

PROTECTIVE ROLE OF *ARTEMISIA AFRA* AQUEOUS EXTRACT ON TISSUE ANTIOXIDANT DEFENSE SYSTEMS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract

Changes in antioxidant capacity in the body as a result of oxidative stress play an important role in the development of diabetic complications. The aim of this study was to evaluate the effect of aqueous extract of *Artemisia afra* Jacq. ex Willd. on antioxidant defense systems in the liver and kidney of streptozotocin-induced diabetic rats. Administration of the extract to diabetic rats for 21 days significantly reduced blood glucose levels and increased body weight. The diabetic animals exhibited decreased levels of glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD) and reduced glutathione (GSH) in the liver and kidney, which were restored to near normal levels following treatment with the herb. The increased levels of lipid peroxidation observed in the tissues of diabetic rats were also reverted back to near normalcy after administering the extract. These findings revealed the protective role of *A. afra* on tissues by reducing oxidative stress which could be attributed to its flavonoids content. The efficacy of the plant compared favourably well with glibenclamide, a standard hypoglycemic drug.

Key words: *Artemisia afra*, antioxidant, diabetes, flavonoids, free radicals, oxidative stress

Introduction

Diabetes is a metabolic disorder characterized by chronic hyperglycaemia leading to various dysfunctions in the body. Free radical generation is currently suggested to play an important role in the causation and complications of the disease (Senthilkumar and Subramanian, 2007). These radicals are continually produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. In healthy individuals, the generation of free radical appears to be approximately in balance with the antioxidant defense system comprising both enzymatic and non-enzymatic antioxidants. In diabetes however, there are alterations in the endogenous free radical scavenging mechanisms which may lead to the production of reactive oxygen species, resulting in oxidative damage and tissue injury (Oberley, 1988). Implication of oxidative stress in the pathogenesis of diabetes is suggested not only by oxygen-free radicals but also due to non-enzymatic protein glycosylation and auto-oxidation of glucose (Mullarkey et al., 1990); alterations in antioxidant enzymes (Strain, 1991) as well as formation of lipid peroxides (Baynes, 1991). Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes, are thought to be the etiology of diabetic complications (Baynes, 1991).

The management of diabetes is considered a global problem and there is no successful and definite therapy yet. A few chemotherapeutic drugs have been in use to manage the disease since the accidental discovery of the hypoglycemic action of sulfonamides (Robinson and Johnston, 1997). The thrust of such management measures is to achieve an effective blood glucose control or utilization, with a view to delaying or averting the onset of complications. The application of these measures is however limited due to their high cost and associated side effects (Upadhyay et al., 1996; Reynolds, 1997). Recently, attention is being focused on the identification of natural antioxidants from plants to replace synthetic ones. Findings from scientific reports have shown that plants contain various substances that possess antioxidant activity (Chanwitheesuk et al., 2005).

Artemisia afra Jacq. ex Willd. (Asteraceae) is locally known as Unhlonyane in Xhosa and African wormwood in English. It is one of the widely used medicinal plants in Southern Africa against many ailments. The plant has been employed to treat cough, cold, headache, chills, dyspepsia, loss of appetite, gastric derangement, colic, gout, asthma, malaria, diabetes, bladder and kidney disorders, influenza, convulsion, fever, heart inflammation, rheumatism and as a purgative (Watt and Breyer-Brandwijk, 1962; Thring and Weitz, 2006). Phytochemical analyses of *A. afra* have revealed the presence of tannins, saponins, triterpenes, α - and β -amyrin, friedelin as well as the alkanes ceryl cerotinate and n-nonacosane (Silbernagel et al., 1990). The major components of its essential oil are α - and β -thujone, 1, 8-cineole and α - pinene (Graven et al., 1992; Viljoen et al., 2006).

The present study was undertaken to evaluate the antioxidant activity of aqueous leaf extract of *Artemisia afra* on the liver and kidney of streptozotocin-induced diabetic rats and the efficacy was compared with glibenclamide, a standard hypoglycemic drug.

Materials and methods

Chemicals

Streptozotocin was procured from Sigma Chemical Co., St. Louis, MO, USA while the assay kits used for biochemical analyses were products of Randox Laboratories Limited, Ardmore, Co Antrim, United Kingdom. All other chemicals and reagents used were of analytical grade.

Plant material and preparation of extract

Fresh and mature leaves of *A. afra* were collected around the University of Fort Hare, Alice (Eastern Cape Province, South Africa). The plant was authenticated by Prof DS Grierson of Botany Department and a voucher specimen was prepared and deposited in the University herbarium. An aqueous extract of the plant was prepared as previously described by Sunmonu and Afolayan (2010). The dried plant material recovered was reconstituted in distilled water to give the required doses of 50 and 100 mg/kg body weight used in the experiment.

Animals

Male albino rats of Wistar strain with a mean weight of 154 ± 4.20 g were used for the experiment. The animals were obtained and reared as described by Sunmonu and Afolayan (2011), after seeking approval from the Ethical Committee on the Use and Care of Animals of the University of Fort Hare, South Africa.

Induction of diabetes

Diabetes was induced in the rats according to the procedure described by Sunmonu and Afolayan (2010). The animals were allowed to drink 5% glucose solution overnight to overcome streptozotocin-induced hypoglycemia. Control rats were injected with citrate buffer alone. After 48 h, fasting blood glucose levels were estimated and levels above 250 mg/dl in streptozotocin-treated rats confirmed diabetes in the animals.

Animal grouping and extract administration

Thirty male rats were randomized into five groups of six animals and were orally administered appropriately for 21 days. Group 1 (normal control) and Group 2 (diabetic control) received distilled water. Groups 3 and 4 are diabetic rats treated with 50 and 100 mg/kg body weight/day of *A. afra* extract respectively while Group 5 comprised diabetic rats administered with glibenclamide (600 µg/kg body weight/day). The body weights of the animals were determined at the beginning and end of the experimental period.

Collection of blood and preparation of tissue homogenate

Twenty four hours after the last dose, blood sample was collected from the tail vein of the animals for estimation of glucose level after which the rats were humanely sacrificed by ether anaesthetization. After dissecting the animals, the liver and kidney were excised carefully and rinsed immediately in ice cold physiological saline. Known weights of the tissues were homogenized in Tris-HCl buffer (pH 7.4) and the resulting homogenates were used for analyses.

Biochemical assays

Tissue protein was estimated according to the method of Lowry et al. (1951) using bovine serum albumin as standard. Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund (1974). Glutathione reductase (GR) activity in the tissues was measured according to the method of Goldberg and Spooner (1983) while glutathione peroxidase (GPx) was assayed using the method of Paglia and Valentine (1967). Lipid peroxidation (LPO) was determined by the formation of malondialdehyde (MDA)-thiobarbituric acid reactive substances (TBARS) adduct according to the method of Ledwozyw et al. (1986). The released malondialdehyde served as the index of lipid peroxidation. Reduced glutathione was estimated by the method of Ellman (1959).

Statistical analysis

Data were expressed as mean \pm SD for six animals in each group and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. *p* values of less than 0.05 were considered statistically significant.

Results

Effect of *A. afra* on hyperglycemia and body weight

Table 1 summarizes blood glucose levels and body weight in the control and experimental groups of animals. The diabetic rats showed significant increase in serum glucose levels and significant decrease in body weight compared to the normal control. Administration of aqueous extract of *A. afra* restored glucose levels and body weight to near normal range.

Table 1: Effect of *A. afra* on blood glucose levels and body weight in diabetic rats (n = 6, $\bar{X} \pm SD$)

Body weight (g)			
Treatments	Glucose (mg/dl)	Initial	Final
Normal control	73.13 \pm 3.89 ^a	154.18 \pm 4.96 ^a	185.48 \pm 4.42 ^a
Diabetic control	318.98 \pm 6.31 ^b	154.40 \pm 4.11 ^a	157.57 \pm 4.42 ^b
Diabetic + 50 mg/kg <i>A. afra</i>	77.64 \pm 2.13 ^a	154.85 \pm 4.19 ^a	175.40 \pm 4.14 ^c
Diabetic + 100 mg/kg <i>A. afra</i>	75.53 \pm 3.31 ^a	154.73 \pm 4.10 ^a	173.33 \pm 4.41 ^c
Diabetic + glibenclamide	75.30 \pm 2.75 ^a	154.37 \pm 3.99 ^a	174.97 \pm 3.89 ^c

Values along the same column with different superscripts are significantly different (p<0.05).

Effect of *A. afra* on lipid peroxidation and GSH content

As reported in Table 2, there was a significant elevation in MDA and a significant decrease in GSH levels in the liver and kidney of diabetic rats. Treatment with the herb resulted in a marked improvement in these indices at the end of the experiment as they were reverted back to normalcy after 21 days.

Table 2: Effect of *A. afra* on malondialdehyde and reduced glutathione levels in the liver and kidney of diabetic rats (n = 6, $\bar{X} \pm SD$)

Malondialdehyde (MDA) (nmol/mg protein)		Reduced glutathione (GSH) (nmol/g tissue)	
Treatments			
	Liver	Kidney	
Normal control			
3.61 ^a	28.85 \pm 1.85 ^a	0.84 \pm 0.06 ^a	1.26 \pm 0.22 ^a
Diabetic control			
2.36 ^b	16.08 \pm 1.24 ^b	1.36 \pm 0.16 ^b	2.05 \pm 0.27 ^b
Diabetic + 50 mg/kg <i>A. afra</i>			
2.33 ^a	26.27 \pm 1.76 ^a	0.85 \pm 0.07 ^a	1.28 \pm 0.10 ^a
Diabetic + 100 mg/kg <i>A. afra</i>			
2.36 ^a	27.10 \pm 1.50 ^a	0.84 \pm 0.08 ^a	1.26 \pm 0.19 ^a
Diabetic + glibenclamide			
2.31 ^a	25.40 \pm 1.60 ^a	0.88 \pm 0.06 ^a	1.30 \pm 0.19 ^a

Values along the same column with different superscripts are significantly different (p<0.05).

Effect of *A. afra* on the activities of antioxidant enzymes

Tables 3 and 4 show the activities of enzymic antioxidants (GPx, GR and SOD) in the liver and kidney respectively. Significantly decreased activities of these enzymes were observed in the tissues of diabetic rats. However, these activities were significantly improved following administration of the extract for 21 days. Generally, the effect of the extract compared favourably well with glibenclamide, a standard hypoglycemic drug.

Table 3: Effect of *A. afra* on the activities of antioxidant enzymes in the liver of diabetic rats (n = 6, $\bar{X} \pm SD$)

Glutathione reductase (GR) Treatments	Glutathione peroxidase (GPx) (U/I/mg protein)	Superoxide dismutase (SOD) (U/I/mg protein)(Units/mg protein)
Normal control 8.33 ± 0.62 ^a	49.68 ± 3.06 ^a	60.50 ± 3.61 ^a
Diabetic control 4.34 ± 0.57 ^b	20.39 ± 2.16 ^b	26.16 ± 1.81 ^b
Diabetic + 50 mg/kg <i>A. afra</i> 7.38 ± 0.69 ^a	44.88 ± 3.07 ^a	55.42 ± 3.36 ^a
Diabetic + 100 mg/kg <i>A. afra</i> 7.56 ± 0.59 ^a	47.37 ± 2.88 ^a	57.46 ± 3.50 ^a
Diabetic + glibenclamide 7.33 ± 0.49 ^a	43.98 ± 3.16 ^a	54.83 ± 3.60 ^a

Values along the same column with different superscripts are significantly different (p<0.05).

Table 4: Effect of *A. afra* on the activities of antioxidant enzymes in the kidney of diabetic rats (n = 6, $\bar{X} \pm SD$)

Glutathione reductase (GR) Treatments	Glutathione peroxidase (GPx) (U/I/mg protein)	Superoxide dismutase (SOD) (U/I/mg protein)(Units/mg protein)
Normal control 13.35 ± 0.81 ^a	33.57 ± 2.66 ^a	43.60 ± 2.16 ^a
Diabetic control 8.30 ± 0.75 ^b	11.18 ± 2.16 ^b	15.06 ± 1.31 ^b
Diabetic + 50 mg/kg <i>A. afra</i> 12.29 ± 0.62 ^a	29.78 ± 3.04 ^a	38.92 ± 2.63 ^a
Diabetic + 100 mg/kg <i>A. afra</i> 12.74 ± 0.57 ^a	32.47 ± 2.58 ^a	40.96 ± 2.15 ^a
Diabetic + glibenclamide 11.93 ± 0.94 ^a	28.98 ± 3.16 ^a	38.33 ± 2.87 ^a

Values along the same column with different superscripts are significantly different (p<0.05).

Discussion

Many of the complications of diabetes have been linked to oxidative stress and antioxidants have been considered as treatments (Reaven et al., 1995; Cunningham, 1998). Hence, it could be suggested that antioxidant action may be an important property of plant medicines associated with diabetes. The present study has clearly demonstrated that aqueous extract of *Artemisia afra* has antioxidant activity; and the efficacy is comparable to glibenclamide, a standard hypoglycemic drug. Increase in blood glucose level is an important characteristic feature of diabetic state. The extract from *A. afra* produced significant hypoglycemic effect in diabetic rats and by day 21, the glucose levels tended towards normalcy as found in the control. Microchemical analyses of the plant have indicated the presence of saponins (Silbernagel et al., 1990) which had been reported to possess hypoglycemic activity in diabetic rabbits (Abdel-Hassan et al., 2000). Therefore, the hypoglycemic activity of *A. afra* observed in this study could be attributed to the presence of saponin.

The reduction in body weight observed in diabetic rats could be attributed to excessive breakdown of tissue proteins (Chatterjea and Shinde, 2002). The improvement in body weight in the *A. afra* treated rats could be due to increase in metabolic activity. This clearly indicates that the plant extract increased glucose metabolism and thus enhanced body weight in diabetic rats. Similar observation was reported by Ravi et al. (2004a). According to these authors, *Eugenia jambolana* seed kernels enhanced body weight of diabetic rats.

Most tissue damages are mediated by free radicals which attack membranes through peroxidation of unsaturated fatty acids (Stringer et al., 1989). Diabetes induction with streptozotocin in rats results in an increase in lipid peroxidation, which is an evidence of intensified free radical production (Maritim et al., 2003). In this study, the levels of malondialdehyde (MDA) were increased in the liver and kidney of diabetic rats which is an indication of increased free radical generation leading to oxidative stress in the tissues. Administration of the extract significantly reduced MDA level, which suggests that the extract might possess

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antioxidant activity. This is an indication that the herb has a protective effect by ameliorating tissue oxidative stress. Similar observation was reported by Ravi et al. (2004b) using *E. jambolana* seed kernel in STZ treated rats. The evidence presented in this study suggests that the antioxidative role of *A. afra* could be attributed to its flavonoids content which act as strong free radical scavengers.

Glutathione is an important antioxidant which plays the role of an intracellular radical scavenger and is a substrate for many xenobiotic elimination reactions (Gregus et al., 1996). Decreased levels of reduced glutathione (GSH) observed in the liver and kidney of diabetic rats may be a result of increased oxidative stress. GSH has the ability to manage oxidative stress with adaptional changes in enzymes regulating GSH metabolism (Arulselvan and Subramanian, 2007). The treatment of the rats with *A. afra* extract significantly increased the GSH levels in their tissues and consequently improved the antioxidant status. This increase may, in turn, activate GSH dependent enzymes such as glutathione peroxidase and glutathione reductase.

Increased concentrations of ROS have been implicated in many of diabetic complications (Halliwell and Gutteridge, 1989). The present findings indicate significantly reduced tissue antioxidant enzymic activities in diabetic rats and its attenuation by *A. afra* treatment. GPx is involved in the reduction of hydrogen peroxide to water by using glutathione as a hydrogen donor (Sies, 1993). Reduced activity of this enzyme in the liver and kidney of diabetic rats has been observed in the current study. Our result is consistent with Ravi et al. (2004b) who reported a reduction in the activity of tissue antioxidant enzymes in diabetic rats. The reduced activity of GPx may result in accumulation of toxic products due to oxidative damage; and its recovery following treatment with *A. afra* extract indicates the protective effect of the herb on antioxidants.

The decreased activities of SOD and GR in both liver and kidney during diabetes as observed in this study may be due to increased production of reactive oxygen radicals which are capable of reducing the activities of these enzymes (Wohaieb and Godin, 1987). SOD is an important defense enzyme which catalyses the dismutation of superoxide radicals while GR is required for the conversion of oxidized (GSSG) to reduced glutathione (GSH). Oral administration of *A. afra* increased the activities of these enzymes which may help to scavenge the free radicals generated during diabetes. This is indicative of the protective role of the herb on antioxidant enzymes in the tissues. Generally, the effect of the treatment with *A. afra* compared favourably with the effect of glibenclamide, a known standard drug for diabetes.

Conclusion

Aside its hypoglycemic action, findings from the present study clearly revealed antioxidant property of aqueous extract of *A. afra* which could protect tissues from oxidative damage. This is an indication that the extract could be a very useful therapeutic agent for treating radical-related pathological damages.

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References

1. Abdel-Hassan, I.A., Abdel-Barry, J.A. and Mohammeda, S.T. (2000). The hypoglycaemic and antihyperglycaemic effect of *Citrillus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. *J. Ethnopharmacol.* 71: 325–330.
2. Arulselvan, P. and Subramanian, S.P. (2007). Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic β -cells in experimental diabetes in rats. *Chem.-Biol. Interact.* 165: 155-164.
3. Baynes, J.W. (1991). Role of oxidative stress in development of complications in diabetes. *Diabetes* 40: 405-412.
4. Chanwitheesuk, A., Teerawutgulrag, A. and Rakariyatham, N. (2005). Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem.* 92: 491-497.
5. Chatterjea, M.N. and Shinde, R. (2002). *Textbook of Medical Biochemistry*, 5th edn., Jaypee Brothers, Medical Publishers Ltd., New Delhi.
6. Cunningham, J.J. (1998). Micronutrients as nutraceutical intervention in diabetes mellitus. *J. Am. Coll. Nutr.* 17: 7-10.
7. Ellman, G.L. (1959). Tissue sulphydryl groups. *Arch. Biochem. Biophys.* 82: 70-77.
8. Goldberg, D.M. and Spooner, R.J. (1983). Glutathione reductase. In: Bergmeyer, H.V., Bergmeyer, J. and Grassl M (Eds) *Method of Enzymatic Analysis*. Weinheim: Deerfield Beach, pp 258–265.
9. Graven, E., Deans, S., Mavi, S., Gundidza, M.G. and Svoboda, K.P. (1992). Antimicrobial and antioxidative property of the volatile (essential) oil of *Artemisia afra* Jacq.. *Flav. Frang. J.* 7: 121–123.

10. Gregus, Z., Fekete, T., Halasz, E. and Klaassen, C.D. (1996). Lipoic acid impairs glycine conjugation of benzoic acid and renal excretion of benzoyl glycine. *Drug Metab. Dispos.* 24: 682-688.
11. Halliwell, B. and Gutteridge, J.M.C. (1989). *Free Radicals in Biology and Medicine*, second ed. Clarendon Press, Oxford, UK.
12. Ledwozyw, A., Michalak, J., Stephien, A. and Kadziolka, A. (1986). The relationship between plasma triglycerides, cholesterol, total lipids and lipid peroxidation products during human atherosclerosis. *Clin. Chim. Acta.* 155: 275-286.
13. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurements with the Folin's reagent. *J. Biol. Chem.* 193: 265-275.
14. Maritim, A.C., Sanders, R.A. and Watkins, J.B. (2003). Diabetes, oxidative stress, and antioxidants: A review. *J. Biochem. Mol. Toxicol.* 17 (1): 24-38.
15. Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* 47: 469-474.
16. Mullarkey, C.J., Edelstein, D. and Brownlee, L. (1990). Free radical generation by early glycation products: A mechanism for accelerated atherogenesis in diabetes. *Biochem. Biophys. Res. Commun.* 173: 932-939.
17. Oberley, L.W. (1988). Free radicals and diabetes. *Free Rad. Biol. Med.* 5: 113-124.
18. Paglia, D.E. and Valentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70: 158-169.
19. Ravi, K., Sivagnanam, K. and Subramanian, S. (2004a). Anti-diabetic activity of *Eugenia jambolana* seed kernels on streptozotocin-induced diabetic rats. *J. Med. Food* 7 (2): 187-191.
20. Ravi, K., Ramachandran, B. and Subramanian, S. (2004b). Protective effect of *Eugenia jambolana* seed kernels on tissue antioxidants in streptozotocin-induced diabetic rats. *Biol. Pharm. Bull.* 27 (8): 1212-1217.
21. Reaven, P.D., Herold, D.A., Barnett, J. and Edelman, S. (1995). Effects of vitamin E on susceptibility of low-density lipoprotein and low-density lipoprotein subfractions to oxidation and on protein glycation in NIDDM. *Diabetes Care* 8: 807-816.
22. Reynolds, J.E.F. (1997). *Martindale-The Extra Pharmacopoeia*, 30th ed., The Pharmaceutical Press, London.
23. Robinson, G. and Johnston, D.E. (1997). Metabolic Disorder: Diabetes, in: S. Tomlinson, A.M. Heagerty, A.P. Weetman (Eds.) *Mechanisms of disease: An Introduction to Clinical Science*, 1st edn., Cambridge University Press, Cambridge.
24. Senthilkumar, G.P. and Subramanian, S. (2007). Evaluation of antioxidant potential of *Terminalia chebula* fruits studied in streptozotocin-induced diabetic rats. *Pharm. Biol.* 45 (6): 511-518.
25. Sies, H. (1993). Damage to plasmid DNA by singlet oxygen and its protection. *Mutat. Res.* 299: 183-191.
26. Silbernagel, E., Spreitzer, H. and Buchbauer, G. (1990). Non-volatile constituents of *Artemisia afra*. *Monatsch Chem.* 121 (5): 433-436.
27. Strain, J.J. (1991). Disturbances of micronutrient and antioxidant status in diabetes. *Proc. Nutr. Soc.* 50: 591-604.
28. Stringer, M.D., Gorog, P.G., Freeman, A. and Kakkar, V.V. (1989). Lipid peroxides and atherosclerosis. *Br. Med. J.* 298: 281-284.
29. Sunmonu, T.O. and Afolayan, A.J. (2010). Protective effect of *Artemisia afra* Jacq. on isoproterenol-induced myocardial injury in Wistar rats. *Food & Chem. Toxicol.* 48: 1969 - 1972.
30. Thring, T.S.A. and Weitz, F.M. (2006). Medicinal plants use in the Bredasdorp/Elim region of the southern Overberg in the Western Cape Province of South Africa. *J. Ethnopharmacol.* 103: 261-275.
31. Upadhyay, O.P., Singh, R.M. and Dutta, K. (1996). Studies on antidiabetic medicinal plants used in Indian folk-lore. *Aryavaidyan* 9: 159-164.
32. Viljoen, A.M., Van Vuuren, S.F., Gwebu, T., Demirci, B., Baser, K. and Husnu, C. (2006). The geographical variation and antimicrobial activity of African wormwood (*Artemisia afra* Jacq.) essential oil. *J. Ess. Oil Res.* 18: 19-25.
33. Watt, J. and Breyer-Brandwijk, M.G. (1962). *The medicinal and poisonous plants of southern and eastern Africa*, 2nd Ed Livingstone, Edinburgh.
34. Wohaieb, S.A. and Godin, D.V. (1987). Alterations in free radical tissue defense mechanisms in streptozotocin-induced diabetes in rats. *Diabetes* 36: 1014-1018.