Clinical and Experimental Health Sciences

Alterations in Enzyme Activity of Carbonic Anhydrase, 6-phosphogluconate Dehydrogenase and Thioredoxin Reductase in Rats Exposed to Doxorubicin and Morin

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ABSTRACT

Objectives: Carbonic anhydrase (CA), 6-phosphogluconate dehydrogenase (6PGD) and thioredoxin reductase (TrxR) enzymes are the essential biological molecules needed for metabolic processes in all living cells. This study was designed to investigate the activities of CA, 6PGD and TrxR enzymes in the brain, kidney, liver, heart and testis tissues of the rats exposed to doxorubicin (DOX) and morin.

Methods: Male Wistar albino rats were randomly divided into three groups as control, morin and DOX, each of them containing 7 rats. At the end of the experimental procedure, CA, 6PGD, and TrxR enzyme activities in tissues of rats were determined spectrophotometrically.

Results: In our study, we observed that DOX activated CA enzyme in liver and kidney tissues while inhibiting CA enzyme in the other tissues, activated 6PGD enzyme in the kidney, liver and heart tissues, and inhibited the TrxR enzyme in all the tissues. In addition, morin activated CA enzyme in the liver tissue while inhibiting CA enzyme in the brain, heart and testis tissues. Morin activated 6PGD enzyme activity while it inhibited TrxR enzyme in all the tissues.

Conclusion: The findings showed that doxorubicin and morin had similar properties in the tissues as to their effect on enzyme activities.

Keywords: Doxorubicin; Morin; Carbonic Anhydrase; 6-phosphogluconate Dehydrogenase; Thioredoxin Reductase

1. INTRODUCTION

Enzymes, the largest and most diverse group of proteins, play an important role in the metabolism of all organisms. All chemical reactions and metabolic pathways in the living cells are catalyzed and regulated by enzymes (1). Carbonic anhydrase (CA) is a zinc-containing monomeric metalloenzyme which catalyzes the reversible reaction of CO_2 to bicarbonate (2,3). This enzyme found in all living species is very important in terms of the reactions which it catalyzes (4). This reaction plays a critical role in various metabolic biosynthetic pathways such as metabolism of CO_2 and its transport between tissues that allow its excretion, secretion of electrolytes in various tissues and organs, pH regulation and homeostasis, gluconeogenesis, lipogenesis, ureogenesis, osteoporosis, tumorigenesis, and epilepsy (5,6). The pentose phosphate pathway is one of the most important components of cellular metabolism and the enzyme, 6-phosphogluconate dehydrogenase (6PGD), catalyzes the third reaction of this pathway (7). As a result of oxidative decarboxylation of 6-phosphogluconate, ribulose 5-phosphate and NADPH are the products of this reaction (8). It has been reported that the 6PGD enzyme increases in transcriptional and translational levels in various cancer types, and this is accompanied by an increase in activity. it has been stated that this causes resistance to chemotherapy and increased metastasis of cancer cells (9). While ribose 5-phosphate, one produce of the pentose phosphate pathway, serves as the building block for nucleic acid synthesis, NADPH is an antioxidant which is suppressed production of reactive oxygen species and contributes to the maintenance of the cell redox homeostasis as well as its role in lipid biosynthesis (10). Thioredoxin reductase enzyme (TrxR) forms the thioredoxin system together with thioredoxin and NADPH (11,12). Thioredoxin system, combined with the glutaredoxin system, performs some important biological functions such as protection against oxidative stress, DNA synthesis, regulation of receptor and transcription factors (13). Thioredoxin system is a broad-spectrum thiol reduction system and plays an important role in maintaining intracellular redox balance. This system is very active in the lung, liver, colorectal and gastric cancer types and it is of great importance in proliferation and survival of abnormal cells in tumor formation (11,14). Doxorubicin (DOX), an anthracycline antibiotic, is one of the most effective chemotherapeutic drugs used in the treatment of solid tumors and hematological malignancies such as leukemia, lymphomas, and breast cancer (15). DOX shows its effect by breaking into among DNA bases, by inhibiting the topoisomerase II enzyme and by increasing the formation of free radicals that damage DNA and membranes (16,17). Although it is a very effective chemotherapeutic agent,

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the use of DOX is restricted due to toxicity caused by the use process and its aftermath (18). Another limitation arises from the resistance to the DOX. These two conditions limit the use of DOX in treatment (19). However, the protective effects of some natural products against DOX-induced cardiotoxicity have been reported (20). Phenolic compounds form an important class of plant-based secondary metabolites. Flavonoids which are polyphenolic phytochemicals are biologically active and have antioxidant, antidepressant, anti-inflammatory effects and enzyme inhibition proterties (21-23). It has been reported morin (3, 5, 7, 2', 4'-pentahydroxyflavone), a bioflavonoid found in many plants and fruits, can regulate some metabolic enzyme activities and protect metabolism against oxidative stress due to its antioxidant properties (24, 25). Within the scope of the study, changes in CA, G6PD and TrxR activities in the liver, heart, kidney, and brain tissues of rats exposed to morin, which is polyphenolic phytochemical, and DOX, used as a cancer drug were investigated.

2. METHODS

2.1. Drugs and chemicals

DOX was obtained as Adrimisin® (50 mg/25 mL injectable solution) from Saba İlaç San. ve Tic. A.Ş, Turkey. Morin hydrate and the other chemicals were purchased from Sigma-Aldrich (St Louis, MO) and Merck (EMD Millipore Corporation, USA). The administration dose of DOX in rats was determined according to the literature (26).

2.2. Animals

The Wistar albino male rats used in this study were provided by Ataturk University Medical Experimental Application and Research Center. 10-week-old rats with a body weight between 200-250 g were kept in special cages in a controlled breeding room (24±1°C), relative humidity of 45±5% under a regular 12 h on/off sight cycle throughout the experiment. The rats were fed (pellet diet and water) *ad libitum.* This study was designed conforming to ethical norms approved by the Animal Experimentation Ethics Committee of Ataturk University.

2.3. Experimental design

The animals were randomly categorized into three groups consisting of 7 rats in each group.

Group C (Control group): Healthy control rats were administered normal saline daily using oral gavage for 10 days.

Grup M (Morin Hydrate 100 mg/kg): The rats were administered morin hydrate at a dose of 100 mg/kg b.wt daily using oral gavage for 10 days.

Group D (DOX 40 mg/kg): The rats were injected intraperitoneally with a single dose of Dox (40 mg/kg b.wt, i.p.) on the 8th day.

At the end of the study period (10th day), the rats were decapitated under mild sevoflurane anesthesia (Sevorane liquid 100%; Abbott Laboratory, Istanbul, Turkey). The liver, testis, heart, kidney, and brain tissues were evaluated from rats for biochemical analysis.

2.4. Preparation of homogenate

The rat tissues were washed with 0.9% NaCl solution and then cut into small pieces by means of a scalpel. The pieces were powdered by grinding in a mortar in the presence of liquid nitrogen. The powder was homogenized with 20 mM Tris-HCl (pH 7.5) solution and taken into Eppendorf tubes. After that, the homogenate was centrifuged at 12,900 rpm for 30 min at +4°C. The supernatant was removed and stored on ice.

2.5. Measurement of carbonic anhydrase activity

The CA activity was measured by the method of esterase activity. CA activity was assayed according to the method of Verpoorte et al (27). This method is based on the hydrolysis of p-nitrophenyl acetate to p-nitrophenol by CA. p-nitrophenol has a maximum absorbance at 348 nm. Thus, the enzyme activity was measured spectrophotometrically at 348 nm.

2.6. Measurement of 6-phosphogluconate dehydrogenase activity

The 6PGD activity was measured according to the method of Beutler (28). The basis of the method is the formation of NADP by reducing $NADP^+$ in the presence of 6-phosphogluconate. The resulting NADPH shows a maximum absorbance at 340 nm. Thus, the activity was measured spectrophotometrically at 340 nm.

2.7. Measurement of thioredoxin reductase activity

The TrxR activity was measured according to the method of Holmgren and Bjornstedt (29). This method is based on the reduction of 5,5'-Dithiobis- (2-Nitrobenzoic Acid) which is the artificial substrate of the enzyme in the presence of NADPH and the maximum absorbance of the resulting 5-thio-2-nitrobenzoic acid at 412 nm.

2.8. Statistical analysis

SPSS 16.0 program was used for statistical evaluation. Kruskal–Wallis test was used to determine the difference between the groups obtained semi-quantitative in the histopathological examination. Detection of different groups was determined by Mann–Whitney U-test. Statistical significance was considered $p < 0.05$. One-way ANOVA (Tukey) SPSS (version 12.0; SPSS, Chicago, IL) statistical program was used for biochemical analysis. All values were given as mean ± standard error (±S.E.M.), while the results at $p < 0.05$ were considered as significant.

3. RESULTS

The wistar albino male rats used in this study were randomly categorized into three groups consisting of 7 rats in each group. In group C, the rats were administered normal saline daily by oral gavage for 10 days. In group M, the rats were administered morin hydrate at a dose of 100 mg/kg b.wt daily by oral gavage

for 10 days. In group D, the rats were injected a single dose of DOX (40 mg/kg b.wt, i.p.) on the 8th day of the experiment. At the end of the 10th day, the rats were decapitated under mild sevoflurane anesthesia. After that, the liver, testis, heart, kidney, and brain tissues from the rats were evaluated for biochemical analysis. The spectrophotometer was used to measure the enzyme activities in all the tissues.

In accordance with our findings, CA activities was significantly decreased in the brain, heart, and testis tissues of the rats in the groups M and D when compared with that of the rats in the group C ($p<0.05$). CA activities in the brain and testis tissues of the rats were inhibited in the presence of DOX and morin, but the inhibitory effect of DOX was higher than that of morin. In contrast, the inhibitory effect of morin was higher than that of DOX in the heart tissue ($p<0.05$). In the kidney tissue, CA enzyme was activated in presence of DOX, but morin did not show any effect on CA activity when compared with that of the rats in the group C ($p<0.05$). In the liver tissue, CA enzyme activity was increased in presence of both morin and DOX when compared with the group C, but this increased CA enzyme activity was higher in the group M than the group D ($p<0.05$, Figure 1).

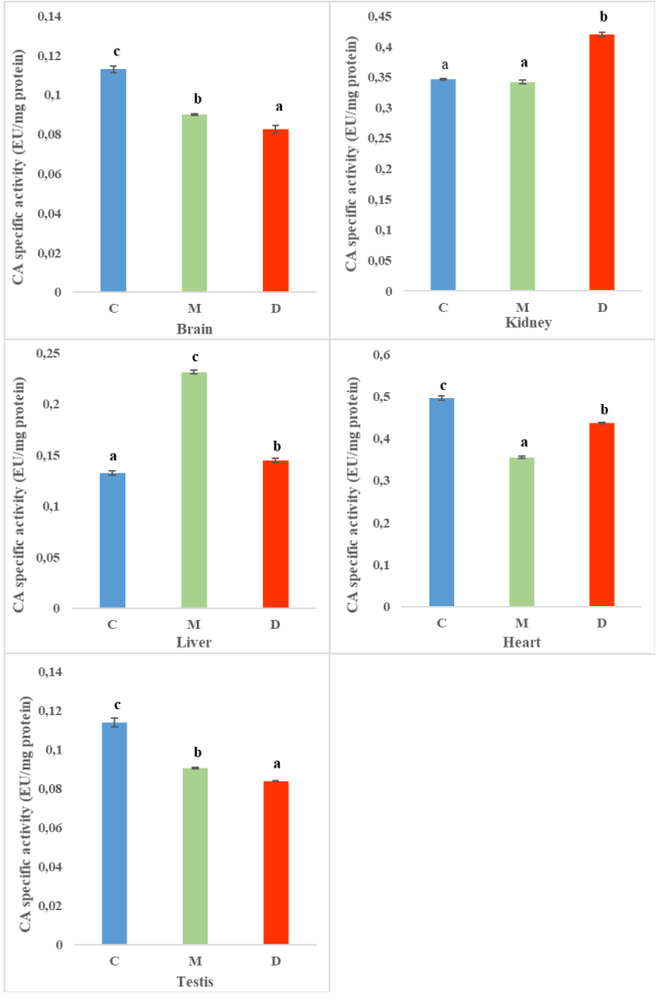

Figure 1. *The effects of morin and DOX on CA enzyme activity in different tissues (C: Control, M: Morin, D: Doxorubicin). Data represent the means ±S.E.M. of seven rats in each group. Results were derived from one-way ANOVA followed by Tukey's post hoc test. Graphs (a–c) show significant (P<0.05) differences among groups for each tissue.*

Within the scope of the study, the effects of morin and DOX on 6PGD enzyme activity were investigated. According to the measurement results, morin activated statistically the enzyme in all the tissues compared with that of the rats in the group C ($p<0.05$). DOX did not show any effect on 6PGD activities in the brain and testis tissues of the rats in the group D, but it activated 6PGD enzyme in the kidney, liver and heart tissues of the rats in the group D. However, 6PGD activities in the kidney, liver and heart tissues of the rats in the group D were lower than those of the rats in the group M ($p<0.05$, Figure 2).

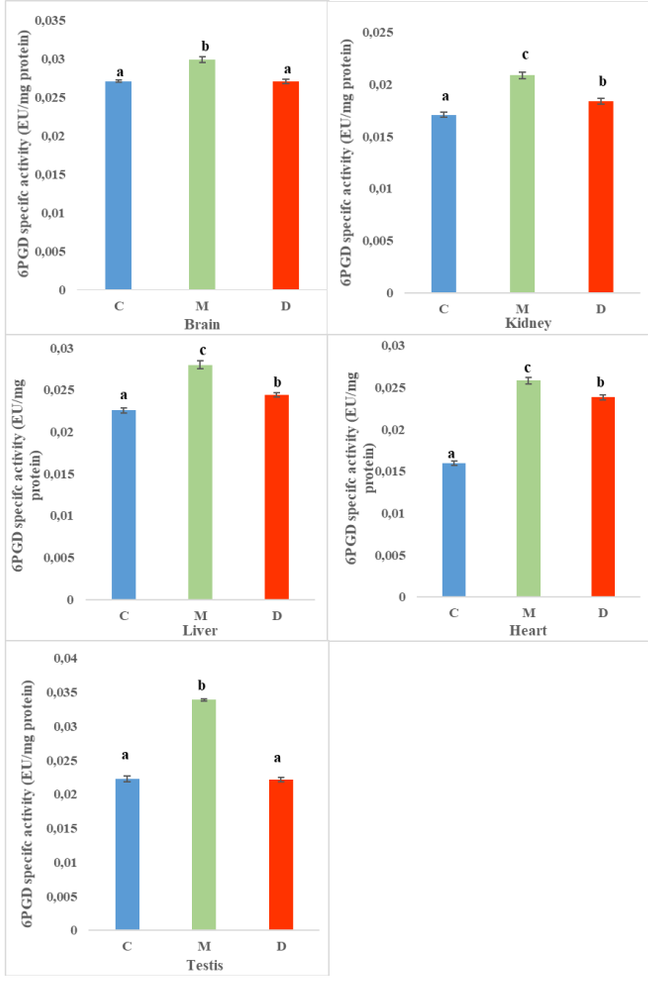

Figure 2. *The effects of morin and DOX on 6PGD enzyme activity in different tissues (C: Control, M: Morin, D: Doxorubicin). Data represent the means ±S.E.M. of seven rats in each group. Results were derived from one-way ANOVA followed by Tukey's post hoc test. Graphs (a–c) show significant (P<0.05) differences among groups for each tissue.*

In this study, the effects of morin and DOX on TrxR enzyme activity were investigated. It was determined that morin and DOX inhibited the enzyme in all the tissues when compared with that of the rats in the group C. The inhibitory effects of

DOX and morin on TrxR enzyme were statistically significant in all the tissues ($p<0.05$). Moreover, the inhibitory effect of DOX was higher than that of morin in the other tissues except for the brain tissue ($p<0.05$). In the brain tissue, there were no significant differences between the group M and D in terms of the inhibition effect of DOX and morin on TrxR enzyme ($p<0.05$, Figure 3).

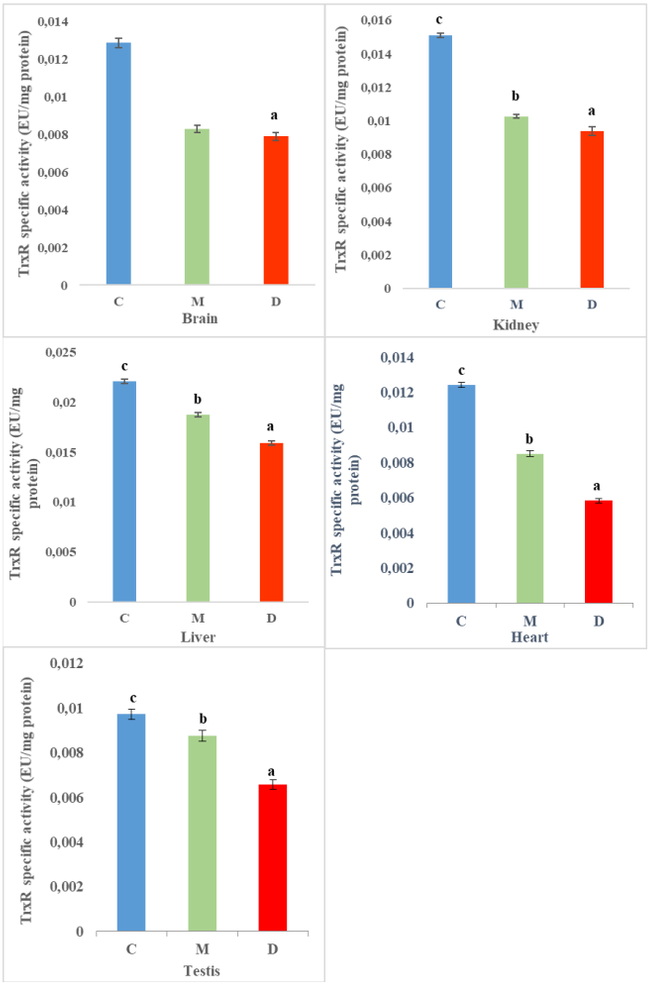

Figure 3. *The effects of morin and DOX on TrxR enzyme activity in different tissues (C: Control, M: Morin, D: Doxorubicin). Data represent the means ±S.E.M. of seven rats in each group. Results were derived from one-way ANOVA followed by Tukey's post hoc test. Graphs (a–c) show significant (P<0.05) differences among groups for each tissue.*

4. DISCUSSION

Morin (3,5,7,2',4'-pentahydroxyflavone), a member of the flavanol group, is a flavonoid isolated from members of the Moraceae family and has been reported to have many biological activities (30). In several studies, anti-epileptic (31), neuroprotective (32), anti-inflammatory (33), antioxidaznt (34), anti-fibrotic (35), anti-diabetic (36), anti-arthritic (37) and anti-mutagenesis (38) effects of morin have been reported. The anti-tumor effects of morin have been discovered in a variety of cancers, especially in breast cancer and leukemia. In addition, it has been shown that morin is capable of promoting the apoptosis and inhibiting the proliferation in prostate cancer cell line LNCaP (39). DOX, an antibiotic of anthracycline group, is one of the most potent broad-spectrum antitumor agents. DOX is widely used in the treatment of cancer types such as solid tumor, leukemia, and lymphoma (40,41). However, its clinical use is limited due to its serious toxicity. In previous studies, it has been reported that DOX can cause cardiotoxicity (42), hepatotoxicity (43), pulmonary toxicity (44) and nephrotoxicity (45). The present study was designed to investigate the effects of DOX, a drug that is frequently used in chemotherapy, and morin, a flavanol that is determined its biological activities by many researchers, on the activities of CA, 6PGD and TrxR enzymes which are important in the events of metabolism.

CA is an enzyme found in almost all cell types and subcellular organelles, from unicellular cyanobacteria to mammals (46). Carbonic anhydrase isoenzymes with 16 isoforms in mammals are involved in numerous pathological and physiological processes such as gluconeogenesis, lipogenesis, tumor formation, and virulence of some pathogens (47). In addition, it has recently been reported that CA inhibitors may have potential to use as anti-obesity (48), anticancer (49) and anti-infective (50) drugs as well as their roles as diuretic and antiglaucoma drugs (47). In some studies, it has been reported that the activation of CA enzyme may offer new approaches to the treatment of Alzheimer's disease (51).

According to the results of our study, the activity of CA enzyme in the brain, heart and testis tissues of the rats was significantly inhibited while CA enzyme in the liver tissues of the rats was activated in the present of morin. As for the kidney tissue, morin did not show any effect on CA enzyme. In previous studies, the effects of morin on CA isoforms were investigated under *in vitro* conditions and it was reported that morin inhibited the enzyme isoforms I, II, III and IV (52). Some studies also indicated that taxifolin and naringenin, which are some natural flavonoids, may inhibit CA-I and II isoenzymes under *in vitro* or *in vivo* conditions (53,54). As will be understood, our conclusions are in agreement with most literary data obtained. Additionally, the liver tissue contains a monooxygenase enzyme system in which endogenous and exogenous compounds are metabolized (55). Thus, it can be said that the metabolites have different effects on the enzyme activity as a result of the metabolism of morin. When the effects of DOX on the enzyme activity were examined, the inhibition effect of DOX was similar to that of morin. It was observed that DOX inhibited the enzyme activity in the other tissues with the exception of the liver and kidney tissues. In liver and kidney tissues, DOX increased CA enzyme activity. Katzenmeyer et al. reported that 7-deoxydoxorubicinolone and 7-deoxydoxorubicinone metabolites were found in the liver tissues of the rats while doxorubicinol was the major DOX metabolite in the plasma samples of the rats treated with doxorubicin (56). Therefore, the effect of DOX on CA enzyme activity may be different because of the different metabolite concentrations in the different tissues. The results from our research show that morin and DOX may

have similar effects on CA enzyme activity in the brain, liver, heart, and testis tissues.

6PGD is the third enzyme in the oxidative pentose phosphate pathway. This pathway is involved in the redox balance and rapid proliferation of cancer cells by connecting glycolysis to the anabolic biosynthesis (57). 6PGD activation leads to redox homeostasis, glycolysis, and anabolic biosynthesis, which are advantageous for the survival and proliferation of tumor cells (10,58). In addition, 6PGD activity has been reported to increase in many cancers including colon, breast, cervical, and thyroid cancers (59). The effects of some drugs on certain enzymes of carbohydrate metabolism in MCF-7 cells in culture have been investigated and it has been reported that DOX activates the enzyme (60). Moreover, it has been reported that 6PGD activity increases in anaplastic thyroid carcinoma cell in response to doxorubicin which is the most commonly used chemotherapeutic agent in patients with anaplastic thyroid carcinoma, and cancer cells have been reported to cause resistance to the drug. 6PGD inhibition has been found to sensitize effectively the cells to doxorubicin by eliminating this resistance (8). According to the results of our study, it was observed that DOX activates the enzyme in the kidney, liver, and heart tissues. Thus, it can be seen that the cells are resistant to the effects of DOX through 6PGD enzyme activation. Similarly, morin increased 6PGD enzyme activity in all the tissues. Therefore, morin can help in the development of resistance by increasing the activity of 6PGD enzyme in DOX-exposed cells.

The cellular control of the thiol redox state is mainly exerted by the thioredoxin and glutathione systems (61). The thioredoxin system, which comprises TrxR, thioredoxin, and NADPH, regulates crucial cell functions such as proliferation and viability (62). It has been reported that TrxR and thioredoxin expression have increased in some types of cancer (63), indicating that the thioredoxin system may play an important role in tumor formation and progression (64). Thus, it has been reported that TrxR enzyme may serve as a therapeutic target in the treatment of cancer. Furthermore, it has been reported that mouse lung carcinoma cells return to normal morphology as a result of reducing the expression of TrxR enzyme. Besides, there have been a decrease in the rate of proliferation and a decrease in the expression of some proteins related to cancer (65). In our study, it was determined that morin and DOX inhibited significantly TrxR enzyme in all the tissues. In several studies, some researchers have investigated the effects of DOX on TrxR enzyme in rats and they have found that DOX can decrease the enzyme activity in the skin (66). To the best of our knowledge, the effect of morin on TrxR enzyme activity was determined for the first time in this study. In conclusion, the present study has demonstrated that morin might have anticancer properties and one of the effective ways of DOX used in cancer treatment might be TrxR inhibition.

5. CONCLUSION

Within the scope of the study, doxorubicin which is widely used in the treatment of some cancers and morin, a bioflavonoid, have been applied to rats. Changes in CA, 6PGD and TrxR activities, which are metabolically important enzymes in brain, kidney, liver, heart, and testis tissues of rats, were examined. The findings showed that doxorubicin and morin had similar properties in the tissues as to their effect on enzyme activities.

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REFERENCES

[1] Schomburg I, Chang A and Schomburg, D. BRENDA, enzyme data and metabolic information. Nucleic Acids Res 2002; 30(1): 47-49.

[2] Sarioglu N, Bilen C, Sackes Z, Gencer N. The effects of bronchodilator drugs and antibiotics used for respiratory infection on human erythrocyte carbonic anhydrase I and II isozymes. Arch Physiol Biochem 2015; 121(2): 56-61.

[3] Li S, An L, Duan Q, Araneta MF, Johnson CS, Shen J. Determining the rate of carbonic anhydrase reaction in the human brain. Sci Rep 2018; 8(1): 2328.

[4] Pastorekova S, Parkkila S, Pastorek J, Supuran CT. Carbonic Anhydrases: Current State of the Art, TherapeuticApplications and Future Prospects. J Enzyme Inhib Med Chem 2004; 19(3): 199-229.

[5] Erzengin Z, Bilen C, Ergun A, Gencer N. Antipsychotic agents screened as human carbonic anhydrase I and II inhibitors. Arch Physiol Biochem 2014; 120(1): 29-33.

[6] Güleç Ö, Arslan M, Gencer N, Ergun A, Bilen C, Arslan O. Synthesis and carbonic anhydrase inhibitory properties of new spiroindoline-substituted sulphonamide compounds. Arch Physiol Biochem 2017; 123(5): 306-312.

[7] 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. J Biochem Mol Toxic 2018; 32(9): e22193.

[8] Ma L and Cheng Q. Inhibiting 6-phosphogluconate dehydrogenase reverses doxorubicin resistance in anaplastic thyroid cancer via inhibiting NADPH-dependent metabolic reprogramming. Biochem Biophys Res Commun 2018; 498(4): 912-917.

[9] Sarfraz I, Rasul A, Hussain G, Shah MA, Zahoor AF, Asrar M, Selamoglu Z, Ji X, Adem Ş, Sarker SD. 6-Phosphogluconate dehydrogenase fuels multiple aspects of cancer cells: From cancer initiation to metastasis and chemoresistance. BioFactors 2020; 1-13.

[10] Shan C, Elf S, Ji Q, Kang HB, Zhou L, Hitosugi T, Xie J. Lysine acetylation activates 6-phosphogluconate dehydrogenase to promote tumor growth. Mol Cell 2014; 55(4): 552-565.

[11] Zou Q, Chen YF, Zheng XQ, Ye SF, Xu BY, Liu YX, Zeng HH. Novel thioredoxin reductase inhibitor butaselen inhibits tumorigenesis by down-regulating programmed death-ligand 1 expression. J Zhejiang Univ Sci B 2018; 19(9): 689-698.

[12] Tuladhar A, Hondal RJ, Colon R, Hernandez EL, Rein KS. Effectors of thioredoxin reductase: Brevetoxins and manumycin-A. Comp Biochem Phys C 2019; 217: 76-86.

[13] Lillig CH and Holmgren A. Thioredoxin and related molecules–from biology to health and disease. Antioxid Redox Sign 2007; 9(1): 25-47.

[14] Fernandes AP. Expression profiles of thioredoxin family proteins in human lung cancer tissue: correlation with proliferation and differentiation. Histopathology 2009; 55(3): 313-320.

[15] Wu R, Yao PA, Wang HL, Gao Y, Yu HL, Wang L, Gao JP. Effect of fermented Cordyceps sinensis on doxorubicin-induced cardiotoxicity in rats. Mol Med Rep 2018; 18(3): 3229-3241.

[16] Wang S, Kotamraju S, Konorev E, Kalivendi S, Joseph J, Kalyanaraman B. Activation of nuclear factor-kappaB during doxorubicin-induced apoptosis in endothelial cells and myocytes is pro-apoptotic: the role of hydrogen peroxide. Biochem J 2002; 367(Pt 3): 729.

[17] Wen SH, Su SC, Liou BH, Lin CH, Lee KR. Sulbactam-enhanced cytotoxicity of doxorubicin in breast cancer cells. Cancer Cell Int 2018; 18(1): 128.

[18] Benzer F, Kandemir FM, Kucukler S, Comaklı S, Caglaya C. Chemoprotective effects of curcumin on doxorubicin-induced nephrotoxicity in wistar rats: by modulating inflammatory cytokines, apoptosis, oxidative stress and oxidative DNA damage. Arch Physiol Biochem 2018; 124(5): 448-457.

[19] Martin HL, Smith L, Tomlinson DC. Multidrug-resistant breast cancer: current perspectives. Breast cancer: Targets and Therapy 2014; 6: 1-13.

[20] Yu J, Wang C, Kong Q, Wu X, Lu JJ, Chen X. Recent progress in doxorubicin-induced cardiotoxicity and protective potential of natural products. Phytomedicine 2018; 40: 125-139.

[21] Ahmad A, Kaleem M, Ahmed Z, Shafiq H. Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections—A review. Food Res Int 2015; 77: 221-235.

[22] Adem S, Comakli V, Kuzu M, Demirdag R. Investigation of the effects of some phenolic compounds on the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase from human erythrocytes. J Biochem Mol Toxic 2014; 28(11): 510-514.

[23] Adem S, Akkemik E, Aksit H, Guller P, Tüfekci AR, Demirtas İ, Ciftci M. Activation and inhibition effects of some natural products on human cytosolic CAI and CAII. Med Chem Res 2019; 28(5): 711-722.

[24] Lee HS, Jung KH, Hong SW, Park IS, Lee C, Han HK, Hong SS. Morin protects acute liver damage by carbon tetrachloride (CCl 4) in rat. Arch Pharm Res 2008; 31(9): 1160-1165.

[25] Al-Numair KS, Chandramohan G, Alsaif MA, Veeramani C, Newehy AE. Morin, a flavonoid, on lipid peroxidation and antioxidant status in experimental myocardial ischemic rats. Afr J Tradit Complement Altern Med 2014; 11(3): 14-20.

[26] Rashid S, Ali N, Nafees S, Ahmad ST, Arjumand W, Hasan SK, Sultana S. Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats. Toxicol Mech Method 2013; 23(5): 337-345.

[27] Verpoorte J, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrases B and C. J Biol Chem 1967; 242(18): 4221-4229.

[28] Beutler E. Red cell metabolism. Manual of biochemical methods. London: Academic press; 1971.

[29] Holmgren A, Bjornstedt M.Thioredoxin and thioredoxin reductase. Method Enzymol 1995; 252: 199-208.

[30] Bieg D, Sypniewski D, Nowak E, Bednarek I. Morin decreases galectin-3 expression and sensitizes ovarian cancer cells to cisplatin. Arch Gynecol Obstet 2018; 298(6): 1181-1194.

[31] Kandhare A, Mukherjee A, Bodhankar S. Anti-epileptic effect of morin against experimental pentylenetetrazol-induced seizures via modulating brain monoamines and oxidative stress. Asian Pac J Trop Biomed 2018; 8(7): 352-359.

[32] Thangarajan S, Vedagiri A, Somasundaram S, Sakthimanogaran R, Murugesan M. Neuroprotective effect of morin on lead acetate-induced apoptosis by preventing cytochrome c translocation via regulation of Bax/Bcl-2 ratio. Neurotoxicol Teratol 2018; 66: 35-45.

[33] Zhou Y, Cao ZQ, Wang HY, Cheng YN, Yu LG, Zhang XK, Guo XL. The anti-inflammatory effects of Morin hydrate in atherosclerosis is associated with autophagy induction through cAMP signaling. Mol Nutr Food Res 2017; 61(9): 1-10.

[34] Choi JS, Burm JP. Enhanced nimodipine bioavailability after oral administration of nimodipine with morin, a flavonoid, in rabbits. Arch Pharm Res 2006; 29(4): 333-338.

[35] MadanKumar P, NaveenKumar P, Devaraj H, NiranjaliDevaraj S. Morin, a dietary flavonoid, exhibits anti-fibrotic effect and induces apoptosis of activated hepatic stellate cells by suppressing canonical NF-κB signaling. Biochimie 2015; 110: 107-118.

[36] Razavi T, Kouhsari S, Abnous K. Morin exerts anti-diabetic effects in human HepG2 cells via down-regulation of miR-29a. Exp Clin Endocrinol Diabetes 2018; 23(5): 337-345.

[37] Yue M, Zeng N, Xia Y, Wei Z, Dai Y. Morin Exerts Anti-Arthritic Effects by Attenuating Synovial Angiogenesis via Activation of Peroxisome Proliferator Activated Receptor-γ. Mol Nutr Food Res 2018; 62(21): 180-202.

[38] Choi HJ, Choi J-S. Effects of morin pretreatment on the pharmacokinetics of diltiazem and its major metabolite, desacetyldiltiazem in rats. Arch Pharm Res 2005; 28 (8): 970-976.

[39] Li B, Jin X, Meng H, Hu B, Zhang T, Yu J, Wang J. Morin promotes prostate cancer cells chemosensitivity to paclitaxel through miR-155/GATA3 axis. Oncotarget 2017; 8(29): 47849.

[40] Injac R, Strukelj B. Recent advances in protection against doxorubicin-induced toxicity. Technol Cancer Res T 2008; 7(6): 497-516.

[41] Ahmed F, Urooj A, Karim AA. Protective effects of Ficus racemosa stem bark against doxorubucin-induced renal and testicular toxicity. Pharmacogn Mag 2013; 9(34): 130-134.

[42] Fouad AA, Yacoubi MT. Mechanisms underlying the protective effect of eugenol in rats with acute doxorubicin cardiotoxicity. Arch Pharm Res 2011; 34(5): 821-828.

[43] You J-S, Pan T-L, Lee Y-S. Protective effects of Danshen (Salvia Miltiorrhiza) on adriamycin-induced cardiac and hepatic toxicity in rats. Phytother Res 2007; 21(12): 1146-1152.

[44] Minchin RF, Johnston MR, Schuller HM, Aiken MA, Boyd MR. Pulmonary toxicity of doxorubicin administered by in situ isolated lung perfusion in dogs. Cancer 1988; 61(7): 1320-1325.

[45] Yilmaz S, Atessahin A, Sahna E, Karahan I, Ozer S. Protective effect of lycopene on adriamycin-induced cardiotoxicity and nephrotoxicity. Toxicology 2006; 218(2-3): 164-171.

[46] Henry RP. Multiple Roles of carbonic anhydrase in cellular transport and metabolism. Annu Rev Physiol 1996; 58(1): 523-538.

[47] Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008; 7(2): 168-181.

[48] Supuran CT. Carbonic anhydrase inhibitors in the treatment and prophylaxis of obesity. Expert Opin Ther Pat 2003; 13(10): 1545-1550.

[49] Švastová E, Huĺíková A, Rafajová M, Zat'ovičová M, Gibadulinová A, Casini A, Pastoreková S. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. FEBS Lett 2004; 577(3): 439-445.

[50] Mogensen EG, Janbon G, Chaloupka J, Steegborn C, Fu MS, Moyrand F, Levin LR. Cryptococcus neoformans senses CO2 through the carbonic anhydrase Can2 and the adenylyl cyclase Cac1. Eukaryot Cell 2006; 5(1): 103-111.

[51] Sun M-K, Alkon DL. Carbonic anhydrase gating of attention: memory therapy and enhancement. Trends Pharmacol Sci 2002; 23(2): 83-89.

[52] Ekinci D, Karagoz L, Ekinci D, Senturk M, Supuran CT. Carbonic anhydrase inhibitors: *in vitro* inhibition of α isoforms (hCA I, hCA II, bCA III, hCA IV) by flavonoids. J Enzyme Inhib Med Chem 2013; 28(2): 283-288.

[53] Gocer H, Topal F, Topal M, Küçük M, Teke D, Gülçin İ, Supuran CT. Acetylcholinesterase and carbonic anhydrase isoenzymes I and II inhibition profiles of taxifolin. J Enzyme Inhib Med Chem 2016; 31(3): 441-447.

[54] Kuzu M, Özkaya A, Şahin Z, Dağ Ü, Comakli V, Demirdağ R. *In Vivo* Effects of Naringenin and Lead on Rat Erythrocyte Carbonic Anhydrase Enzyme. Turk J Pharm Sci 2017; 14(1): 9-12.

[55] Kuzu M,Ciftci M. Purification and characterization of NADPH-cytochrome P450 reductase from Lake Van fish liver microsomes and investigation of some chemical and metals' effects on the enzyme activity. Turk J Chem 2015; 39(1): 149-158.

[56] Katzenmeyer JB, Eddy CV, Arriaga EA. Tandem laser-induced fluorescence and mass spectrometry detection for high-performance liquid chromatography analysis of the *in vitro* metabolism of doxorubicin. Anal Chem 2010; 82(19): 8113-8120.

[57] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. Nat Rev Cancer 2011; 11(2): 85-95.

[58] Lin R, Elf S, Shan C, Kang HB, Ji Q. Zhou L, Xie J. 6-Phosphogluconate dehydrogenase links oxidative PPP, lipogenesis and tumour growth by inhibiting LKB1–AMPK signalling. Nat Cell Biol 2015; 17(11): 1484.

[59] Yang X, Peng X,Huang J. Inhibiting 6-phosphogluconate dehydrogenase selectively targets breast cancer through AMPK activation. Clin Transl Oncol 2018; 20(9): 1145-1152.

[60] Mitchell I, Deshpande N. Drug effects on certain enzymes of carbohydrate metabolism in MCF-7 cells in culture. Clin Oncol 1984; 10(3): 253-260.

[61] Bindoli A, Rigobello MP, Scutari G, Gabbiani C, Casini A, Messori L. Thioredoxin reductase: a target for gold compounds acting as potential anticancer drugs. Coord Chem Rev 2009; 253(11-12): 1692-1707.

[62] Arnér ESJ, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000; 267(20): 6102-6109.

[63] Berggren M, Gallegos A, Gasdaska JR, Gasdaska P, Warneke J, Powis G. Thioredoxin and thioredoxin reductase gene expression in human tumors and cell lines, and the effects of serum stimulation and hypoxia. Anticancer Res 1996; 16(6B): 3459-3466.

[64] Cassidy PB, Edes K, Nelson CC, Parsawar K, Fitzpatrick FA, Moos PJ. Thioredoxin reductase is required for the inactivation of tumor suppressor p53 and for apoptosis induced by endogenous electrophiles. Carcinogenesis 2006; 27(12): 2538-2549.

[65] Yoo MH, Xu XM, Carlson BA, Gladyshev VN, Hatfield DL. Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. J Biol Chem 2006; 281(19): 13005-13008.

[66] Korać B, Buzadžić B. Doxorubicin toxicity to the skin: possibility of protection with antioxidants enriched yeast. J Dermatol Sci 2001; 25(1): 45-52.

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