TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2017) 47: 675-680

doi:10.3906/sag-1604-145

Effects of Ankaferd BloodStopper on dermal healing in diabetic rats

Sertaç AKTOP[1,*], Burcu ÇEVRELİ[2], Deniz GENÇ[3], Emel SERDAROĞLU KAŞIKÇI[4], Nil ÇOMUNOĞLU ÜSTÜNDAĞ[5], Noushin ZİBANDEH[3], Elif Merve ÖZCAN[1], Gökhan GÖÇMEN[1], Mehmet Kamil GÖKER[1], İsmail Tayfun UZBAY[4], Tunç AKKOÇ[3]

[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Marmara University, İstanbul, Turkey
[2]Experimental Research Unit, Faculty of Engineering and Natural Sciences, Üsküdar University, İstanbul, Turkey
[3]Department of Pediatric Allergy and Immunology, Faculty of Medicine, Marmara University, İstanbul, Turkey
[4]Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University, İstanbul, Turkey
[5]Department of Medical Pathology, Cerrahpaşa Faculty of Medicine, İstanbul University, İstanbul, Turkey

Received: 29.04.2016 • **Accepted/Published Online:** 11.09.2016 • **Final Version:** 18.04.2017

Background/aim: Diabetes mellitus inhibits wound-induced angiogenesis, impairs the wound healing process, and leads to the development of chronic wounds. Ankaferd BloodStopper (ABS) is a new and promising local haemostatic agent. Although the mechanism of ABS-mediated haemostasis is well established, little is known about the associated histological and biochemical tissue reactions. The aim of this study was to evaluate the effects of this new-generation local haemostatic agent on short-term soft-tissue healing in streptozotocin (STZ)-treated rats.

Materials and methods: The 24 Wistar albino rats used in this study were divided into STZ-treated (STZ, n = 12) and nontreated groups (control, n = 12). Four days prior to surgery, rats in the STZ group were subcutaneously administered 60 mg/kg STZ intraperitoneally, while rats in the control group were administered 1 mL saline/kg. An incision was made in the dorsal dermal tissue of all rats, and either ABS or no haemostatic agent (NHAA) was applied to the wound before suturing. All of the rats were euthanised on postoperative day 4. Blood and skin samples were evaluated biochemically and histologically.

Results: The results showed that STZ treatment impaired soft-tissue healing, assessed by measuring glutathione and lipid peroxidation levels. Moreover, while good histological results were obtained in the control group treated with ABS, there were fewer benefits in the STZ-treated group.

Conclusion: ABS's benefits in the control group seemed to lose their effectiveness under STZ medication.

Key words: Streptozotocin, Ankaferd BloodStopper, glutathione, lipid peroxidation, vascular endothelial growth factor, transforming growth factor-beta

1. Introduction

Diabetes mellitus (DM) is a chronic, life-long metabolic disease occurring worldwide. Affected individuals require continuous follow-up and suffer numerous disease-related complications. The high levels of blood glucose that characterise DM contribute to an increase in free radical production, enhanced oxidative stress, and changes in antioxidant capacity (1–3).

Streptozotocin (STZ) is the chemical agent most frequently used to induce experimental diabetes in animal models. By binding to glucose receptors in the plasma membrane, STZ blocks glucose-induced insulin secretion (2,3) and alters blood insulin and glucose concentrations.

Among the many effects of free radical production is lipid peroxidation (LPO). Oxidation of membrane polyunsaturated fatty acids disrupts cell structure and function and induces cytotoxic, hepatotoxic, mutagenic, and genotoxic effects related to the release of aldehydes (4,5).

The tripeptide glutathione (GSH) is found in the cells of all organisms. In humans, GSH concentrations are highest in the liver; in hepatocytes, GSH accounts for 90% of nonprotein sulfhydryl groups. As an important reducing agent and antioxidant, GSH maintains the cellular oxido-reduction balance and protects cells against the toxic effects of oxidants, whether of endogenous or exogenous origin (5–7).

Ankaferd BloodStopper (ABS) (Ankaferd Health Products Ltd., İstanbul, Turkey) is a medicinal extract from the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica* (7,8). ABS is not only an effective haemostatic agent but also

* Correspondence: sertacaktop@hotmail.com

exhibits tissue antioxidant properties (5,9). In Turkey, ABS is approved for the management of postsurgical dental bleeding and external haemorrhage (5). ABS forms an encapsulated protein providing focal points for erythrocyte aggregation, without affecting other components of the coagulant system.

Studies on wound healing often involve assessments of collagen, a protein crucial to restoring the integrity of the skin (10,11), and angiogenesis, in which vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β) play prominent roles (12,13). VEGF controls a variety of endothelial cell functions involved in angiogenesis and protects these cells from apoptosis (14). Fibroblast growth factor-2 (FGF-2) and TGF-β induce VEGF expression in vascular endothelial cells (15). In addition, TGF-β is an important regulator of tissue morphogenesis and a potent inhibitor of proliferation for most cell types (13).

Several studies have shown that ABS promotes tissue healing (5,9,16–21). Therefore, in this study, we addressed the effectiveness of ABS in diabetes, in which wound healing is severely impaired. Specifically, we evaluated the effects of ABS on short-term dermal soft-tissue healing in rats with STZ-induced diabetes.

2. Materials and methods

2.1. Animals and treatment

Twenty-four male Wistar albino rats weighing 280–450 g were divided into STZ-treated and control groups (n = 12 each). The animals were obtained from the Department of Experimental Research Unit, Üsküdar University (İstanbul, Turkey), where the surgery and postoperative care were performed. All experimental protocols were approved by the Animal Care and Use Ethical Committee of Marmara University (no: 40.2013.mar)

The 12 control rats were injected subcutaneously with 1 mL saline/kg intraperitoneally and the 12 rats in the STZ group were injected subcutaneously with a single dose of STZ (60 mg/kg, freshly dissolved in 1 mL of saline) intraperitoneally 4 days before surgery. In the latter group, the animals were considered to be diabetic, based on blood glucose levels ≥250 mg/dL. All 24 rats underwent surgery and on postoperative day 4 were euthanised by the injection of a high dose of anaesthetic.

The animals were anaesthetised with a combination of 90 mg/kg ketamine (Ketalar, Pfizer İlaçları Ltd. Şti, İstanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer HealthCare, Leverkusen, Germany). Surgery was performed under aseptic conditions.

The dorsal skin was shaved, and an incision 2 cm long and perpendicular to the head-to-tail direction was made. In 6 of the 12 animals in each group, the wounds were sutured without application of haemostatic agent and left to heal naturally. The incisions of the other 6 animals were treated with 0.25 ml ABS, applied before suturing.

The dorsal skin was excised completely from the euthanised animals, and the wounded tissue area was prepared for histological evaluation at the Department of Medical Pathology, Cerrahpaşa Faculty of Medicine, İstanbul University (İstanbul, Turkey). Blood samples taken from the euthanised animals were used in biochemical evaluations, performed at the Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University (İstanbul, Turkey).

2.2. Biochemical tests

Blood GSH concentrations were determined according to the method of Beutler, using metaphosphoric acid for protein precipitation and 5'5'-dithiobis-2-nitro-benzoic acid for colour development (22). Blood LPO levels were assayed by measuring serum malondialdehyde levels, determined as thiobarbituric acid reactive substances according to the method described by Yagi (23).

2.3. Histological evaluation

Surgical specimens were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin blocks. The 4-μm-thick sections cut from the blocks were stained with haematoxylin and eosin. In addition, 4- to 6-μm-thick sections were cut, floated on positively charged microscope slides, and labelled with an indirect avidin-biotin-peroxidase complex for automated immunohistochemistry analysis (Ventana Medical Systems, Tucson, AZ, USA). Tissue sections were deparaffinised, rehydrated in decreasing concentrations of alcohol, and washed with distilled water. Antigen retrieval was achieved by incubating the slides in 10 mM sodium citrate at 36 °C for 30 min. The slides were then incubated for 1 h with TGF-β rabbit polyclonal antibody (1:500, Abcam, Cambridge, UK), VEGF rabbit polyclonal antibody (1 μg/mL, Abcam), and collagen 4 rabbit polyclonal antibody (1/1000, Abcam) and then counterstained with haematoxylin. Positive controls for TGF-β, VEGF, and collagen consisted of human placental tissue, haemangioma tissue, and human epidermal keratinocyte lysate, respectively. The negative control consisted of PBS instead of the primary antibody.

2.3.1. Histological scoring

Immunostaining was scored by a pathologist blinded to the clinical data and was evaluated using a double-headed BHS Olympus microscope. The intensity of staining was graded as 0, negative; 1+, weak; 2+, moderate; and 3+, strong. The extent of immunostaining was graded as 0 (0%), negative, 1+ (0%–25%), 2+ (26%–50%), and 3+ (51%–100%).

2.4. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences, version 22.0 (SPSS, Chicago, IL, USA). The results were evaluated using the Shapiro–Wilk test for data with a normal distribution and are expressed as means ± standard deviation. Quantitative data that fulfilled the parametric criteria were analysed using Student's t test; nonparametric data were analysed using the Mann–Whitney U test. A P value <0.05 was considered to indicate statistical significance.

3. Results

3.1. Biochemical results

3.1.1. Comparison of GSH and LPO levels in blood samples from the control and STZ groups

There were no significant differences in the blood samples from ABS versus NHAA rats in either the control or STZ group. The GSH values were significantly higher in the control group than in the STZ group for both the NHAA- and the ABS-treated rats (P = 0.0001; P = 0.003, respectively). LPO values were significantly higher in the NHAA blood samples of the STZ group than those of the control group (P = 0.0001). The LPO values in the ABS-treated animals were slightly, but not significantly, higher in the STZ group than in the control group (Table 1).

3.2. Histological results

3.2.1. Comparison of histological scores in tissues of the control and STZ groups

In the control group, the scores for both the intensity and extent of collagen 4 staining were significantly higher in the ABS-treated than in the NHAA-treated tissues of the control group and the ABS-treated tissues of the STZ group (P = 0.021 and P = 0.021, respectively). The VEGF intensity scores within the control group were significantly higher in ABS-treated than in NHAA-treated tissues (P = 0.034) (Table 2).

Table 1. GSH and LPO levels in blood samples on postoperative day 4.[a]

		Control group (n = 12)			STZ group (n = 12)			
		Mean	±	SD	Mean	±	SD	P
GSH (mg/g p)	ABS (n = 6)	31.35	±	4.62	18.58	±	3.1	0.0001*
	NHAA (n = 6)	42.03	±	10.14	20.52	±	3.01	0.003*
P			0.51			0.298		
LPO (nmol MDA/mg p)	ABS (n = 6)	5.95	±	1.7	7.8	±	1.23	0.06
	NHAA (n = 6)	5.88	±	0.67	8.52	±	0.82	0.0001*
P			0.922			0.264		

Abbreviations: SD: standard deviation; ABS: Ankaferd BloodStopper; NHAA: no haemostatic agent administered, p: protein, GSH: glutathione, LPO: lipid peroxidation

[a]Values are means ± SD.

*P < 0.05 according to Student's t test

Table 2. Histologic scores of tissue samples on postoperative day 4.

	Collagen 4 extent	Collagen 4 intensity	VEGF extent	VEGF intensity	TGF-β extent	TGF-β intensity
Control - NHAA	-	-	++++	++++	+	+
Control - ABS	++*	++*	++++	++++++*	++	+
STZ - NHAA	-	-	++++	+++++	++	++
STZ - ABS	-	-	++++	+++++	++	+

Abbreviations: ABS: Ankaferd BloodStopper; NHAA: no haemostatic agent administered; VEGF: vascular endothelial growth factor; TGF-β: transforming growth factor-beta

*Statistically significant according to Student's t test or the Mann–Whitney U test

4. Discussion

Many studies have investigated the healing potential of particular agents in animal models of diabetes, examining clinical effects or histological outcomes (24–27). ABS has been used as a local haemostatic agent in surgery, but it was also shown to promote healing (5,9). Its effects on soft-tissue healing have been investigated (17,19–21), but not in a STZ model of diabetes. Therefore, the aim of the present study was to histologically assess the effects of ABS on collagen 4, blood GSH levels, and LPO to evaluate its potential effects in early-stage soft-tissue healing on STZ-treated rats.

The STZ group was injected with a single dose of STZ (60 mg/kg, freshly dissolved in 1 mL of saline solution) intraperitoneally for 4 days before surgery. Many other successful STZ administration protocols are described in the literature (2,28–30).

Collagen, which is beneficial for endoepidermal growth and therefore healing, is a major functional extracellular matrix protein in the dermal layer of the skin (20). Collagen 4, VEGF, and TGF-β are commonly used markers of healing potential (24,26,27,30–32) and were evaluated in this study as well. Wound healing comprises four primary stages that occur in a partly sequential, partly overlapping process. During the proliferation (third) phase, fibroblasts migrate from the surrounding connective tissue, proliferate, and begin to synthesise a matrix of ground substance, fibronectin, and extracellular proteins such as collagen, elastin, and integrins. In addition, macrophages release numerous growth factors that promote angiogenesis, including basic FGF and VEGF (33).

Huri et al. applied chitosan and ABS as haemostatic agents to the excision area in 40 Wistar rats with partial nephrectomy and found no significant differences in their effects on haemostasis. They suggested that ABS was just as effective as other haemostatics, none of which led to fibrosis, adhesion, or calcification, and all achieved good histopathological results (34).

Satar et al. compared ABS and oxidised cellulose in experimentally injured rats. The bleeding time was shorter in the ABS-treated group, as determined in liver sections. The favourable histopathological changes included fewer signs of inflammation than in the oxidised-cellulose-treated group on days 7 and 14 (17).

In a study based on a rat skin defect model, Akalin et al. reported higher collagen deposition scores and lower inflammatory scores in the ABS group. The fibroblast proliferation scores were higher in the ABS group on day 14 (35). The authors suggested that ABS can be safely used for the repair of full-thickness wounds. Yüce et al. also recommended ABS to promote wound healing (19).

Our study demonstrated that, in the control, nondiabetic animals, collagen 4 extent and intensity were significantly higher in ABS tissues than in NHAA tissues ($P = 0.021$). VEGF scores were also significantly higher in ABS tissues ($P = 0.034$). These histologically based observations suggest that ABS supports wound healing. Moreover, the extent and intensity of collagen staining were significantly higher in the ABS tissues of the control group than in those of the STZ group ($P = 0.021$), indicating that ABS did not compensate for the effects of STZ treatment on wound healing.

Isler et al. examined the effects of ABS on bone healing during the first 7 days and noted that ABS decreased inflammation and necrosis and increased new bone formation (16). Bulut et al. evaluated ABS and routine antibiotic prophylaxis (AP) on the early healing of bone defects in diabetic rats but were unable to detect significant differences in new bone formation between the AP- and ABS-treated animals (18).

ROS are produced in metabolic and physiological processes, but in healthy individuals their harmful oxidative effects are prevented (35) via enzymatic and nonenzymatic antioxidative mechanisms. A shift in the oxidative and antioxidative balance towards the former, as occurs in many disorders including DM, results in oxidative stress (22,36).

An increase in the concentration of the end products of LPO is the most prominent evidence of free radical involvement in human disease (37). However, rather than accelerating the bulk peroxidation of cell membrane lipids (22), oxidative stress is likely to cause cell damage, which in turn leads to a secondary increase in LPO (38). Thus, LPO is often a late event that accompanies rather than causes cell death (39). In our study, LPO levels were significantly increased in the NHAA blood samples but not in the ABS blood samples of the STZ group ($P = 0.0001$, $P = 0.06$, respectively). Oxidative stress has been associated with insulin resistance (29) and thus may develop in response to STZ treatment, resulting in poor soft-tissue healing (25,29,40–42). The absence of a significant difference in the blood samples of the ABS-treated rats in the control versus STZ groups suggests that ABS reduces the oxidative stress that occurs in DM.

Koluman et al. revealed the presence of several antioxidant molecules (including tocotrienols, vitamin E, tryptophan, estriol, galangin, apigenin, oenin, 3,4-divanillyltetrahydrofuran, TBHQ, thymol, BHA, BHT, lycopene, glycyrrhetinic acid, and tomatine), which may have clinical implications in the pharmacobiological actions of ABS. They concluded that the antioxidant content of ABS should be investigated in future studies (43).

Conversely, GSH is part of an integrated antioxidant system that protects cells and tissues from oxidative damage (44). In DM, a decrease in tissue GSH could be due to a decrease in the synthesis of GSH or to an increase in its degradation by oxidative stress (40). We found that STZ treatment caused a significant reduction in blood GSH values, both in ABS-treated and NHAA-treated rats ($P = 0.0001$, $P = 0.03$, respectively).

Aktop et al. found that warfarin treatment in rats inhibited antioxidant capacity, similar to the treatment effects of STZ treatment (5). Similar to our own results, in the control group of their study, there were no statistically significant differences in GSH and LPO levels in ABS-treated versus NHAA-treated tissues ($P = 0.051$, $P = 0.922$). The same authors evaluated catalase and superoxide dismutase activities in warfarin-treated rats and found positive effects of ABS on soft-tissue healing (9). In our study ABS administration improved soft-tissue healing, consistent with the histologically determined increase in collagen 4 levels, but there were no significant benefits with respect to blood GSH and LPO levels.

Aydin et al. applied ABS to the healing tendons of rats but found no beneficial effects, as determined histologically (21). Evren et al. reported significantly increased fibrosis and necrosis in the auricular cartilages of New Zealand rabbits treated with ABS (45).

In conclusion, STZ treatment may impair soft-tissue healing in rats by altering the antioxidant–oxidative stress balance. ABS had histologically confirmed benefits on wound healing in control rats but not in STZ-treated rats. Nonetheless, ABS seems to reduce the oxidative stress associated with diabetic metabolism, based on GSH and LPO levels that were similar to the control levels.

References

1. Baynes YW, Thorpe R. Role of oxidative stress in diabetic complications. Diabetes 1999; 48: 1-9.
2. Koroglu P, Senturk GE, Yucel D, Ozakpinar OB, Uras F, Arbak S. The effect of exogenous oxytocin on streptozotocin (STZ)-induced diabetic adult rat testes. Peptides 2015; 63: 47-54.
3. Kanter M, Aktas C, Erboga M. Protective effects of quercetin against apoptosis and oxidative stress in streptozotocin induced diabetic rat testis. Food Chem Toxicol 2012; 50: 719-725.
4. İseri S, Gedik İ, Erzik C, Uslu B, Arbak S, Gedik N, Yegen B. Oxytocin ameliorates skin damage and oxidant gastric injury in rats with thermal trauma. Burns 2008; 34: 361-369.
5. Aktop S, Emekli-Alturfan E, Özer C, Gönül O, Garip H, Yarat A, Göker K. Effects of ankaferd blood stopper and celox on skin glutathione and lipid peroxidation levels in warfarintreated rat. Journal of Marmara University Institute of Health Sciences 2012; 2: 32-42 (article in Turkish with an abstract in English).
6. Mann K. The challenge of regulating anticoagulant drugs: focus on warfarin. Am Heart J 2005; 149: 536-542.
7. Abacıoğlu S, Aydın K, Büyükcam F, Kaya U, Işık B, Karakılıç ME. Comparison of the efficiencies of buffers containing Ankaferd and Chitosan on hemostasis in an experimental rat model with femoral artery bleeding. Turk J Hematol 2016; 33: 48-52.
8. Goker H, Haznedaroglu IC, Ercetin S, Kirazli S, Akman U, Ozturk Y, Firat HC. Haemostatic actions of the folkloric medicinal plant extract Ankaferd Blood Stopper. J Int Med Res 2008; 36: 163-170.
9. Aktop S, Emekli-Alturfan E, Gönül O, Göçmen G, Garip H, Yarat A, Göker K. Effect of Ankaferd Blood Stopper on Skin Superoxide Dismutase and Catalase Activities in Warfarin-Treated Rats. Clin Appl Thromb Hemost 2015 Sep 8. DOI: 10.1177/1076029615604049.
10. Yamamoto N, Nishioka S, Sasai Y. Polarization microscopic investigation of collagen and acid glycosaminoglycans in the skin of progressive systemic sclerosis (PSS). Acta Histochem 1995; 97: 195-202.
11. Linares HA, Kischer CW, Dobrkovsky M, Larson DL. The histiotypic organization of the hypertrophic scar in humans. J Invest Dermatol 1972; 59: 323-331.
12. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev 2004; 25: 581-611.
13. Massagué J, Blain SW, Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. Cell 2000; 103: 295-309.
14. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol 2005; 23: 1011-1027.
15. Ferrari G, Pintucci G, Seghezzi G, Hyman K, Galloway AC, Mignatti P. VEGF, a prosurvival factor, acts in concert with TGF-beta1 to induce endothelial cell apoptosis. Proc Natl Acad Sci USA 2006; 103: 17260-17265.
16. Işler SC, Demircan S, Cakarer S, Cebi Z, Keskin C, Soluk M, Yüzbaşioğlu E. Effects of folk medicinal plant extract Ankaferd Blood Stopper on early bone healing. J Appl Oral Sci 2010; 18: 409-414.
17. Satar NY, Akkoc A, Oktay A, Topal A, Inan K. Evaluation of the hemostatic and histopathological effects of Ankaferd Blood Stopper in experimental liver injury in rats. Blood Coagul Fibrinolysis 2013; 24: 518-524.
18. Bulut E, Baş B, Altunkaynak BZ, Bekçioğlu B, Erdem Koç G, Gönülol E, Önger ME, Kaplan S. Efficacy of Ankaferd Blood Stopper on bone healing in diabetic rats: a stereological and histopathological study. Biotech Histochem 2014; 89: 535-543.

19. Yüce S, Celal C, Yenidunya S, Muslu B. New hemostatic agent: the effect of Ankaferd Blood Stopper on healing wounds in experimental skin incision model. Turk J Med Sci 2014; 44: 288-294.

20. Akalin C, Kuru S, Barlas AM, Kismet K, Kaptanoglu B, Demir A, Astarci HM, Ustun H, Ertas E. Beneficial effects of Ankaferd Blood Stopper on dermal wound healing: an experimental study. Int Wound J 2014; 11: 64-68.

21. Aydın BK, Altan E, Acar MA, Erkoçak ÖF, Ugraş S. Effect of Ankaferd blood stopper® on tendon healing: an experimental study in a rat model of Achilles tendon injury. Joint Diseases and Related Surgery 2015; 26: 31-37.

22. Beutler E. Glutathione: Red Cell Metabolism. A Manual of Biochemical Methods. 2nd edition. New York, NY, USA: Grune and Stratton, 1975; pp. 112-114.

23. Yagi K. Assay for blood plasma or serum methods in enzymology. Methods Enzymol 1984; 105: 328-337.

24. Tomizawa YJ. Clinical benefits and risk analysis of topical hemostats: a review. Artif Organs 2005; 8: 137-142.

25. Saravanan G, Ponmurugan P. Ameliorative potential of S-allyl cysteine on oxidative stress in STZ induced diabetic rats. Chemico-Biological Interactions 2011; 189: 100-106.

26. Ertürküner SP, Basar M, Tuncdemir M, Seckin I. The comparative effects of perindopril and catechin on mesangial matrix and podocytes in the streptozotocin induced diabetic rats. Pharmacological Reports 2014; 66: 279-287.

27. Kandhare AD, Ghosh P, Bodhankar SL. Naringin, a flavanone glycoside, promotes angiogenesis and inhibits endothelial apoptosis through modulation of inflammatory and growth factor expression in diabetic foot ulcer in rats. Chem Biol Interact 2014; 219: 101-112.

28. Singh J, Kakkar P. Modulation of liver function, antioxidant responses, insulin resistance and glucose transport by *Oroxylum indicum* stem bark in STZ induced diabetic rats. Food Chem Toxicol 2013; 62: 722-731.

29. Lima TI, Monteiro IC, Valença S, Leal-Cardoso JH, Fortunato RS, Carvalho DP, Teodoro BG, Ceccatto VM. Effect of exercise training on liver antioxidant enzymes in STZ-diabetic rats. Life Sci 2015; 1: 64-71.

30. Kuo CW, Shen CJ, Tung YT, Chen HL, Chen YH, Chang WH, Cheng KC, Yang SH, Chen CM. Extracellular superoxide dismutase ameliorates streptozotocin-induced rat diabetic nephropathy via inhibiting the ROS/ERK1/2 signaling. Life Sci 2015; 135: 77-86.

31. Chen F, Zhang H, Zhu J, Liu K, Cheng H, Li G, Xu S, Lv W, Xie Z. Puerarin enhances superoxide dismutase activity and inhibits RAGE and VEGF expression in retinas of STZ-induced early diabetic rats. Asian Pac J Trop Med 2012; 5: 891-896.

32. Iglesias-de la Cruz MC, Ziyadeh FN, Isono M, Kouahou M, Han DC, Kalluri R, Mundel P, Chen S. Effects of high glucose and TGF-beta1 on the expression of collagen IV and vascular endothelial growth factor in mouse podocytes. Kidney Int 2002; 62: 901-913.

33. Brisset AE, Hom DB. The effects of tissue sealants, platelet gels, and growth factors on wound healing. Curr Opin Otolaryngol Head Neck Surg 2003; 11: 245-250.

34. Huri E, Akgül T, Yücel O, Astarcı M, Üstün H, Germiyanoğlu C. The second step in vitro trial of Ankaferd® Bloodstopper®: comparison with the other hemostatic agents. Turk J Med Sci 2011; 41: 7-15.

35. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001; 54: 176-186.

36. Llesuy S, Evelson P, González Flecha P, Peralta J, Carreras M, Poderoso J, Boveris A. Oxidative stress in muscle and liver of rats with septic syndrome. Free Radic Biol Med 1994; 16: 445-451.

37. Lowry OH, Rosbrough NJ, Farr AL, Randall RJ. J. Protein measurement with the Folin phenol reagent. Biol Chem 1951; 193: 265.

38. Mylorie AA, Collins H, Umbles C, Kyle J. Erythrocyte superoxide dismutase activity and other parameters of cupper status in rats ingesting lead acetate. Toxicol Appl Pharmacol 1986; 82: 512-520.

39. Aebi H. Catalase. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. New York, NY, USA: Academic Press, 1974; pp. 673-677.

40. Loven D, Schedl H, Wilson H, Daabees TT, Stegink LD, Diekus M. Effect of insulin and oral glutathione on glutathione levels and superoxide dismutase activities in organs of rats with streptozotocin induced diabetes. Diabetes 1986; 35: 503-507.

41. Ewis SA, Abdel-Rahman MS. Effect of metformin on glutathione and magnesium in normal and streptozotocin-induced diabetic rats. J Appl Toxicol 1995; 15: 387-390.

42. Saxena AK, Srivastava P, Kale RK, Baquer NZ. Impaired antioxidant status in diabetic rat liver: effect of vanadate. Biochem Pharmacol 1993; 45: 539-542.

43. Koluman A, Akar N, Malkan UY, Haznedaroglu IC. Qualitative/chemical analyses of Ankaferd hemostat and its antioxidant content in synthetic gastric fluids. Biomed Res Int 2016; 2016: 8957820. doi: 10.1155/2016/8957820.

44. Skalli O, Gabbiani G. The biology of the myofibroblast relationship to wound contraction and fibrocontractive disease. In: Clark RAF, Henson PM, editors. The Molecular and Cellular Biology of Wound Repair. New York, NY, USA: Plenum Press, 1988; pp. 373-402.

45. Evren C, Uğur MB, Yıldırım B, Bektaş S, Yiğit VB, Çınar F. Unpredicted effects of Ankaferd® on cartilage tissue. Int J Clin Exp Med 2015; 8: 922-927.