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Investigation of *In Vivo* Effects of Carbon Tetrachloride (CCl_4) and Quercetin on Some Metabolic Enzyme Activities in Rat Erythrocyte

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Keywords
Carbon tetrachloride, Quercetin, Glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, Glutathione reductase, Glutatyon S-transferaz.

Abstract: In the study; the purpose was to investigate the *in vivo* impact of carbon tetrachloride (CCl_4) and quercetin (Qu) on activities of important metabolic enzymes such as Glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD), glutathione reductase (GR) and glutathione S-transferase (GST) in rat erythrocytes. At the experimental stage, rats were divided into 4 groups. 1.Group (Control): Pure olive oil at a dose determined according to their body weight (1mL/kg) was given to the rats in this group, 2.Group (CCl4: 1.0 mL/kg (ip)(1:1), 3.Group (Ku: 25) mg/kg (ip), 4.Group (CCl4(1.0 ml/kg (ip)+ Ku (25 mg/kg (ip) was injected. The study was continued for 3 days. The results revealed that the activities of; G6PD ($p<0.01$), 6PGD ($p<0.01$), GR($p<0.001$) and GST ($p>0.05$) enzyme activities were decreased in the CCl_4 group compared to the control group. It was determined that enzyme activities were higher in CCl_4+Qu applied groups compared to CCl_4 group. The application of Qu caused an increase in the enzyme activity value. This can be accepted as an indication that the inhibition caused by CCI_4 has disappeared. Consequently; It is thought that Qu may be effective in preventing oxidative damage due to CCl_4 administration.

Sıçan Eritrositlerinde Karbon Tetraklorürün(CCl_4) ve Kuersetinin Bazı Metabolik Enzim Aktiviteleri Üzerine *İn Vivo* Etkisinin İncelenmesi

Anahtar Kelimeler
Karbon tetraklorür, Kuersetin, Glukoz 6-Fosfat Dehidrogenaz, 6-fosfoglukonat dehidrogenaz, Glutatyon redüktaz, Glutatyon S-transferaz.

Öz: Çalışmada; Sıçan eritrositlerinde karbon tetraklorür (CCl_4) ve Kuersetinin(Ku)'in, Glukoz 6-fosfat dehidrogenaz (G6PD), 6-fosfoglukonat dehidrogenaz (6PGD), glutatyon redüktaz (GR) ve glutatyon S-transferaz (GST) gibi önemli metabolik enzim aktiviteleri üzerine *in vivo* etkilerinin incelenmesi hedeflendi. Deneysel aşamada sıçanlar 4 gruba ayrıldı. 1.Grup(Kontrol): Bu gruptaki ratlara vücut ağırlıklarına göre belirlenen dozda(1 mL/kg)saf zeytin yağı, 2.Grup(CCl_4: 1.0 mL/kg (i.p.)(1:1), 3.Grup(Ku: 25 mg/kg (i.p),4.Grup(CCl_4(1.0 ml/kg (i.p.)+ Ku (25 mg/kg (i.p.) enjekte edildi.Çalışma 3 gün sürdürüldü. Sonuçlar değerlendirildiğinde; G6PD ($p<0,01$), 6PGD ($p<0,01$) GR ($p<0,001$) ve GST ($p>0,05$) enzim aktiviteleri kontrol grubuna oranla CCl_4 grubunda azalmıştır. CCl_4+Ku uygulanan gruplarda ise enzim aktivitelerinin CCl_4 grubuna oranla yüksek olduğu belirlenmiştir. Ku uygulanması, enzim aktivite değerinin yükselmesine neden olmuştur. Buda CCI_4'ün neden olduğu inhibisyonun ortadan kalktığının göstergesi olarak kabul edilebilir. Sonuç olarak, CCl_4 uygulamasına bağlı olarak ortaya çıkan oksidatif hasarı önlemede Ku'nın etkili olabileceği düşünülmektedir.

1. INTRODUCTION

Carbon tetrachloride (CCl_4) is a colorless, non-flammable, quickly evaporating, fragrant dense liquid, and is widely used in the production of petroleum products, varnishes, lacquers, resin solvents and organic compounds [1]. It is frequently used in dry cleaning, firefighting, grain disinfection and insect control [2]. Biologically inactive CCl_4 is transformed into reactive toxic metabolites for their activation and takes part in target cells [3,4,5]. CCl_4 exerts its toxic effect with the formation of a free radical, the trichloromethyl radical. The peroxyl radical, which is formed as a result of the combination of this radical with oxygen, is a strong lipid peroxidation initiator that plays a role as the primary mechanism in the formation of cell damage by disrupting the cell membrane structure [3,6,7,8]. Malondialdehyde (MDA), which is formed as the end product of lipid peroxidation, is frequently used in the determination of oxidative damage [9]. In CCl_4 poisoning; analogous to oxidative damage as an experimental model [10] (Figure 1).

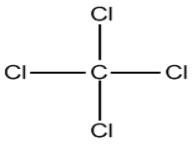

Figure 1. Molecular structure of carbon tetrachloride (CCl_4) [11].

Quercetin (Qu) is one of the most widely studied bioflavonoids in the flavonols class [12] and is one of the most common flavonoids found and many dietary sources [13]. Qu, occurs mainly in the leaves and other parts of the plant in the form of glycosides or aglycones formed by the attachment of one or more sugar groups to phenolic groups by glycosidic bonds. Qu has powerful anti-oxidative and cytoprotective impact on oxidant-induced endothelial cell apoptosis due to its chemical structure [12]. Qu, which is included in various herbal teas today; It is used in biochemistry, food chemistry, paint chemistry, medical chemistry, paint industry and cosmetics[14,15]. Qu, prevents oxidative damage and cell death by prohibit lipid peroxidation and scavenging free oxygen radicals [16,17]. Qu, exerts its possible biological properties through its antioxidant activity [18] (Figure 2).

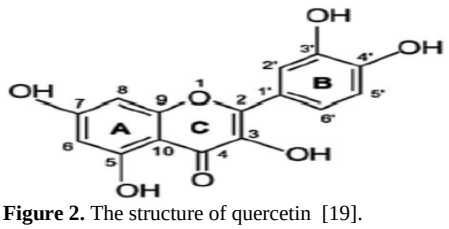

Figure 2. The structure of quercetin [19].

G6PD and 6PGD are known as metabolic enzymes that catalyze the first and third steps of the pentose phosphate pathway. As an agent that protects cells against oxidative stress, NADPH is known as an important molecule for biochemical processes such as reducing biosynthesis reactions [20,21]. NADPH is the most important molecule that reduces the disulfide form of glutathione, as well as being considered as a cellular reducer, which is significant for nitric oxide biosynthesis, detoxification of xenobiotics and pharmaceuticals [22-24]. GR preserve the cell counter the harmful effects of these molecules by reducing oxidized molecules thanks to the –SH groups in its construction. In addition, membrane proteins such as GSH, the reduced form of GR, hemoglobin and spectrin, and the preserve of thiol groups of various enzymes, DNA and protein synthesis, detoxification of xenobiotics and some metabolic end products, and transport of amino acids [25,26]. The GR enzyme catalyzes the regeneration reaction of glutathione in the presence of NADPH, thereby maintaining the intracellular redox balance [27]. The binding reactions of GSH to endogenous and exogenous hydrophobic electrophiles are carried out by GST isoenzymes. GSTs can non-catalytically bind many exogenous and endogenous compounds such as fatty acids, hormones, flavonoids, bilirubin and xenobiotics [28,29].The purpose of the presented study was to investigate the *in vivo* effects of CCl_4 and Qu treatments on some metabolic enzymes G6PD, 6PGD, GR and GST activities in rat erythrocytes.

2.MATERIALS AND METHODS

2.1.Chemicals

The chemicals used in the study were purchased from Sigma and Merck.

2.2. Experimental Design

Twenty-eight Wistar albino male rats weighing 200-300 g were used.They were fed ad-libitum with standard laboratory chow and water at a constant temperature of 20 ± 2ºC and twelve (12 hours) light-dark cycle (light; 07:00-19:00, dark 19:00-07:00). The rats were randomly divided into 4 groups of 7 in each group. The study was approved by the Bingol University Experimental Animal Ethics Committee. (BUHADEK: 4.10.2018-2018/08-08/01).

1. Group (Control): Pure olive oil i.p. given through in a dose determined according to the body weight of the rats (1 mL/kg) was given to the rats in this group [30].
2.Group (CCl_4): The rats in this group were mixed with 1 mL/kg of CCl4 in olive oil at a ratio of 1:1 and administered i.p. was applied as. [31].
3.Group (Qu): The rats in this group were administered i.p. at a dose of 25 mg/kg by using the stock solution prepared in dimethyl sulfoxide (DMSO) [32].
4.Group (CCl_4 + Qu): The rats in this group were administered 1 mL/kg CCl4 (1:1 in olive oil) + 25 mg/kg Qu (using the stock solution prepared in DMSO) i.p. At the end of the 3rd day, blood samples were taken from the rats under anesthesia.

2.3. Preparation of Hemolysate

Blood samples were taken into EDTA tubes. Fresh blood samples were taken into EDTA tubes. Afterwards, plasma and leukocytes were eliminated by centrifugation for 15 minutes (2500 xg). Red cells packed with KCl solution (0.16 M) were washed three times. Blood

samples were centrifuged at (2500 xg) and supernatants were discarded. Erythrocytes were hemolyzed using 5-fold distilled water. Samples were centrifuged at +4°C (10000 xg) for 30 minutes to eliminated cell membranes and insoluble molecules. The supernatant was saved for analysis [33].

2.4. Determination of Enzyme Activities

G6PD and 6PGD enzyme activity was measured spectrophotometrically according to the Beutler method at 340 nm. This method is based on the principle that NADPHs in the reaction medium absorb light at 340 nm. In reactions catalyzed by both G6PD and 6PGD, enzymes react with $NADP^+$ as a co-substrate and convert to NADPH. This increase is followed spectrophotometrically, the amount of absorption per minute is found and converted into enzyme units [34-36]. The method proposed by Carlberg and Mannervik was used for the measurement of GR enzyme activity. This method depends on the reduction of NADPH in the reaction catalyzed by the GR enzyme. This decrease was monitored by spectrophotometry at 340 nm to determine enzyme activity [37] and the activity of the GST enzyme was determined according to the Habig method. This method is based on the conversion of 1-chloro-2,4-dinitrobenzene (CDNB) to S-glutathione dinitrobenzene (DNB-SG). The product of this reaction, DNB-SG, shows maximum absorbance at 340 nm [38].

3. STATİSTİCAL ANALYSİS

Statistical evaluation was made using the SPSS 20 program. Data were expressed as mean ± SD. The Kruskal Wallis test followed by the Mann Whitney U test was used to define the diverse among the groups. The diverse among the groups was remerkable important when $p< 0.05$.

4. RESULTS AND DISCUSSION

According to the results of the current study; G6PD enzyme activity was statistically lower in the CCl_4 group compared to the control group ($p<0.01$). Qu applied group; G6PD enzyme activity was found close to the control group.In the CCl_4+Qu group, the G6PD enzyme activity was discover to be higher than the CCl_4-treated group (Figure 3)

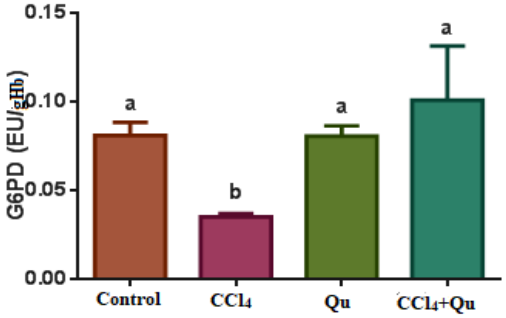

Figure 3. The in vivo effect of CCl_4 and Qu on rat erythrocyte G6PD enzyme activity (a,b differs between groups with different letters) ($p<0.01$).

When the 6PGD enzyme activity was assessed, a statistically significant reduction was observed in the CCl_4 treated group compared to the control group ($p<0.01$). The 6PGD enzyme activity was found to be higher in the Qu-treated group than in the CCl_4 group. Enzyme activity rised in CCl_4+Qu group compared to CCl_4 group. These results show that Qu reduces the inhibitory effects of CCl_4 on 6PGD enzyme activity(Figure 4).

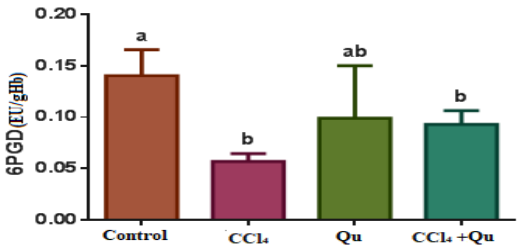

Figure 4. In vivo effect of CCl_4 and Qu on rat erythrocyte 6PGD enzyme activity (a,b, difference between groups with different letters) ($p<0,01$).

When the GR enzyme activity is evaluated; it was observed that the enzyme activity was reduced in the CCl_4 applied group compared to the control group($p<0,001$). In the CCl_4 +Qu applied group, it was identified that the GR enzyme activity was increased compared to the CCl_4 group (Figure 5).

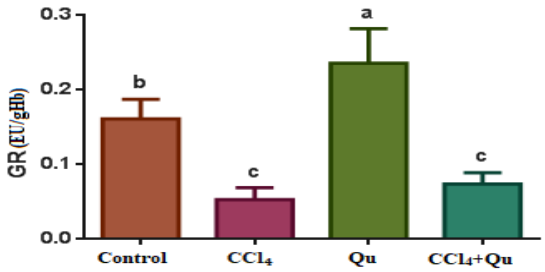

Figure 5. In vivo effect of CCl_4 and Qu on rat erythrocyte GR enzyme activity (a,b,c there is difference between groups with different letters) ($p<0,001$).

When the GST enzyme activity was assessed; it was determined that there was a decrease in the CCl_4 group compared to the control group, and a partial increase was observed in the CCl_4+Qu group compared to the CCl_4 group. No statistical differences were discovered between the groups ($p>0,05$) (Figure 6).

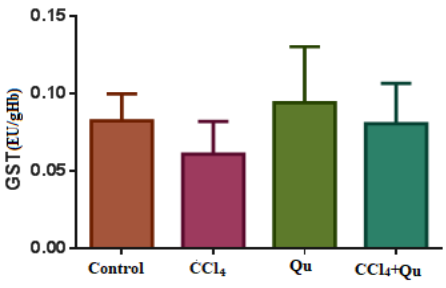

Figure 6. In vivo effect of CCl_4 and Qu on rat erythrocyte GST enzyme activity ($p>0,05$).

When the results of this study are evaluated in general; G6PD, 6PGD and GR enzyme activities are inhibited by CCl_4. As a result of prevention of G6PD and 6PGD enzyme activities, cells cannot produce enough NADPH in the pentose phosphate pathway to detoxify endogenous and exogenous oxidized molecules. At the same time, inhibition of GR and GST enzyme activities may cause disruption in intracellular GSH metabolism, thus rendering cells vulnerable to oxidative stress. In the study; G6PD enzyme activity was decreased in CCl_4 group compared to control group ($p<0.01$). Enzyme activity in the Qu group was discover to be close to the control group. In the CCl_4+Qu group, the enzyme activity was discover to be higher than in the CCl_4 group. This suggests that quercetin may have a decreasing effect on the inhibition of CCl_4 in G6PD enzyme activity. When 6PGD enzyme activity is evaluated; The decrease in enzyme activity ($p<0.01$) in the group treated with CCl_4 compared to the control group can be considered as an indicator of inhibition of the 6PGD enzyme by CCl_4 application. The rise in the enzyme activity value in the CCl_4+Qu applied group can be considered as an indication that the inhibition effect is partially eliminated. While it was observed that GR enzyme activity reduction statistically important ($p<0.001$) in the carbon tetrachloride group compared to the control group, it was concluded that the enzyme activity increased partially in the CCl_4 +Qu administered group. When the GST enzyme activity was assesment, a partial reduction was determined in the CCl_4 group compared to the control group. It was seen that the increases or decreases between the groups were not statistically significant. In the literature review, no study was found that examined the effects of CCl_4 and Qu on metabolic enzymes in rat erythrocytes. In a different study, it was determined that carbon tetrachloride (CCl_4) intoxication caused a decrease in enzyme activities such as NADPH-cytochrome c reductase, NADH-cytochrome reductase, coumarin hydroxylase, 7-ethoxy coumarin-O-deethylase, UDP-glucuronyl transferase and glutathione-S-transferase [39]. In a different study by Sheweita et al., it was determined that CCl_4 application caused inhibition of GST enzyme activity [40]. In a study by Elbe et al., the antioxidative effects of Qu in CCl_4-caused cardiac detriment were histologically and biochemically investigated. While SOD activity were remarkably declined in CCl_4 administered group, Qu administration caused considerable rise in SOD activity. In CCl_4 toxicity, it was concluded that the use of Qu could provide beneficial effects owing to its antioxidative properties [41]. In a different study investigating the antioxidant defense system in tissue damage caused by CCl_4 and ethyl alcohol in goose liver and kidneys, which are among the important antioxidant and metabolic enzymes due to damage to the liver and kidneys, glutation peroxidase(GSH-Px), catalase (CAT),glucose-6-phosphate dehydrogenase (G6PD) activities were decreased [42]. In a different study by Jeon et al., it was reported that carbon tetra chloride administration caused a decrease in the activities of important metabolic enzymes glutathione S-transferase and glutathione reductase in the rat liver [43]. In a different study researchers evaluated teh effects of CCl_4 and Qu treatment. They demonstrated that Qu isolated from *Delonix elata* L. plant has significant prophylactic effects on liver function markers AST, ALT, ALP, serum bilirubin and total protein, as well as antioxidant enzymes SOD, CAT, GPx and GST [44]. Literature also stated that after CCl_4 exposure, antioxidant enzyme activities such as SOD, CAT and GPx decreased significantly in the rat kidney, CCl_4 exposure caused oxidative stress by inhibiting antioxidant enzyme activities [45]. There are many other studies revealing various drugs and substances inhibit activities of metabolic enzymes [46-48].

5. CONCLUSION

When the results of this study are evaluated in general; G6PD, 6PGD and GR enzyme activities are inhibited by CCl4. In the CCl_4+Qu applied groups, the enzyme activities were higher than the CCl_4 group. While the GST enzyme activity decreased in the CCI4-administered group, it partially increased in the Qu-treated group. These results indicated that Qu reduces the inhibition impact of CCl_4 on G6PD, 6PGD and GR enzyme activities. Accordingly, it is thought that Qu may be effective in preventing oxidative damage due to CCl_4 administration. At the same time, there is a need for further studies in which different Quercetin dosages will be used.

CONFLICTS OF INTEREST

There is no conflict between the authors.

REFERENCES

[1] Vural DF. Karbontetraklorür (CCl_4) Uygulanmış Sıçanların Gastrocnemius Kasına Melatoninin Etkisi. Ankara Üniversitesi Fen Bilimleri Enstitüsü. Yüksek Lİsans Tezi.2015.

[2] Akkoyun-Bayraoğlu M**,** Akkoyun HT. Effect Of Carbon Tetrachlorıde (CCl_4) And Ellagıc Acıd On Rat Erythrocyte G6PD, 6PGD, GR, GST AND TrxR Enzyme Actıvıtıes. Fresenius Environmental Bulletin. 2019;28(9), 6779-6785

[3] Akkuş İ. Serbest Radikaller ve Fizyopatolojik Etkileri. Mimoza Yayınları, Konya.1995.

[4] Murray RK, Mayes PA, Granner DK, and Rodwell VW. Harper'ın Biyokimyası. Barış Kitapevi. 1993.

[5] Kayaalp O. Rasyonel Tedavi Yönünden Tıbbi Farmakoloji. 10. Baskı, Hacettepe Taş. 2002.

[6] Kurt H, Başaran A, Musmul A. Sıçanlarda Karbon Tetraklorit (CCl_4)'in Oluşturduğu Oksidatif Stresin Kateşin ile Önlenmesi. Kocatepe Tıp Dergisi. 2004; 5(2),29-34.

[7] León OS, Menéndez S, Merino N, Castillo R, Sam S, Pérez L, Cruz E, Bocci V. Ozone oxidative preconditioning: a protection against cellular damage by free radicals. Mediators Inflamm. 1998;7(4),289-94.

[8] Süha-Yalçin A, Koçak-Toker N, Uysal M, Aykaç G, Sivas A, Oz H. Stimulation of lipid peroxidation and impairment of glutathione-dependent defence system in the liver of rats repeatedly treated with

carbon tetrachloride. J Appl Toxicol. 1986; 6(4),303-6.
[9] Tekeli H,Bildik A. Karbon Tetraklorür İle Oluşturulan Karaciğer Hasarında Glutatyon (GSH) Ve Glutatyon S-Transferaz (GST) Aktivitesi Üzerine N-Asetil Sisteinin Etkisi. Balikesir Saglik Bil Derg. 2016; 5(2).
[10] Akkoyun HT, Bengü AŞ,Ulucan A,Bayramoğlu-Akkoyun M,Arihan O. Protectıve Effect Of Ellagıc Acıd Agaınst Carbon Tetrachlorıde (CCl_4) Induced Oxıdatıve Braın Injury In Rats. Fresenius Environmental Bulletin. 2018; 27(5),3148-3155.
[11] Demiralp T. Sıçanlarda Karbon Tetraklorürün (CCl_4) Böbrekte Oluşturduğu Hasara Karşı Dekspantenolün (Provitamin B5) Koruyucu Etkisinin Biyokimyasal Açıdan Araştırılması. Yüksek Lisans Tezi-2021. T.C. İnönü Üniversitesi. Sağlık Bilimleri Enstitüsü.
[12] Choi YJ, Kang JS, Park JH, Lee YJ, Choi JS, Kang YH. Polyphenolic flavonoids differ in their antiapoptotic efficacy in hydrogen peroxide treated human vascular endothelial cells. J Nutr. 2003;133,985–991.
[13] Pavanato A, Tunon MJ, Sanchez-Campos S, Marroni CA, Llesuy S, Gonzalez- Gallego J, Marroni N. Effects of quercetin on liver damage in rats with carbon tetrachloride-induced cirrhosis. Digestive Diseases and Sciences. 2003;48(4), 824-829.
[14] Ergüzel ET. Quercetin (3, 3', 4', 5, 7-pentahidroksiflavon)'in Bakır (II) ve Çinko (II) Komplekslerin Kararlılık Sabitlerinin Tayini. Yüksek Lisans. İstanbul: Marmara Üniversitesi. 2006.
[15] Emek H. Dönmez Burukoğlu D. Bayçu C, Musmul A. Erişkin erkek sıçanlarda karbon tetraklorür ile oluşturulan testis hasarı üzerine kuersetinin etkisi, Osmangazi Tıp Dergisi. 2017;39(65-73).
[16] Cho JY, Kim IS, Jang YH, Kim AR, Lee SR. Protective effect of quercetin, a natural flavonoid against neuronal damage after transient global cerebral ischemia. Neurosci Lett. 2006;404(3),330–335
[17] Schültke E, Kamencic H, Zhao M, Tian GF, Baker AJ, Griebel RW, Juurlink BH. Neuroprotection following fluid percussion brain trauma: a pilot study using quercetin. J Neurotrauma. 2005;22(12),1475–1484
[18] Terao J,Piskula M. Flavonoids as inhibitors of lipid peroxidation in membranes. In: Flavonoids in Health and Disease. Rice-Evans, C. and Packer, L., eds. Marcel Dekker: New York. 1998; 277–93.
[19] Mitchell LJ. Spectrophotometry of Molybdenum, Tungsten and Chromium chelates of quercetin. PhD Thesis, Oregeon State University: USA. 1965.
[20] Temel Y, Ayna A, Hamdi Shafeeq I, Ciftci M. *In vitro* effects of some antibiotics on glucose-6-phosphate dehydrogenase from rat (*Rattus norvegicus*) erythrocyte. Drug Chem Toxicol. 2020;43(2),219-223.
[21] Temel Y, and Kocyigit U M. Purification of glucose-6-phosphate dehydrogenase from rat (Rattus norvegicus) erythrocytes and inhibition effects of some metal ions on enzyme activity, Journal of biochemical and molecular toxicology. 2017; 31(9),e21927.
[22] Krebs HA. and Eggleston LV. The regulation of the pentosephosphate cycle in rat liver. In: G. Weber, ed. Advances in Enzyme Regulation. Oxford: Pergamon Press Ltd. 1978; 12, 421–433.
[23] Nelson DL and Cox MM. Lehninger Principles of Biochemistry. 4th ed. New York: WH. Freeman & Co, ABD. 2005; 558–561.
[24] Stanton RC. Glucose-6-phosphate dehydrogenase, NADPH, and cell survival. IUBMB Life. 2012; 64 (5), 362–369
[25] Temel Y, Bozkuş T, Karagözoğlu Y, Çiftci M. "Glutatyon Redüktaz (GR) Enziminin Japon Bıldırcın (Coturnix coturnix japanica) Eritrositlerinden Saflaştırılması ve Karakterizasyonu." Iğdır Univ. J. Inst. Sci. Tech. 2017; 7(3),143-150.
[26] Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol. Chem. 2009;390, 191–214,
[27] Couto N, Wood J, Jill B. "The role of glutathione reductase and related enzymes on cellular redox homoeostasis network." Free radical biology and medicine. 2016; 95, 27-42.
[28] Işık M, Demir Y, Kırıcı M, Demir R, Şimşek F, Beydemir Ş. Changes in the anti-oxidant system in adult epilepsy patients receiving anti-epileptic drugs. Archives of physiology and biochemistry. 2015; 121(3), 97-102.
[29] Temel Y, Koçyigit UM, Taysı MŞ, Gökalp F, Gürdere MB, Budak Y, Çiftci M. Purification of glutathione S-transferase enzyme from quail liver tissue and inhibition effects of (3aR, 4S, 7R, 7aS)-2-(4-((E)-3-(aryl) acryloyl) phenyl)-3a, 4, 7, 7a-tetrahydro-1H-4, 7-methanoisoindole-1, 3 (2H)-dione derivatives on the enzyme activity. Journal of biochemical and molecular toxicology. 2018; 32(3), e22034.
[30] Karaca Ö, Pekmez H, Kuş MA, Akpolat N, Ögetürk M, Kuş İ. Deneysel Karbon Tetraklorür Toksisitesi Sonucu Karaciğerdeki IŞP70 İmmunoreaksiyon Artışı Üzerine Melatonin Hormonunun Etkisi. F.Ü.Sağ.Bil.Tıp Derg. 2011; 25 (2): 73 - 76.
[31] Makni M, Chtourou Y, Garoui EM, BoudawaraT, Fetoui H. Carbon tetrachloride- induced nephrotoxicity and DNA damagein rats: Protective role of vanillin. Human and Experimental Toxicology. 2012; 31, 844-852.
[32] Yuan Y, Ma S, Qi Y, Wei X, Cai H, Dong L, Guo Q. Quercetin inhibitedcadmium-induced autophagy in the mouse kidneyvia inhibition of oxidative stress. Journalof Toxicologic Pathology. 2016; 29(4), 247-252.
[33] Bayramoğlu-Akkoyun M, Bengü AŞ, Temel Y, Akkoyun HT, Ekin S, Ciftci M. The effect of astaxanthin and cadmium on rat erythrocyte G6PD, 6PGD, GR, and TrxR enzymes activities in vivo and on rat erythrocyte 6PGD enzyme activity in vitro. J Biochem Mol Toxicol. 2018;32(8),e22170.

[34] Beutler E. Redcell metabolism. Manual of biochemical methods Acad Pres London. 1971;12,68–70.
[35] Bayindir S, Temel Y, Ayna A, Ciftci M. The synthesis of N-benzoylindoles as inhibitors of rat erythrocyte glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Journal of biochemical and molecular toxicology. 2018; 32(9), e22193.
[36] Bayindir S, Ayna A, Temel Y, Ciftci M. The synthesis of new oxindoles as analogs of natural product 3,3-bis(indolyl) oxindole and *in vitro* evaluation of the enzyme activity of G6PD and 6PGD. Turkish Journal of Chemistry. 2018b;42(2),332-345.
[37] Carlberg I, Mannervik B.Purification and characterization of glutathione reductase from calf liver. Glutathione reductase assays. Methods Enzymol. 1981;113,484-4.
[38] Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry. 1974; 249(22), 7130-719.
[39] Kumaravelu, P, Dakshinamoorthy DP, Subramaniam S, Devaraj H, Devaraj NS. Effect of eugenol on drug-metabolizing enzymes of carbon tetrachloride-intoxicated rat liver. Biochemical pharmacology. 1995; 49(11), 1703-1707.
[40] Sheweita SA, Abd El-Gabar M, Bastawy M. Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: role of antioxidants. Toxicology. 2001; 165(2-3), 217-224.
[41] Elbe H, Eşrefoğlu M, Taslidere A, Taşlıdere E, Ateş B. Sıçanlarda karbon tetraklorür ile indüklenen kardiyak hasar üzerine melatonin ve quercetin'in tedavi edici etkileri. İnönü Üniversitesi Turgut Özal Tıp Merkezi Dergisi. 2016; 23(2), 171-176.
[42] Güven A. and Yılmaz S. The effect of carbon tetrachloride (CCl_4) and ethanol (C_2H_5OH) on the determination of levels glutathione peroxidase, catalase, glucose-6-phosphate dehidrogenase and lipid peroxidation liver and kidney in the goose. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2005; 11(2), 113-117.
[43] Jeon TI, Hwang SG, Park NG, Jung YR, Im Shin S, Choi SD, Park DK. Antioxidative effect of chitosan on chronic carbon tetrachloride induced hepatic injury in rats. Toxicology. 2003; 187(1), 67-73.
[44] Krishnappa P, Venkatarangaiah K, Rajanna S, Kumar S, Kashi Prakash Gupta R. Antioxidant and prophylactic effects of Delonix elata L., stem bark extracts, and flavonoid isolated quercetin against carbon tetrachloride-induced hepatotoxicity in rats. BioMed Research International. 2014;1155-507851
[45] Akkoyun HT. Karbontetraklorür'ün Neden Olduğu Sıçan Böbrek Dokusu Hasarlarına Karşı Kuersetin'in Koruyucu Etkisi. Journal of the Institute of Science and Technology.2019;9(2),708-716.
[46] Ayna A, Khosnaw L, Temel Y, Ciftci M. Antibiotics as Inhibitor of Glutathione S-transferase: Biological Evaluation and Molecular Structure Studies. Current drug metabolism.2021; 22(4), 308-314.
[47] Ayna A, Moody PC. Crystal structures of a dual coenzyme specific glyceraldehyde-3-phosphate dehydrogenase from the enteric pathogen Campylobacter jejuni. Journal of Molecular Structure.2021;1242, 130820.
[48] Ayna A, Moody PC. Activity of fructose-1, 6-bisphosphatase from Campylobacter jejuni. Biochemistry and Cell Biology.2020; 98(4), 518-524.