Original Article

Sodium Metabisulphite, a Preservative Agent, Decreases the Heart Capillary Volume and Length, and Curcumin, the Main Component of *Curcuma Longa*, Cannot Protect It

(curcumin / heart / rat / stereology / sulphite)

A. NOORAFSHAN[1, 2], R. ASADI-GOLSHAN[1], S. MONJEZI[3], S. KARBALAY-DOUST[1, 2]

[1]Histomorphometry and Stereology Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Anatomy Department, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Department of Basic Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract. Sodium metabisulphite is used as an antioxidant agent in many pharmaceutical formulations. It is extensively used as a food preservative and disinfectant. It has been demonstrated that sulphite exposure can affect some organs. Curcumin, the main element of *Curcuma longa*, has been identified to have multiple protective properties. The present study extends the earlier works to quantitative evaluation of the effects of sulphite and curcumin on the heart structure using stereological methods. In this study, 28 rats were randomly divided into four experimental groups. The rats in groups I to IV received distilled water (group I), sodium metabisulphite (25 mg/kg/day) (group II), curcumin (100 mg/kg/day) (group III), and sodium metabisulphite+curcumin (group IV), respectively, for 8 weeks. The left ventricle was subjected to stereological methods to estimate the quantitative parameters of the myocardium. A 20 % decrease was observed in the total volume of ventricular tissue in the sulphite-treated animals compared to the distilled water treatment ($P < 0.02$). Also, the volume and length of the capillaries were reduced by 43 % on average in the sulphite-treated rats in comparison to the distilled water-treated animals ($P < 0.02$). However, no significant change was seen in the mean and total volume of the myocardium and the cavity and diameter of the capillaries after sulphite ingestion. Treatment with curcumin did not protect the animals against the structural changes of the ventricle. Sulphite, as a preservative food agent, reduced the length and volume of the ventricular capillaries and curcumin could not protect them.

Introduction

Sulphite salts are extensively used as food preservatives (due to their antimicrobial activity) and have been accepted as safe by the Food and Drug Administration since 1959 (Gunnison and Jacobsen, 1987; Kencebay et al., 2013). Sodium metabisulphite is one of these salts. It is a white powder with light odour of rotten egg. Previous studies have reported that ingested sulphite is absorbed by the gastrointestinal tube and distributed essentially to all body organs (Gunnison and Benton, 1971; Gunnison and Jacobsen, 1987). However, researchers have focused mainly on histopathological evaluation of the heart after sulphite ingestion and the quantitative aspect of the heart structure has received less attention (Nair and Elmore, 2003; Dänicke et al., 2008). Curcumin (diferuloyl methane), the main element of *Curcuma longa*, is known to have multiple properties, including anti-oxidant, anti-inflammatory, anti-hypertensive, and anti-tumour effects. *Curcuma longa* (turmeric) can be easily added to foods and might be considered as an available protective agent (Noorafshan and Ashkani-Esfahani, 2013).

There are several studies bringing evidence of the cardiac protective effects of curcumin. Chen et al. (2013) showed that curcumin pre-treatment improved cardiac contractility and attenuated myocardial injury through reducing the inflammatory process in the heart and oxidative stress in the myocardium. Yang et al. (2013) also demonstrated that curcumin has protective effects on the cardiac function in rats with sepsis. Kapakos et al. (2012) also highlighted the cardiovascular protective role of curcumin with an emphasis on the molecular basis of this effect. The present study aims to evaluate the structural changes of the heart and to find quantitative responses to the following questions using stereological methods: How much does the volume of

Received March 18, 2014. Accepted May 30, 2014.

This work was performed and financially supported by grant No. HSRC-92-1126 at Histomorphometry and Stereology Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran.

Corresponding author: Saied Karbalay-Doust, Histomorphometry and Stereology Research Centre, Shiraz University of Medical Sciences, Zand Ave., Shiraz, Iran, Postal code: 71348-45794. Phone and Fax: (+98) 711-2304372; e-mail: karbalas@sums.ac.ir

Abbreviations: WHO – World Health Organization.

the left ventricle change after sulphite exposure? How much does the volume of the myocardium and connective tissue change after sulphite treatment? How much does the volume and length of the capillaries change after sulphite exposure? Does curcumin protect the heart structure after sulphite treatment?

Material and Methods

Animals

The present study was conducted in 28 adult male Sprague-Dawley rats (weight 200–250 g, the rats were purchased from the laboratory animal centre of Shiraz University of Medical Sciences, Shiraz, Iran). All the trials were performed according to the guidelines of the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (Approval No. HSRC-92-1126). The animals were randomly divided into four experimental groups (N = 7). The rats in groups I to IV received daily gastric gavages of distilled water (group I), sodium metabisulphite (group II, 25 mg/kg/day, Sigma-Aldrich, St. Louis, MO), curcumin (group III, 100 mg/kg/day, Merck KGaA, Darmstadt, Germany) and sodium metabisulphite+curcumin (group IV), respectively, for 8 weeks (Akdogan et al., 2011; Ercan et al., 2013; Noorafshan and Ashkani-Esfahani, 2013). The amount of 5–6 mg sodium metabisulphite was dissolved in 1 ml of the vehicle for each rat. The amount of 20–25 mg curcumin was dissolved in 1 ml of the vehicle for each rat. The dose of sodium metabisulphite used in this study was chosen according to the preceding information of the World Health Organization (WHO), which has determined the acceptable daily intake level of sulphites as 0.7 mg/kg body weight, expressed as sulphur dioxide. With this in mind, it is significant to note that the mean per capita of sulphite consumption from food and beverages is estimated as 19 mg sulphur dioxide equivalents per day. This limit is reported to be 163 mg sulphur dioxide equivalents in the 99th percentile of the population (Elmas et al., 2005). The amount of the ingested sulphite is related to the dietary regime and cannot be definitely estimated. Therefore, 25 mg/kg/day was used in this work. Additionally, the dose of curcumin was selected according to our earlier results that confirmed 100 mg/kg/day as the appropriate dose of curcumin with no side effects on the liver, kidney, and blood levels of aspartate aminotransferase, alanine aminotransferase, urea nitrogen, and creatinine (Noorafshan and Ashkani-Esfahani, 2013). Therefore, in the present study "curcumin as a preservative agent" was evaluated in connection with higher than usual dosage of sodium metabisulphite.

Estimation of the ventricle volume

The stereological equipment consisted of a Nikon E-200 microscope (Nikon, Tokyo, Japan) and a microcator (Heidenhain MT-25, Tranreut, Germany) joined to a computer, a monitor, and the stereology software designed at Shiraz University of Medical Sciences, Shiraz, Iran. The stereological probes (point grids and counting frames) were superimposed onto the images of the tissue sections that appeared on the monitor.

The left ventricle including interventricular septum was removed and immersed in neutral buffered formaldehyde (Mühlfeld et al., 2010). The volume of the left ventricle was estimated according to the isotropic Cavalieri method. Isotropic uniform random sectioning was necessary for stereological estimation and the sections were obtained using the isector method (Nyengaard and Gundersen, 1992; Mühlfeld et al., 2010). Briefly, the left ventricle was embedded in a spherical paraffin block (Fig. 1). Starting at a random position outside the left ventricle, the tissue was totally cut into 4 and 24 µm thickness serial sections. Briefly, two 4 µm were sectioned and then two 24 µm were cut and collected. This process was continued until the whole ventricle was sectioned. The tissue was stained with Heidenhain's AZAN trichrome. After sampling of 8–12 sections, the volume of the left ventricle was estimated using the following formula:

$$V_{(heart)} = \sum P_{(heart)} \times a(p) \times T$$

Where "$\sum P_{(heart)}$" represented all the points hitting the sectional profile of the ventricular tissue (181 points per animal on average), "(a/p)" was the area associated with each point projected on the heart tissue (here 2.12 mm^2), and "T" was the distance between the sections (0.804 mm) (Fig. 1).

Estimation of the volume of the myocardium, capillaries, and connective tissue

The volume density of the myocardium, connective tissue, and capillaries was estimated using the point-counting method (Gundersen et al., 1988a, b; Nyengaard, 1999). Briefly, a grid of points was superimposed upon the images of the ventricle sections viewed on the monitor. Then, the volume density "V_V(structure/ref)" of the favoured parameters was obtained using the following formula:

$$V_V(\text{structure/ref}) = P(\text{structure}) / P(\text{ref})$$

Where "P(structure)" and "P(ref)" represented the total number of the points hitting the structures of interest (myocardium capillaries or connective tissues) and the left ventricle sections, respectively (Fig. 1).

The total volume of the vessels and the connective tissue was estimated by multiplying the volume density by the final volume of the ventricle (Gundersen et al., 1988a, b; Nyengaard, 1999).

Estimation of the mean volume of the cardiomyocyte

Volume-weighted mean volume of the cardiomyocyte was estimated using the point-sampled intercept method

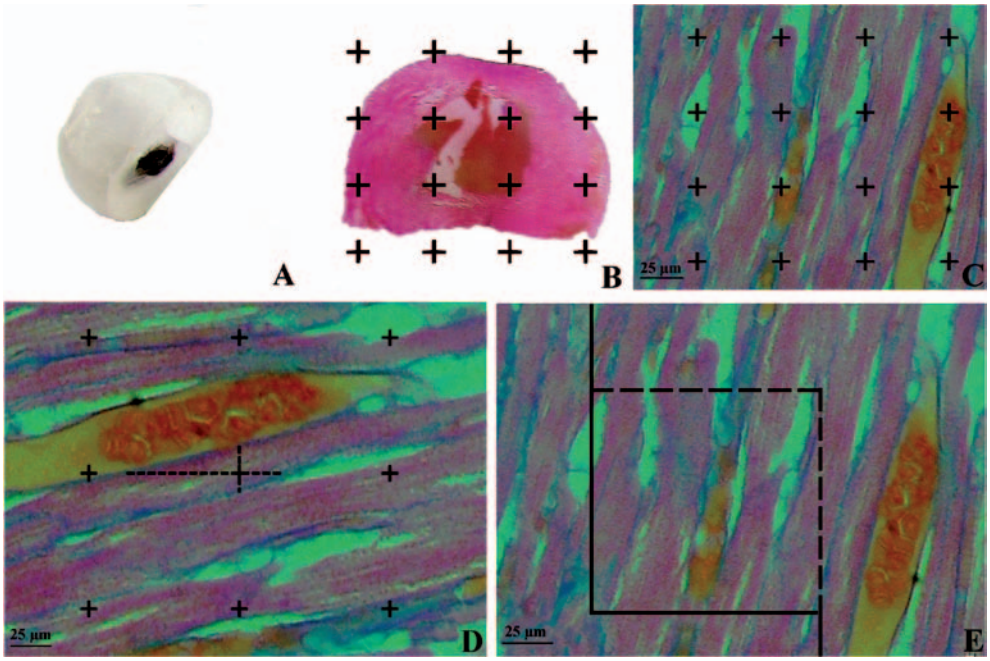

Fig. 1. **A**. Isector method. The left ventricle was embedded in a spherical paraffin block. Then, starting at a random position outside the left ventricle, the tissue was totally cut into 4 and 24 µm thickness serials to obtain 8 to 12 sections. **B**. Cavalieri method. Using the point-counting method, the area of each section was estimated. **C**. Point-counting method. To estimate the volume density of the myocardium, connective tissue, and vessels, the number of the points hitting the structure was divided by the total number of points. **D**. Point sampled intercept. The intercept lengths on the isotropic sections were measured in two directions. **E**. Length density and diameter of the vessels. The profiles of the vessels that were located inside the frame and did not touch the left and lower borders of the frame were counted. The diameter of the vessels was estimated from the broadest diameter orthogonal to the longest axis of the vessels that approximately touched the centre of the vessels. Heidenhain's AZAN trichrome stain.

(Gundersen et al., 1988a, b; Nyengaard, 1999). Briefly, a grid of points was uniformly superimposed on the 4 µm heart sections in a random manner. Some points were landed on the cardiomyocytes. Then, the length of the random intercept "l_0" passing through the point was measured and the volume was estimated using the following formula:

$$V = \frac{\pi}{3} \times \bar{l}_0^3$$

Estimation of the length and diameter of the capillaries

The length density of the capillaries was estimated using the following formula:

$$L_V(\text{capillaries /heart}) = 2\Sigma Q/(\Sigma P \times a/f)$$

Where "ΣQ" denoted the total number of the capillary profiles counted per heart, "ΣP" was the total number of the counted frame, and "a/f" was the area of the counting frame (2400 µm^2) (Fig. 1).

The diameter of the capillaries was estimated from the broadest diameter orthogonal to the longest axis of the capillaries that approximately touched the centre of the capillaries (Fig. 1) (Gundersen et al., 1988a, b; Nyengaard, 1999).

Statistical analysis

The data were shown as mean ± SD and analysed by application of Mann-Whitney U-test. P values less than 0.05 were considered as statistically significant. Coefficient of variation (CV = SD/mean) offers a way of comparing variations in different study groups.

Results

No qualitative histopathological changes were seen in any groups (Fig. 2). The results of the quantitative evaluations are shown in Fig. 3. The ventricular tissue includes the myocardium, connective tissues and microvessels. A 20 % decrease was observed in the total volume of the ventricular tissue in the sulphite-treated rats in comparison to the distilled water-treated animals (P = 0.02). The volume of the myocardium and connective tissue did change, but the volumes of the capillaries were decreased. However, no significant change was seen in the volume of the ventricular cavity (Fig. 3).

Also, no significant difference was found among the study groups regarding the mean volume of the cardiomyocyte (Fig. 3).

In addition, the volume and length of the capillaries were reduced by 43 % on average in the sulphite-treated rats in comparison to the distilled water-treated animals

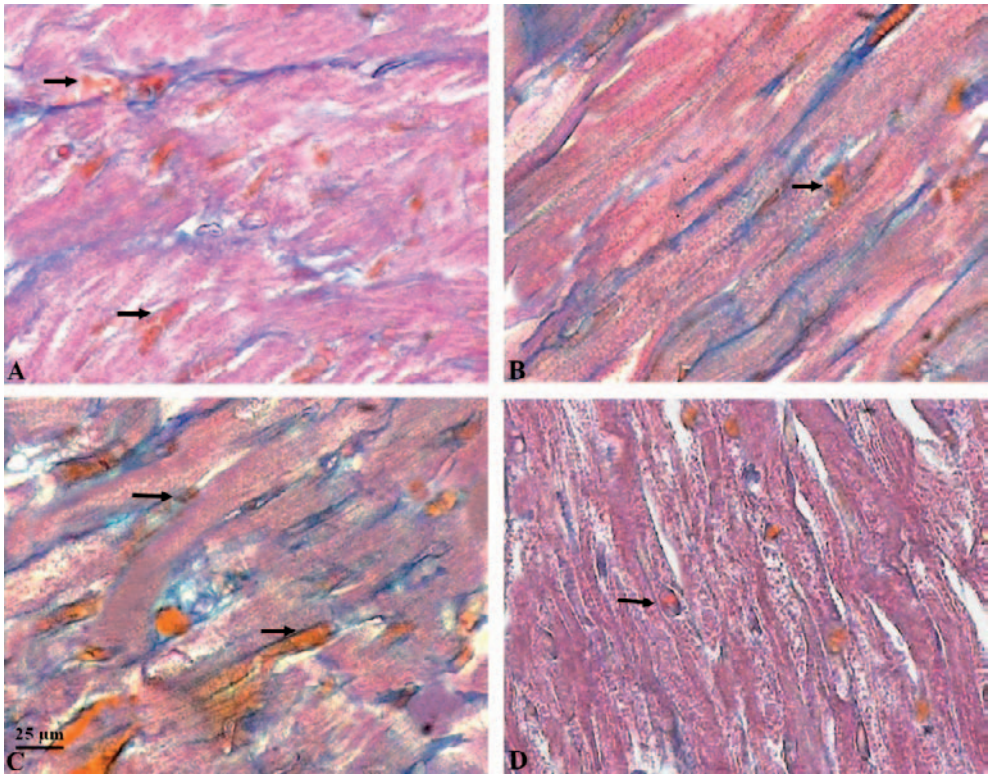

Fig. 2. Histopathological evaluation of the heart in the groups treated with distilled water (**A**), sulphite (**B**), curcumin (**C**) and sulphite+curcumin (**D**). Cardiomyocytes appear normal and their striations are apparent. No sign of ischaemia and necrosis are seen. No increase in fibrous tissue is observable. A lower profile of capillaries (arrows) in the sulphite- and sulphite+curcumin-treated groups can be observed. Heidenhain's AZAN trichrome stain.

(P = 0.02) (Fig. 3). The diameter of the capillaries did not change after sulphite ingestion.

Treatment of the animals with curcumin did not change the quantified parameters of the ventricle including myocardium, connective tissue and microvessels of the heart. Co-treatment of curcumin with sulphite could not protect the alterations of capillaries induced by sulphite (Fig. 3).

Discussion

The present study explored the effects of sodium metabisulphite and curcumin on the heart structure. The study results revealed a decrease in the ventricular volume after sulphite ingestion. The ventricle is mainly composed of myocardium and capillaries and sulphite mainly affects the capillaries. Nair et al. (2003) and Dänicke et al. (2008) reported no histopathological alterations in the heart structure after sulphite treatment in their evaluations. It should be mentioned that qualitative studies might not reflect all the changes. The qualitative evaluation of microscopic slides that were prepared in the present study also showed no signs of significant changes. Hence, quantitative methods are more useful in these circumstances. In addition to reporting the changes in a quantitative way, stereological methods can be used to define the extent of changes.

The evaluation showed that the volume of the left ventricle decreased and the total heart weight was reduced, although not significantly. The change in the heart weight might not reflect the exact changes due to the sulphite effects. The reason might be the remaining heart appendages or small amounts of the blood clots in the tissue. The study by Yang et al. (2012) proved the vasodilator effect of sodium metabisulphite. In contrast, a previous study showed vasoconstriction effects of sodium metabisulphite on the rats' aortic rings (Wills et al., 1989). The above-mentioned studies have focused on the aortic ring, and to the best of our knowledge limited studies have been conducted on the capillary changes after metabisulphite exposure. The study by Lavoie and Chessex (1993) demonstrated that sulphite used in parenteral nutrition modified local vaso-reactivity. These authors reported that sulphite could affect the capillary endothelium. This effect can be explained by the fact that free oxygen radicals have an inhibitory effect on prostacyclin synthase. Inhibition of the synthesis and action of prostaglandins (including PGE2) can reduce angiogenesis (Lavoie and Chessex, 1993). Finetti et al. (2008) reported that PGE2 stimulated capillary formation in the aortic rings that were strictly abridged by inhibitors of signalling molecules or by a receptor antagonist. The hazardous effects of sulphite have also been shown by Sakamoto et al. (1992). They demonstrated that bronchial airway microvascular leakage was induced by metabisulphite, as it was quantified by the extravasation of Evans blue dye (Sakamoto et al., 1992).

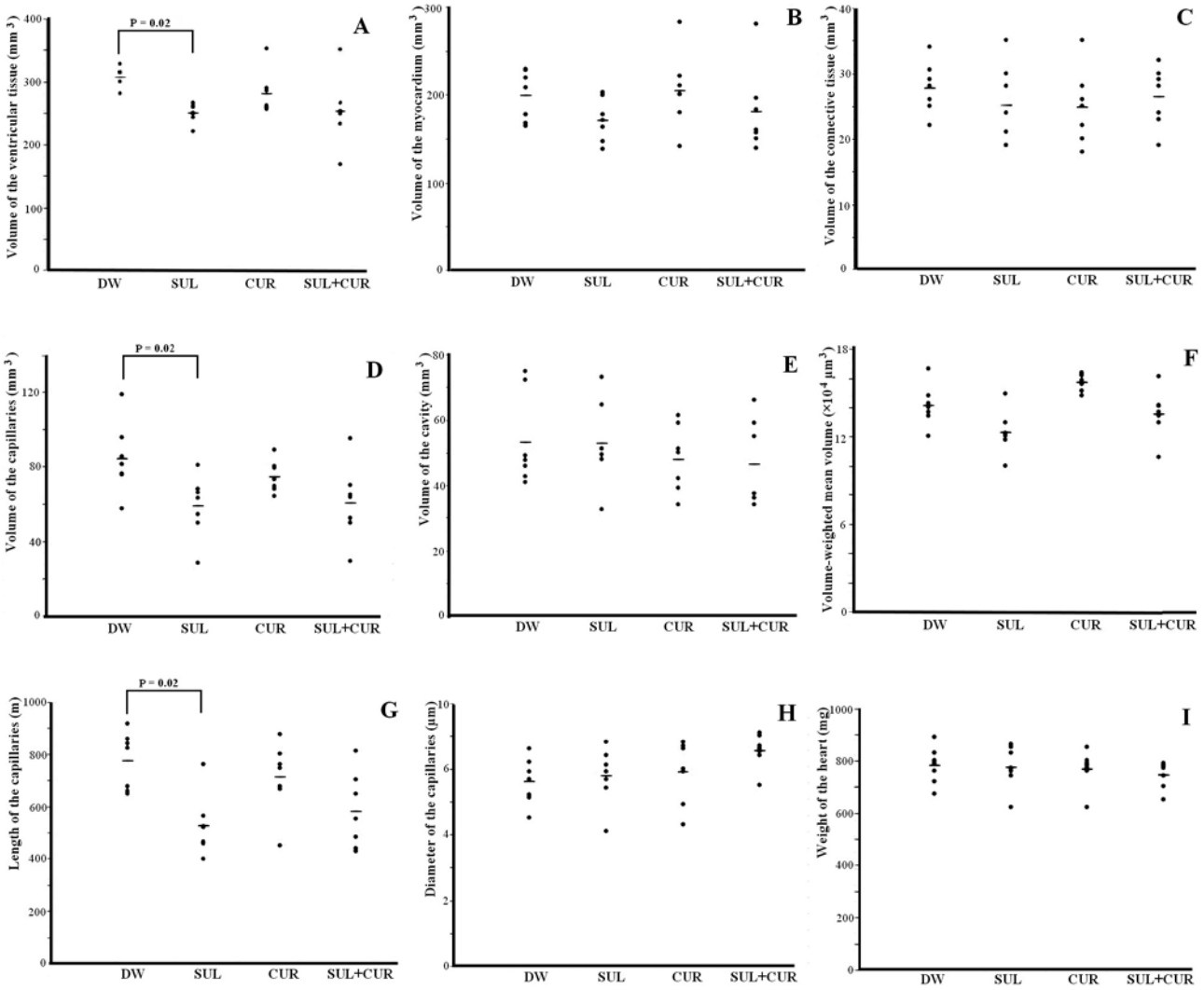

Fig. 3. The scatter plots of the quantified parameter of the hearts in groups treated with distilled water (DW), sulphite (SUL), curcumin (CUR) and sulphite+curcumin (SUL+CUR). Volume (mm^3) of the ventricular tissue (**A**), myocardium (**B**), connective tissue (**C**), capillaries (**D**), ventricular cavity (**E**), volume-weighted mean volume (µm) of the myocytes (**F**), length (m) of the capillaries (**G**), diameter (µm) of the capillaries (**H**) and weight (mg) of the heart (**I**). Each dot represents an animal and the horizontal bar indicates the mean of the group. P = 0.02, sulphite vs. distilled water.

Evaluation of the co-treatment of curcumin and sulphite was another goal of the current study. A previous study by Thaloor et al. (1998) showed that curcumin inhibited angiogenesis in the umbilical vein by its effects on endothelial cells. They also indicated that curcumin might exert its suppressor effect at both transcriptional and posttranscriptional levels and by modulating protease activity throughout endothelial morphogenesis (Thaloor et al., 1998). In the same line, Sawatpanich et al. (2010) demonstrated the potential use of anti-angiogenic action of curcumin in the maintenance of normal vasculature of the kidney in diabetes mellitus. They also reported that curcumin exerted an inhibitory effect on the vascular endothelial growth factor expression. Since sulphite mainly affects the capillaries' endothelium, curcumin could not exert any significant protection.

Conclusion: Sulphite, as a preservative food agent, reduced the length and volume of the ventricular capillaries of the rats' hearts. However, no changes were observed in the cardiomyocytes of the sulphite-treated animals. Also, curcumin could not protect against the structural changes of the heart capillaries.

Acknowledgements

The authors are grateful to the Research Improvement Centre of Shiraz University of Medical Sciences and Ms. A. Keivanshekouh for English revisions in the manuscript. The authors declare no conflict of interest.

References

Akdogan, I., Kocamazm, E., Kucukatay, V., Yonguc, N. G., Ozdemir, M. B., Murk, W. (2011) Hippocampal neuron number loss in rats exposed to ingested sulfite. *Toxicol. Ind. Health* **27**, 771-778.

Chen, T. H., Yang, Y. C., Wang, J. C., Wang, J. J. (2013) Curcumin treatment protects against renal ischemia and reper-

fusion injury-induced cardiac dysfunction and myocardial injury. *Transplant. Proc.* **45**, 3546-3549.

Dänicke, S., Beineke, A., Goyarts, T., Valenta, H., Beyer, M., Humpf, H. U. (2008) Effects of a Fusarium toxin-contaminated triticale, either untreated or treated with sodium metabisulphite (Na2S2O5, SBS), on weaned piglets with a special focus on liver function as determined by the 13C-methacetin breath test. *Arch. Anim. Nutr.* **62**, 263-286.

Elmas, O., Aslan, M., Cağlar, S., Derin, N., Agar, A., Alicigüzel, Y., Yargiçoğlu, P. (2005) The prooxidant effect of sodium metabisulfite in rat liver and kidney. *Regul. Toxicol. Pharmacol.* **42**, 77-82.

Ercan, S., Basaranlar, G., Gungor, N. E., Kencebay, C., Sahin, P., Celik-Ozenci, C., Derin, N. (2013) Ghrelin inhibits sodium metabisulfite induced oxidative stress and apoptosis in rat gastric mucosa. *Food Chem. Toxicol.* **56**, 154-161.

Finetti, F., Solito, R., Morbidelli, L., Giachetti, A., Ziche, M., Donnini, S. (2008) Prostaglandin E2 regulates angiogenesis via activation of fibroblast growth factor receptor-1. *J. Biol. Chem.* **283**, 2139-2146.

Gundersen, H. J., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sørensen, F. B., Vesterby, A., West, M. J. (1988a) The new stereological tools: dissector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS* **96**, 857-881.

Gundersen, H. J., Bendtsen, T. F., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J. R., Pakkenberg, B., Sørensen, F. B., Vesterby, A., West, M. J. (1988b) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS* **96**, 379-394.

Gunnison, A. F., Benton, A. W. (1971) Sulfur dioxide: Sulfite. Interaction with mammalian serum and plasma. *Arch. Environ. Health* **22**, 381-384.

Gunnison, A. F., Jacobsen, D. W. (1987) Sulfite hypersensitivity. A critical review. *CRC Crit. Rev. Toxicol.***17**, 185-214.

Kapakos, G., Youreva, V., Srivastava, A. K. (2012) Cardiovascular protection by curcumin: molecular aspects. *Ind. J. Biochem. Biophys.* **49**, 306-315.

Kencebay, C., Derin, N., Ozsoy, O., Kipmen-Korgun, D., Tanriover, G., Ozturk, N., Basaranlar, G., Yargicoglu-Akkiraz, P., Sozen, B., Agar, A. (2013) Merit of quinacrine in the decrease of ingested sulfite-induced toxic action in rat brain. *Food Chem. Toxicol.* **52**, 129-136.

Lavoie, J. C., Chessex, P. (1993) Biologic effects of parenteral bisulfite on human vascular tissue. *Pediatr. Res.* **33**, 347-351.

Mühlfeld, Ch., Nyengaard, J. R., Mayhew, T. M. (2010) A review of state-of-the-art stereology for better quantitative 3D morphology in cardiac research. *Cardiovasc. Pathol.* **19**, 65-82.

Nair, B., Elmore, A. R., Cosmetic Ingredients Review Expert Panel. (2003) Final report on the safety assessment of sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite. *Int. J. Toxicol.* **22(Suppl 2)**, 63-88.

Noorafshan, A., Ashkani-Esfahani, S. (2013) A review of therapeutic effects of curcumin. *Curr. Pharm. Des.* **19**, 2032-2046.

Nyengaard, J. R., Gundersen, H. J. G. (1992) The isector: A simple and direct method for generating isotropic, uniform random sections from small specimens. *J. Microsc.* **165**, 427-431.

Nyengaard, J. R. (1999) Stereological methods and their application in kidney research. *J. Am. Soc. Nephrol.* **10**, 1100-1123.

Sakamoto, T., Elwood, W., Barnes, P. J., Chung, K. F. (1992) Pharmacological modulation of inhaled sodium metabisulphite-induced airway microvascular leakage and bronchoconstriction in the guinea-pig. *Br. J. Pharmacol.* **107**, 481-487.

Sawatpanich, T., Petpiboolthai, H., Punyarachun, B., Anupunpisit, V. (2010) Effect of curcumin on vascular endothelial growth factor expression in diabetic mice kidney induced by streptozotocin. *J. Med. Assoc. Thai.* **93(Suppl 2)**, S1-8.

Thaloor, D., Singh, A. K., Sidhu, G. S., Prasad, P. V., Kleinman, H. K., Maheshwari, R. K. (1998) Inhibition of angiogenic differentiation of human umbilical vein endothelial cells by curcumin. *Cell Growth Differ.* **9**, 305-312.

Wills, M. H., Johns, R. A., Stone, D. J., Moscicki, J. C., Difazio, C. A. (1989) Vascular effects of 2-chloroprocaine and sodium metabisulfite on isolated rat aortic rings. *Reg. Anesth*. **14**, 271-273.

Yang, C., Wu, K., Li, S. H., You, Q. (2013) Protective effect of curcumin against cardiac dysfunction in sepsis rats. *Pharm. Biol.* **51**, 482-487.

Yang, Z., Zhang, Y., Meng, Z. (2012) The vasodilator mechanisms of sodium metabisulfite on precontracted isolated aortic rings in rats: signal transduction pathways and ion channels. *Food Chem. Toxicol.* **50**, 3114-3119.