of phenylalanine to phenyllactic acid by *L. sanfranciscensis DSM20451T* and *L. plantarum TMW1.468* (Li et al., 2017; Valerio et al., 2016). Phenylpyruvic acid and phenyllactic acid are oxidized to phenylacetic acid in the organism. This product is conjugated with amino acids and excreted from the body (Fernandez-Lafuente et al., 1998). A portion of phenyllactic acid that is not excreted can be used in protein synthesis after conversion to phenylalanine via phenylpyruvic acid (Eıdusont & Dunn, 1956). The reduction of aromatic alpha-keto acids to aromatic lactic acids in mammalian tissues is accomplished by 80% aromatic alpha-keto acid reductase and 20% lactate dehydrogenase. Lactate dehydrogenase also catalyzes the reduction of p-hydroxyphenylpyruvic acid and phenylpyruvic acid (Webert & Zannonı, 1966).

Warburg effect is seen in cancer cells. Pyruvic acid, which is produced as a result of glucose metabolism, is converted to lactic acid by the enzyme lactate dehydrogenase. The lactic acid is converted to alanine and glucose. In this way, cancer cells obtain the building blocks (amino acids and DNA) and energy they need. Since phenylpyruvic acid uses the same enzyme in its metabolism, it can inhibit the production of lactic acid that occurs in cancer cells. Therefore, the formation of glycine, glutumate and aspartate synthesized from alanine and glucose can be suppressed. This may cause an antitumoral effect. In this study, it was aimed to investigate the antitumoral effects of sodium phenylpyruvate in cell cultures of lung adenocarcinoma (A549) and breast adenocarcinoma (MDA-MB-231).

2. MATERIALS AND METHODS

Study design

Sodium phenylpyruvate (MA 186.14 g) was obtained from Sigma (CAS 114-76-1). In the study, the cell culture method by Doğan and Mutlu (Doğan & Mutlu, 2019, s. 361-370). was used by modifying it to the laboratory.

Lung adenocarcinoma (A549), mammary adenocarcinoma (MDA-MB-231), and normal healthy primary cell (HUVEC) lines (Kafkas University Central Research Laboratory) were inoculated into DMEM (Dulbecco's Modified Eagle Medium, Sıgma) containing 10% FBS (Fetal Bovine Serum, Sıgma) and 1% antibiotic (Penstrep, Thermo Fisher) and incubated at 37 °C in a free humidity incubator containing 5% CO_2. After incubation, cells that grew at the rate of 70% were removed by trypsin (Sigma) and each well was inoculated into microplates containing $1x10^3$ cells. Cell lines were incubated for 48 hours under the same conditions. At the end of the period, control, 0.0375 mM, 0.075 mM, 0.15 mM, 0.3 mM, 0.6 mM, 1.2 mM and 2.4 mM doses and 100 µL volumes from daily prepared solutions of sodium phenylpyruvate were added to the microplate wells containing 1x103 cells, respectively. The same inoculation was done on normal healthy cells. Low doses of phenylpyrvate were tested in cell culture (lung cancer cell) and effective doses used were determined. To determine the densities of the cells in the wells at 24, 48 and 72^{th} hours, 10 µL of CVDK-8 Cell Viability Test kit (Eco-Tech) was added to each well and 1 hour later, the absorbance of the microplates was measured at 450 nm in the spectrophotometer.

Determination of doubling times of cells

Cell densities at the inoculation time of cell lines inoculated into wells were determined using the formula time · log (2) / log (final concentration) – log (first concentration) together with the cell densities obtained at the end of 72 hours of experimental applications (Roth, 2006).

Statistical analysis

Data set was created by transferring absorbance values and cell folding times to the software IBM SPSS 26.0.0 (IBM SPSS, 2017). Shapiro-Wilk normality test first was applied to the created data set, and then variance homogeneity test was applied to the obtained results. According to the normality results of the data, Post-Hoc tests were performed in accordance with the parametric data in line with the results of the One-Way ANOVA test (Bonferroni or Tamhane's T2 PostHoc). $P<0.05$ was considered significant in all statistical analyzes. The absorbance values of the cell densities obtained from the experimental groups were calculated compared to the control groups. Mean ± standard deviation values were used because the obtained data are parametric in cell density percentage and doubling time figures.

3. RESULTS

The densities of lung adenocarcinoma cell (A549), breast adenocarcinoma cell (A549) and normal healthy primary cell (HUVEC: Human umbilical vein endothelial cells) 24, 48 and 72 hours after the administration of sodium phenylpyruvate are presented in Figures 1, 2 and 3, respectively. It was determined that the cell densities of all groups decreased according to the dose and time compared to the control group.

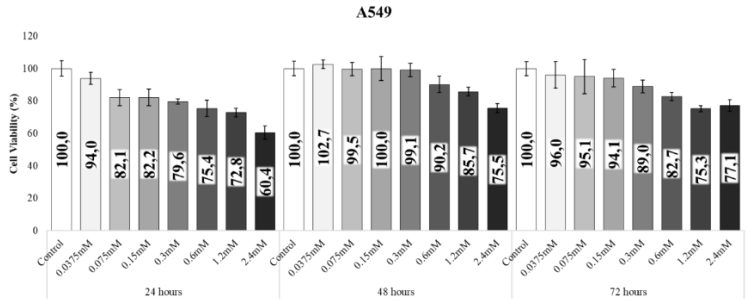

Figure 1. Effects of sodium phenylpyruvate on the densities of lung adenocarcinoma cell (A549) 24, 48 and 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (the cell density of each hour group is compared with its control group).

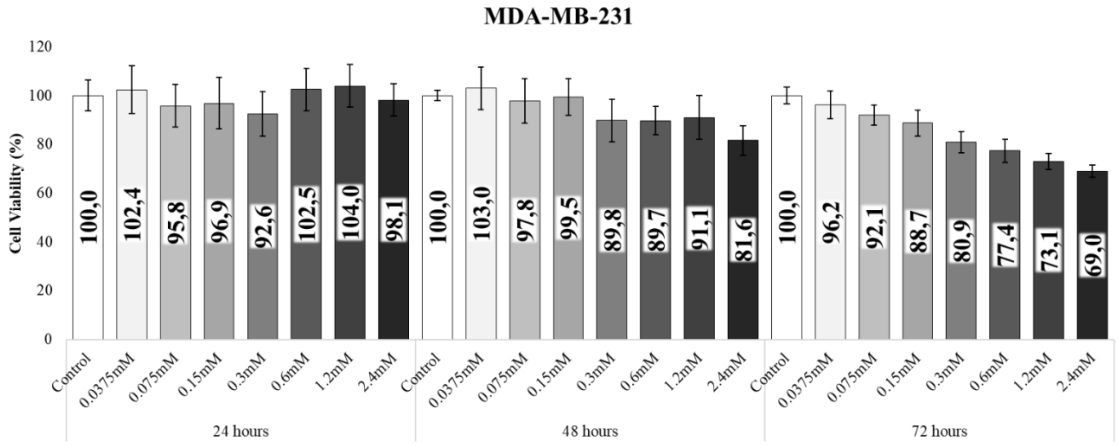

Figure 2. Effects of sodium phenylpyruvate on the densities of breast adenocarcinoma cells (MDA-MB-231) 24, 48 and 72 hours after the application at concentrations varying between 0.0375-2.4 mM (the cell density of each hour group is compared with its control group).

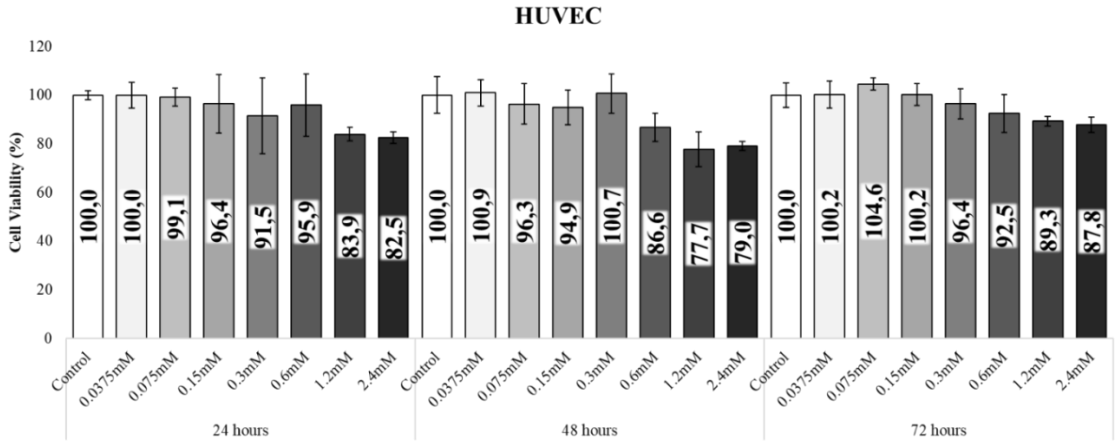

Figure 3. Effects of sodium phenylpyruvate on the densities of HUVEC 24, 48 and 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (the cell density of each hour group is compared with its control group).

The effects on the folding of Lung (A549), breast adenocarcinoma and normal healthy primary cells 24, 48 and 72 hours after the administration of sodium phenylpyruvate are

presented in Figures 4, 5 and 6, respectively. In general, the rise of the dose increases the doubling times in the groups.

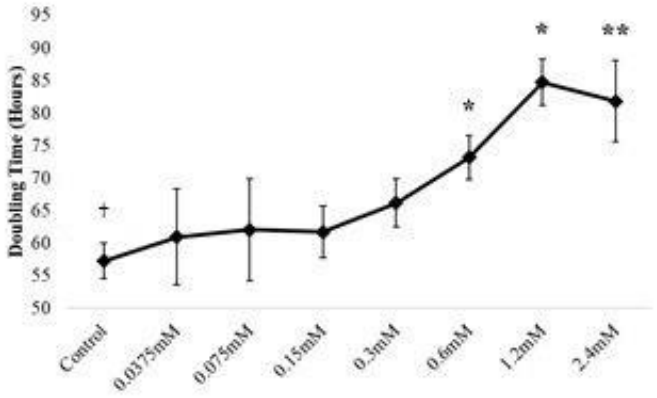

Figure 4. Effects of sodium phenylpyruvate on the folding times of lung adenocarcinoma cells (A549) 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (One-Way ANOVA: $p<0.001$, PostHoc Tests (Bonferroni/Tamhane's T2): †-*$p<0.001$, †-**$p<0.01$).

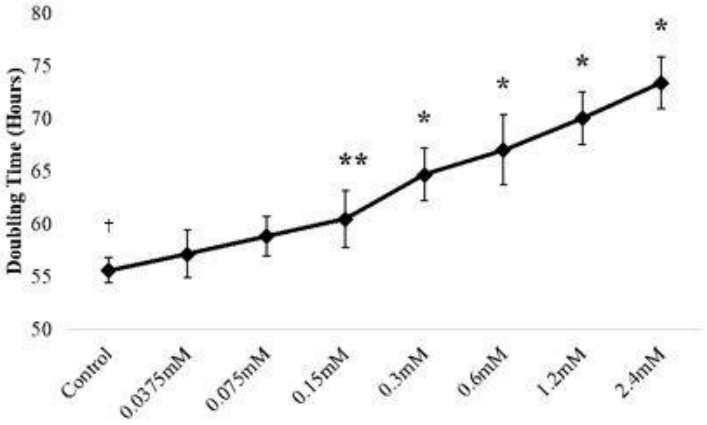

Figure 5. Effects of sodium phenylpyruvate on the folding times of mammary adenocarcinoma cells (MDA-MB-231) 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (One-Way ANOVA: $p<0.001$, PostHoc Tests (Bonferroni/Tamhane's T2): †-*$p <0.001$, †-**$p<0.01$).

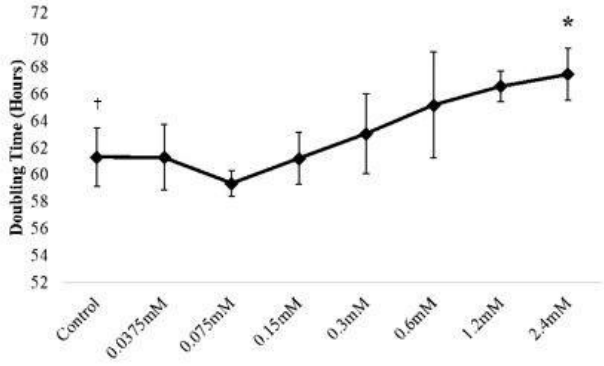

Figure 6. Effects of sodium phenylpyruvate on the folding times of HUVEC 72 hours after the application at concentrations ranging from 0.0375-2.4 mM (One-Way ANOVA: p<0.001, PostHoc Tests (Bonferroni/Tamhane's T2): †-*p<0.001).

A statistical comparison of the effects of sodium phenylpyruvate on the densities of lung adenocarcinoma, breast adenocarcinoma (A549) and normal healthy primary cell 24, 48 and 72 hours after the application of its concentrations ranging from 0.0375-2.4 mM is presented in Table 1, 2 and 3.

Table 1. Statistical evaluation of the effects of sodium phenylpyruvate doses on densities in lung carcinoma cell lines.

Dose (mMol)	Lung adenocarcinoma (A549) densities			P-values
	24 Hours	48 Hours	72 Hours	
Control (0)	†0.479±0.026	†0.444±0.022	†0.446±0.021	
0.0375	0.450±0.020	0.456±0.013	0.428±0.040	*NSD*‡
0.075mM	*0.393±0.027	0.442±0.020	0.424±0.052	*NSD*‡
0.150 mM	*0.382±0.010[a]	0.444±0.036[d]	0.420±0.026	*p<0.05*‡
0.3 mM	*0.381±0.008[a]	0.440±0,020[c]	0.397±0,020	*p<0.001*‡
0.6 mM	*0.361±0.027[a]	0.400±0,024[d]	**0.369±0.012	*p<0.05*‡
1.2 mM	*0.349±0.015[a]	**0.381±0.013[c]	*0.336±0.009	*p<0.001*‡
2.4 mM	*0.289±0.022[a]	*0.335±0.014[c]	*0.344±0.017[c]	*p<0.001*‡
p-values	*p<0.001*‡	*p<0.001*‡	*p<0.001*‡	

‡One-Way ANOVA, PostHoc Tests (Bonferroni/Tamhane's T2): [ab, af, bf, eb, df]p<0.001, [ac, ae]p<0.01, [ad, bc, ce, cd]p<0.05, †-*p<0.001, †-**p<0.01, †-***p<0.05, NSD: No significant difference (Dots were used to indicate statistical differences for horizontal direction and letters for vertical direction). Symbols also apply to Tables 1, 2 and 3.

Table 2. Statistical evaluation of the effects of sodium phenylpyruvate doses on densities in breast adenocarcinoma cell lines.

Dose (mMol)	Breast adenocarcinoma (MDA-MB231) densities			P-values
	24 Hours	48 Hours	72 Hours	
Control (0)	0.851±0.058	†1.055±0.025	†1.038±0.038	
0.0375	0.871±0.090[a]	1.086±0.099[b]	0.999±0.063[d]	*p<0.001*‡
0.075mM	0.815±0.079[a]	1.031±0.104[b]	***0.956±0,045[c]	*p<0.001*‡
0.150 mM	0.824±0.095[a]	1.049±0.085[b]	*0.921±0.058[c]	*p<0.001*‡
0.3 mM	0.787±0.083[a]	0.947±0.098[c]	*0.840±0.049[e]	*p<0.01*‡
0.6 mM	0.872±0,083	0.946±0066[a]	*0.803±0,053[c]	*p<0.01*‡
1.2 mM	0.884±0,079[a]	0.960±0.101[d]	*0.759±0.036[b]	*p<0.001*‡
2.4 mM	0.835±0.060[c]	**0.860±0.069[b]	*0.717±0.029[a]	*p<0.001*‡
p-values	*NSD*‡	*p<0.001*‡	*p<0.001*‡	

‡One-Way ANOVA, PostHoc Tests (Bonferroni/Tamhane's T2): [ab, af, bf, eb, df]p<0.001, [ac, ae]p<0.01, [ad, bc, ce, cd]p<0.05, †-*p<0.001, †-**p<0.01, †-***p<0.05, NSD: No significant difference (Dots were used to indicate statistical differences for horizontal direction and letters for vertical direction). Symbols also apply to Tables 1, 2 and 3.

Table 3. Statistical evaluation of the effects of sodium phenylpyruvate doses on densities in HUVEC cell lines.

Dose (mMol)	HUVEC densities and hours			P-values
	24 Hours	48 Hours	72 Hours	
Control (0)	0.360±0,008	†0.529±0.044	†0.717±0.040	
0.0375	0.360±0.022[a]	0.534±0,032[b]	0.718±0,045[f]	*p<0.001*‡
0.075mM	0.357±0.015[a]	0.510±0.050[e]	0.750±0.019[b]	*p<0.001*‡
0.150 mM	0.347±0.050[a]	0.502±0.042[b]	0.719±0.037[f]	*p<0.001*‡
0.3 mM	0.329±0.063[a]	0.533±0.047[b]	0.691±0.050[f]	*p<0.001*‡
0.6 mM	0.345±0.053[a]	0.459±0.034[d]	0.663±0.063[f]	*p<0.001*‡
1.2 mM	0.302±0.011[a]	*0.411±0.042[e]	0.640±0.016[b]	*p<0.001*‡
2.4 mM	0.297±0.010[a]	**0.418±0.011[b]	***0.630±0025[f]	*p<0.001*‡
p-values	*NSD*‡	*p<0.001*‡	*p<0.001*‡	

‡One-Way ANOVA, PostHoc Tests (Bonferroni/Tamhane's T2): [ab, af, bf, eb, df]p<0.001, [ac, ae]p<0.01, [ad, bc, ce, cd]p<0.05, †-*p<0.001, †-**p<0.01, †-***p<0.05, NSD: No significant difference (Dots were used to indicate statistical differences for horizontal direction and letters for vertical direction). Symbols also apply to Tables 1, 2 and 3.

4. DISCUSSION

Cell culture and animal experiments are used in drug development studies for cancer. Cell cultures reduce the number of animals to be used in research. This is one of the important advantages of two-dimensional (2D) cell culture that grows in flat layers on plastic surfaces (Herreros-Pomares et al., 2021; Foglietta et al., 2020). However, two-dimensional cell cultures cannot fully simulate the natural environment in studies with cancer stem cells. For this reason, three-dimensional cell cultures (3D) taken from cancer cell lines and enriched with cancer stem cells have been developed. 3D cell cultures are better at imitating tumor cells than 2D cell cultures. However, 2D cell cultures are easier to prepare and cost-effective (Herreros-Pomares et al., 2021). In this study, 2D cell culture was used due to its advantages.

Phenylpyruvic acid can be produced by some microorganisms. In plants and bacteria, it is used in the shikimic acid pathway for the biosynthesis of L-phenylalanine and L-tyrosine (Coban et al., 2014; Hou et al., 2015; Fernandez-Lafuente et al., 1998; Marais et al., 1996). Shikimic acid hardly penetrates into bacteria. Therefore, rumen mıcroorganism (*Aerobacter aerogenes, Pseudomonas aeruginosa, Bacillus subtilis, Saccharomyces cerevisiae*) synthesize small amounts of aromatic amino acids. It has been reported that phenylalanine from phenylpyruvic acid and tryptophan from indolpyruvic acid can be produced by rumen bacteria, protozoa and their mixtures (Khan et al., 2002).

Phenylalanine is an essential amino acid that constitutes 4-6% of the amino acid content of proteins. Phenylalanine is primarily metabolized by hydroxylation (75%) to tyrosine. Phenylalanine is converted to phenylethylamine by quadradic decarboxylation. This substance is converted to phenylacetylglutamate over phenylacetate and excreted in the urine. Phenylalanine can also be metabolized by transamination. Phenylpyruvate emerges as a result of this reaction. Phenylpyruvate is converted to either o-hydroxyphenylacetate or phenylactate. It can be excreted by conversion to phenylacetylglutamate directly through phenylacetate (Erdem, 2013). Significant amounts of phenylpyruvic acid and phenylacetic acid were detected in the urine of rabbits after oral or subcutaneous administration of 2.0 g of phenylalanine or equivalent amount of phenylpyruvic acid. In the metabolism of phenylalanine, oxidation events occur primarily in the side chains. The benzene ring is less oxidized. Benzene is then decomposed in a small amount (Chandler & Lewis, 1932). It has been shown that some microorganism (*Lactobacillus plantarum CECT-221*) produce phenyllactic

acid (antibacterial) when phenylpyruvic acid is added to the medium (Li et al., 2007; Valerio et al., 2016; Rodríguez-Pazo et al., 2013).

Significant intrinsic targets (epigenetic changes, transcriptional and signal transduction dysregulation, abnormal pathway and metabolic activity) and extrinsic targets (changes in the tumor microenvironment, and differentiation and composition of immune cells and fibroblasts) of drugs have been identified in the tumor cell. Detection of the characterization of cancer genomes has made great advances in the development of drug targets. However, the function of most cancer genes has not been fully elucidated. Persistent cells are usually able to escape chemotherapeutic stress and in some cases become reresponsive to therapy after drug discontinuation. This means that there is a non-genetic mechanism of drug tolerance (Hahn et al., 2021). Therefore, targeting genes alone may not be sufficient for most cancer treatments. Metabolic vulnerabilities are important among non-gene therapeutic targets. In this study, non-gene metabolic vulnerabilities of cancer cells were targeted.

Since cancer cells can constantly reproduce, their metabolism is fast. Therefore, cancer cells are addicted to glucose, glutamine, glycine, serine and aspartate. These amino acids (glutamine, glycine, aspartate) are involved in the biosynthesis of structures such as protein, pyrimidine, purines, phospholipids and glutathione. In some tumors, increased expression of serine synthetase and phosphoglycerate dehydrogenase has been known. The suppression of serine metabolism in these tumor cells leads to tumor regression (Hahn et al., 2021).

Aromatic keto acid reductase and lactate dehydrogenase enzymes are involved in the reduction of alpha-keto acids such as phenylpyruvic acid (eg, to phenyllactic acid) in mammalian tissues. The lactate dehydrogenase enzyme is inhibited by sodium dodecyl sulfate (Webert & Zannonı, 1966). NADH-dependent L-lactate dehydrogenase enzyme has been detected in *L. casei*. This enzyme catalyzes phenylpyruvic acid to phenyllactic acid. Their genes have been able to be transferred to *E. coli*. It has been shown that the recombinant *E. coli BL21* converts phenylpyruvic acid to L-phenyllactic acid (Li et al., 2018). Lactate dehydrogenase has also been demonstrated in Lactic acid bacteria *Pediococcus acidilactici DSM 20284* and lactic acid producing *Bacillus coagulans* (Mu et al., 2012). Phenyllactic acid is excreted in the body by conjugation with aliphatic amino acids.

Tumor cells metabolize glucose to pyruvic acid with the effect of Warburg, and then convert it to lactic acid through the enzyme lactate dehydrogenase. The resulting lactic acid is converted to alanine (pyruvic acid and glucose) and enters into the synthesis of some aliphatic

amino acids. These amino acids are used in DNA and glucose synthesis. When phenylpyruvic acid is given, the enzyme lactate dehydrogenase uses phenylpyruvate instead of pyruvic acid to produce phenylactate. The resulting phenylacetate is excreted by conjugation with aliphatic amino acids. Due to the decrease in the level of aliphatic amino acids, DNA synthesis can be suppressed and the growth of tumor cells can be inhibited. In this study, it was determined that phenylpyruvic acid inhibites the growth of tumor cells in lung and breast cancer cell lines. The findings strengthen the suspicion that phenylpyruvic acid can suppress the Warburg effect. Sodium phenylpyruvate showed the most tumorostatic effect in the Lung Adenocarcinoma cell line after 24 hours and in the Breast Adenocarcinoma cell line after 72 hours at a dose of 2.4 mM (Figure 1, 2, 3). It was also seen that the cell folding times increase (Figure 4, 5, 6). Statistical differences were significant between suppressions at some doses (Table 1, 2, 3). Therefore, the results obtained in the study support the hypothesis. In the study, phenylpyruvic acid reduces the density of normal and cancer cells. These effects may be due to its chemical structure. In the study, a linear curve could not be obtained in cell densities due to dose increases in some groups. This can be caused by operating errors.

4-Phenylbutyrate, similar to phenylpyruvic acid, affects various cellular processes. This substance is known as a biological response modulator that can cause cellular differentiation or apoptosis in various neoplasms such as prostate, ovarian, melanoma, glioma and leukemia. Phase I and II are on trial in treatment studies of Phenylbutyrate and its metabolic product, phenylacetate, and Hodgkin and non-Hodgkin acute myeloid leukemia (Liu et al., 2004). The chemical resemblance of phenylpyruvic acid to phenylbutyrate and its conversion to phenylacetate as a result of metabolism is consistent with the results of this study. DNA degradation initiates cancer. However, impaired metabolic functions can have a significant impact on cancer progression. The results of these studies raise the suspicion that aromatic amino acid deficiency or the inability to metabolize aromatic amino acids to alpha-keto acids may play a role in the development of tumors.

Exposure of human fibroblasts to UV in a medium with phenylpyruvic acid increases single-stranded breaks in DNA (Hargreaves et al., 2007). It was determined that phenylpyruvic acid significantly reduces the glucose-6-phosphate dehydrogenase activity in rats (Rosa et al., 2012). Cytotoxic effects of phenylpyruvic acid detected in this study may also be due to its local chemical or oxidant effects. Phenylpyruvic acid can damage cells by reacting with cell structures. Since some amount of phenylpyruvic acid is converted to phenylalanine, it can be

used in the proliferation of cells. Since some of the phenylpyruvic acid is converted to phenylalanine, it can be used as an amino acid in the proliferation of cells.

As a result, it can be suggested that sodium phenylpyruvate has an cytotoxic effect in cell culture. This may bring up the use of aromatic amino acids and phenylpyruvate (or other aromatic alfa-keto acids and their derivatives) in the treatment or the synthesis of a new group of antitumoral drugs. For this reason, it is of great importance to support or confirm the results of research with studies on many cancer cell lines and experiments on animals.

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Evaluation of Graft Harvesting Operations from Anterior and Posterior Iliac Donor Sites by Finite Element Analysis

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Öz

Greft donör bölgeleri, kemik rezervi ve fonksiyonel açıdan değerlendirildiğinde iliak bölgesi öne çıkmaktadır. Ancak, çeşitli iliak donör bölgelerinden gerçekleştirilen greft alım operasyonlarında karşılaşılan komplikasyonlar birçok araştırmacı tarafından raporlanmıştır. Bu komplikasyonları azaltmak, operasyon başarısını artırmak veya iliak bölgeyi biyomekanik açıdan derinlemesine tahlil etmek amacıyla literatürde çok sayıda modelleme ve Sonlu Elemanlar Analizi (SEA) çalışması gerçekleştirilmiştir. Ancak, anterior ve posterior iliak greft alımı cerrahi operasyonlarının biyomekanik açıdan karşılaştırılması literatürdeki eksiklerdendir. Bu çalışmanın amacı anterior ve posterior iliak donör bölgelerinden yapılan cerrahi operasyonların, hacim analizi ile sundukları kemik rezervi açısından ve SEA ile biyomekanik açıdan karşılaştırılmasıdır. Çalışmanın sonuçlarına göre, posterior iliak greft alımı, anterior operasyona göre %264 daha fazla trabeküler kemik rezervi sağlamaktadır. Bununla birlikte, kortikal kemik için bu oran %132'dir. Modeller biyomekanik açıdan karşılaştırıldığında ise, anterior osteotomi modelinde, posterior osteotomi modeline kıyasla %8,6 daha fazla maksimum von Mises gerilmesi elde edilmiştir. Elde edilen bulgular, posterior greft alımı operasyonunun morbidite oranı, eklem kırık riski ve greft rezervi açısından avantaj sunduğunu, anterior greft alımının ise eklem stabilitesi ve operasyon kolaylığı açısından tercih edilebileceğini göstermektedir.Ancak; alınan greft miktarı, hastanın kemik kalitesi, anatomik farklılıklar, yaş ve cinsiyet gibi faktörler, elde edilen sonuçları etkileyeceğinden, modelleme ve analizlerin hastaya özel olarak gerçekleştirilmesinin operasyon başarısını artıracağı değerlendirilmektedir.

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Abstract

When the graft donor areas are evaluated in terms of bone reserve and functional aspects, it can be said that the iliac site has outstanding properties. However, complications of graft harvesting operations performed from various iliac donor sites have been reported by many researchers. Numerous studies have been carried out in the literature to reduce these complications, and to increase the success of the operation. However, biomechanical comparison of anterior and posterior iliac graft

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Hemi-pelvis
Finite Element
Graft Harvesting
Donor Site

harvesting operations is one of the gaps in the literature. This study aims to assess both biomechanical behavior and bone graft reserve comparison of the two surgical operation alternatives. According to the FEA results of the study, posterior iliac graft harvesting provides 264% more trabecular bone reserve than anterior operation. However, this rate is 132% for cortical bone. When the models are compared, anterior osteotomy model has a 8.6% higher von Mises strain compared to the posterior osteotomy model. Results of the present study has shown that the region with the highest stress value in the cortical bone is the sacroiliac joint for both models. While posterior graft harvesting operation offers advantages in terms of morbidity rate, joint fracture risk and graft reserve, anterior operation can be preferred in terms of operational ease and the sacroiliac joint stability. However, since results obtained may be affected by the factors such as the amount of graft harvested, the patient's bone quality, anatomical differences, age and gender, it has been evaluated that the success of the operation may be enhanced by carrying out a patient-specific approach for modeling and analysis steps.

1. INTRODUCTION

Bone graft harvesting is a frequently used method for maxillofacial surgery and orthopaedic applications. If the adequate bone volume does not exist for dental implant fixation, harvested bone is used for reconstruction before the operation. Orthopaedic operations are other common usage areas of autologous bone transplantation in order to recover bone injuries. For the aforementioned surgical applications, the iliac crest is one of the most preferred regions due to its large trabecular bone reserve. There are two main options for iliac crest harvesting operations where can be from anterior and posterior sites. However, in the existing literature, many intraoperative and postoperative complications have been reported. It had been reported in a case report presented on iliac crest harvesting that iliac vessel injury was occurred due to the dislodgement of soft tissue retractor (Escalas & Dewald, 1977). In another study presented on the comparison of different graft reserve sites in terms of early postoperative complications, it was reported that iliac bone harvesting had shown more risk than of the rib. In intraoperative and early postoperative stages, complications such as gait disorders, more blood loss, and longer painful period were reported for iliac crest harvesting. However, even these complications, the iliac crest is one of the most popular bone harvesting sites due to its large trabecular bone reserve. Additionally, it was also documented that iliac graft harvesting showed much more advantages considering the late postoperative period (Laurie et al., 1984). Even though iliac crest bone harvesting is a frequently applied technique, it causes some serious problems such as pain, nerve and arterial injury, cosmetic deformity, blood loss and infection (Kurz et al., 1989). It was reported that the high complication rate of iliac harvesting (varies between 9.4% and 49%) could be reduced

by developing a surgery protocol (Banwart et al., 1995). These complications can be mainly classified as posterior and anterior. The complications of posterior iliac graft harvesting had been reported as local hematoma, severe retroperitoneal blood loss, superficial infection and osteomyelitis, bowel hernia and perforation, sacro-iliac joint instability, ectopic bone formation, arterial and nerve injuries, etc. On the other hand, drawbacks of anterior graft operation had been documented as herniation of muscle and abdominal tissues,heterotropic bone formation, ilium fracture, pain, infection, etc.(Dosoglu et al., 1998). In the literature, some limits have been suggested in terms of graft harvesting operations. Anterior Superior Iliac Spine (ASIS) and Posterior Superior Iliac Spine (PSIS) points are two main references which are recommended for anterior and posterior operations, respectively (Kilinc et al., 2017). These reference points are of great importance in order to provide adequate distance between the osteotomy line and bone surface. In the posterior region where the iliac graft harvesting is mostly preferred, it has been stated that the sacro-iliac joint should not be damaged during the operation which causes decrease in stability of the pelvis. However, over-harvesting from the posterior region may cause pelvic instability due to ligament damage. Thus, pelvis fracture risk increases (Chan et al., 2001). In a clinical study, approximately one third of 92 patients who underwent iliac graft harvesting operation reported their complaints and minimally invasive surgery had been recommended by planning the osteotomy (Hill et al., 1999). It has been reported that the periosteum and muscle joints are exposed to a considerable dissection in the conventional iliac graft harvesting operations, and complications can be significantly reduced via less invasive methods such as trephine and bone abrasion techniques (Abdulrazaq et al., 2015; Burstein et al., 2000). In another study conducted on minimally invasive surgical techniques, it has been reported that it is necessary to improve surgical equipments in terms of clinical use (Steffen et al., 2000).

There are many finite element studies on this subject in the literature. Kawahara et al. (2003) applied 480 N vertical force to the half of the upper surface of the L3 vertebra in the spinopelvic finite element semi-model, and performed the analyzes by fixing it from the lower part of the pelvis. In another study, the effect of synovial state on force transmission in the sacroiliac joint was investigated using a FEA model fixed from the distal femur and 500 N force applied from the upper surface of the L3 vertebra. It was reported that to synovial state is a very effective factor (Shi et al., 2014). In another study related to the hip joint, the hemi-pelvis model was limited from the midline and fixed from the upper part, and analyzes were

performed by applying hip force (Bachtar et al., 2006). In a Finite Element Model (SEM) established on the proximal femur and acetabulum, the ligaments were defined as spring elements and the model was fixed from the upper part and loaded from the femur shaft (Rudman et al., 2006). In a study showing that the boundary conditions significantly affect the FEA results, the behavior of the model was compared.under free and fixed boundary conditions of the ligaments (Li et al., 2007). Clinical and theoretical comparison of sacral fractures was made using the model in which force is applied from the upper part of the sacrum by fixing bilaterally from the acetabulum. Limitations of the study were noted as asymmetrical hip anatomy and neglected asymmetry-based muscle abnormalities (Linstrom et al., 2009). In the study on the application of cemented acetabular prostheses to the patient-specific pelvis bone, the pelvis was fixed from the upper part and hip force was applied (Zhang et al., 2010). It was noted that for FEM, where hip forces act through the acetabulum and are fixed from the upper part of the sacrum, the material properties should be determined individually and the forces that may cause pelvic fractures due to instability should be investigated (Bohme et al., 2012). In another study in which similar boundary conditions were preferred, a more detailed model was established by defining the ligaments. However, as the limitations, it was reported that the geometry and material properties of the pelvis and ligaments were modeled for a single situation, parametric studies were needed, and bone cartilage and ligament material properties were accepted as linear-elastic (Bohme et al., 2014). In a study where FEM boundary conditions were created by fixing the Distal Femur and by applying 600 N load to the upper surface of the Sacrum, pelvic fractures were theoretically evaluated and the importance of pelvic stability was emphasized (Lei et al., 2015).In a finite element study involving femoral and pelvis models, it was reported that small changes in the femoral neck angle can increase the loads balanced by the hip joint cartilage (Egea et al., 2014). It was stated that the method developed in the study, which was obtained with the mapping technique of the personal pelvis model without segmentation, was effective and reliable. As the boundary conditions, different forces were applied to the L5 vertebra from its upper surface (65 N, 115 N, 230 N, and 345 N) and the distal femur was fixed. The friction force between the femoral head and acetabular cartilage has been determined to be 0.01 (Salo et al., 2015).In a finite element study examining the effect of different boundary conditions on pelvic load transfer, the model with synovial connections (cartilages) and femoral contact yielded more realistic results compared to other simplified models, but the

absence of muscle elements was the main limitation of the study (Hu et al., 2017). Although there are studies in the literature in which the lower extremity was completely modeled (Filardi, 2015; Mo et al., 2017), simpler models were mostly established and the boundary conditions were achieved by fixing the sacro-iliac and pubic joints (Cardiff et al., 2014; Hsu et al., 2007; Hsu et al., 2006; Liu et al., 2015; Mircheski & Gradisar, 2016; Nie et al., 2014; Phillips et al., 2006; Phillips et al., 2007).

However, in finite element studies conducted on the iliac bone, it is seen that the FEA boundary conditions used for the pelvic region vary. While some studies have established more detailed models in which many tissues interact, some publications have required the use of simpler models and boundary conditions for various reasons. Although certain simplifications are made in determining the FEM and boundary conditions, virtual surgery simulations should be specifically evaluated for each patient using comparative studies considering clinical issues. In this study, the mechanical behavior of the two different iliac harvesting surgery methods (anterior and posterior) was compared using Finite Element analysis and clinical recommendations were made according to the results obtained.

2. MATERIAL AND METHOD

Biomedical Modelling

Artificial hemi-pelvis bone was used in this study. Computed tomography (CT) images of the hemi-pelvis bone to be grafted were obtained in DICOM format and transferred to MIMICS 21.0 (Materialise, Leuven, Belgium) program. Three-dimensional models of cortical and trabecular bones were created by processing medical images (Figure 1.a, b). Then, virtual surgical simulations of anterior and posterior graft osteotomies were performed (Figure 1.c, d).

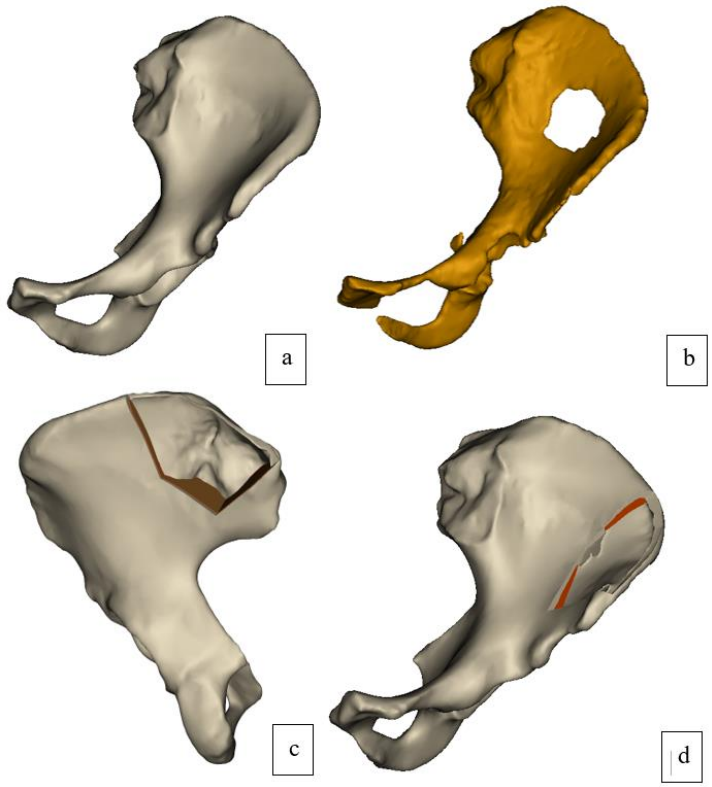

Figure 1. Hemi-pelvis bone model (a)Cortical bone (b)Trabecular bone (c)Posterior iliac graft harvested model (d)Anterior iliac graft harvested model

Finite Element Model

For the trabecular and cortical layers of the anterior and posterior models with virtual surgery, a volumetric mesh was constructed in the 3-Matic program and saved in *.cdb format. The volumetric mesh files were assembled in ANSYS FE modeler module, the regions where the boundary conditions will be applied were created as separate surfaces and the final geometry was obtained. The bone material properties are defined as linear isotropic by transferring the model to ANSYS "Static Structural" module. Elasticity modulus (E) and Poisson ratio (v) values were calculated as 15.1 GPa and 0.3 for the cortical segment, respectively, considering different values in the literature; for trabecular bone, it was accepted as 0.445 GPa and 0.22 (Cai et al., 2020; Guo & Li, 2020; Song et al., 2016). Anterior and posterior models have 407530 and 307191 10-node regular tetrahedral element (SOLID 187), respectively. The skewness ratio was obtained as 0.25 and 0.29 for the models, respectively. With reference to the many studies in the literature, the boundary conditions were applied by fixing the sacroiliac and pubic joints (Hsu et al., 2007; Hsu et al., 2006; Liu et al., 2015; Phillips et al.,

2006; Phillips et al., 2007), and applying the average hip joint strength to 2236 N (Bergmann et al., 2016).

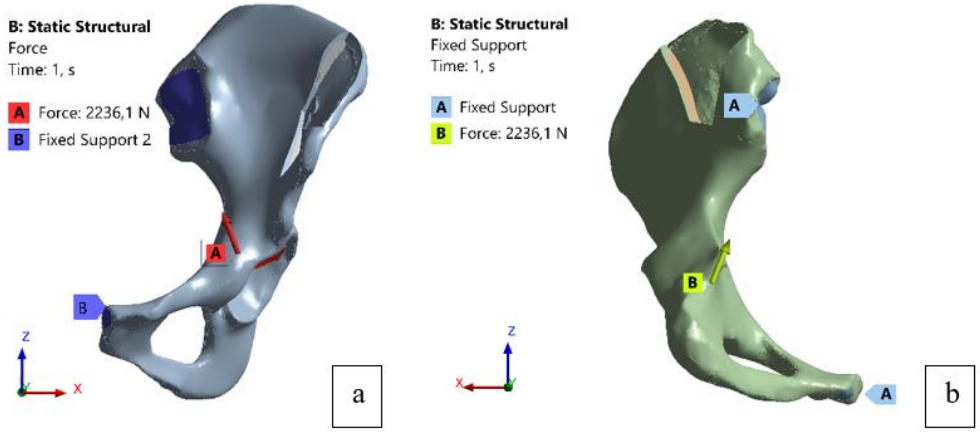

Figure 2. Boundary conditions of Finite Element Models (a)Anterior Osteotomy Model (b)Posterior Osteotomy Model

3. THE RESEARCH FINDINGS AND DISCUSSION

The bone volume amounts obtained as a result of virtual bone graft removal are given in Table 1. According to the results, the total amount of grafts taken for anterior and posterior osteotomy was calculated as 18.81 ml and 60.03 ml, respectively. In both models, the trabecular bone reserve is greater than the cortical bone. In addition, the trabecular bone obtained as a result of posterior osteotomy offers 264% more reserve compared to the other model. For the cortical bone, this rate was calculated as 132%.

Table 1. Graft Amounts Harvested From Anterior and Posterior Osteotomies

	Bone Volume (cm^3)	
	Anterior Osteotomy	Posterior Osteotomy
Cortical Bone	6,02	14,01
Trabecular Bone	12,61	46,02

According to Finite Element Analysis (FEA) results, when the stress values in the cortical bone in the models applied with the same boundary conditions were examined, it is seen (in Figure 3. a,b) that the anterior osteotomy model (AOM) has shown 8.6% more maximum von Mises stress value compared to the posterior osteotomy model (POM). However, the opposite

is true in trabecular bone. The AOM has shown 48.57% less maximum von Mises stress in trabecular bone compared to the POM (Figure 3. c,d).

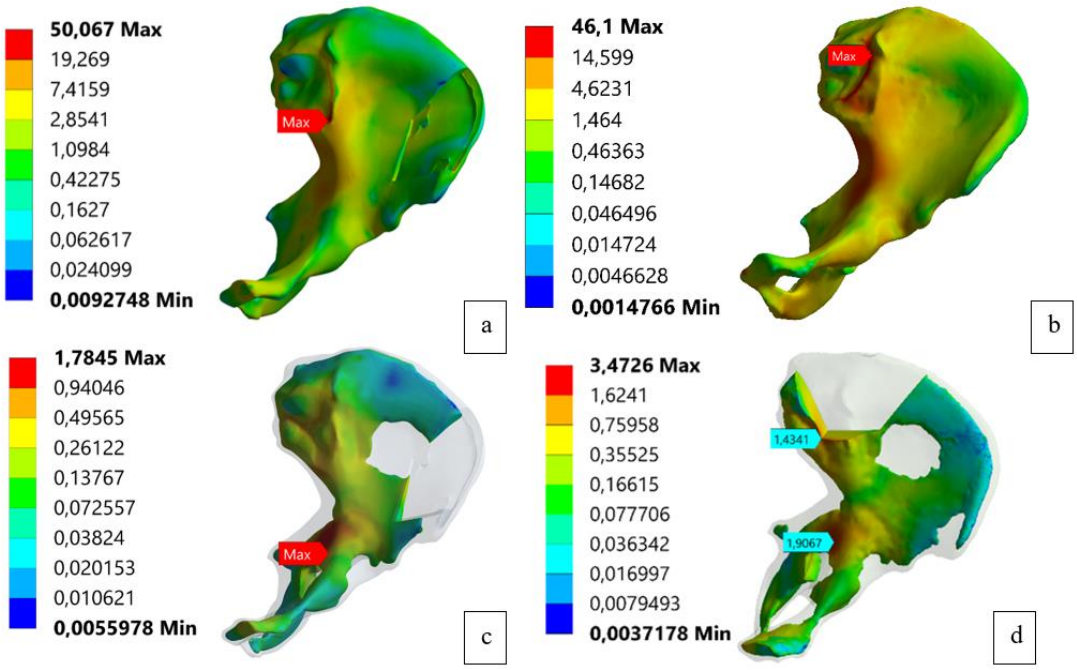

Figure 3. Von Mises stress distribution of cortical and trabecular bones (a,c) Anterior Osteotomy Model, (b,d) Posterior Osteotomy Model

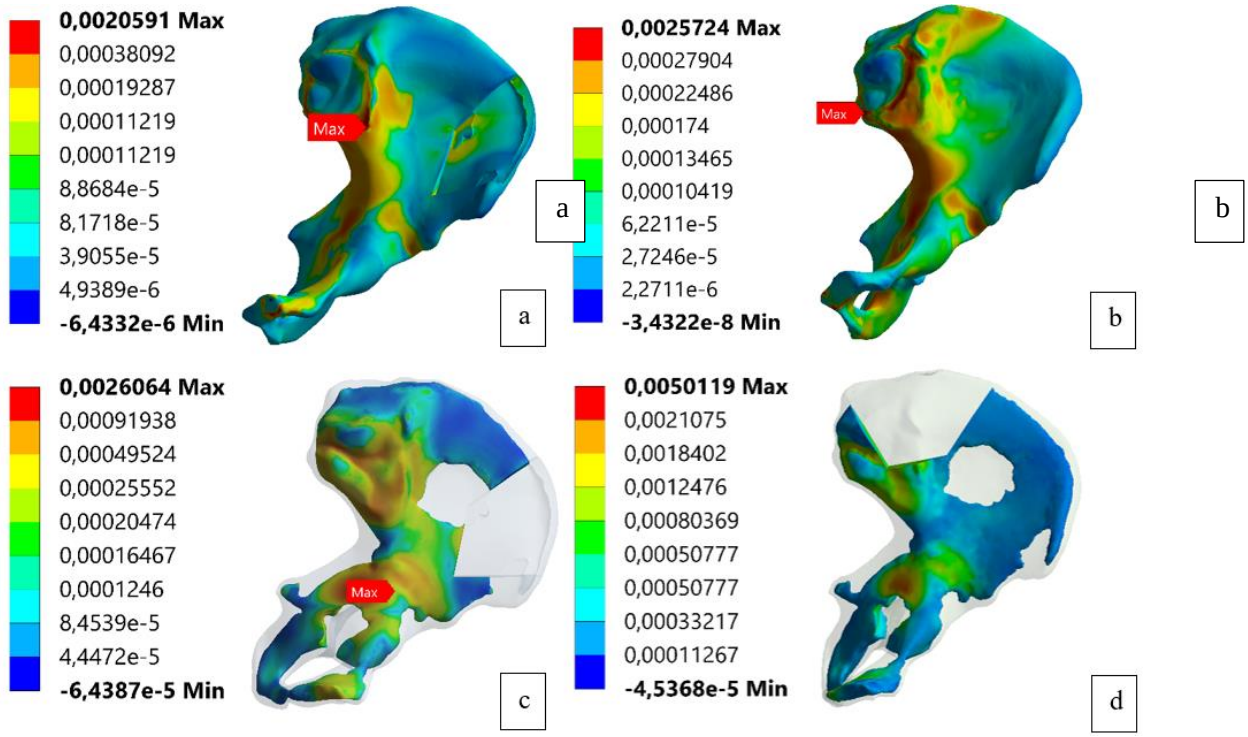

Figure 4. Principal strain distribution of cortical and trabecular bones (a,c) Anterior Osteotomy Model, (b,d) Posterior Osteotomy

When the models were examined in terms of maximum principal strains, its value in cortical bone in AOM was obtained as 2059.1 με, while it was found as 2572.4 με in POM. The maximum principal unit shape change values obtained in trabecular bone were 2606.4 με and 5011.1 με, respectively.

More accessibility of the anterior iliac crest may be a reason for preference, but it has been reported in the literature that the morbidity rate in the anterior donor site is higher than the posterior donor site and the posterior graft harvesting carries less risk of complications (Ahlmann et al., 2002; Kessler et al., 2005). The findings obtained within the scope of this study support these reports. In both models, the region with the highest stress value in the cortical bone is the sacroiliac joint. This can be explained by joint fracture, which is a complication of iliac graft operation (Suda et al., 2019). In addition, it can be said that osteotomy planes are associated with the possibility of damage in terms of intersection points. Especially, these intersection lines to be obtained close to the ASIS and PSIS references include stress concentrations and increase the probability of damage. On the other hand, in terms of maximum principal unit shape changes, values obtained in the same region can be associated with sacroiliac joint instability (COVENTRY & TAPPER, 1972). Pain, a common postoperative complication (Cansiz et al., 2019; Kono et al., 2018), can be reduced by reconstructing the graft site with bone cement (Zhang et al., 2018).

Since the study is conducted under certain constraints, a more comprehensive biomedical model can increase the sensitivity of the results obtained. Using the references of the biomechanical studies in the literature (Henyš & Čapek, 2019; Sensoy et al., 2018; Şensoy et al., 2019; Şensoy et al., 2020; Wang et al., 2019) the bone material model used in this study assumed as linear isotropic and homogeneous, which is one of the limitations in the present study. Another limitation is the absence of a soft tissue model and direct application of muscle strength to the model. In future studies, establishing the material model as heterogeneous and anisotropic (Enns-Bray et al., 2016; Guo & Li, 2020; Kharmanda et al., 2020; Latypova et al., 2017) will better reflect the actual tissue properties of the patient, thus the accuracy of the results can be increased. Since the results obtained will be affected by factors such as the amount of grafts harvested, the bone quality of the person, anatomical differences, age and gender, it is considered that the success of the operation can be increased patient-specific modeling, patient-specific virtual surgery as well as the patient-specific FEA. On the other

hand, the osteotomy guideline-assisted surgical approach suggested in the literature for different anatomical regions (Sensoy et al., 2018; Zhang et al., 2019) can also be applied for this operation. Therefore, the minimally invasive operation to be performed can reduce the pain and the risk of pelvic fracture after the operation. In future studies, considering the aforementioned limitations and the actual surgical facts, it is planned to evaluate the proposed osteotomy guideline-supported graft operation with FEA, both with in-vitro experiments.

4. RECOMMENDATIONS

When anterior and posterior osteotomy alternatives are evaluated, it is seen that both methods have prominent advantages and risks. While anterior graft removal offers advantages in terms of ease of operation and joint stability, posterior graft removal operation can be preferred in terms of joint fracture risk and graft reserve. ASIS and PSIS reference points play an important role in relation to the amount of graft to be used in this choice. Because the osteotomy starting point, which can be preferred close to these points, will cause the iliac bone to be damaged by the inguinal and iliolumbar ligaments. However, since the operations are performed individually, the geometry of the incision line and the amount of grafts taken are parameters that can affect the results. Here, determining the method with minimum risk for the patient as a result of personal analyzes may be an approach that increases the success of the operation. In addition, since the presence of sharp edges at the intersection areas of the osteotomy plane causes stress accumulation, obtaining the corner regions in curvilinear form with a personalized surgical guide in these areas may be an approach to reduce the risk of fracture.

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Investigation of the Effect of Boric Acid on Antioxidant System, HDL Levels and PON Activity in Rats Fed with High Fat Diet

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Öz

Son yıllarda yapılan araştırmalar, bu çağın en tehlikeli ve karmaşık sağlık sorunlarından biri olduğu bilinen obezitenin tedavisinde bor bileşiklerinin etkili olabileceğini ortaya koymuştur. Bu çalışma da diyetteki bor moleküllerinin obezite üzerindeki tedavi edici etkisi araştırılmıştır. Bu amaçla, yüksek yağlı diyetle beslenen farelerde bor bileşiklerinin Toplam Antioksidan Seviye (TAS), Toplam Oksidan Seviye (TOS), Yüksek Yoğunluklu Lipoprotein (HDL) seviyeleri ve Paraoksonaz (PON) aktivitesi üzerine etkisini incelenmiştir. Çalışma materyali, ortalama canlı ağırlığı 226,95 ± 5,75 g olan 4-5 aylık 40 Sprague Dawley sıçanıydı. Hayvanlar Grup I (normal diyet), Grup II (Yüksek yağ), Grup III (Yüksek yağ+ Borik Asit) ve Grup IV (Borik Asit) olmak üzere 4 gruba ayrıldı. Denemeden altı hafta sonra hayvanlardan kan örnekleri alındı ve örneklerden TAS, TOS, HDL seviyeleri ve PON aktivitesi ölçüldü. Ayrıca hayvanların canlı ağırlık değişimleri kaydedildi. Çalışma sonunda içme suyuna ek olarak verilen borik asidin TAS düzeyini ve PON aktivitesini azalttığını ancak istatistiksel olarak anlamlı olmadığını, TOS ve HDL düzeylerini yükselttiğini belirledik. Grup 3'de kontrol grubu dışında ki gruplardan daha fazla PON aktivitesi saptandı. Ayrıca borik asit uygulanan grubun ağırlık ortalaması da düşürdüğü gözlendi. Sonuç olarak borik asit ve yüksek yağlı diyet antioksidan sistem üzerine olumlu etki göstermedi fakat HDL düzeyinin artmasına ve PON aktivitesinin azalmasına neden oldular. Ratlar borik asit ve yüksek yağlı ile birlikte beslendiğinde PON aktivitesinde bir nispi artış gözlendi. PON ve HDL biribirinden bağımsız hareket ettiler. Ayrıca borik asit vücut ağırlığını düşürmede etkili olabilir

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Abstract

Recent studies have revealed that boron compounds can be effective in the treatment of obesity, which is known to be one of the most dangerous and complex health problems of this age. In this study, the therapeutic effect of boron molecules in the diet on obesity was investigated. For this purpose, the effects of boron compounds on Total Antioxidant Status (TAS), Total Oxidant Status (TOS), High Density Lipoprotein (HDL) levels and paraoxonase (PON) activity were investigated in mice fed a high-fat diet. The study material was 40 Sprague Dawley rats 4-5 months old with a mean live weight of 226.95 ± 5.75 g. Animals were divided into 4 groups as Group I (normal diet), Group II (High fat), Group III (High fat + Boric Acid) and Group IV (Boric Acid). Six weeks after the experiment, blood samples were taken from the animals and TAS, TOS, HDL levels and PON activity were measured from the samples. An increase in PON activity was observed when rats were fed a high-fat diet with boric acid. In addition, the live weight changes of the animals were recorded. At the end of the study, we determined that boric acid given in addition to drinking water decreased TAS level and PON activity, but it was not statistically significant and increased TOS and HDL levels. In Group 3, more PON activity was detected than the groups other than the control group. In addition, it was observed that the weight average of the group treated with boric acid decreased.

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As a result, boric acid and high-fat diet did not have a positive effect on the antioxidant system, but they caused an increase in HDL level and a decrease in PON activity. A relative increase in PON activity was observed when rats were co-fed with boric acid and high fat. PON and HDL acted independently of each other. In addition, boric acid may be effective in lowering body weight.

1. INTRODUCTION

Obesity which is defined as high and abnormal deposition of fat that may endanger adipose tissues is a complex and multifactorial disease in which social-behavioral disturbances are seen in addition to deformation in metabolic systems (Forster et al., 1988). Its etiology includes higher energy intake than expenditure, impaired lipid metabolism, stress, genetic vulnerability, low activity, and social factors. Considering the age we live in speed and change are influential in every aspect of our lives, including our nutrition and lifestyle habits. Many studies have reported that fast, excessive or irregular feeding by reducing feeding time triggers obesity (Bentley et al., 2018; Liu et al., 2015; Murakami et al., 2016).

Obesity is defined as a disease that decreases lifespan of living beings because it causes several health problems including cancer and cardiovascular disturbances. World Health Organization (WHO) reports that obesity is among the most risky diseases of our time and threatens our future (Forster et al., 1988).

Research has shown that increased workload on many tissues and increased metabolism which parallels excessive weight gain associated with obesity increase oxidative stress level. In addition, inadequate antioxidant intake and increased lipid content are thought to be effective in the development of obesity by leading to an increase in the amount of free radicals (Moylan & Reid, 2007; Tabur et al., 2010) . In short, oxidative stress in living organisms due to obesity is also seen as an effective factor in the progression of obesity.

Various methods are used to prevent obesity. Reducing body weight can be considered as the main purpose of these methods. Some studies on weight loss have shown that boric acid may be effective in reducing body weight by using supplemented boron compounds (Fail et al., 1998; Yildiz et al., 2013). Furthermore, healing the deteriorating lipid profile may be effective in struggle obesity(Doğan et al., 2017).

Boron is a semi-metal located at 3A in the periodic table and symbolized by B (Bolaños et al., 2004). Boron is generally found in human and in animals in the form of boric acid. It can be found in transparent crystal structure which is colorless, odorless and easily soluble in water as well as it can take the form of white granular powder (Sutherland et al., 1998; Woods,

1994). In addition to industrial use boron takes place in many processes in the metabolism of living beings like mineral, lipid and energy metabolism, and immune and endocrine systems. In addition, it has been reported that, it is also effective in the activity of many enzymes although the mechanism has not been fully appreciated (Armstrong et al., 2001; Dupre et al., 1994; Hunt, 1998; Hunt et al., 2009; Kurtoglu et al., 2018).

Boron enters the human body exogenously through respiration, skin, food and beverages; absorbed from mucosal surfaces at low concentrations and is converted to boric acid at appropriate pH values. It is distributed to various tissues of the body (nails, hair, teeth, liver, spleen, etc) and most of it (90-95%) is excreted in 24 hours with urine. It is accumulated in small amounts in bone tissue (Murray, 1998; Shuler, 1990; U.S. Environmental Protection Agency, n.d.). Inadequate boron intake by the organism has been stated to cause a decrease in functions of many biomolecules, such as calcium (Ca), magnesium (Mg), and vitamin D (Chapin et al., 1998).

In some studies with boric acid, enhancing effects of this mineral on antioxidant system were mentioned (Ince et al., 2010; Türkez et al., 2007). In studies using high fat diets, elevation in the oxidant system, reduction in High Density Lipoprotein (HDL) level which is a lipoprotein, and inhibition of HDL-dependent paraoxonase (PON) enzyme activity were reported (Olusi, 2002; Piva et al., 2011; Sorenson et al., 1995).

PON is a calcium-dependent ester hydrolase (Durrington et al., 2001) which can hydrolyse paraoxon, a potent inhibitor of cholinesterases (Türkez et al., 2007). It is defined as a glycoprotein because about 15.8% of the PON enzyme consists of carbohydrates. It has a cyclic structure due to interactions of disulfide bonds in the protein structure (Gan et al., 1991; La Du et al., 1999). PON enzyme, is integrated to HDL_2 and HDL_3 and hydrophobic nature of N-terminal of PON enzyme causes easy binding of HDL to lipids. The PON gene family has 3 members as PON_1, PON_2, and PON_3 (Primo-Parmo et al., 1996). PON_1 has antioxidant effects due to its LDL (low density lipoprotein) protective property from oxidation and the ability to neutralize many radicals. PON_1 has also been shown to play an important role in the metabolism of lipid peroxides by HDL (Gan et al., 1991).

There is no conclusive evidence that boron and its compounds, which are known to have many benefits in terms of protecting people's health, are essential elements. But due to the important role boron has in the metabolism and in regulation of many enzymes, bor element is included in trace elements and it is thought to be a probable essential element.

(Bauer & Pettersson, 1974; Cui et al., 2004; Hunt, 1998; Kettner & Shenvi, 1984; Tanaka & Fujiwara, 2008).

The aim of this study was to investigate the effects of boron supplements on some biochemical parameters such as Antioxidant System, Enzyme and HDL, levels and PON activity as an alternative treatment method in obesity.

2. MATERIAL AND METHOD

Material: Study material was 4-5 months old 40 Sprague Dawley rats with a mean live weight of 226,95 ± 5,75 g which were grown in Experimental Animals Laboratory of Kafkas University. The study was approved by the Ethics Committee of the Animal Experiments of Kafkas University (Decision no: KAÜ-HADEK: 2012.67).

Experimental Design**:** Animals were divided into 4 groups each containing 10 rats as follows; Group I (control; normal food and drinking water), Group II (high-fat diet), Group III received a high-fat diet and boric acid (5.72 mg/L H_3BO_3 (Sigma:B6768) dissolved in distilled water) containing 1mg/L boric acid in drinking water according to previously reported (Aysan et al., 2011). Group IV was fed with a normal diet and boric acid (H_3BO_3 in drinking water) containing a final concentration of 1 mg/L of boron. At the end of 6-week period, the intracardiac blood samples were collected into the serum tubes under ether anesthesia. Blood samples were centrifuged at 3000 rpm for 10 minutes and the serum samples were obtained. The samples were maintained at -20 °C until further analyses.

Biochemical Analyses**:** Serum total antioxidant status (TAS), total oxidant status (TOS) and HDL levels were determined using commerical kit respectivly (RelAssay, Gaziantep-Türkiye, Roche 657621). Serum PON activity was determined by a method which has previously been reported (Eckerson et al., 1983).

Statistical Analysis

Parametric tests were performed after it was determined that the data were parametric as a result of the Kolmogorov-Smirnov test. Statistical analyses were done using SPSS Windows 16.0 package program. Variance analysis (ANOVA) and Duncan test were used for statistical analysis of the data. The results are presented as mean ± standard error. Results with $P < 0.05$ were considered significant.

3. FINDINGS

Table 1. Content of feed used in the experiment of diets

Ingredients	(%)	The calorie composition of the experimental diets (%)		
Corn	60.50	**Contents of Nutrients**	**Normal**	**High-fat**
Gluten Meal	33.45	**Dry matter**	89.6	83.8
Vegetable fat	3.30	**Crude protein**	23.90	24.30
Marble dust	1.0	**Crude fat**	20.63	53.83
Dicalcium phosphate	0.5	**Crude cellulose**	7.17	7.12
Sodium chloride	0.5	**Crude ash**	8.43	8.37
DL-Methiyonine	0.10	**ME (kcal/kg^{-1})**	2600	5616
Lizin	0.15			
Vitamin-mineral premix[a]	0.5			

Mix supplied per 1.0 kg: 20,000,000 IU Vitamin A, 3000,000 IU Vitamin D3, 25 g Vitamin E, 4 g Vitamin B1, 8 g Vitamin B2, 5 g Vitamin B6, 20 mg Vitamin B12, 20 g nicotinamid, 12 g calcium-o-pantothenic acid, 200 g choline chloride, 50 g manganese, 50 g iron, 50 g zinc, 10 g copper, 0.8 g iodine, 0.15 g cobalt, 0.15 g selenium.

Table 2. The levels of TAS, TOS, HDL and PON activity in experimenteal groups

Parameters	Group I	Group II	Group III	Group IV	P
TAS (mmol TroloxEqv./L)	1,31±0,08^{a}	0,78±0,05^{b}	0,88±0,06^{b}	0,88±0,04^{b}	0,001
TOS (µmol H_2O_2Eqv./L)	3,5±0,24^{b}	4,74±0,60^{ba}	5,94±0,49^{a}	5,34±087^{ba}	0,063
PON (U/L)	50,43±7,45^{a}	26,96±5,21^{bc}	41,49±6,54^{ab}	21,66±5,17^{c}	0,009
HDL (mg/dL)	34±3,8^{b}	36±1,9^{ab}	44±2,61^{a}	43±2,48^{a}	0,036

Different superscript letters (a,b,c) within the same row indicate significant differences. Group I: Control, Group II :High-fat diet (**HF**), Group III: High-fat diet + Boric acid (**HF**+**BA**), Group IV: Boric acid (**BA**)

Table 3. Weight average of the groups by week (grams)

Weeks	Group 1	Group 2	Group 3	Group 4
1. week	230,8	232,8	223,4	220,8
2. week	240,1	367,5	258,7	230,1
3. week	230,4	266,3	257,8	220,4
4. week	217,7	270	265	207,2
5. week	222,1	272	284,87	212,1
6. week	225,8	285,44	288,88	215,8

Feed and water of the animals were given in equal amounts per hour at 24 hour intervals. Group weights were weighed weekly.

4. DISCUSSION AND CONCLUSION

The effects of boron on antioxidant oxidative stress is one of the current controversial issues. In addition, the lowering effects of boron compounds on body weight are promising for the problems caused by excessive weight(Atakisi et al., 2019; Fail et al., 1998). Increased oxidative stress with increased weight gain leads to an increase in the amount of free radicals and deterioration of homeostasis between free radicals and antioxidants in the body against antioxidants. Increased metabolic and mechanic workload on tissues increases oxygen consumption of these tissues and this increased oxygen consumption increases reactive oxygen species (ROS) and therefor amount of free radicals (Moylan & Reid, 2007; Roh & So, 2017).

In a study conducted by Ozata et al. (2002), there was a significant decrease in activity of erythrocyte selenium-dependent glutathione peroxidase (GSH-Px) and copper-zinc superoxide dismutase (Cu-Zn SOD) in obese subjects and erythrocyte TBARS (thiobarbituric acid reactive substance level) was increased significantly compared with the control group(Ozata et al., 2002; Roh & So, 2017). Our study showed increase in ROS amount and reduction in antioxidant system in the groups that had high-fat diet.

Farshad et al (2012) studied Cu-Zn SOD, GSH-Px, and catalase (CAT) activities in women with abdominal obesity and found decreased activities of Cu-Zn SOD, GSH-Px, and CAT activities compared with women who had normal body weights (Amirkhizi et al., 2014). Cu-Zn SOD, GSH-Px, and CAT enzymes act as first-line defense mechanisms in the body against ROS in the organism. Decreased activity of these enzymes is believed to be due to increased amount of oxidants or prooxidants. Decreased TAS amount in our study is thought to be due to increased TOS level due to oxidative stress produced by prooxidant or oxidant levels mediated by obesity.

Research about effects of boron and boron compounds on oxidative system has gained momentum during recent years. However this studies investigating the effect of boron supplements on the antioxidant system are often contradictory. (Garcia-Gonzalez et al., 1991; Ince et al., 2010; Türkez et al., 2007). A study about the effects of boron and boric acid on blood samples exposed to heavy-metals revealed that malonil dialdehyde (MDA) levels which were increased after exposure to heavy metals decreased with addition of 5 ppm boric acid. In addition, the authors reported increases in SOD, CAT, and GSH-Px activities which were decreased due to effects of heavy metals and boric acid might have antioxidant effects (Türkez

et al., 2007). Similarly, Kucukkurt vd. demonstrated that boron applications have positive results on the antioxidant system in mice showing arsenic toxicity(Kucukkurt et al., 2015). Sharma et al. reported that boron supplements have positive effects on Vitamin D level and antioxidant system(Sharma et al., 2020). Another study which explored effects of boric acid and borax on antioxidative system of rats reported that boron compounds decrease MDA level, which is a product of lipid peroxidation and increase GSH amount and CAT and SOD activities (Ince et al., 2010). TAS and TOS can be evaluated as cumulative markers of oxidative stress (Ghiselli et al., 2000) Türkez et al. showed in their studies that boron containing compounds increase the amount of TAS and decrease the amount of TOS(Türkez et al., 2007). Similar results were observed in the study by Cengiz et al. They reported that boron supplementation increased the levels of GSH, TAS, and decreased the levels of MDA and TOS(Cengiz et al., 2020).

In contrast to Ince (2013) could not find any effect of borax dehydrate on antioxidant system in the study which examined acute effects of different doses (100 mg/kg and 200 mg/kg) of borax dehydrate on antioxidant system (Ince et al., 2010). Gonzalez et al. studied *Anabaena sp.* PCC 7119[1] and found increased amounts of SOD and CAT enzymes in erythrocytes in boric acid insufficiency(Garcia-Gonzalez et al., 1991). Karabal et al. studied Anatolian and Hamidiye barleys by adding 5 mM and 10 mM boric acid to water. Compared with the control group MDA amount was higher in the study groups and MDA amount increased with increasing boric acid amounts. (Karabal et al., 2003).

Findings from our study demonstrated that antioxidant capacity decreased and oxidant capacity did not change after boric acid addition. This difference is thought to be due to dosage given to the patients and duration of the study.

Recent studies have demonstrated that boric acid might be effective in decreasing body weight (Atakisi et al., 2019; Fail et al., 1998; Yildiz et al., 2013). A study reported that addition of various doses (1200, 2500, 5000, 10000, 20000 ppm) of boric acid to drinking water decreased body weight and this decrease was positively correlated with dosage (Fail et al., 1998). Results of aforementioned studies are in parallel with our study. Weight of the group which took 1mg/L boric acid added to drinking water decreased during the study. But addition of boric acid with high-fat diet did not change body weight. These results support the notion that boric acid when used solely may decrease body weight.

This suggests that boric acid might have a direct or indirect effect on fat metabolism and boron compounds have been administered effectively in the control of obesity. β-catenin are positive effectors for Wnts. Wnt-10b-mediated Wnt signaling acts as a molecular switch that governs adipogenesis. By inhibition of Wnt signaling, differentiation of preadipocytes is inhibited through the adipogenic transcription factors CCAAT/enhancer binding protein a (C/EBPa) and peroxisome proliferator-activated receptor (PPARϕ). It has been reported that it can inhibit adipogenesis in this way(Prestwich & MacDougald, 2007; Ross et al., 2000). Studies have shown that boron can inhibit genes and proteins related to adipogenesis by inhibiting Wnt / β -catenin, Akt and other extracellular pathway (Doğan et al., 2017). In studies on lipid profile, it was reported that boron-containing diet caused a significant decrease in LDL and total colesterol (TC) levels, and a nonsignificant increase in HDL levels was observed. (Kuru et al., 2019) In another study in which similar results were obtained, it was emphasized that boron supplementation decreased LDL level and increased HDL level (Hall et al., 1989). It has also been shown by Başoğlu that borax applied in addition to foods affects the lipid profile. Insulin, serum glucose, and apolipoprotein-B levels decreased and lipidemia decreased in dogs over the 4 week of the study (Basoglu et al., 2000). Some studies show that boron has negative effect on the lipid profile. In a study on this subject 0.45 g/L boric acid solution was given to animals by adding 2 mg boron to drinking water. After 2 weeks, serum levels of HDL and triacylglycerol were decreased (Naghii & Samman, 1997). In our study, addition of boric acid increased HDL level.

PON is an enzyme responsible for the hydrolysis of organophosphate and statins found in HDL dependent. Recent developments have revealed its LDL and cholesterol lowering effect in cardiovascular disease (CVD). In addition, it has the ability to specifically reduce oxidized phospholipids. (Meneses et al., 2019). An in vitro study by Aviram A et al demonstrated that inhibition of PON enzyme by copper sulfate ($CuSO_4$) increased oxidation of HDL and caused elevation of MDA. In addition, HDL level was positively correlated with PON activity(Aviram et al., 1998). These findings suggest that PON enzyme might have protective effects like an antioxidant molecule.

Aslan et al. as a result of the study conducted by, it was reported that HDL and PON activity decreased when oxidative stress increased in obese individuals (Aslan et al., 2011). It shows a decrease in the activation of the PON enzyme, which is a strong antioxidative agent, with the oxidant load and advanced glycation reactions that occur in obesity(Cakir et al., 2018;

Cervellati et al., 2018; Yu et al., 2017). This may explain the decrease in PON activity measured in the high-fat diet group in our study. PON is a Ca^{+2} dependent enzyme. The thiol groups in its structure are the target site of many metal ions and can be inhibited especially by metals with high concentrations(Debord et al., 2003; Deveci et al., 2015; Gonzalvo et al., 1997). Inhibition of metal ions can explain the purpose of PON activity in boric acid days in our study. However, with the combination of boric acid and high fat diet, PON activity increased compared to the obese group. Alak et al. They reported that PON level and antioxidant effect of boric acid decreased at high doses, but increased PON level and antioxidant level at low doses. In the same study, they reported that myeloperoxidase (MPO) showed an inverse correlation with the PON enzyme(Alak et al., 2020). MPO, another HDL-dependent enzyme, is responsible for LDL oxidation and may lead to impaired activation of the PON enzyme(Y. Huang et al., 2013). co-execution of boric acid with a high-fat diet may also reduce boric acid absorption. In this case, the antioxidant effect of low-dose boric acid may cause increased PON activation, decreased MPO level, and decreased heavy metal load. This information may help explain the increase in PON observed in Group 3 compared to Group 2 and Group 4.

Although PON is an HDL-dependent antioxidant enzyme(Kunutsor et al., 2016), there are many parameters that affect its expression. therefore, it is not entirely correct to directly link its activation to HDL(Costa et al., 2005). further, some studies suggest that PON1 activity behaves independently of HDL(Cervellati et al., 2018; Mackness et al., 2003). In particular, the increase in BMI (body mass index) deteriorates the stabilization between PON and HDL. In this regard, Thomàs-Moyà et al. reported in their study that the main reason for the decrease in PON1 in obese individuals was not the decrease in HDL. instead, it suggests that the resulting obesity and oxidative stress have an effect on the PON level(Thomàs-Moyà et al., 2008). Morever most of the PON activity is found in HDL-3. In this case, another theory is that PON activity may be dependent on lower fractions of HDL(Pérez-Méndez et al., 2014). This information may help us to explain the PON enzyme's HDL independent results.

Changes in lipoprotein levels which have roles in body homeostasis may be seen by excessive weight gain. Olusi et al. showed that while MDA levels of people with high BMI increased, Cu-Zn SOD activity and HDL levels decreased.(Olusi, 2002). These results are consistent with our study. Similarly a study on 48 volunteers showed decrease in HDL with increasing BMI (Piva et al., 2011). Huang et al (2013) studied rats and gave normal forage to the control group and fatty diet (430 kcal/100 g) (%10 protein, %15 bacon, %10 sucrose) for

176 days. No difference was found in HDL levels between the control and the study groups (W. Huang et al., 2013). Despite previous studies that have shown decreased HDL levels we found increased HDL levels compared with the control group ($P<0.05$). HDL lowering effect of boric acid suggests that butter will be a better explanation for this subject. Studies on butter demonstrated that butter with high cis-9 and cis-11 conjugated linoleic acids increase HDL levels (de Almeida et al., 2014; Roy et al., 2007). This studies suggest that butter produced with traditional methods as a source of high-fat diet will not decrease HDL level and may even increase HDL level by trials using different amounts of butter.

In addition studies have shown a positive correlation between PON activity and HDL level (Aviram et al., 1998; Deveci et al., 2015). But, our findings suggest that HDL values increase and PON activity decrease in the groups taking high-fat diet and boric acid diet compared with the control group in contrast with the previous studies.

5. SUGGESTIONS

As a result, boric acid and high-fat diet did not have a positive effect on the antioxidant system, but they caused an increase in HDL level and a decrease in PON activity. A relative increase in PON activity was observed when rats were co-fed with boric acid and high fat. PON and HDL acted independently of each other. In addition, boric acid may be effective in lowering body weight. This study will provide up-to-date information to the literature in terms of a more comprehensive evaluation of the effect of boric acid on obesity and the antioxidant system.

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Biological Properties of a Newly Isolated Bacteriophage (NL1) that Infects *Escherichia coli* O157:H7 Strain

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Öz

Escherichia coli O157: H7 suşu ve daha önce sporadik vakalardan izole edilmiş enteroinvaziv *Escherichia coli* suşları**,** kirli çevresel odaklardan ve hayvansal atıklarla kontamine olmuş sulardan izole edilmiş bakteriyofajların konakçı spektrumunu belirlemek için standart suş olarak kullanılmıştır. NL1 Olarak isimlendirilen, *Escherichia coli* O157:H7'ye spesifik bakteriyofajın fizyolojik özellikleri belirlenmiştir. İzolasyon basamağından sonra bakteyofajlar pürifiye edilmiş ve titreleri artırılmıştır. Konakçıların çapraz antijenik özellikleri *Escherichia* phage NL1 fajı ile araştırılmıştır. Fajların adsorpsiyon süresi, latent periyot, patlama boyutu, enfeksiyon değerinin çokluğu (MOI) gibi tek aşamalı büyüme eğrisi parametreleri belirlenmiştir. Sonuçlar, bakteriyofaj NL1'in *E.coli* O157:H7 (RSKK 09007) ve *E.coli O:164* RSKK 324'ü tamamen lize ettiğini göstermiştir. Bu çalışmada, faj NL1'in sadece *E.coli* O157:H7 üzerindeki etkileri araştırılmıştır. Hesaplanan en yüksek titre, optimal enfeksiyon çokluğu ($2.16x10^8$) olarak belirlenmiştir Latent periyodun 20 dakika sürdüğü ve fajın yaklaşık 90 dakikada patlama boyutuna ulaştığı belirlenmiştir.

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Abstract

Escherichia coli O157: H7 strain was used as the standard strain for bacteriophages isolated from polluted environmental foci and animal waste-contaminated waters, and some of the enteroinvasive *Escherichia coli* strains previously isolated from sporadic cases were used to determine the host diversity of phages. The physiological properties of bacteriophages specific to *Escherichia coli* O157:H7, designated *Escherichiaphage* NL1, were studied. After the isolation step, bacteriophages were purified and their titer was increased. Cross-antigenic properties of the hosts were investigated with *Escherichia* phage NL1 phage. One-step growth curve parameters such as adsorption time of phages, latent period, burst size, multiplicity of infection value (MOI) were determined. Results showed that bacteriophage NL1 completely lysed the *E.coli* O157:H7 (RSKK 09007) and *E.coli* O:164 (RSKK 324). In this study the effects of phage NL1 only on *E.coli* O157:H7 were investigated. The calculated highest titer was determined as optimal multiplycity of infection ($2.16x10^8$). It was determined that the latent period lasted for 20 minutes and the phage was reached the burst size at approximately 90 minutes.

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1. INTRODUCTION

Enterohemorrhagic *Escherichia coli* (EHEC) is a pathogen that can cause worldwide epidemics in humans and animals. The most important component of their virulence is the production of one or both Shiga-like toxins (Stx I and II) (O'Brien et al., 1992). Strains belonging to the O157 serogroup, specifically O157:H7, have been associated with epidemics and sporadic cases around the world. The Japanese Ministry of Health and Welfare recommends the use of kanamycin, fosfomycin, and a new quinolone antimicrobial agent, norfloxacin (Ministry of Health and Welfare, 1996). However, these various antimicrobial agents, including the novel quinolone, caused the production of Stx under certain conditions (Walterspiel et al., 1992). It has also been shown that, unlike antibiotics, phage therapy does not induce Shiga toxin production. These findings suggest that some newly isolated bacteriophages have biocontrol potential and are therapeutic agents for pathogenic *E. coli* (EHEC and EPEC) strains (Viscardi et al., 2008). Today, the most basic treatment for *E.coli* is oral rehydration (Bhan et al., 1994). This simple and inexpensive measure has saved countless lives, but it does not affect the natural course of the disease and has no antibacterial activity. *E.coli* vaccine studies are still in the early development phase (Savarino et al., 2002). Water and sanitation programs can be improved and drinking water quality can be improved, but they are very expensive methods for developing countries. Some researchers have investigated the use of O157-specific phages in food sanitation (Kudva et al., 1999, O'Flynn et al., 2004). Experimentally, when meat was contaminated with O157 strains, high titers of bacteriophages were required to reduce bacterial density. Similar high phage titers were required to clear chicken skin of Salmonella and Campylobacter contaminations (Goodridge et al., 2003). The biggest obstacle to the practical use of colyphages can be considered as the destruction of the intestinal commensals by phage cocktails, however, in an experiment conducted in mice, it was shown that four orally administered phage cocktails did not affect the amount of intestinal commensals (Chibani-Chennoufi et al., 2004). Similarly, volunteers orally exposed to T4 phage maintained a commensal *E. coli* population (Bruttin and Brüssow, 2005).

The fact that resistance to antibiotics has become an important problem today will lead to a longer interest in bacteriophage studies. In our study, the phage that we predict can be used in the biocontrol of *E.coli* O157: H7, which can also cause food-borne outbreaks, and some properties of this phage have been determined.

2. MATERIALS AND METHODS

Collection of samples and bacterial strains

Liquid samples (sewage and contaminated water) were collected in sterile capped bottles. Samples were taken from 20 different foci in Kars (Turkey).

Bacterial strains were obtained from the Ministry of Health, General Directorate of Public Health, National Type Culture Collection Laboratory (Ankara/Turkey) (Table 1).

Table 1. Bacterial strains

Species	Isolate
Escherichia coli O157:H7	RSKK 09007
Escherichia coli O:28	RSKK 314
Escherichia coli O:164	RSKK 324
Escherichia coli O:112	RSKK 688
Escherichia coli O:124	RSKK 318
Escherichia coli O:143	RSKK 322
Escherichia coli O:152	RSKK 323

Isolation of bacteriophage

After the collected samples were centrifuged at 5000 g for 20 min, the supernatant was filtered through 0.22 millipore membrane filters. 10 ml of the single colony of the *E.coli* strain, which was cultivated the day before, incubated at 37 °C for 3 hours in LB broth, and 10 ml of the filtrate were mixed in the test tube. The mixture was transferred into 100 ml of LB broth supplemented with 0.1 M calcium chloride and 0.1 M magnesium sulfate, and the mixture was incubated at 37 °C for 16 hours. During this process, the number of bacteriophages remaining in incubation with their host was expected to increase. After the incubation period, the mixture was filtered through 0.22 millipore membrane filters by centrifuging again (5000 g, 10 min) to get rid of bacteria (Clokie and Kropinski, 2009)

Spot test

An overnight fresh bacterial culture (*Escherichia coli* O157: H7) incubated on agar medium was added to 4.5 ml of LB broth and vortexed, and 100 µl of the obtained culture was transferred to a sterile tube. Then, 4 ml of soft agar was added to the bacterial culture and

spread on LB agar. 10 µl of bacteriophage suspension was dripped onto the prepared agar preparations and incubated at 37 °C for 18 hours in aerobic environment, and plaques formed on the agar were observed the next day. This procedure was applied separately for all strains. The experiment was repeated until plaque formation was observed (Clokie and Kropinski, 2009)

Purification of bacteriophages

In order to dilute the phage titer of the liquid samples in which the presence of phage was determined, sequential dilutions up to 10-8 were prepared, 1 ml of bacteriophage sample taken from the dilution tubes was mixed with 100 µl of fresh target bacteria culture and kept at room temperature for 10 minutes. Then, using the double layer agar method, 4 ml of soft LB agar was added and mixed, and it was spread on LB agar and incubated at 37 °C for 18 hours. After 18 hours of incubation, plaque formations on petri dishes were examined, bacteriophage plaques from petri dishes with single plaque formation were cut with a sterile pasteur pipette and transferred into LB broth. The cut bacteriophage plates were mixed in the broth medium and the bacteriophages were allowed to diffuse into the medium. This bacteriophage suspension was diluted again and incubated at 37 °C for 18 hours using the double layer agar method. This process was repeated for three days. The final medium phage mixture was used as purified bacteriophage stock.

Determination of lysis titer of bacteriophage

The bacteriophage stock was diluted to 10^{-8}. Bacterial strains incubated in slant agar aerobically for 24 hours at 37°C, and after incubation, they vortexed with 4.5 ml of LB broth, and 100 µl taken from it and transferred to another sterile tube. 1 ml of bacteriophage dilutions was added to each bacterial culture and 3 ml of soft LB agar was added on them and poured into petri dishes containing LB agar, so the double layer agar method was applied. The number of bacteriophages in the starting stock was determined by calculating pfu/ml on 30-300 plaque-formed petri dishes (Li et al.,2016, Clokie and Kropinski, 2009).

Host range

Strains susceptible to bacteriophages were determined by applying the "spot test" protocol on each of the bacterial strains.

Determination of optimal MOI of bacteriophages

The host bacteria were incubated at 37 ^{0}C for 16 hours and adjusted to 1x10^7 cfu/ml with a spectrometer. 3x10^7 pfu/ml phage solution and *E.coli* were mixed together to the MOI

would be 10, 1, 0.1, 0.01 and 0.001, respectively. Each tubes were kept at 37 ^{0}C for 3 hours and centrifuged at 5,000 g for 20 minutes and the supernatants were collected. They were filtered through 0.22 μm pores and the highest calculated titer was determined as the optimal multiplycity of infection (Li et al.,2016).

Determination of one-step growth parameters

Bacteriophage samples were mixed with *E.coli* strains (3x108 cfu/ml) with a MOI of 1 and kept at 55 ^{0}C for 10 minutes. After centrifugation at 10,000 g for 5 minutes, cells were collected, transferred to 1 ml of fresh medium and mixed. This process was repeated 3 times, so that non-absorbed phage particles were removed. 10 μl of the sample was taken into 100 ml broth medium and incubated at 55 ^{0}C for 100 min. The phage titer in the culture was tested with the double layer agar technique at 10 min intervals (Lin et al., 2011).

3. RESULTS AND DISCUSSION

Isolation and purification

The lytic bacteriophage of the host bacterium was isolated from environmental samples. The plaques observed in the double layer agar method were smooth-edged and 1.00-1.10 mm in diameter.

Determination of bacteriophage titer

Petri dishes consisting of 30 plaques (dilution ratio=10^{-6}) were selected for the *E.coli* O157:H7 (RSKK 09007) phage. It was calculated with formula;

Pfu/ml= Number of plaques / amount of phage stock (ml) x Dilution rate

The stock titer for *E.coli* O157:H7 (RSKK 09007) phage was calculated as 3x10^7 pfu/ml.

Host range

Results showed that bacteriophage NL1 completely lysed the *E.coli* O157:H7 (RSKK 09007) and *E.coli* O:164 RSKK 324. In this study, the effects of phage NL1 only on *E.coli* O157:H7 were investigated.

One-step growth curve

Results showed that the highest phage titer of 2.16x10^8 was obtained when the MOI value was 1 (Table 2).

Table 2: Optimal multiplycity of infection (MOI) of *E.coli* O157:H7 phage

***** The calculated highest titer was determined as Optimal multiplycity of infection

MOI	**Phage concentration**	**Bacteria solution concentration**	**Phage lysis titer after 6 hours**
10	$3x10^7$	$1x10^7$	$4.22x10^7$
1	$3x10^7$	$1x10^7$	***$2.16x10^8$**
0.1	$3x10^7$	$1x10^7$	$4.44x10^7$
0.01	$3x10^7$	$1x10^7$	$2.12x10^7$
0.001	$3x10^7$	$1x10^7$	$1.00x10^7$
0.0001	$3x10^7$	$1x10^7$	$8.00x10^6$

E.coli O157:H7 culture and NL1 phage were mixed at MOI 1 and the titer of NL1 phage was determined every 10 minutes. As seen in **figure 1**, it was determined that the latent period lasted for 20 minutes and the phage was reached the burst size at approximately 90 minutes.

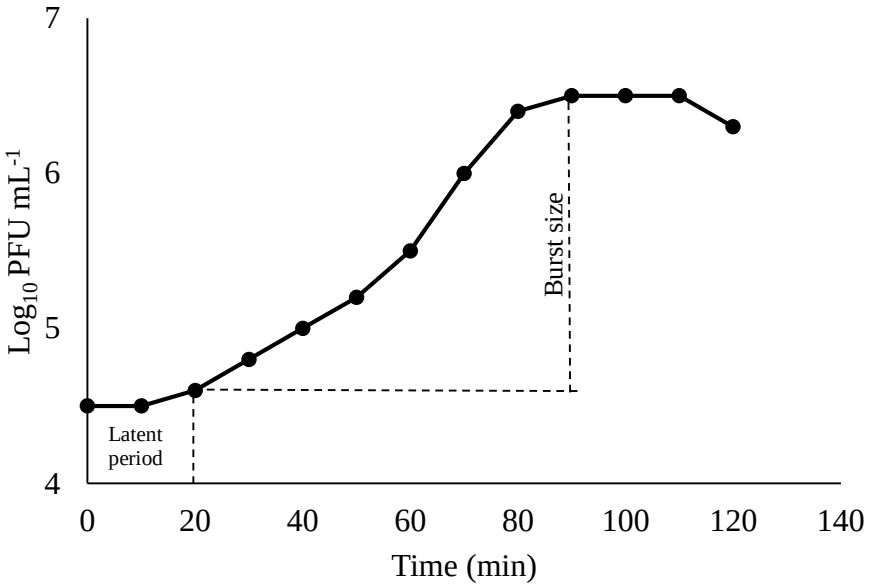

Figure 1. One-step growth curve of NL1

The increasing importance of the resistance problem in bacteria has led to the importance of anti-infective models in modern medicine and biotechnology. There can be resistance to bacteriophages, but this has been poorly documented in the scientific literature to date. The increase in antibiotic-resistant bacteria, as well as the shortcomings in the development of effective new classes of antibiotics, brought up phage's use in the treatment

of infections (Sulakvelidze et al., 2001). As decontamination with phages and local phages are important in treatments, phage diversity is also very important. While developing resistance to a phage, the other phage or phages destroy the target bacteria. Therefore, local phages to be isolated against a bacterium is important (Boyd and Brüssow, 2002).

Various preparations have been prepared and used for phage therapy since the discovery of phages. United States The Food and Drug Administration (US FDA) confirmed the use of listeriaphages on the surfaces of meat and poultry products in 2006 (Kim and Kathariou, 2009). Today, experimental studies are being carried out to protect dental health (Chkonia I. et al., 2012), fight against mastitis (Donjacour A. and Paros M., 2012), meningitis and sepsis (Pouillot F. and Gabard J., 2012), to ensure milk hygiene (Endersen E. et al., 2012).

In our study, the characterized *Escherichia* phage NL1 was found to have broad host range activity infecting *E. coli* isolates that belonged to two different pathotypes of EIEC and EHEC. In some studies, it has been reported that isolated phages show a wide host spectrum. In a study, it was shown that the phage acting on *Klebsiella* is effective on different serotypes. In their study, Monahar et al. reported that applying bacteriophage cocktail on bacterial strains of different species significantly reduced the bacterial load within 24 hours (Monahar et al., 2018). In another study, it was determined that the absorption of T4 phage into the polycaprolactone (PCL) film used packaging raw beef contaminated with *E. coli* O157:H7 showed approximately 30 times more inhibitory effect (Choi et al.2021). In some studies, it has been stated that phages inhibit *E.coli* biofilm formation (Ribeiro et al. 2018, Alves et al. 2014, Dorlan 2009).

It has been shown that the use of antibiotics and phages together may have a synergistic effect and it has been stated that it can prevent antibiotic resistance that will occur with the use of antibiotics or phages alone (Valério et al. 2017, Zhang 2012, Kirby 2012).

In our study, the phage we named NL1 showed lytic effect in *E.coli* O157:H7 (RSKK 09007) and *E.coli* O:164 RSKK 324 strains. *E.coli* O157:H7 is known to cause foodborne outbreaks and it was thought that NL1 could be used in the treatment of E. coli. Since it is known that bacteriophage cocktails are more effective with a synergistic effect, it has been evaluated that NL1 can also be used in phage cocktails. Again, it is thought that it may have a synergistic effect when used with antibiotics. Based on the lytic effect of NL1 on both *E.coli* O157:H7 (RSKK 09007) and *E.coli* O:164 (RSKK 324) strains, cross-antigenic structures between the two strains can be investigated and this new phage can be used for typing.

4. CONCLUSION

In this study, properties different from the physiological properties of previously isolated phages specific to *E. coli* O157:H7 strain were determined. The results of this study need to be expanded and advanced for the biocontrol of *E. coli* O157:H7, one of the important pathogens that can cause epidemics. Determining the identity of the isolated NL1 phage by molecular characterization methods will guide other studies.

Conflict of Interest

The authors wish to declare that they have no conflict of interest

Authors contribution

The authors contributed equally to the study.

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