Biosci. Biotechnol. Biochem., **75** (11), 2083–2086, 2011

Artemisia afra Jacq. Ameliorates Oxidative Stress in the Pancreas of Streptozotocin-Induced Diabetic Wistar Rats

Anthony Jide AFOLAYAN† and Taofik Olatunde SUNMONU

Phytomedicine Research Center, Department of Botany, University of Fort Hare, Alice 5700, South Africa

Received October 22, 2010; Accepted August 17, 2011; Online Publication, November 7, 2011
[doi:10.1271/bbb.100792]

Diabetes is characterized by hyperglycemia resulting from defects in pancreatic insulin secretion and/or impaired target cell responsiveness to insulin, and *Artemisia afra* Jacq. is widely used in South Africa to treat the disease, but the mechanism of action is yet to be elucidated. This study explored the effect of oral administration of aqueous leaf extract of *A. afra* on the pancreas of streptozotocin-induced diabetic rats. We found that the extract significantly reduced blood glucose levels, accompanied by an increase in the serum insulin concentration. Moreover, the antioxidant enzymic activities of glutathione peroxidase, glutathione reductase, and superoxide dismutase also improved significantly after treatment with the extract. Increased pancreatic lipid peroxidation in the diabetic rats was also normalized by the extract. This study indicates that *A. afra* possesses hypoglycemic and antioxidant activities. Our findings suggest that the herb might exert its anti-diabetic activity by regenerating pancreatic beta cells, thereby stimulating the release of insulin.

Key words: *Artemisia afra*; antioxidant enzymes; diabetes; lipid peroxidation; pancreas

Diabetes mellitus (DM) is a metabolic disorder characterized by high blood glucose levels that result from defects in pancreatic insulin secretion and/or impaired target cell responsiveness to insulin.[1)] Studies have also revealed that free radicals might also play a vital role in the pathogenesis of this disease.[2,3)] Diabetes is the most common of the endocrine disorders, affecting more than 150 million people worldwide, with a projection of 300 million people by 2025.[4)]

DM is a pathologic condition resulting in severe metabolic imbalances and non-physiological changes in many tissues, particularly in the pancreas, where oxidative stress plays an important role in etiology.[2)] The pancreas is responsible for the secretion of insulin, the hormone that regulates blood glucose levels. Streptozotocin is frequently used to induce DM in experimental animals through its toxic effects on pancreatic β cells.[5)] It is a potent methylating agent for DNA and acts as a nitric oxide donor in pancreatic β cells.[6,7)] Studies have shown that pancreatic damage occurs following streptozotocin injection as a result of antioxidant deficit and increased oxidative stress through free-radical generation.[8,9)]

Artemisia afra Jacq. ex Willd. (Asteraceae) is an indigenous South African plant known as *unhlonyane* in Xhosa and African wormwood in English. It is a widely used medicinal plant because of its acclaimed healing properties for many ailments, including diabetes. It is an erect, shrubby, perennial plant growing up to 2 m tall with a leafy, hairy stem. The leaf shape is narrowly ovate, feathery, and finely divided, and the leaf grows up to 8 cm long and 4 cm wide. It is widespread in all the provinces of South Africa except for the Northern Cape, and it is easily identifiable by its characteristic aromatic odor.[10)] Phytochemical analyses of *A. afra* have revealed the presence of tannins, saponins, triterpenes, α- and β-amyrin, friedelin, and the alkanes ceryl cerotinate and *n*-nonacosane.[11)] Anti-diabetic activity has been reported for *A. afra*, but no detailed study has been reported on its mechanism of action.

The present study investigated the effect of oral administration of aqueous leaf extract of *A. afra* on the pancreas of streptozotocin-induced diabetic rats with reference to oxidative stress indices, with a view to determining the possible mechanism of the anti-diabetogenic action of the extract.

Materials and Methods

Chemicals. Streptozotocin was purchased from Sigma Chemical (St. Louis, MO). The assay kits used for biochemical assays were from Randox Laboratories (Ardmore, UK). All the other chemicals and reagents used were of analytical grade.

Plant material and authentication. Freshly picked *A. afra* comprising mature leaves and stems were collected from the University of Fort Hare (Alice, Eastern Cape Province, South Africa) in June 2009. The plant was authenticated by Professor D.S. Grierson, a botanist in the Department of Botany at the University of Fort Hare, and a voucher specimen (Sunmed. 2009/01) was prepared and deposited at the Giffen Herbarium of the university.

Preparation of plant extract. An aqueous extract of the plant was prepared as previously described by Sunmonu and Afolayan.[12)] The dried powder obtained was reconstituted separately in distilled water to give the doses of 50 and 100 mg/kg of body weight used in the experiment. These doses were selected based on the fact that they correspond to those employed by traditional healers in the study area in the treatment of diabetes.

Animals used. Male albino rats of the Wistar strain at a mean weight of 155 ± 5.32 g were used. The animals were obtained and reared as described by Sunmonu and Afolayan,[12)] following approval by the Ethical Committee on the Use and Care of Animals of the University of Fort Hare.

Induction of diabetes. The animals were fasted for 18 h, followed by a single intravenous injection of freshly prepared solution of

† To whom correspondence should be addressed. Tel: +27-822022167; Fax: +27-866282295; E-mail: Aafolayan@ufh.ac.za

streptozotocin (60 mg/kg of body weight) in 0.1 M citrate buffer (pH 4.5) to induce diabetes. They were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After 72 h of streptozotocin injection, fasting blood glucose levels were estimated, and levels above 250 mg/dL confirmed diabetes resulting from a damaged pancreas.

Animal grouping. Twenty-four male rats were randomized into four groups of six animals, and they were orally administered appropriately for 14 d. Group 1 (normal control) and group 2 (diabetic control) received distilled water, while groups 3 and 4, also comprising diabetic rats, were treated with 50 and 100 mg/kg of body weight/d respectively of *A. afra* extract.

Collection of blood and preparation of pancreatic homogenate. On day 15, the rats were sacrificed humanely under ether anaesthesia, and the jugular vein was cut with a sterile surgical blade for blood collection into non-heparinized tubes. The blood was centrifuged at 1,282 $g \times 5$ min, and the serum was carefully aspirated with a Pasteur pipette into sample bottles for biochemical assay. After dissection of the animals, the pancreas was excised carefully and rinsed immediately with ice-cold physiological saline, followed by evaluation of the pancreas to body weight ratio. A portion of the pancreatic tissue was homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) containing potassium chloride (1.17% w/v). The homogenate was centrifuged at 800 g for 5 min to separate the nuclear debris. The supernatant obtained was further centrifuged at 10,500 g for 20 min to produce the post-mitochondrial supernatant, which was used in analysis.

Biochemical assays. The concentration of glucose in the serum was determined as described by Barham and Trinder.[13] The serum insulin concentration was measured using an insulin radioimmunoassay kit (CEA-JRE-SORIN, Monaco, France). Protein was estimated using bovine serum albumin as standard.[14] Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund.[15] Glutathione reductase (GR) activity in the pancreas was measured by the method of Goldberg and Spooner,[16] and glutathione peroxidase (GPx) was assayed by the method of Paglia and Valentine.[17] Pancreatic lipid peroxidation (LPO) was determined by the formation of malondialdehyde (MDA)-thiobarbituric acid reactive substances (TBARS) adduct by the method of Ledwozyw *et al.*[18] The released malondialdehyde served as an index of lipid peroxidation.

Statistical analysis. Data were expressed as mean ± SD for six replicates, and were subjected to one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test for multiple comparison. Values were considered statistically significant at $p < 0.05$.

Results

Effects of A. afra *on serum glucose and insulin levels and pancreas weight*

The untreated diabetic animals showed significantly raised serum glucose levels and reduced serum insulin concentrations as compared to the normal control (Table 1).

The administration of aqueous extract of *A. afra*, however, significantly restored glucose and insulin levels in the diabetic rats to near normal. The pancreas/body weight ratio of the diabetic rats was also significantly reduced after 14 d as compared with the control (Table 1). The aqueous extract of this herb restored the pancreas/body weight ratio to near the normal range.

Effects of A. afra *on lipid peroxidation and enzymatic antioxidants*

We observed a significant elevation in pancreatic MDA levels in the diabetic rats (Fig. 1), but treatment with the extract resulted in a marked decrease in these values at the end of the experiment. We also found that the activities of enzymatic antioxidants (GPx, GR, and SOD) were significantly reduced in the pancreas of the diabetic animals (Figs. 2–4), but they were significantly improved following treatment with the extract for 14 d.

Table 1. Effects of Oral Administration of Aqueous Extract of *A. afra* on Blood Glucose, Insulin Concentration, and Pancreas/Body Weight Ratio in Diabetic Rats ($n = 6 \pm$ SD)

Groups	Glucose (mg/dL)	Insulin (ng/mL)	Pancreas/Body ratio (%)
Normal control	75.31 ± 4.98	0.79 ± 0.04	0.27 ± 0.04
Diabetic untreated	$308.89 \pm 6.13^{*}$	$0.36 \pm 0.03^{*}$	$0.13 \pm 0.05^{*}$
Diabetic + 50 mg/kg *A. afra*	$79.46 \pm 2.31^{**}$	$0.69 \pm 0.03^{**}$	$0.23 \pm 0.04^{**}$
Diabetic + 100 mg/kg *A. afra*	$77.35 \pm 3.53^{**}$	$0.70 \pm 0.02^{**}$	$0.24 \pm 0.04^{**}$

$^{*}p < 0.05$ statistically significant compared with the normal control animals
$^{**}p < 0.05$ statistically significant compared with the diabetic control animals

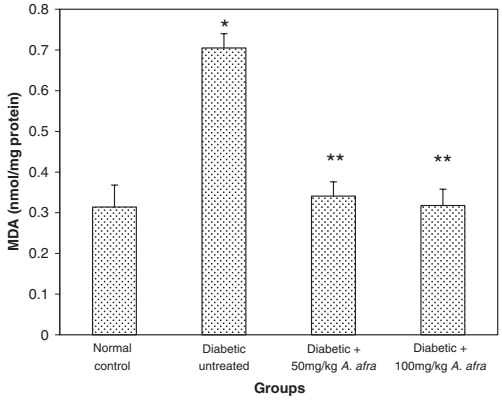

Fig. 1. Effect of *A. afra* Aqueous Extract on Lipid Peroxidation (MDA) in the Pancreas of Diabetic Rats.

Data are expressed as mean ± SD for six determinations. $^{*}p < 0.05$ compared with the control animals; $^{**}p < 0.05$ compared with the diabetic control animals.

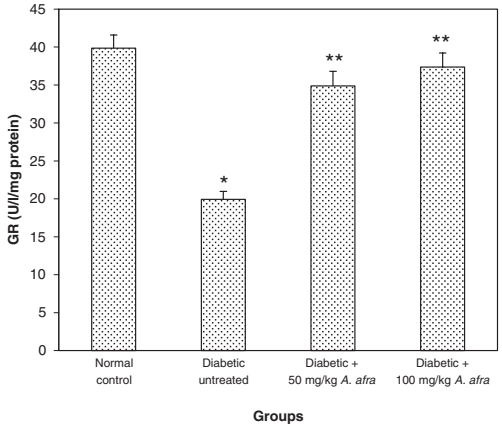

Fig. 2. Effect of *A. afra* Aqueous Extract on Glutathione Reductase (GR) Activity in the Pancreas of Diabetic Rats.

Data are expressed as mean ± SD for six determinations. $^{*}p < 0.05$ compared with the control animals; $^{**}p < 0.05$ compared with the diabetic control animals.

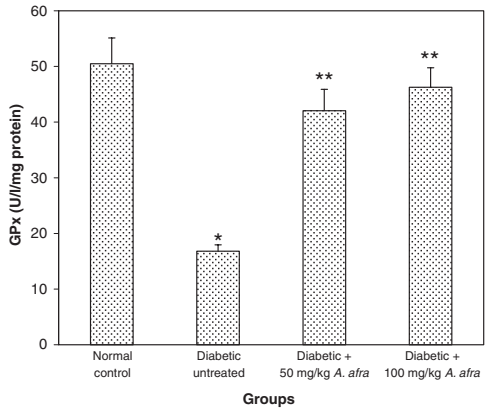

Fig. 3. Effect of *A. afra* Aqueous Extract on Glutathione Peroxidase (GPx) Activity in the Pancreas of Diabetic Rats.

Data are expressed as mean ± SD for six determinations. $^{*}p < 0.05$ compared with the normal control animals; $^{**}p < 0.05$ compared with the diabetic control animals.

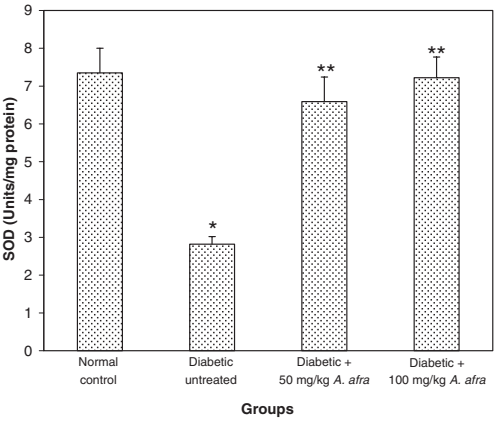

Fig. 4. Effect of *A. afra* Aqueous Extract on Superoxide Dismutase (SOD) Activity in the Pancreas of Diabetic Rats.

Data are expressed as mean ± SD for six determinations. $^{*}p < 0.05$ compared with the normal control animals; $^{**}p < 0.05$ compared with the diabetic control animals.

Discussion

Induction of diabetes by streptozotocin has been reported to involve pancreatic damage arising from an antioxidant deficit and increased oxidative stress through free-radical generation.[8,9] This can be further confirmed by increases in the blood glucose concentration. An increase in the organ-to-body weight ratio is an indication of inflammation, while a decrease might be attributed to cell constriction.[19] The reduction in the pancreas/body weight ratio observed in our study suggests constriction of the pancreatic beta cells, an indication of damage to the pancreas. This probably accounts for its reduced functional capacity, as reflected in alterations in lipid peroxidation and antioxidant enzyme activities. Treatment of diabetic rats with *A. afra* extract enhanced pancreatic weight, and this can be attributed to regeneration of β-cells.

The results of the present investigation indicate that aqueous extract of *A. afra* possesses hypoglycemic activity in that it substantially reduces blood glucose levels in streptozotocin-induced diabetic rats. This can be attributed to the saponin content reported to be present in *A. afra* extract,[11] and saponin has exhibited hypoglycemic activity in diabetic rabbits.[20] Treatment of diabetic rats with *A. afra* extract caused reversal of serum glucose to near normal levels, and this was confirmed by the elevated level of serum insulin. The observed increase in the insulin concentration following treatment with the extract can be attributed to increased secretion by regenerated pancreatic β-cells.

The elevated pancreatic LPO in the diabetic rats can be attributed to enhanced production of reactive oxygen species, which leads to oxidative stress. Lipid peroxidation has been reported to play an important role in diabetic pancreatic damage.[21] In the present study, administration of *A. afra* extract potentially lowered the MDA level, which suggests that the extract possesses antioxidant principles producing such a response. This is an indication that the herb has a protective effect in that it alleviates pancreatic oxidative stress in diabetic rats. A similar observation was reported for diabetic rats administered aqueous extracts of *Punica granatum* and *Artemisia campestris*.[22,23] According to these authors, the extracts can be effective in correcting hyperglycemia and preventing diabetic complications. This may be an indication that the plant extracts have the potential to repair a damaged pancreas. Thus, the evidence presented here suggests that *A. afra* can regenerate pancreatic β-cells that are damaged as a result of streptozotocin injection. Of particular interest is the fact that the effect of the extract at the doses studied compared favorably to the control animals.

DM is associated with increased formation of free radicals and a reduction in pancreatic antioxidant potential. As a result, the balance between free-radical formation and protection against them, which is normally present in the cells, is disturbed.[24] An imbalance in the oxidant/antioxidant defense system results in alterations in the activities of antioxidant enzymes such as SOD, GR, and GPx.[25] This indicates that streptozotocin-induced diabetes disrupts the activities of pancreatic antioxidant enzymes. The decreased activities of these enzymes in the pancreas of diabetic rats might be due to the production of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals.[26,27]

GPx is involved in the elimination of hydrogen peroxide at lower concentrations, and GR is required for the conversion of oxidized glutathione (GSSG) to the reduced (GSH) form. A decrease in the activities of these enzymes in the diabetic state can result from radical-induced inactivation and glycation of the enzymes.[28,29] In this study, *A. afra* potentiated antioxidative defense by increasing the activities of these enzymes in the diabetic rats. This can be attributed to the strong antioxidative properties of the extract. Similar observations have been reported for administration of *Catharanthus roseus* and *Punica granatum* extracts to diabetic rats.[8,22]

The natural cellular antioxidant enzyme SOD plays a vital role in oxygen defense metabolism by intercepting and reducing superoxide to hydrogen peroxide.[26,30] The observed reduction in SOD activity in the pancreas of the streptozotocin-treated rats can be attributed to inactivation by hydrogen peroxide or by glycosylation of the enzyme, which have been reported to occur in

diabetes.[31] Administration of *A. afra* extract significantly increased the activity of the enzyme and thus prevented oxidative damage to the pancreas. This is an indication of the antioxidant activity of the extract due to scavenging of free radicals.

The findings of the present study indicate that aqueous leaf extract of *A. afra* can be effective in correcting hyperglycemia and alleviating oxidative stress in the pancreas of streptozotocin-induced diabetic rats. We suggest that the extract exerts its anti-diabetic activity by stimulating the release of insulin by the pancreas.

Acknowledgment

This research was supported by grants from Govan Mbeki Research and Development Centre of the University of Fort Hare and by the National Research Foundation of South Africa. We are also grateful to the University of Ilorin, Nigeria, for postdoctoral fellowship support of Dr. T. O. Sunmonu.

References

1) Kumar PJ and Clark M, "Textbook of Clinical Medicine," Saunders, London, pp. 1099–1121 (2002).
2) Baynes JW and Thorpe SR, *Curr. Opin. Endocrinol.*, **3**, 277–284 (1996).
3) Feillet-Coudray C, Rock E, Coudray C, Grzelkowska K, Azais-Braesco V, and Dardevet D, *Clin. Chim. Acta*, **284**, 31–43 (1999).
4) King H, Aubert R, and Herman W, *Diabetes Care*, **21**, 1414–1431 (1998).
5) Kim MJ, Ryu GR, Chung JS, Sim SS, Min-Dos-Rhie DJ, Yoon SH, Hahn SJ, Kim MS, and Jo YM, *Pancreas*, **26**, 292–299 (2003).
6) Lukic ML, Stosic-Grujicic S, and Shahin A, *Dev. Immunol.*, **6**, 119–128 (1998).
7) Spinas GA, *News Physiol. Sci.*, **14**, 49–54 (1999).
8) Singh SN, Vats P, Suri S, Shyam R, Kumria MML, Ranganathan S, and Sridharan K, *J. Ethnopharmacol.*, **76**, 269–277 (2001).
9) Szkudelski T, *Physiol. Res.*, **50**, 537–546 (2001).
10) van Wyk BE, van Oudtshoorn B, and Gericke N, "Medicinal Plants of South Africa," Briza Publications, Pretoria, pp. 7–22 (1997).
11) Silbernagel E, Spreitzer H, and Buchbauer G, *Monatsch. Chem.*, **121**, 433–436 (1990).
12) Sunmonu TO and Afolayan AJ, *Food Chem. Toxicol.*, **48**, 1969–1972 (2010).
13) Barham D and Trinder P, *Analyst*, **97**, 142–145 (1972).
14) Lowry OH, Rosenbrough NJ, Farr AL, and Randall RJ, *J. Biol. Chem.*, **193**, 265–275 (1951).
15) Marklund S and Marklund G, *Eur. J. Biochem.*, **47**, 469–474 (1974).
16) Goldberg DM and Spooner RJ, "Glutathione Reductase," eds. Bergmeyer HV, Bergmeyer J, and Grassl M, Weinhein, Deerfield Beach, pp. 258–265 (1983).
17) Paglia DE and Valentine WN, *J. Lab. Clin. Med.*, **70**, 158–169 (1967).
18) Ledwozyw A, Michalak J, Stephien A, and Kadziolka A, *Clin. Chim. Acta*, **155**, 275–286 (1986).
19) Moore KL and Dalley AF, "Clinical Oriented Anatomy," Woller Klumner Corporation, Philadelphia, pp. 287–299 (1999).
20) Abdel-Hassan IA, Abdel-Barry JA, and Mohammeda ST, *J. Ethnopharmacol.*, **71**, 325–330 (2000).
21) Ilhan N, Halifeoglu I, and Ozercan HI, *Cell Biochem. Funct.*, **19**, 207–212 (2001).
22) Bagri P, Ali M, Aeri V, Bhowmik M, and Sultana S, *Food Chem. Toxicol.*, **47**, 50–54 (2009).
23) Sefi M, Fetoui H, Makni M, and Zeghal N, *Food Chem. Toxicol.*, **48**, 1986–1993 (2010).
24) Nazirogilu M and Butterworth P, *Can. J. Appl. Physiol.*, **30**, 172–185 (2005).
25) Maritim AC, Sanders RA, and Watkins JB, *J. Nutr. Biochem.*, **14**, 288–294 (2003).
26) Vincent AM, Russell JW, Low P, and Feldman EL, *Endocr. Rev.*, **25**, 612–628 (2004).
27) Kaleem M, Asif M, Ahmed QU, and Bano B, *Singapore Med. J.*, **47**, 670–675 (2006).
28) Zhang XF and Tan BK, *Clin. Exp. Pharmacol. Physiol.*, **27**, 358–363 (2000).
29) Rajasekaran S, Sivagnanam K, and Subramanian S, *Pharmacol. Rep.*, **57**, 90–96 (2005).
30) Lin YF, Tsai HL, Lee YC, and Chang SJ, *Nutr.*, **135**, 2457–2461 (2005).
31) Ravi K, Ramachandran B, and Subramanian S, *Biol. Pharm. Bull.*, **27**, 1212–1217 (2004).