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Ameliorative Actions of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) on Biomarkers of Diabetes and Diabetic Nephropathy in Rats: Comparison to Aspirin

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Abstract: Garlic and ginger have been shown to have positive effects in the streptozotocin (STZ)-induced rat model of diabetes. Diabetic rats were treated with either raw garlic or ginger extract (500 mg kg^{-1} intraperitoneally (IP)), or aspirin (10 mg kg^{-1} IP) for 8 weeks. The dramatic weight loss and increased water and food intake as well as urine output of diabetic rats was improved by ginger while garlic treatment of diabetic animals resulted in a modest weight gain and decreased food (but not water) intake and urine output. Blood glucose and serum creatinine, fructosamine and uric acid were significantly elevated in diabetic rats and were significantly lowered by ginger and garlic. In contrast, serum protein, albumin and insulin levels decreased significantly in diabetic rats while the ginger and garlic-treated diabetic rats had increased serum levels of protein, albumin and insulin. Total urine protein, albumin and albumin/creatinine ratio were significantly elevated in diabetic animals and both garlic and ginger treatments resulted in significant decrease. In contrast, urine uric acid was significantly decreased in diabetic rats and significantly elevated by ginger and garlic. Glycated haemoglobin (GHb) levels increased over 3-fold in erythrocytes of diabetic rats with both ginger and garlic-treated rats showing a significant decrease in GHb levels. Aspirin-treated diabetic animals only exhibited a significant decrease in blood glucose after 8 weeks of treatment with no other parameters being significantly changed compared to diabetic controls. This study suggested that ginger and garlic are effective in reversing diabetic symptoms especially in the kidney.

Key words: Garlic, ginger, aspirin, diabetes, diabetic nephropathy

INTRODUCTION

Diabetes mellitus, a disease characterized by hyperglycemia, is caused by absolute or relative insulin deficiency often associated with insulin resistance (Yang *et al.*, 2011). Chronic hyperglycemia leads to long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves and cardiovascular system (Xu *et al.*, 2005; Aronson, 2008; American Diabetes Association, 2009; Tesfaye and Gill, 2011). Diabetes mellitus (DM) affects over 165 million people and causes nearly 7% of all deaths annually worldwide (IDF, 2011; Yang *et al.*, 2011).

In DM, damage to small blood vessels with resulting microvascular disease can lead to the diabetic nephropathy which is characterized by progressive loss of kidney function due to reduced glomerular filtering capacity leading to proteinuria and albuminuria. Between 25 and 40% of diabetic patients have nephropathy and 30-40% of newly diagnosed cases of end-stage renal disease (ESRD) requiring dialysis are attributed to DM

(Ritz, 2006; Tang, 2010). Thus, prevention or reversal of diabetic nephropathy would improve the prognosis of many DM patients.

Metabolic syndrome, the precursor of DM in man and DM have been described in recent years as inflammatory diseases. Activation of the innate immune system and low grade chronic inflammation are believed to be closely involved in the pathogenesis of type 2 diabetes (Navarro and Mora, 2006). Studies on type 2 DM have indicated that inflammatory markers and pro-inflammatory cytokines are increased in diabetes and levels of these agents have been positively correlated with measures of insulin resistance (Festa *et al.*, 2000; Pickup *et al.*, 2000). Anti-inflammatory drugs have been shown to have positive effects on inflammation in diabetes (Yuan *et al.*, 2001; Sethi *et al.*, 2011).

Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) exhibit many beneficial medical properties including alleviation of diabetic symptoms (Anwar and Meki, 2003; Akhani *et al.*, 2004; Abdulrazaq *et al.*, 2011; Madkor *et al.*, 2011). We have

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previously reported that both garlic and ginger ameliorate many biochemical and clinical abnormalities in streptozotocin (STZ)-induced diabetic rats (Al-Amin *et al.*, 2006; Thomson *et al.*, 2007). In particular, we observed that both spices were able to reduce urine output and proteinuria in this model of type I DM. In addition, we have previously reported that both garlic and ginger attenuate the progress of structural nephropathy in STZ-induced diabetic rats (Al-Qattan *et al.*, 2008). Therefore, the present study was designed to further investigate the effects of garlic and ginger on diabetic nephropathy in STZ-induced diabetic rats with particular emphasis on markers of nephropathy. In light of the close link between inflammation and DM, aspirin was included in the study to compare the effects of a specific anti-inflammatory agent with garlic and ginger.

MATERIALS AND METHODS

Preparation of garlic and ginger extracts: Aqueous garlic and ginger extracts were prepared from fresh garlic bulbs and ginger roots purchased from the local market as previously described (Ali and Mohammed, 1986; Al-Amin *et al.*, 2006). The aqueous extracts of garlic and ginger were stored in small aliquots at -20°C and thawed daily for use. The frozen garlic and ginger extracts were analyzed by GC-MS by modified methods of Kubec *et al.* (2009) for garlic and Usman *et al.* (2013) for ginger. The major components in the frozen extracts as determined by GC-MS were vinyl dithins (39%) and S-allyl cysteine (30%) for garlic and 4, 6, 8 and 10- gingerols (57%) and α-zingiberene (23%) for ginger (Fig. 1).

Preparation of acetylsalicylic acid (Aspirin): A stock solution of aspirin was prepared according to the method of Cerskus *et al.* (1980). Briefly, a stock of 100 mg mL^{-1} of aspirin (acetylsalicylic acid, ASA), was prepared initially in 8 mL of distilled water which was titrated with 3.1 M sodium carbonate to maintain the pH at 7. After complete dissolution, the solution was made up to 10 mL. A 1:1 dilution was made fresh daily before injection.

Animal model and experimental design: Two month old male Sprague-Dawley rats weighing 150-200 g were used for the study. The study conformed with the National Research Council (1996), Guide for the Care and Use of Laboratory Animals (1996). All animals were maintained on a 12 h light/dark cycle and a temperature of 22 ±1°C. All animals received diet and water ad libitum. For induction of diabetes, a number of rats were weighed, fasted overnight and injected intraperitoneally (IP) with a single dose of 60 mg streptozotocin (STZ)/kg body weight (BW) in a constant volume of 0.5 mL citrate buffer. Blood glucose was determined in each rat by tail-vein puncture before STZ injection and one week after STZ injection to determine baseline blood glucose levels. The blood glucose level was quantitated using a One Touch UltraEasy glucometer (LifeScan, UK). Rats with a blood glucose concentration > 16.5 mmol L^{-1} after STZ injection were considered diabetic.

The diabetic rats were divided into 4 groups and were treated for 8 weeks as follows: diabetic control (untreated diabetic group); garlic-treated group (injected IP daily with 500 mg kg^{-1} BW aqueous garlic extract); ginger-treated group (injected IP daily with 500 mg kg^{-1} BW aqueous ginger extract) and aspirin-treated group (injected IP daily with 10 mg kg^{-1} BW aspirin). A normal (non-diabetic) control group was included for comparative purposes and received IP saline only. Each group contained 10 to 14 rats. The blood glucose level was also measured in weeks 4 and 8. After 8 weeks, all rats were sacrificed under sodium pentobarbitone anesthesia (10 mg kg^{-1}, May and Baker, UK). Blood was collected from each rat by cardiac puncture, allowed to clot for 30 min, centrifuged at 1,000 x g and the serum was stored at -80°C in small aliquots for later analysis. For determination of glycated hemoglobin, red blood cells (RBSs) were prepared by collecting 0.5mL whole blood in tubes containing 0.1 mL citrate (3.8%) as an anticoagulant. The mixture was centrifuged at 1,000 x g for 10 min. The plasma and buffy coat were carefully removed and the RBC pellet was resuspended and used for analysis on the same day.

Water and food intake, body weight and urine collection: Water and food intake was recorded daily. All rats were weighed before the start of the experiment and weekly throughout the experimental period. Twenty-four hour urine samples were collected by housing the rats in metabolic cages for a 24 h period before STZ administration and during the 4 and 8th weeks of the experimental period. The collected urine samples were centrifuged at 1,000 x g for 10 min to remove any insoluble material and the clear urine (supernatant) was stored at -40°C for later analysis.

Biochemical assays: Protein concentration of serum and urine was determined by the Coomassie Blue dye binding method using bovine serum albumin as standard (Bradford, 1976). Serum insulin was determined by ELISA using a kit supplied from SPIbio (France). Erythrocyte glycated hemoglobin (GHb) levels were determined by an affinity chromatography method using kits supplied by
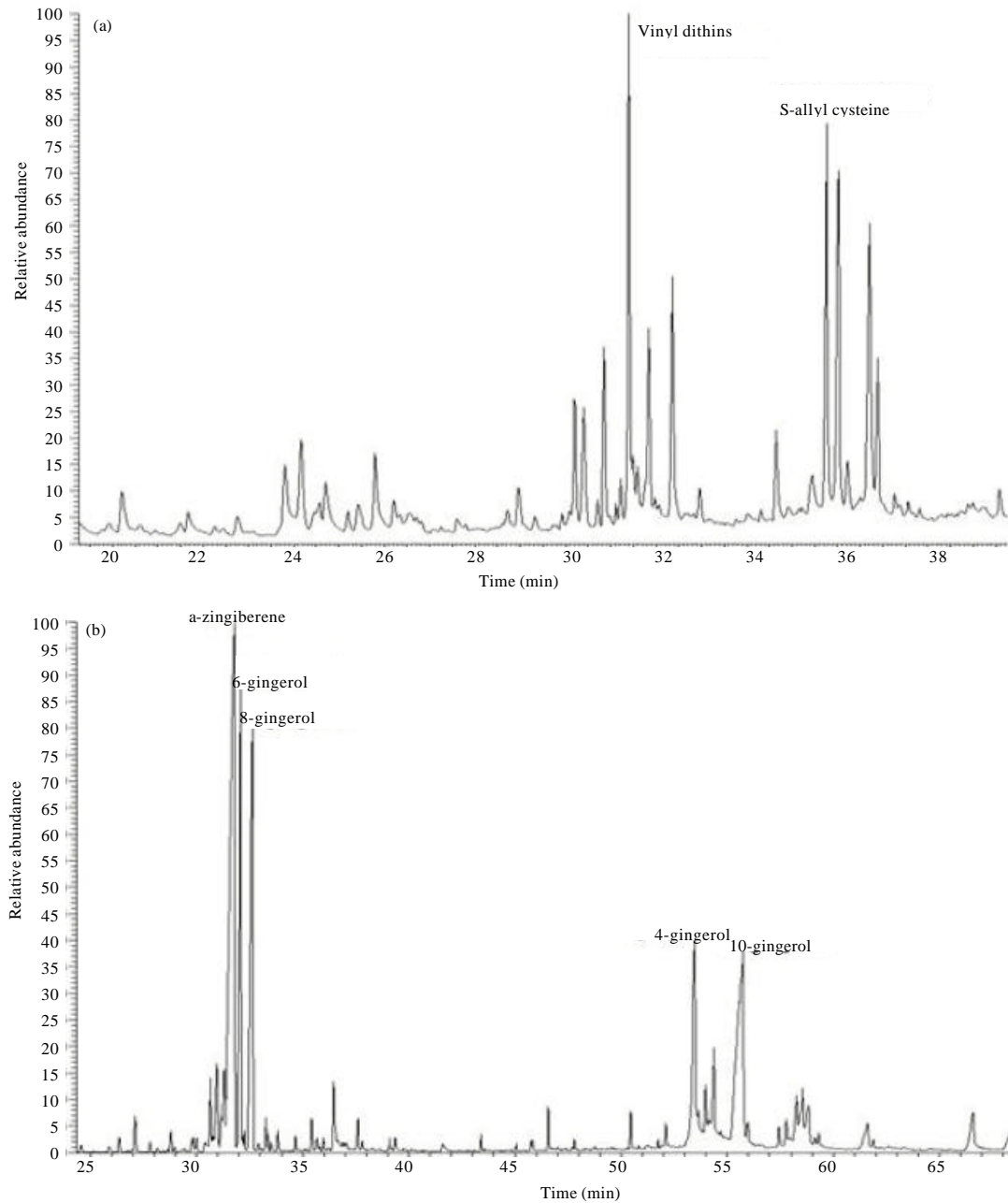
Fig. 1(a-b): GC-MS chromatograms of frozen (a) garlic and (b) ginger extracts, a-zingiberene = α- zingiberene

Helena Laboratories (USA). Serum fructosamine was determined by the method of Chung *et al.* (1988). Serum and urine albumin and uric acid levels were determined using kits supplied by BioAssay Systems (USA). Serum and urine creatinine was determined using kits supplied by Randox (USA).

Statistical analysis: The data are expressed as Mean±SEM. The one-way test of analysis of variance (ANOVA) with post hoc STD was employed to examine differences among readings between groups. A $p < 0.05$ level was selected as significant. All statistical analyses were performed using SPSS (Version 19). In all figures

and tables, a = Significantly different from normal saline control, b = Significantly different from diabetic control and c = Not significantly different compared to diabetic control.

RESULTS

Effects on physical parameters: Normal saline-treated rats doubled in weight during the 8 weeks of the experiment (Fig. 2). In contrast, diabetic control rats lost significant weight during the experiment so that after 8 weeks the diabetic control animals had lost about 40% of their body weight. In comparison, ginger- and garlic-treated diabetic rats recovered in terms of weight after an initial loss so that at the end of the experiment the treated rats exhibited a modest but significant 15-20% weight gain. In contrast, the weights of aspirin-treated diabetic rats were not significantly different from diabetic controls.

We also observed that diabetic control rats had significantly increased water and food intake compared to normal controls (Table 1). Ginger treatment of diabetic animals resulted in significantly decreased food and water intake in comparison to diabetic control animals. In contrast, food intake but not water intake of garlic-treated diabetic rats was significantly lower than diabetic controls. Aspirin treatment had no significant effect on food and water intake of diabetic rats.

Table 2 summarizes the urine output data for all groups. Clearly, urine output in diabetic control rats was approximately 10 times the output in normal saline controls at week 1 and remained at this level throughout the experiment. In contrast, the ginger- and garlic-treated diabetic animals began to produce less urine at week 4 and this trend continued so that at week 8 the urine output in ginger- and garlic-treated animals was approximately 23 and 15% less than in diabetic control rats, respectively. Thus, the decrease in urine output in the garlic- and ginger-treated diabetic rats was significantly different from the diabetic controls. This amelioration of diuresis in the garlic- and ginger-treated animals supports partial reversal of diabetic symptoms in these animals. In contrast, aspirin treatment had no effect on urine output in diabetic animals.

Effects on biochemical parameters: Glucose levels in whole blood of STZ-induced diabetic rats were assessed early in the treatment period (week 1), midway through the treatment (week 4) and at the end of the treatment period (week 8). As can be seen in Fig. 3, glucose levels in untreated diabetic control rats continued to increase throughout the experimental period while glucose levels in garlic-and ginger-treated diabetic rats decreased
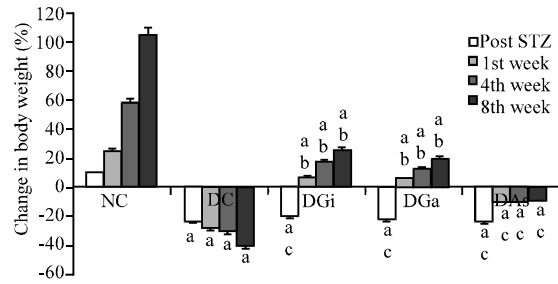

Fig. 2: Effects of garlic, ginger and aspirin on body weight of diabetic rats, weights were taken for normal control rats, STZ-induced diabetic rats, garlic-treated STZ-induced diabetic rats, ginger-treated STZ-induced diabetic rats and aspirin-treated STZ-induced diabetic rats. The animals were weighed immediately after STZ injection (Post STZ), 1 week after STZ injection and at weeks 4 and 8 of the experiment. Changes in body weights are plotted as percentiles. The data is expressed as Mean±SEM. a = Significantly decreased compared to normal saline rats, b = Significantly increased compared to diabetic control and c = Not significantly different from diabetic control
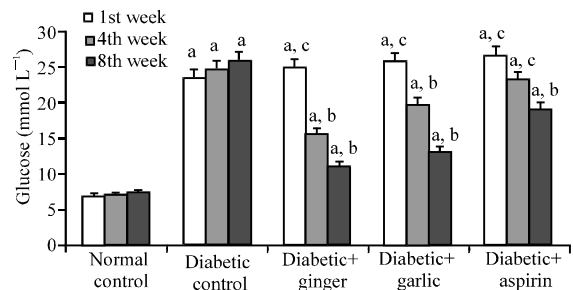

Fig. 3: Effects of garlic, ginger and aspirin on blood glucose of diabetic rats, blood glucose levels (mmol L^{-1}) were measured at weeks 1, 4 and 8 of the experiment in normal control rats, STZ-induced diabetic rats, garlic-treated STZ-induced diabetic rats, ginger-treated STZ-induced diabetic rats and aspirin-treated STZ-induced diabetic rats. The data is expressed as Mean±SEM. a = Significantly increased compared to normal saline rats, b = Significantly decreased compared to diabetic control, c = Not significantly different from diabetic control and d = Not significantly different from normal saline rats

Table 1: Effect of ginger, garlic and aspirin on daily water and food intake in diabetic rats

Group	Water intake (mL)	Food intake (g)
Normal control	40.25±2.73	25.51±1.21
Diabetic control	250.00±5.03[a]	42.17±1.08[a]
Diabetic+ginger	200.50±3.12[a,b]	32.07±1.10[a,b]
Diabetic+garlic	240.75±4.23[a,c]	35.32±0.83[a,b]
Diabetic+aspirin	239.52±3.19[a,c]	40.07±0.34[a,c]

Data is expressed as Mean±SEM, [a]Significantly increased compared to normal saline rats, [b]Significantly decreased compared to diabetic control, [c]Not significantly different from diabetic control

Table 2: Effect of ginger, garlic and aspirin on urine output in diabetic rats (mL/24 h)

	Week		
Group	1	4	8
Normal control	8.00± 0.77	9.05± 1.05	9.91± 0.82
Diabetic control	93.10± 0.72[a]	94.50± 0.70[a]	95.00± 0.71[a]
Diabetic+ginger	94.00± 1.02[a,c]	87.93± 1.09[a,b]	72.85± 1.04[a,b]
Diabetic+garlic	95.13± 1.29[a,c]	88.50±1.07[a,b]	80.50± 1.09[a,b]
Diabetic+aspirin	93.75±0.92[a,c]	90.38±1.07[a,c]	89.61±0.96[a,c]

Data is expressed as Mean±SEM, [a]Significantly increased compared to normal saline rats, [b]Significantly decreased compared to diabetic control, [c]Not significantly different from diabetic control

significantly close to normal levels by 8 weeks. By week 8, aspirin also demonstrated a slight but significant hypoglycemic effect although the blood glucose levels in aspirin-treated diabetic rats remained significantly higher than normal saline controls as well as ginger- and garlic-treated animals.

In parallel with hyperglycemia, induction of diabetes with STZ resulted in over a 10-fold reduction in serum insulin levels compared to normal controls (Fig. 4). Both ginger and garlic-treatment of diabetic rats resulted in significant increase in serum insulin levels, with ginger- and garlic-treated diabetic rats exhibiting about 48 and 28% recovery of insulin levels, respectively. In contrast, aspirin-treatment of diabetic animals had no effect on insulin levels compared to diabetic control animals.

Figure 5 summarizes the levels of serum fructosamine and GHb in erythrocytes of untreated diabetic rats and diabetic rats treated with ginger, garlic or aspirin. The data clearly shows elevation of GHb in diabetic control rats compared to normal control animals. Garlic and ginger treatments significantly lowered GHb compared to untreated diabetic rats while aspirin-treated rats had GHb levels nearly the same as untreated diabetic rats. Similarly, serum fructosamine levels in diabetic controls were more than double the levels in normal controls. Ginger and garlic treatments significantly lowered serum fructosamine levels while aspirin-treated diabetic rats had serum fructosamine levels similar to diabetic controls. Thus, these 2 indices of hyperglycemia are lowered by garlic and ginger but not aspirin treatment of STZ-induced diabetic rats.
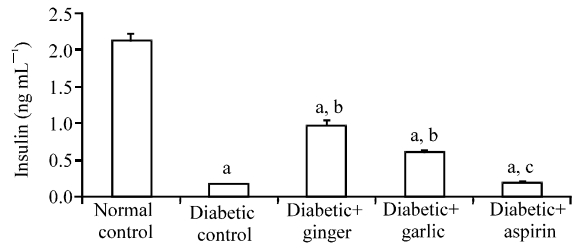

Fig. 4: Effects of garlic, ginger and aspirin on serum insulin levels of diabetic rats, serum insulin (ng mL^{-1}) was determined at 8 weeks for normal control rats, STZ-induced diabetic rats, garlic-treated STZ-induced diabetic rats, ginger-treated STZ-induced diabetic rats and aspirin-treated STZ-induced diabetic rats. The data is expressed as Mean±SEM. a = Significantly increased compared to normal saline rats, b = Significantly decreased compared to diabetic control and c = Not significantly different from diabetic control
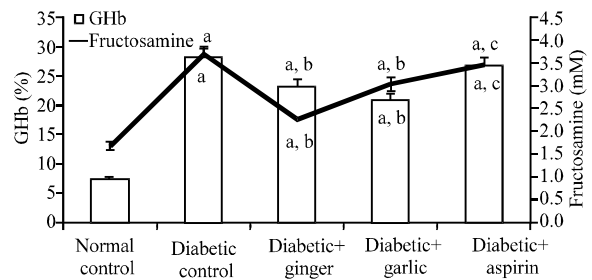

Fig. 5: Effects of garlic, ginger and aspirin on erythrocyte glycated hemoglobin (GHb, %) and serum fructosamine (mM) of diabetic rats, erythrocyte glycated hemoglobin (GHb, %) and serum fructosamine (mM) were determined at 8 weeks for normal control rats, STZ-induced diabetic rats , garlic-treated STZ-induced diabetic rats, ginger-treated STZ-induced diabetic rats and aspirin-treated STZ-induced diabetic rats. The data is expressed as Mean±SEM. a = Significantly increased compared to normal saline rats, b = Significantly decreased compared to diabetic control and c = Not significantly different from diabetic control

Total serum protein decreased by about 50% in diabetic rats compared to normal control rats while the ginger and garlic-treated diabetic rats had significantly higher serum protein levels (Fig. 6). At 8 weeks, urine protein was increased over 4-fold in diabetic control

Table 3: Effect of ginger, garlic and aspirin on serum and urine albumin, serum creatinine and urine albumin/creatinine ratio in diabetic rats

Group	Serum albumin (g dL^{-1})	Urine albumin (g/24 h)	Serum creatinine (µM)	Albumin/creatinine ratio (µg mg^{-1})
Normal control	2.69±0.04	0.012±0.007	62.69±1.04	0.452±0.06×10^{-3}
Diabetic control	1.31±0.18[a]	0.192±0.072[a]	100.31±1.18[a]	7.21±0.07×10^{-2}[a]
Diabetic+ginger	2.01±0.33[a,b]	0.074±0.009[a,b]	72.01±1.33[a,b]	4.21±0.04×10^{-3}[a,b]
Diabetic+garlic	2.12±0.22[b,d]	0.081±0.018[a,b]	80.12±1.22[a,b]	4.09±0.06×10^{-3}[a,b]
Diabetic+aspirin	1.49±0.12[a,c]	0.095±0.007[a,b]	91.49±2.12[a,c]	6.93±0.05×10^{-2}[a,c]

Data is expressed as Mean±SEM, [a]Significantly increased compared to normal saline rats, [b]Significantly decreased compared to diabetic control, [c]Not significantly different from diabetic control
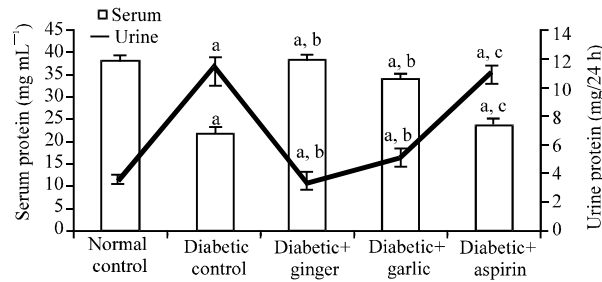

Fig. 6: Effects of garlic, ginger and aspirin on serum and urine protein of diabetic rats, serum protein (mg mL^{-1}) and urine protein (mg/24 h) were quantitated at 8 weeks for normal control rats, STZ-induced diabetic rats, garlic-treated STZ-induced diabetic rats, ginger-treated STZ-induced diabetic rats and aspirin-treated STZ-induced diabetic rats. The data is expressed as Mean±SEM. a = Significantly increased compared to normal saline rats, b = Significantly decreased compared to diabetic control and c = Not significantly different from diabetic control

Fig. 7: Effects of garlic, ginger and aspirin on uric acid levels in serum and urine of diabetic rats, Uric acid levels in serum and urine (mg dL^{-1}) were quantitated at 8 weeks for normal control rats, STZ-induced diabetic rats, garlic-treated STZ-induced diabetic rats, ginger-treated STZ-induced diabetic rats and aspirin-treated STZ-induced diabetic rats. The data is expressed as Mean±SEM. a = Significantly increased compared to normal saline rats, b = Significantly decreased compared to diabetic control and c = Not significantly different from diabetic control
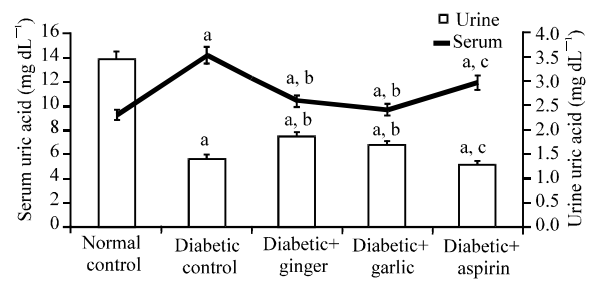
animals compared to normal saline controls (Fig. 6). Treatment with ginger or garlic resulted in a significant decrease in urine protein by the 4th week of the experiment (data not shown), with this effect persisting to week 8. In contrast, urine protein levels in aspirin-treated diabetic rats remained elevated similar to diabetic control animals.

Albumin levels were determined in both serum and urine of diabetic rats. As shown in Table 3, serum albumin levels were significantly decreased in diabetic control animals compared to normal controls. Treatment with ginger and garlic but not aspirin, resulted in significant elevation of serum albumin in diabetic rats. In contrast, urine albumin levels were significantly elevated in diabetic control rats compared to normal controls and significantly decreased by ginger, garlic or aspirin treatment.

The urine albumin/creatinine ratio, a measure of microalbuminuria, was determined for all experimental groups of rats. Diabetic control rats had a 16 fold increased albumin/creatinine ratio compared to normal controls. Treatment with ginger or garlic resulted in a significantly reduced albumin/creatinine ratio while aspirin did had no effect on the albumin/creatinine ratio. In addition, serum creatinine levels were significantly elevated in STZ-induced diabetic rats and were significantly lowered by ginger and garlic but not aspirin treatments (Table 3).

The uric acid levels in serum and urine of diabetic rats are shown in Fig. 7. In contrast to the albumin results, serum uric acid levels were significantly elevated in diabetic control rats compared to normal controls and were significantly lowered by both ginger and garlic but not aspirin treatment. In comparison, urine uric acid levels were significantly lowered in diabetic control rats compared to normal controls. Treatment with garlic or ginger but not aspirin significantly increased urine uric acid levels compared to diabetic controls.

DISCUSSION

Diabetes mellitus and associated hyperglycemia leads to several complications in diabetic patients, including diabetic nephropathy with a progressive increase in proteinuria and a decline in glomerular filtration rate. In recent years, medicinal herbs (natural products or functional foods) have been subjected to intensive research to assess and evaluate their efficacy in the treatment of DM. We have previously shown that raw extracts of both garlic and ginger are effective in ameliorating diabetic indicators, including proteinuria, in the STZ-induced rat model of DM (Al-Amin *et al.*, 2006; Thomson *et al.*, 2007).

Anti-diabetic effects of garlic and ginger: As in previous reports by our group and others, the current study confirmed the hypoglycemic effects of garlic and ginger with very significant reduction in blood glucose levels observed in garlic- and ginger-treated diabetic rats. This result is consistent with previous studies showing that garlic and ginger are effective in reducing blood sugar in animal models of diabetes (Anwar and Meki, 2003; Srinivasan 2005; Al-Amin *et al.*, 2006; Thomson *et al.*, 2007; Islam and Choi, 2008; Madkor *et al.*, 2011).

Hyperglycemia in the STZ-rat model of type I DM may be partially explained by the marked reduction of serum insulin observed in these diabetic animals in the current study and by others (Mariee *et al.*, 2009; Madkor *et al.*, 2011). Here we have observed that both garlic and ginger treatments resulted in significantly increased serum insulin levels suggesting that both of these natural spices have an insulin-increasing effect in STZ-induced diabetic rats. The insulin-increasing effect of garlic is in agreement with both Mariee *et al.* (2009) and Madkor *et al.* (2011) who both reported that garlic administration to STZ-induced diabetic rats increased serum insulin levels. In contrast, Madkor *et al.* (2011) reported that ginger treatment did not increase serum insulin levels. In the current study we observed that ginger was more potent then garlic in increasing serum insulin which is in agreement with Islam and Choi (2008) who reported that ginger but not garlic increased serum insulin in a type 2 rat model of diabetes.

In humans and animal models, prolonged hyperglycemia in DM has been shown to result in specific symptoms including polyuria, polydipsia, polyphagia and loss of body weight (American Diabetes Association, 2009; Kiran *et al.*, 2012). In agreement, the untreated diabetic control rats in the present study exhibited polyuria, polydipsia and polyphagia with decreased body weight. As reported previously, treatment of STZ-induced diabetic rats with garlic or ginger extracts for 8 weeks resulted in a significant decrease in urine output and increase in body weight. Treatment with ginger or garlic significantly reduced food intake of diabetic rats. However, only ginger treatment significantly reduced water consumption in STZ-induced diabetic rats. These observations are in agreement with our previous reports (Al-Amin *et al.*, 2006; Thomson *et al.*, 2007). The different effects of ginger and garlic on water consumption may be due to garlic's nature as a fervent herb that promotes extra water consumption. In contrast, Madkor *et al.* (2011) reported that garlic but not ginger significantly reduced food intake in a rat model of type 2 diabetes.

In diabetic patients, levels of GHb in red blood cells (HbA1c) are used as a measure of glycemic control (McCance *et al.*, 1994; WHO, 2011; Lyons and Basu, 2012). Similarly, GHb is measured in animal models of DM as an index of the severity of hyperglycemia (Yue *et al.*, 1982; De Tata *et al.*, 1997). In addition, serum fructosamine is used as an index of glycemic control in diabetic patients and animals (Cefalu *et al.*, 1993; Reusch *et al.*, 1993; De Michele *et al.*, 2008; Sako *et al.*, 2008; Youssef *et al.*, 2008). In the present study, both GHb and serum fructosamine were significantly elevated in diabetic control rats and were significantly decreased in garlic- and ginger-treated diabetic rats with garlic exhibiting more potency in lowering GHb and ginger being more potent in decreasing fructosamine. In contrast, in a type 2 DM model, Islam and Choi (2008) did not observe lowering of GHb by either ginger or garlic. In humans, HbA1c yields an estimate of glycemia over the previous 2-3 months and serum fructosamine represents glycemia for 2-3 weeks (Beck *et al.*, 2011). As rat erythrocytes have a life span of about 60 days, we would expect these values to reflect glycemia within a similar time frame. Thus, the results with GHb and fructosamine in the current study are in agreement with the hypoglycemic effects of ginger and garlic in this rat model of type I DM.

Anti-nephropathic effects of garlic and ginger: Diabetic nephropathy leading to end-stage renal disease is observed in approximately one third of diabetic patients (Atkins and Zimmet, 2010). Development of nephropathy is recognized clinically by progression from normoalbuminura to microalbuminuria to macroalbuminria finally leading to kidney failure. Similarly in animal models of diabetes, proteinuria and albuminuria are used as indicators of diabetic nephropathy (Islam and Choi, 2008). In the STZ-induced rat model of type I diabetes, we have previously described marked proteinuria in untreated diabetic rats which was remarkably improved by treatment with either raw garlic or ginger extracts (Al-Amin *et al.*,

2006; Thomson *et al.*, 2007). In the current study, we report significant albuminuria in diabetic control rats that was significantly improved by either garlic or ginger treatment. These results are in agreement with the report of Mariee *et al.* (2009), who observed increased urinary albumin that was decreased by oral garlic treatment (200 & 400 mg kg^{-1}). We also report urinary albumin/creatinine ratio, a commonly used measure of albuminuria in diabetics (Jensen *et al.*, 1997; Jafar *et al.*, 2007). In agreement with urinary albumin, the albumin/creatinine ratio which was markedly increased in diabetic control rats, was lowered by both garlic and ginger treatments of diabetic rats. Thus, the present results confirm the reversal of proteinuria by ginger and garlic and in addition show that STZ-induced diabetic rats exhibit marked albuminuria that is significantly ameliorated by daily treatment with either ginger or garlic.

In diabetes, elevated serum creatinine is used as a clinical indicator of nephropathy (KDOQI, 2007; Grover *et al.*, 2012). We report here that induction of diabetes with STZ caused a significant elevation of serum creatinine levels. Treatment with ginger and garlic resulted in marked lowering of serum creatinine levels in diabetic rats. Similarly, in models of renal failure, increased serum creatinine has been reported that is decreased by ginger or garlic treatment (Deniz *et al.*, 2011; Mahmoud *et al.*, 2012).

Hyperuricemia has been associated with increased risk of cardiovascular disease and has been shown to be particularly prevalent in patients with kidney disease, metabolic syndrome and DM (Riegersperger *et al.*, 2011; Zapolski *et al.*, 2010). Type I diabetic patients with elevated serum uric acid levels have been observed to be more likely to develop diabetic nephropathy (Hovind *et al.*, 2009). In addition, a connection between hyperuricemia and development of type 2 diabetes has been reported (Kodama *et al.*, 2009). Recently Zapolski *et al.* (2010) studied the association between renal function, serum uric acid and markers of pro-inflammatory and pro-thrombotic state in patients with type 2 DM and reported that a significant correlation between renal dysfunction and uric acid. For example, serum creatinine levels and GFR were correlated with serum uric acid. In addition, Yuan *et al.* (2011) have reported a correlation between increased uric acid levels in moderate hyperglycemia.

In the present study, STZ-induced diabetic rats had increased serum uric acid levels and decreased excretion of uric acid. Both of these parameters were ameliorated by treatment with garlic and ginger. Similarly, Saddala *et al.* (2013) also reported increased serum uric acid levels in STZ-induced diabetic rats. In addition, in fructose-fed rats, a model of type 2 DM, serum uric acid levels were increased, with garlic treatment inducing a lowering of serum uric acid levels (Padiya *et al.*, 2011). In contrast, in a model of alcohol-induced renal damage, Shanmugam *et al.* (2010) reported reduced serum uric acid that was increased by ginger treatment. Thus, in agreement with human and animal studies, both ginger and garlic ameliorated hyperuricemia in the STZ-induced rat model of type I diabetes.

The current study confirms the progression of diabetic nephropathy in STZ-induced diabetic rats as indicated by proteinuria, albuminuria, hyperuricemia, increased serum creatinine and urinary albumin/creatinine ratio. The study also clearly shows that aqueous extracts of garlic and ginger are very effective in reversing diabetic nephropathy as indicated by improvement in diabetic nephropathic indicators. These biochemical results are in agreement with our previous reports of improved kidney structure in STZ-induced diabetic rats treated with ginger and garlic (Al-Qattan *et al.*, 2008, 2013).

Aspirin effects: Aspirin, the original anti-inflammatory drug, exerts its anti-inflammatory and analgesic actions through inhibition of cyclooxygenase (COX-1), a key enzyme leading to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever (Vane and Botting, 2003). New insights into the pharmacological actions of aspirin have suggested that induction of nitric oxide is involved in aspirin's anti-inflammatory action (Gilroy, 2005). Since, a significant correlation has been established between diabetic hyperglycemia and inflammation (Pugliese, 2013; Ceriello *et al.*, 2012), the effect of anti-inflammatory agents in diabetes have been investigated with aspirin exhibiting beneficial effects in diabetes (Yuan *et al.*, 2001; Sethi *et al.*, 2011).

In the current study, treatment with aspirin (10 mg kg^{-1} BW) did not affect most of the physical and biochemical parameters that were perturbed in the STZ-induced diabetic rats, although a lowering of blood glucose was observed in aspirin-treated diabetic animals. These results are in contrast to the results of Sethi *et al.* (2011) who reported that aspirin exhibited anti-diabetic activity in an atherogenic diet-induced rat model of DM. In addition, Bhatt and Addepalli (2011) reported that the combination of minocycline and aspirin (50 mg kg^{-1} each orally) had very significant positive effects on indicators of diabetes and DN in STZ-induced Wistar rats. Interestingly, similar to our current results, aspirin alone was not effective in lowering diabetic indicators in the study of Bhatt and Addepalli (2011).

Since ginger has been shown to have anti-inflammatory properties by both ourselves and others (Thomson *et al.*, 2002; Grzanna *et al.*, 2005; Sang *et al.*, 2009; Ramadan *et al.*, 2011; Rani *et al.*, 2011; Li *et al.*, 2012), the anti-diabetic potential of aspirin in comparison to ginger and garlic was investigated in the present study. In addition, we have previously reported that garlic and ginger inhibit COX activity in a manner similar to that of aspirin (Ali and Thomson, 1995; Thomson *et al.*, 2002). However, in the current study in the STZ-induced rat model of DM, aspirin did not exhibit any significant beneficial effect in ameliorating hyperglycemia and the associated biochemical changes or improving nephropathic indicators. Therefore, these results suggest that the effects of ginger and garlic in this model are most likely not due to anti-inflammatory properties.

CONCLUSIONS

In summary, the results of the current study suggest that garlic and ginger are more effective anti-diabetic and anti-nephropathic agents than aspirin in the treatment of diabetes mellitus. In addition, the current study suggests that the beneficial effects of ginger and garlic in the STZ-model of type 1 DN are most likely due to the hypoglycemic and insulin-increasing activities of ginger and garlic rather than anti-inflammatory properties. Future studies should be directed toward elucidation of the mechanisms of the anti-diabetic effects of both garlic and ginger especially as related to diabetic nephropathy.

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