Original Article

Effect of curcumin on the expression of Caspase-3 and Bcl-2 in the spleen of diabetic rats

Hagar A. Hashish, Rania N. Kamal

Department of Anatomy and Embryology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Abstract

Background: The spleen is the largest lymphoid organ, concerns with immunological reaction and filtration of blood. Diabetes mellitus is a primary disorder of carbohydrate metabolism that cause depletion of oxidant defense system. Curcumin has anti-inflammatory, anti-proliferative, anti-apoptotic, anti-bacterial, anti-cancer and potent antioxidant activities. **Aim of the Work:** The purpose of this study was to clarify the effect of induced type I diabetes on the spleen of albino rat and whether these changes could be prevented by curcumin. **Materials and Methods:** Eighteen albino rats were divided randomly into three groups; control nondiabetic rats; untreated diabetic rats and curcumin-treated diabetic rats. After 8 weeks of treatment, animals were sacrificed; spleen was dissected, processed and stained with hematoxylin and eosin and immunohistochemistry for activated Caspase-3 and Bcl-2**. Results:** Diabetes caused decrease in the body weight, degeneration of splenocytes, increased Caspase-3, and reduced Bcl-2 activity. Treatment with curcumin decreased the blood glucose level, prevented the loss of body weight and protected the spleen against diabetic induced structural changes. **Conclusion:** The current results suggested that consumption of curcumin protected the spleen against diabetic induced changes, reduced Caspase-3, and improved Bcl-2 expression. Thus, curcumin may attenuate the pathologic effects observed in the spleen of diabetic rats.

Key words: Bcl-2, Caspase-3, curcumin, diabetes, spleen

INTRODUCTION

The spleen is the largest lymphoid organ and the most vital organ for the immunological reaction against antigen in the blood (Pabst 1993). It is the only organ concerns with filtration of blood (Bao *et al*., 2009). The spleen is composed of heterogeneous group of cells, consisting mostly of lymphocytes (T and B lymphocytes), has an important role in cellular and humoral immunity (Mebius and Kraal, 2005).

Diabetes mellitus is a disorder of carbohydrate metabolism with many mechanisms that involve insulin deficiency or insulin resistance or both (Olefasky 1992). High blood sugar level cause depletion of oxidant defense system (Zivic *et al*., 2008; Hernández Marco *et al*., 2009). Excessive reactive oxygen radicles activate the process of apoptosis through increasing Caspase-3 activity and suppressing Bcl-2 expression (Takahashi *et al*., 2004).

Apoptosis is a form of cell death in tissues. Apoptosis is a vital regulatory mechanism on T and B lymphocyte expression and maturation in peripheral lymphoid organs after antigen recognition (Rathmell and Thompson, 2002).

Address for correspondence:
Dr. Hagar A. Hashish,
Department of Anatomy and Embryology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
E-mail: nada_2612@yahoo.com

Access this article online	
Quick Response Code:	Website: www.jecajournal.com
	DOI: 10.4103/1596-2393.158923

Curcumin is a bright yellow compound found in turmeric, which is derived from the rhizomes of the plant *Curcuma longa* (Waltner-Law *et al*., 2002). Curcumin has anti-inflammatory, antiproliferative, pro-apoptotic, anti-bacterial and anti-cancer activities (Epstein *et al*., 2010). Curcumin is well known to be a potent antioxidant in various *in vivo* and *in vitro* models (Araújo and Leon, 2001).

The current study used streptozotocin (STZ) to induce diabetes in rats for evaluation of apoptosis in the spleen using immunohistochemical stains for activated Caspase-3 and Bcl-2. This study was carried out also to evaluate the effect of curcumin on the changes induced by diabetes in the spleen.

MATERIALS AND METHODS

Animals

Eighteen adult male albino rats 13–18-week-old weighting 200–250 g were obtained from the Faculty of Pharmacy animal house, Mansoura University. The use of experimental animals was prospectively approved by the Ehtical Committee at Mansoura University, Faculty of Medicine. The animals were housed two or three in a cage at a constant temperature 18°C and humidity 45% on a 12-h light/dark cycle. They had free access to standard diet and drinking water.

Experimental Protocol

The animals were divided randomly into three groups.

Group 1

Control nondiabetic ($n = 6$) received single intraperitoneal injection of sodium citrate buffer (pH 4.7). The rats received regular standard diet for 8 weeks.

Group 2

Streptozotocin-induced, untreated diabetic rats ($n = 6$), received a single intraperitoneal injection of STZ (50 mg/kg body weight) dissolved in 0.1 mol/l sodium citrate buffer (pH 4.7). The rats received regular standard diet for 8 weeks (Rodrigues *et al.*, 1997).

Group 3

Streptozotocin-induced diabetic, curcumin-treated group: Curcumin powder, (Sigma Aldrich Company,United States of America), in dose of 300 mg/kg/day was mixed with the regular diet of the rats and this food was given to the rats after establishment of diabetes for 8 weeks.

Two days after STZ treatment, development of diabetes was confirmed by measuring blood glucose levels in tail vein blood samples. The drop of blood was immediately placed onto an ACCU-CHEK glucose test strip and evaluated with the ACCU-CHEK glucose meter (Roche Diagnostic Corporation, Indianapolis, IN, USA). Rats with blood glucose levels of 250 mg/dL or higher were considered to be diabetic.

During the course of the experiment, the body weights of rats in each group were measured weekly. At the end of the experiment, rats were sacrificed by cervical dislocation and their spleens were removed, and used for histopathological analysis.

Histological and Immunohistochemical Procedures

Following fixation, spleens were washed in 0.1 M phosphate buffer solution (PBS) and dehydrated through graded ethanol series, cleared in xylene before embedding in paraffin. Paraffin blocks were cut into 5 μm coronal serial sections, stained with hematoxylin and eosin (H and E) and examined by light microscopy.

For immunohistochemical analyses, 5 μ thick sections were used for the activated Caspase-3 detection system (Biovision activated Caspase-3 [1:100] and Bcl-2 [1:100]). In brief, the deparaffinization procedure was accomplished in Xylene for 1 h. Rehydration was done in 100%, 95%, 80% and 70% alcohol series for 2 min each. After immersion in distilled water for 5 min, sections were washed in PBS for 10 min and exposed to microwave radiation at 500 W for 10 min in citrate buffer (10 mM, pH 6.0) for antigen retrieval. Then the primary antibody was applied in an incubator at 4°C overnight then washed with PBS. Then, the biotinylated secondary antibody was applied, washed with PBS before incubating with the enzyme conjugate and 3,3-diaminobenzidine tetrahydrochloride. The whole procedure was finished after staining the sections with Mayer's hematoxylin.

Image Analysis

Slides were digitized using Olympus digital camera (Olympus LC20- Japan) installed on Olympus microscope (Olympus BX-50, Tokyo, Japan) with 1/2X photo adaptor, using 40X objective. The result images were analyzed on Intel® Core I3® based computer using Video Test Morphology 5.2 software (Russia) with a specific built-in routine for immunohistostaining analysis and stain quantification. The system measured the area percentage of Caspase-3, Bcl-2 positive expression.

Images from five slices per spleen were taken 200 μm apart. Five visions per slice were randomly chosen for assessment of positive cells using image analysis software (JID801D). The average grayscale of the positive cells was automatically calculated. Immunoreactive intensity were expressed by average grayscale. Values <160 was considered high, 160–170 medium and 170–180 low.

Statistical Analysis

Statistical analysis was done using computer software SPSS program (Statistical Package for Social Science) version

10,Chicago,USA. All data were expressed as the mean ± standard deviation. All the data obtained were subjected to statistical analysis using independent samples *t*-test for parametric values and Mann–Whitney test for nonparametric values. The significance level considered was $P \leq 0.05$.

RESULTS

Effect of Curcumin Treatment on Blood Glucose Level

Diabetic animals that experienced 8 weeks duration of the diabetic state showed a significant increase in blood glucose level compared with the control group. Rats treated with curcumin showed a significant decrease in blood glucose level when compared to the diabetic rats [Graph 1].

Changes in Body Weights

Diabetic animals that experienced 8 weeks duration of the diabetic state showed a significant decrease in their body weight compared with the control group. Rats treated with curcumin showed significant weight gain when compared to the diabetic rats [Histogram 1].

Histological and Immunohistochemical Findings

Group 1, control group

Hematoxylin and eosin-stained sections of the spleen revealed normal architecture of the spleen without any detectable abnormalities [Figure 1a]. The parenchyma of the spleen consists of 2 parts White pulp(WP), Red pulp (RP). The white pulp consists of lymphocytes arranged around central arteriole. White pulp stains basophilic in H and E stain. The red pulp is composed of a network of cell cords in series with vascular sinuses (VS). The splenic cords contain macrophage, plasma cells, lymphocytes and other blood cells as erythrocytes and granulocytes. VS are wide vascular channels lined with endothelial cells [Figure 2a].

In immunohistochemistry stained sections, the area percent of positive splenocytes containing Caspase-3 protein was low (35% ± 7%), [Figure 3a and Histogram 2]. The area percent of positive splenocytes containing Bcl-2 protein was high (46% ± 13%) [Figure 4a and Histogram 3].

Group 2, uncontrolled diabetic group

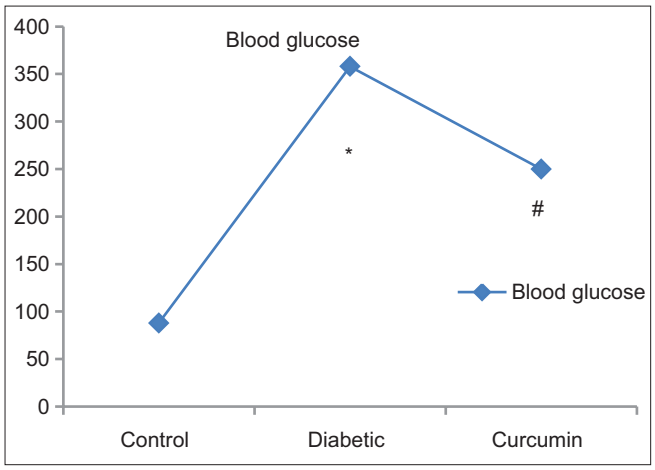
Hematoxylin and eosin-stained sections of the spleen showed fibrotic contracted area causing distortion of the splenic contour. Both red and white pulps were replaced by scar tissue. Also, the contour of the white pulp was lost and appeared irregular in the diabetic group when compared with the control group [Figure 1b]. Some cells appeared degenerated, swollen cell with vacuolated cytoplasm and pyknotic nuclei [Figure 2b].

In immunohistochemistry stained sections, the area percent of positive splenocytes containing Caspase-3 protein was high when compared with the control group (44% ± 11%), [Figure 3b and Histogram 2]. The area percent of positive splenocytes containing Bcl-2 protein was decreased when compared with the control group (31% ± 8%) [Figure 4b and Histogram 3].

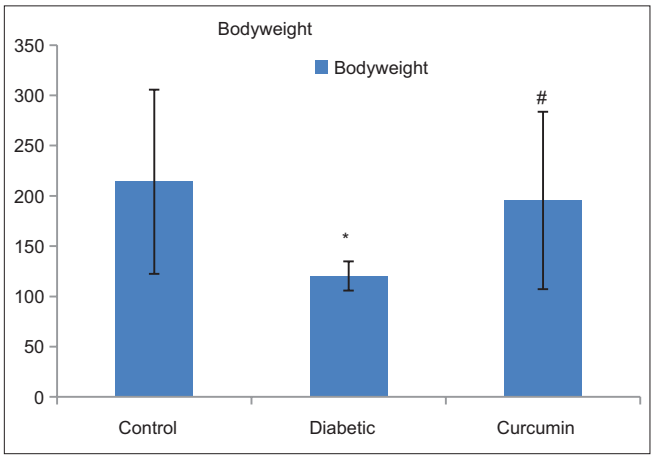
Group 3, curcumin-treated diabetic rats

Hematoxylin and eosin-stained sections of the spleen revealed the architecture of the spleen without detectable abnormalities [Figure 1c]. The parenchyma of the spleen consists of 2 parts white pulp and red pulp. The white pulp appeared basophilic, consists of lymphocytes arranged around central arteriole. The red pulp is composed of cell cords in series with VS. The splenic cords contain macrophage, plasma cells, lymphocytes and other blood cells [Figure 2c].

In immunohistochemistry stained sections, the area percent of positive splenocytes containing Caspase-3



Graph 1: Mean blood glucose levels (mg/dL) in all groups, *significant versus control group, #significant versus diabetic group



Histogram 1: Mean values of body weight (g) ± standard deviations of all groups, *significant versus control group, #significant versus diabetic group

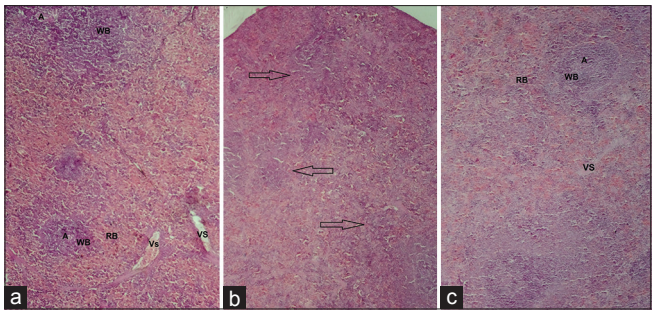


Figure 1: Photomicrographs of H and E stained sections (a): Coronal section in the spleen of control rat showing normal architecture of the spleen. The parenchyma of the spleen consists of white pulp (WP) with central arteriole (A), and red pulp (RP). The red pulp is composed of a network of cell cords in series with vascular sinuses (VS). (b) The diabetic group showed distorted splenic contour. The contours of the white pulp were lost and appeared irregular in the diabetic group when compared with the control group (arrows). (c) The curcumin-treated group, well-defined white pulp can be detected (WB) with central arteriole (A), red pulp (RB). The red pulp is composed of a network of cell cords in series with VS (H and E, ×100)

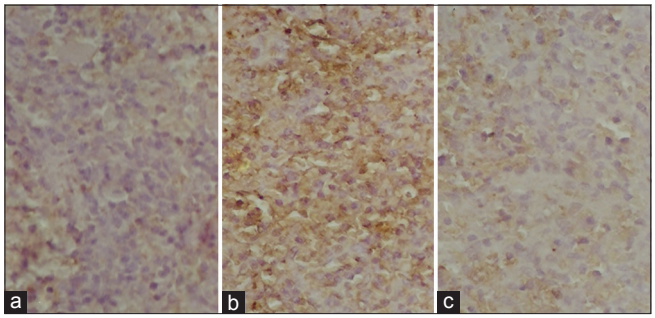
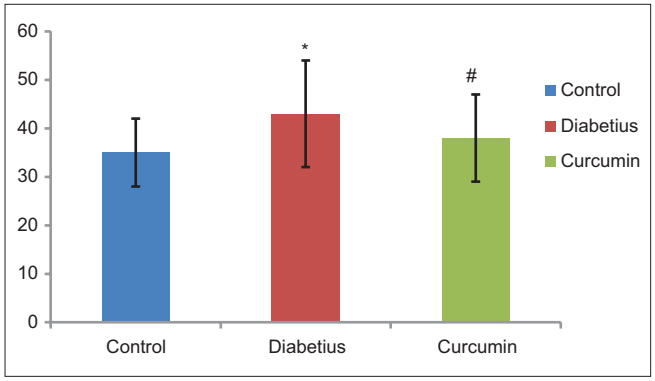


Figure 3: (a) Immunohistochemistry stained sections in control spleen, the area percent of positive splenocytes containing Caspase-3 protein is low. (b) Immunohistochemistry stained sections in diabetic rat, the area percent of positive splenocytes containing Caspase-3 protein is high. (c) Immunohistochemistry stained sections in curcumin-treated rats, the area percent of positive splenocytes containing Caspase-3 protein is low (Caspase-3, ×400)



Histogram 2: Mean area percentage of positive Caspase-3 immune-reactivity (%) ± standard deviations in all groups, *significant versus control group, #significant versus diabetic group

protein was low when compared with the diabetic group (38% ±9%), [Figure 3c and Histogram 2]. The area percent of positive splenocytes containing Bcl-2 protein was increased when compared with the diabetic group (40% ±10%), [Figure 4c and Histogram 3].

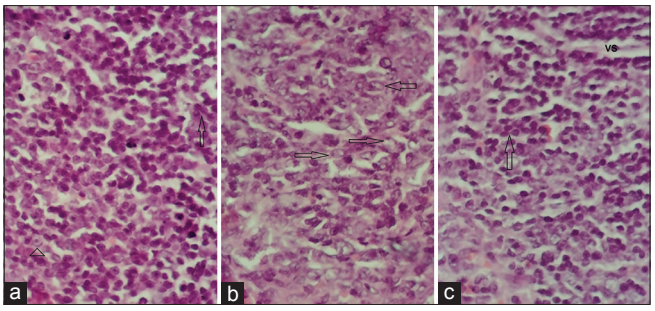


Figure 2: (a) Higher magnification of H and E stained sections of control group, shows the white pulp consists of lymphocytes, neutrophils (arrow), macrophages (arrowhead). (b) The diabetic group shows degenerated cells, with vaculated cytoplasm and pyknotic nuclei (arrows). (c) The curcumin-treated group, the red pulp is composed of a network of cell cords in series with vascular sinuses. Neutrophils can be detected (arrow) (H and E, ×400)

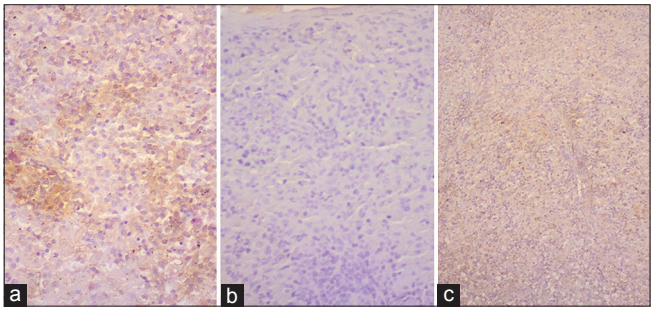
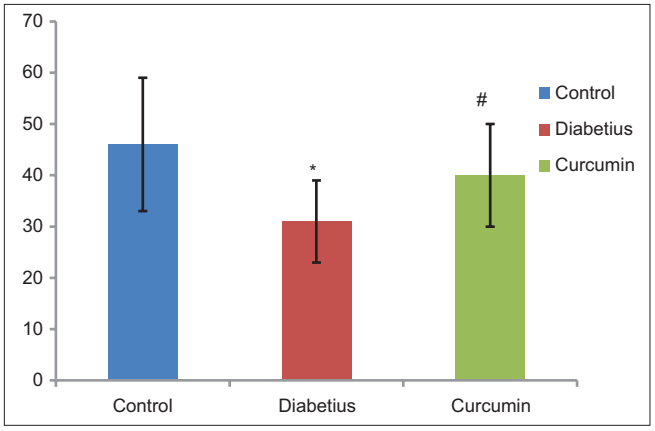


Figure 4: (a) Immunohistochemistry stained sections in control spleen, the area percent of positive splenocytes containing Bcl-2 protein is high. (b) Immunohistochemistry stained sections in diabetic rat, the area percent of positive splenocytes containing Bcl-2 protein is low. (c) Immunohistochemistry stained sections in curcumin-treated rats, the area percent of positive splenocytes containing Bcl-2 protein is high (Bcl-2, ×100)



Histogram 3: Mean area percentage of positive Bcl immune-reactivity (%) ± standard deviations in all groups,*significant versus control group, #significant versus diabetic group

DISCUSSION

This is the first report to our knowledge, showing that curcumin has beneficial effects on splenic tissue abnormalities in diabetes. These results raise the possibility that curcumin may be helpful in improving the immune system in diabetes. Diabetes is a chronic metabolic disorder that continues to present a major worldwide

health problem. Diabetes stands out as one of the most vital medical problems of the 21st century (Malecki 2004).

In the current study, curcumin treatment caused a decrease of the blood glucose level in diabetic rats compared to untreated diabetic ones. Similar results were reported that curcumin may decrease high blood glucose level in diabetic rats. Also, it was found that curcumin protected the rats from diabetic body weight reduction. This tends to agree with the result that curcumin-treated diabetic rats showed even increase in body weight when compared with diabetic rats (Chougala *et al*., 2012).

Histological examination of the spleen of diabetic animals revealed morphological changes in the form of disturbance of splenic contour and extensive paranchymal fibrosis in spleen of diabetic animals. Similar result was reported in diabetic rat spleen which showed disturbed architecture (Aktuğ *et al*., 2010).

As a peripheral lymphoid organ, the spleen stores activated immune cells, and spleen lymphocytes are very sensitive to apoptotic signals. Apoptosis result in the release of cytochrome c from mitochondria and activation of a specific class of cytoplasmic enzymes known as Caspases (Hofmann *et al*., 1997). Caspase-3 is the key inducer of apoptosis. These activated Caspase-3 destroy numerous cellular structures, leading to cell death (Wang *et al*., 2007). The Bcl-2 family proteins are major antagonist of apoptosis. Bcl-2 which could be present in mitochondria has been shown to inhibit cytochrome c release and protect against oxidative-induced apoptosis (Yang *et al*., 1997).

In the current study, it was found that diabetes increased the expression of Caspase-3 immunoreactivity in the spleen. On the reverse, the expression of Bcl-2 was significantly decreased by diabetes. Similar result was reported that the high glucose concentration enhances release of proinflammatory cytokines that mediated apoptosis of rat pancreatic islet cells (Mellado-Gil and Aguilar-Diosdado 2004).

Curcumin is known to have potent antioxidant and anti-inflammatory properties (Hsuuw *et al*., 2005). The antioxidant effect of curcumin has been considered to be mediated via its major effects on eradication of free radicals and/or via preventing lipid peroxidation and it is at least 10 times more active as an antioxidant than Vitamin E (Pandya *et al*., 2000).

In the present study, curcumin was found to decrease structural changes in the spleen. Curcumin-treated rats showed a significant decrease in the expression of Caspase-3 immunoreactivity in the spleen. Curcumin treatment significantly increased the expression of Bcl-2. These finding may be explained according to the fact that curcumin reduce the changes in lysosomal enzymes activities, thereby minimizing the damage caused due to diabetes (Chougala, *et al*., 2012). Curcumin also decreases lipid peroxidation, increases intracellular antioxidant factors (Pandya *et al*., 2000; Strasser *et al*., 2005; Osawa and Kato 2005).

In line with our results, it was found that Caspase-3 level was significantly elevated in diabetic rat pancreas, while treatment of diabetic rats within doses of 50 or 300 mg/kg curcumin reduced pancreatic Caspase-3 content without dose-dependent effect (Kamel *et al*., 2014). Also, our results were in agreement with the statement that treatment with curcumin could reduce apoptotic cell death by regulating the activation of Bcl-2 family proteins (Manna *et al*., 2010).

The specific mechanisms underlying the anti-diabetic action of curcumin are still unknown. It has been suggested by some investigators that curcumin may inhibit hepatic glucose output and/or stimulate insulin secretion from the pancreas (Ali Hussain 2002). In addition, it has been reported recently that anti-diabetic potential of antioxidants such as curcumin may be attributed to protection of glucose transporter. However, the curcumin supplementation may not be sufficient to be used as an ant-idiabetic on its own (Rungseesantivanon *et al*., 2010).

CONCLUSION

In this study, we investigated whether curcumin could protect against structural changes induced by diabetes in spleen of albino rats, and we checked the possible effect of curcumin on the expression of active Caspase-3 and Bcl-2 in spleen of diabetic rats using immunohistochemistry. The current data indicates that curcumin may protect the spleen from STZ-induced diabetic changes.

REFERENCES

1. Pabst R. (1993). Anatomical and physiological conditions for the conservation of post-operative. Spleen function after milzerhaltenden interventions. Chir Gastroenterol 9:19-22.
2. Bao H.J., Li M.Y., Wang J., Qin J.H., Xu C.S., Hei N.N., *et al.* (2009). Architecture of the blood-spleen barrier in the soft-shelled turtle, Pelodiseus sinensis. Anat Rec (Hoboken) 292 (8):1079-87.
3. Mebius R.E., Kraal G. (2005). Structure and function of the spleen. Nature reviews. Immunology 5 (8):606-16.
4. Olefasky J.M. Diabetes mellitus(1992). In: Smith LH, Wyngarden JB, Bennett JC, editors. Text Book of Medicine. WB Saunders, Philadalphia.
5. Zivic S., Vlaski J., Kocic G., Pesic M., Ciric V., Duric Z. (2008). The importance of oxidative stress in pathogenesis of type 1 diabetes – Determination of catalase activity in lymphocytes of diabetic patients. Med Pregl 61 (9-10):458-63.

6. Hernández Marco R., Codo˜ner Franch P., Pons Morales S., Del Castillo Villaescusa C., Boix García L., Valls Bellés V. (2009). Oxidant/antioxidant status and hyperfiltration in young patients with type 1 diabetes mellitus. Pediatr Nephrol 24 (1): 121-7.
7. Takahashi A., Masuda A., Sun M., Centonze V.E., Herman B. (2004). Oxidative stress-induced apoptosis is associated with alterations in mitochondrial caspase activity and Bcl-2-dependent alterations in mitochondrial pH (pHm). Brain Res Bull 62 (6):497-504.
8. Rathmell J.C., Thompson C.B. (2002). Pathways of apoptosis in lymphocyte development, homeostasis, and disease. Cell 109 Suppl: S97-107.
9. Waltner-Law M.E., Wang X.L., Law B.K., Hall R.K., Nawano M., Granner D.K. (2002). Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 277 (38):34933e40.
10. Epstein J., Sanderson I.R., Macdonald T.T. (2010). Curcumin as a therapeutic agent: The evidence from *in vitro*, animal and human studies. Br J Nutr 103 (11):1545-57.
11. Araújo C.C., Leon L.L. (2001). Biological activities of *Curcuma longa* L. Mem Inst Oswaldo Cruz 96 (5):723-8.
12. Rodrigues B., Poucheret P., Betell M. L. and McNeill J. N. 1999. Streptozotocin–Induced Diabetes: Induction, Mechanism(s), and Dose Dependency; in Experimental Models of Diabetes (ed) J H McNeill (USA: CRC Press LLC). p. 3-17.
13. Malecki M.T. (2004). Type 2 diabetes mellitus and its complications: From the molecular biology to the clinical practice. Rev Diabet Stud 1 (1):5-8.
14. Chougala M.B., Bhaskar J.J., Rajan M.G., Salimath P.V. (2012). Effect of curcumin and quercetin on lysosomal enzyme activities in streptozotocin-induced diabetic rats. Clin Nutr 31 (5):749-55.
15. Aktuğ H., Kosova B., Yavasoğlu A., Çetintas V.B. (2010). Evaluation of caspase-3 and bcl-2 expressions in spleen tissue of streptozotocin-induced diabetic rats by immune-histochemistry and quantitative RT-PCR. Ege J Med Ege Tıp Derg 49 (1):1-6.
16. Hofmann K., Bucher P., Tschopp J. (1997). The CARD domain: A new apoptotic signalling motif. Trends Biochem Sci 22 (5):155-6.
17. Wang J.M., Xiao B.L., Zheng J.W., Chen H.B., Zou S.Q. (2007). Effect of targeted magnetic nanoparticles containing 5-FU on expression of bcl-2, bax and caspase 3 in nude mice with transplanted human liver cancer. World J Gastroenterol 13 (23):3171-5.
18. Yang J., Liu X., Bhalla K., Kim C.N., Ibrado A.M., Cai J, *et al.* (1997). Prevention of apoptosis by Bcl-2: Release of cytochrome c from mitochondria blocked. Science 275 (5303):1129-32.
19. Mellado-Gil J.M., Aguilar-Diosdado M. (2004). High glucose potentiates cytokine- and streptozotocin-induced apoptosis of rat islet cells: Effect on apoptosis-related genes. J Endocrinol 183 (1):155-62.
20. Hsuuw Y.D., Chang C.K., Chan W.H., Yu J.S. (2005). Curcumin prevents methylglyoxal-induced oxidative stress and apoptosis in mouse embryonic stem cells and blastocysts. J Cell Physiol 205 (3):379-86.
21. Pandya U., Saini M.K., Jin G.F., Awasthi S., Godley B.F., Awasthi Y.C. (2000). Dietary curcumin prevents ocular toxicity of naphthalene in rats. Toxicol Lett 115 (3):195-204.
22. Strasser E.M., Wessner B., Manhart N., Roth E. (2005). The relationship between the anti-inflammatory effects of curcumin and cellular glutathione content in myelomonocytic cells. Biochem Pharmacol 70 (4):552-9.
23. Osawa T., Kato Y. (2005). Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann N Y Acad Sci 1043:440-51.
24. Kamel R., Hashim A., Ali S. (2014). Palliative effect of curcumin ON STZ-induced diabetes in rats. Int J Pharm Pharm Sci 1491 (6) Suppl 2, 558-63.
25. Manna P., Das J., Ghosh J., Sil P.C. (2010). Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IkappaBalpha/NF-kappaB, MAPKs, and mitochondria-dependent pathways: Prophylactic role of arjunolic acid. Free Radic Biol Med 48 (11):1465-84.
26. Ali Hussain H.E. (2002). Hypoglycemic, hypolipidemic and antioxidant properties of combination of Curcumin from *Curcuma longa*, Linn, and partially purified product from *Abroma augusta*, Linn. in streptozotocin induced diabetes. Indian J Clin Biochem 17 (2):33-43.
27. Rungseesantivanon S., Thenchaisri N., Ruangvejvorachai P., Patumraj S. (2010). Curcumin supplementation could improve diabetes-induced endothelial dysfunction associated with decreased vascular superoxide production and PKC inhibition. BMC Complement Altern Med 10:57.

How to cite this article:
Hashish, H., & Kamal, R. (2015). Effect of curcumin on the expression of Caspase-3 and Bcl-2 in the spleen of diabetic rats. J. Exp. Clin. Anat, 14, 18-23.

Source of Support: Nil, **Conflict of Interest:** None declared.