Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 13(2), 1101-1109, 2023
Journal of the Institute of Science and Technology, 13(2), 1101-1109, 2023

ISSN: 2146-0574, eISSN: 2536-4618

Chemistry **DOI: 10.21597/jist.1195089**

Research Article

Received: 17.11.2022 **Accepted: 27.01.2023**

To Cite: Yılmaz, A., & Çomaklı, V. (2023). Investigation of Effects of Some Metal Ions and Some Pesticides on Glutathione S-Transferase (GST) Enzyme purified from Van Lake Fish (Chalcalburnus Tarichi) Kidney. *Journal of the Institute of Science and Technology*, 13(2), 1101-1109.

Investigation of Effects of Some Metal Ions and Some Pesticides on Glutathione S-Transferase (GST) Enzyme Purified from Van Lake Fish (Chalcalburnus Tarichi) Kidney

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Highlights:

- Purification of C. Tarichii kidney GST
- Enzyme kinetic studies
- Metal ions inhibition

Keywords:

- Glutathione S-transferase
- C. tarichii
- Inhibition
- Some metals
- Pesticide.

ABSTRACT:

Industrial and agricultural processes have brought about the pollution of aquatic systems, and this situation in the water had adverse effects on human health. In order to reduce these negative effects, all mammalian and aquatic species have a defense system. One of these antioxidant systems is the glutathione S-transferase enzyme group, which has detoxification activity against ROS produced by organic pollutants and heavy metals. In this study, the effects of some pesticides and heavy metal ions on the GST enzyme activity purified kidney tissue of the Lake Van fish (C. Tarichii) was analyzed. Firstly, the purification process was carried out as homogenate preparation and glutathione-agarose affinity technique. SDS-PAGE was carried out to check the enzyme purity. The enzyme was obtained in 89% yield (335-fold) with 27.8-specific activity , and kinetic constants was determined for the enzyme; K_m for GSH substrate: 0.429 mM V_{max} for GSH substrate: 0.207 EU/mL^{-1}; K_m for CDNB substrate: 0.276 mM, V_{max} for CDNB substrate: 0.161EU/mL^{-1}. Effects of Met-Hg, Fe^{3+} Cr^{3+} , Cu^{2+}, Mg^{2+}, Zn^{2+}, atrazine, methomyl ,and dinicanozole were studied on the activity of the purified enzyme and the IC50 values were determined as 34.5 mM, 10 µM, 1.5 µM, 2.4 µM, 63 µM, 0.15 mM, 9.8 µM, 2.67 µM , and 1.3 µM, respectively.

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INTRODUCTION

Glutathione S-transferase (GST; EC 2.5.1.18) is an enzyme with antioxidant activity and, plays a critical role in the phase II biotransformation system (Özaslan et al., 2017) besides participating in the detoxification of a great many xenobiotics of exogenous or endogenous origin in living organisms. This detoxification system includes the conjugation of xenobiotics like polyaromatic hydrocarbons, some pollutants, heavy metals, pesticides and herbicides with reduced GSH molecule (Türkkan et al., 2019; Higgins et al., 2011). In addition to its role in detoxification, the GST enzyme has many biological functions, namely cellular peroxide degradation, leukotrienes and prostaglandins, isomerization of steroids, synthesis and transportation of hormones (Hayes et al., 2005; Isik et al., 2015; Sau et al., 2010). Depending on its location in the cell, the enzyme is naturally called cytosolic, mitochondrial, and microsomal. (Hayes et al., 2005). These groups have a large number of isoforms related to the differences in protein structure, amino acid sequence and substrate diversity. For example, according to sequence similarity of amino acids, cytosolic GSTs are divided into 7 classes: Pi, Mu, Sigma, Alpha, Omega, Theta, and Zeta. Mitochondrial GSTs belong to Kappa and microsomal GSTs belong to the MGST class. (Glisic et al., 2014).

Recently years, developments in biotechnology and agricultural processes have caused to contamination of the aquatic environment by pollutants (Topal et al., 2008; Ekinci et al., 2008; Ekinci and Beydemir, 2010; Soyut and Beydemir, 2008). These pollutants not only affect the integrity of ecosystems, but also affect the physiological functions of various animals. (Şen and Kırıkbakan, 2004; Perez-Lopez et al., 2002). Heavy metals, pesticides, and herbicides accumulate in the aquatic species' tissues and lead to the production of reactive oxygen species (ROS). ROS accumulation brings about oxidative damage, degradation of cellular antioxidant capacity, and dysfunction of physiological and biochemical mechanisms (Comakli et al., 2015). Fish, like all living things reduce the harmful effects of ROS, have antioxidant enzyme systems such as süperoxide dismutase(SOD), catalase (CAT), glutathione peroxidase (GPx), GST and glutathione reductase (GR) (Zhang et al., 2009). The role of the GST enzyme in xenobiotic metabolism has been extensively studied in mammals, but this enzyme has not been well studied in aquatic species. (Ozaslan et al., 2017; Comakli et al., 2013; Aksoy et al., 2016; Ozaslan et al., 2018; Huang, et al., 2008; Donham et al., 2005).

Van Lake fish is an endemic species that living in the Van region in Turkiye (Kuzu et al., 2016). For its economic importance in agriculture, there is a need to evaluate the comprehensive effects of the pollutants emitted by agricultural development on this fish. Correspondingly, this study is interested with to perform in vitro investigation of the effects of methyl mercury, Fe^{3+}, Cr^{3+}, Cu^{2+}, Mg^{2+}, Zn^{2+} metal ions, and dinicanozole, atrazine, and methomyl pesticides on the activity of GST purified from the kidney tissue of C. Tarichii

MATERIALS AND METHODS

Materials

GSH-Agarose affinity gel, 1 chloro 2 4-dinitrobenzene, glutathione (reduced), enzyme assay reagents for analysis and chemicals used in electrophoresis were obtained from Sigma-Aldrich. Unless specified.

Obtainment of homogenates

The C. Tarichi kidney tissue (20g) was crushed-powdered with liquid nitrogen , and decomposed cell membranes were suspended in 50 mM Tris-HCl (pH 7.4) containing 1 mM EDTA, DTT, and PMSF.

The prepared homogenate was centrifuged at 11000 rpm for 60 minutes. The supernatant obtained after centrifugation was stored at -80 ºC for further steps.

Purification of Enzyme

The GST enzyme purification procedure was undertaken by the modification of previous procedures (Toribio et al., 1996). The prepared kidney homogenate was applied to an affinity gel equilibrated with 10 mM phosphate buffer containing 0.15 M NaCl (pH 7.4). The column was washed with the same buffer for removal of exceeded materials buffer, and GST was eluted with a gradient of 5-10 mM GSH containing 50 mM Tris/HCl.

Protein determinations

Protein contents in all purification steps were determined according to the Bradford method (Bradford, 1976). For this purpose, bovine serum albumin was used as standard protein.

Determination of enzyme activity assay

The GST enzyme activity determination was undertaken by a minor modification of previous procedures (Habig et al., 1974) using CDNB as a substrate. The assay system included a phosphate buffer (pH 7.2), GSH (1 mM) and CDNB (0.5 mM) in-tub concentrations. The absorbance increase at 340 nm of the formed product was determined spectrophotometrically.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the procedure described by Laemmli to determine the molecular weight of the GST enzyme. (Laemmli, 1970).

In vitro inhibition studies

The inhibitory effect of Fe^{3+}, Cr^{3+}, Cu^{2+}, Mg^{2+}, and Zn^{2+} metal ions and dinicanozole, atrazine, and methomyl pesticides were investigated on the kidney tissue GST enzyme activity The activity values were determined at five different concentrations of each respective chemical. No chemicals were added to the control cuvette, which has 100% enzyme activity. The IC_{50} values of the chemicals were calculated from the activity (%)-inhibitor concentration graph.

RESULTS AND DISCUSSION

In this study, the GST enzyme from Van Lake fish was 335-fold purified at 89% with a yield of 27.8-specific activity. The purification procedure was conducted using GSH-agarose affinity chromatographic method, and purification results have shown in Table 1. To check the purity of the enzyme, SDS-PAGE electrophoresis was performed, and the SDS-PAGE image was photographed (Figure 1).Rf values of standard proteins and GST were tested to determine the molecular weight of the purified enzyme subunits.Then Laemmli method was used (1970) to clarify the enzyme structure. The subunit molecular weight of the enzyme was determined as 28kDa from the Rf – log molecular weight (MW) graph (Figure 2). The K_m and V_{max} values were calculated for each substrate using the Lineweaver-Burk graph (Figures 3 and 4). The K_m constant was 0.429 mM, and the V_{max} value was 0.207 EU/mL^{-1} for GSH. The K_m constant for the CDNB substrate was determined as 0.276 mM and the V_{max} value was determined as 0.161 EU/mL^{-1}. The inhibitory effects of Met-Hg, Fe^{3+}, Cr^{3+}, Cu^{2+}, Mg^{2+}, Zn^{2+} metal ions and atrazine dinicanozole, and methomyl pesticides on the purified kidney GST enzyme activity were investigated. The IC_{50} values of the inhibitor were determined by graphs as demonstrated

in figure 5 and 6 and calculated as 34.5mM, 10µM, 1.5µM, 2.4µM, 63 µM, 0.15mM, 10µM, 9.8 µM, 1.3 µM, 2.67 µM, respectively (Table 2).

Table 1. Purification conditions for obtaining GST from kidney tissue of Van Lake Fish

Sample type	Total volume (mL)	Activity (EU/ml)	Protein (mg/ml)	Total protein (mg)	Total activity	Specific activity (EU/mg)	Yield (%)	Purification coefficient coefficient
Homogenate	22	0.12	1.46	32.12	2.66	0.083	100	1
Affinity	3	0.8	0.023	0.086	2.39	27.8	89	335

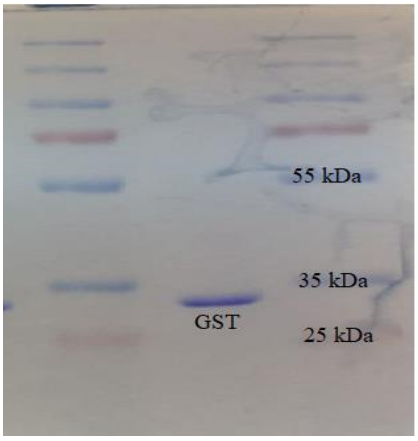

Figure 1. SDS-PAGE photograph showing the purity of the enzyme. Lane 1: standart proteins. Lane 2: Van Lake fish kidney

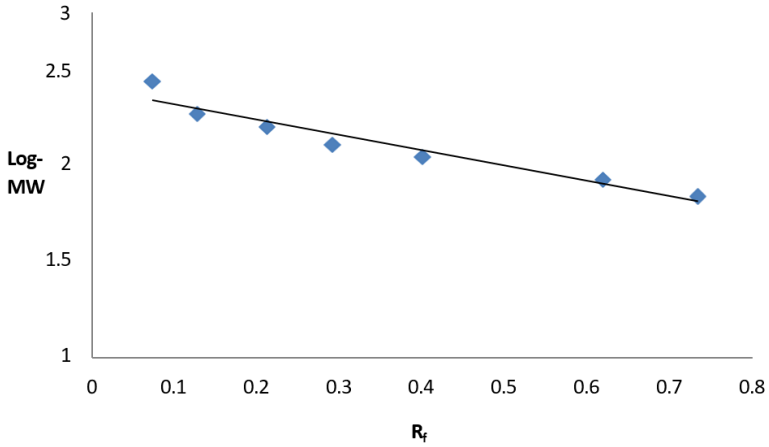

Figure 2. Standard Rf-Log MW graph of GST using SDS-PAGE

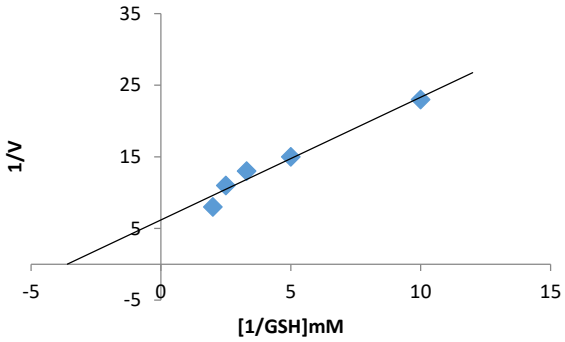

Figure 3. Lineweaver-Burk graph in five different concentrations for GSH substrate

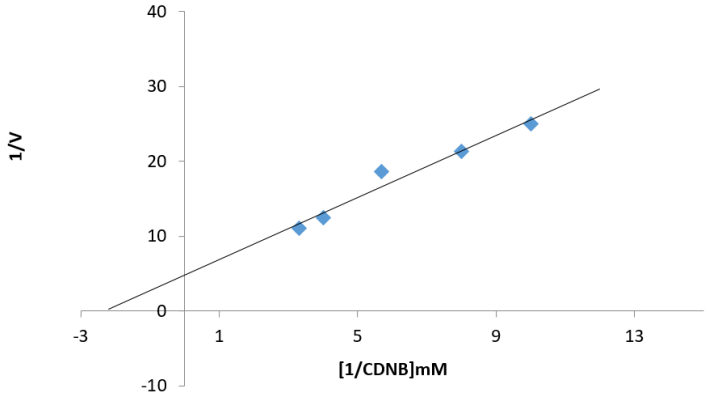

Figure 4. Lineweaver-Burk graph in five different concentrations for CDNB substrate

It is known that the biochemical reactions that take place in living organisms are catalyzed by specific enzymes. Pollution of aquatic environments with various natural and industrial compounds is known as one of the environmental problems. (Schwarzenbach et al., 2006). Reactive oxygen species such as H_2O_2, superoxide and hydroxyl radicals occur in living organisms exposed to metal and pesticide contamination. These stations contribute to the formation of oxidative damage in cells (Ozaslan et al., 2018; Pinto et al., 2003). The GST is a phase II xenobiotic-metabolizing enzyme family and plays critical roles in cellular defence against toxic compounds. Moreover, the GST is a detoxification enzyme against some harmful exogenous molecules such as mutagens compounds, carcinogens, and environmental pollutants (Cui et al., 2015; Tu et al., 2005).

In the present paper, the GST enzyme was purified from the kidney tissue of Chalcalburnus Tarichi using the GSH-agarose affinity technique. The purification results given in Table 1 show that the enzyme was purified 335-fold, with 27.8 EU/mg protein-specific activity and 89% yield.The affinity technique is important, so it takes to advance to obtain high enzyme yield in a very short time used in this study. Fishes is used as a contamination indicator in the aquatic environments for long years. The GST enzyme is studied from different fish tissues such as Chalcalburnus Tarichi (Ozaslan et al., 2017), Rainbow trout (Comakli et al., 2013), Salmo trutta labrax (Perez-Lopez et al., 2002), Oncorhynchus tshawytscha (Donham et al., 2005), Zebrafish (Alena et al., 2018). According to what we know, there is no research containing the effect of metal and pesticides on Chalcalburnus Tarichi kidney GST enzyme in the literature.

Recent studies in the literature been conducted for the purification of the GST enzyme from different sources. Akkemik et al. They purified the GST enzyme from turkey liver 252.7 times with a 45% yield by glutathione-agarose affinity chromatography (Akkemik et al., 2012).In another similar study, the GST enzyme was purified 1143-fold from human erythrocytes with a specific activity of 16.00 EU/mg and a yield of 80%. (Erat and Şakiroğlu, 2013). Additionally, the M. albus liver GST was purified via Sepharose - 6B affinity chromatography with a yield of 14 % (Huang et al., 2008).

The cytosolic GSTs are dimeric proteins (Noble et al., 1998). Single band image obtained in the SDS-PAGE in Figure 1 showed the enzyme as a homodimer. Furthermore, obtained data from SDS-PAGE results from human erythrocytes (Aksoy and Kufrevioglu, 2018), rainbow trout (Comakli et al., 2013), and Salmo trutta labrax (Comakli et al., 2015) support our results. The Km and V_{max} values for CDNB and GSH acting as substrates of the GST enzyme were calculated by the Lineaweaver-Burk graph (Figure 3 and Figure 4). According to the results, the K_m and V_{max} values for the CDNB substrate were 0.276 mM and 0.161 EU/mL, respectively. On the other hand, the Km constant and Vmax for the GSH substrate was 0.429 mM and 0.207 EU/mL, respectively. The obtained data in the present study revealed a higher affinity of the enzyme for the CDNB substrate with a smaller Km value. We also

examined the inhibition effects to understand the toxic effects of metals and pesticides on the enzyme. According to the results obtained, the inhibitory effect of pesticides and metal ions on GST enzyme was observed. The results obtained are given in Table 2. Enzymes are known to catalyze reactions in the living organisms.. The environmental contaminants, namely, metal ions and pesticides, influence metabolism at low concentrations by enhancing or inhibiting enzyme activities. Therefore, researchers have carried out many studies that reveal the effects of metal ions and pesticides on enzyme activities. In previous studies, researchers purified the GST enzyme from turkey liver.They revealed the effects of some ions like Zn^{2+}, Cu^{2+}, Ag^{+}, Mg^{2+}, Hg^{2+}, Fe^{2+}, Ni^{2+}, and Mn^{2+} on GST activity, and recorded that ions inhibited the GST activity with different concentrations (Akkemik et al., 2012).

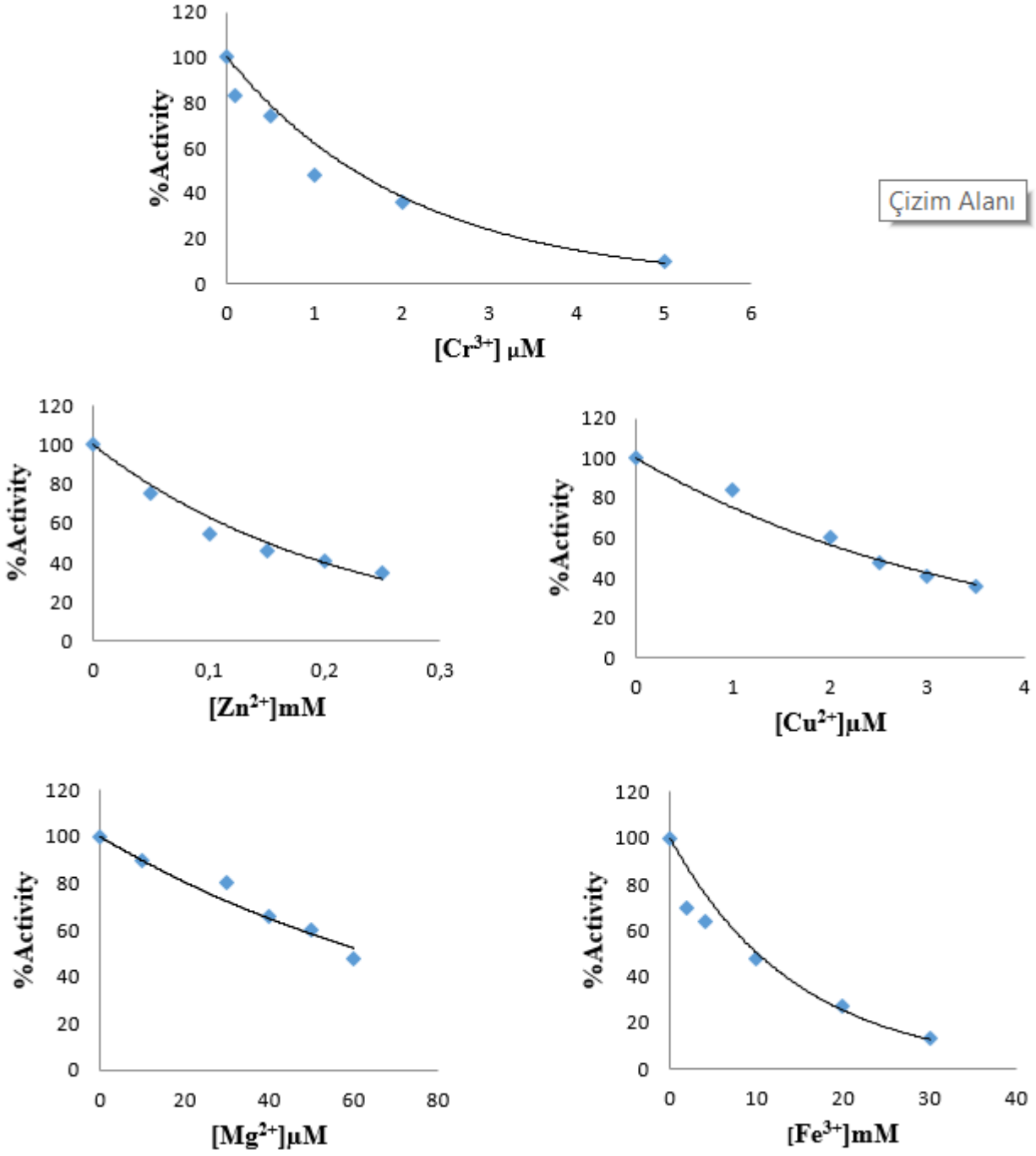

Figure 5. Activity % -metal ions graphs for Van Lake fish kidney GST

Table 2. IC_{50} values for kidney GST of metal ion and pesticide chemicals.

Inhibitor	IC_{50}
Zn^{+2}	0.15 mM
Mg^{+2}	63 µM
Fe^{+3}	10 µM
Cu^{+2}	2.4 µM
Cr^{+3}	1.5 µM
Metil Cıva	34.5 mM
Atrazine	9.8 µM
Methomyl	2.67 µM
Diniconazole	1.3 µM

Mozhdeganloo et al. (2015) have shoved that oxidative damage emerged in the rainbow trout livers exposed to Met-Hg at various concentrations and durations, and their antioxidant defence systems changed (Mozhdeganlo et al., 2015). Aksoy et al. (2018) reported to in vitro effects of some pesticides on GST from Van Lake Fish liver. They found λ-cyhalothrin, dichlorvos, 2,4-DDMA, and glyphosate isopropylamine showed remarkable inhibitory effects (Özaslan et al., 2018).

In this study, we purified the GST enzyme from the liver of Lake Van Fish (C. Tarichi) by one-step glutathione-agarose affinity chromatography. Then, we investigated the in vitro toxic effects of some heavy metal ions and some pesticides on enzyme activity. After the inhibition plots were drawn, we determined the IC50 values of metal ions and pesticides in the range of µM and mM. It is thought that chemicals with a low IC50 value may be toxic not only for some living things but also for all living things.Thus, it might be suggested that responsible parties should pay more attention releasing of these environmentally pollutants.

CONCLUSION

In conclusion, the in vitro inhibitory effects of environmental hazardous pollutants like metal ions and pesticides were investigated on the GST enzyme purified from the kidney tissue of Van Lake Fish. It is well known that the GST enzyme has an important role in the detoxifyingof xenobiotics metabolism. In the literature, there are studies that investigate the in vitro effects of environmental pollutants on the GST enzymeIt is seen that our results are compatible with them. However, it is essential that our findings must be supported by in vivo studies.

ACKNOWLEDGEMENTS

This study was supported by the Agri Ibrahim Cecen University

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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