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Metabolic and Immunologic Alterations of Ginger Rhizome among Streptozotocin-Nicotinamide Induced Diabetic Rats

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ABSTRACT

Introduction: This study was conducted to determine immunological and metabolic effects of different concentrations of ginger rhizome (*Zingiber officinale Roscoe*) in streptozotocin (STZ)-nicotinamide (NA) induced diabetic rats. **Methods:** Forty-eight fasted male Sprague-Dawley rats were induced diabetes using a single intraperitoneal injection of NA(110 mg/kg b.w.) and STZ (65 mg/kg b.w, 15 min after NA). Diabetic rats orally received either different concentrations (250, 500 and 750 mg/kg body weight) of ginger rhizome suspension or glibenclamide (10 mg/kg body weight) for 6 weeks. Two control diabetic and normal groups were gavaged with only distilled water as a vehicle. **Results:** The results indicated that the lower concentrations of ginger modulated body weight, fasting blood glucose, level of triglyceride and tumor necrosis factor-α (TNF-α) (p<0.05). In contrast, ginger could not enhance atherogenic indices due to a decline in the level of HDL-c. Immunological features of ginger were evident by a significant lymphocyte proliferation in all treated groups at stimulation by 5 µg/ml PHA (p<0.001). Level of CD45ra+ (B cell marker) increased significantly in the lowest dose of ginger (p<0.05, 58%), and 250 mg/kg body weight of ginger was found to be safe for not altering the level of CD4+CD25+ marker (p>0.05). **Conclusion:** Ginger indicated better impact on metabolic and immunologic parameters in lower doses of supplementation compared with high doses of treatment.

Key words: Diabetes, ginger, inflammation, lymphocytes, streptozotocin-nicotinamide diabetic rats

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INTRODUCTION

Diabetes mellitus is a general term for heterogeneous disturbances of metabolism with chronic hyperglycaemia which can occur through impaired insulin secretion, impaired insulin action or both. Diabetes is classified mainly into two types (type 1 and type 2), as well as other specific forms such as gestational diabetes (Kerner & Brückel, 2014). The number of people with diabetes is growing rapidly, particularly in developing countries. Similarly, the rise of type 2 diabetes in South Asia is estimated to be more than 150% between 2000 and 2035 (Nanditha *et al*., 2016). A recent study in Malaysia also revealed an overall diabetes prevalence of 22.6% in this country which reflected a twofold increase from the year 2006 (Wan Nazaimoon *et al*., 2013).

Type 2 diabetes is associated with wide-ranging innate immune responses including chronic inflammation and increasing levels of blood cytokines which originate from development of islet dysfunction (Butcher *et al*., 2014). Elevated levels of inflammatory cytokines may lead to clinical and biomedical features of metabolic syndrome such as central obesity, hypertension, dyslipidemia and atherosclerosis (Reaven, 1988). Defect in insulin secretion in diabetes may cause inappropriate immune-responses and hence lymphocytes inactivity (Stentz *et al*., 2004, Butcher *et a*l., 2014). On the other hand, a chronic rise in inflammatory mediators can affect insulin-sensitive tissues and blood vessel walls as well as pancreatic β-cells (Donath *et al*., 2003) which can worsen both inflammatory and diabetic conditions. It has been found that nutritional intervention can interfere with the progress of these ailments via anti-oxidative and anti-inflammatory moderators of foods and plants. Accordingly, the present study reports some nutritional aspects of ginger rhizome to resuscitate the immune system of streptozotocin-nicotinamide induced diabetic rats.

Ginger (*Zingiber officinale Roscoe)* belongs to the family of Zingiberaceae. This plant was first cultivated in South-east Asia, and then made well-known as a general spice and herb in other parts of the world. Gingerols in fresh ginger and shogaols in dry samples are homologous series of phenols which cause the pungency of the plant (Wohlmuth *et al*., 2005); these biologically active components make a significant contribution towards medicinal applications of ginger (Sanwal *et al*., 2010). Some health benefits of ginger and its constituents that include hypoglycemic, immunomodulatory, hypolipidemic, anti-cancer, anti-inflammatory, anti-apoptotic and anti-emetic actions have been recognized (Ali *et al*., 2008). Ginger has been shown to have a positive impact on diabetes mellitus by inhibitory action of key enzymes controlling carbohydrate metabolism and increased insulin release/sensitivity (Li *et al*. 2012). Moreover, the cardiovascular, antioxidant capacities of ginger in molecular aspects and targets have been investigated (Butt & Sultan, 2011). This study was subsequently organised to address the gaps in the literature of the immune-protective role of the ginger rhizome including anti-inflammatory action, lymphocyte proliferative and immune-phenotyping effects rather than its metabolic impact in a model of diabetic rats.

METHODS

Materials

Streptozotocin and nicotinamide were purchased from Merck Millipore (Germany) while the biochemical measurement kits were supplied by Roche (USA). The commercial kits for performing sandwich Enzyme Linked Immunosorbent Assay were purchased from Scientifacts Sdn. Bhd in Malaysia (eBioscience, Austria) (IL-6:BMS625 and TNF-α: BMS622). Spleen cells cultured in media consisted of RPMI 1640 (Gibco BRL, Invitrogen) supplemented with 10%

foetal calf serum (Gibco BRL, Invitrogen). Scintillation fluid, anti-bodies and cocktail kits were purchased from Becton Dickinson, USA.

Preparation of ginger

Young fresh ginger rhizomes (*Zingiber officinale Roscoe*) were purchased from a local market in Selangor, Malaysia. Authentication was performed based on the recommendation of Mosihuzzaman and Choudhary (2008). An expert botanist from Agriculture Park, Universiti Putra Malaysia authenticated the sample as a young fresh ginger rhizome. A voucher specimen was identified by the herbarium of Universiti Putra Malaysia, Selangor, Malaysia (H. Bentong 6030, KLU). The rhizomes were freeze dried to constant weight prior to use for animal treatment; the yield of ginger following freeze-drying was 9.1 g of 100 g sample. Freeze-dried gingers were milled into powder in a mechanical grinder (Retsch SM 200, Rostfrei, Hann, Germany). Ginger powder suspension was prepared using distilled water based on the procedures of a previous study (Madkor, Mansour & Ramadan, 2011), and the experimental rats were gavaged with filtrated juice.

Animal groups and treatments

In this study, Sprague-Dawley rats were selected to perform an *in vivo* study given the simplicity of tests, physiological similarity to humans, ethical aspects, easy handling and lower costs compared to i*n vitro* models. A total of 48 male adult rats weighing 180-200 g were obtained from Chenur Supplier, Selangor, Malaysia. Rats were acclimatised to laboratory conditions (25±3ºC temperature, 50–60% humidity, and a 12-h light-dark cycle) for at least 7 days prior to commencement of the experiment. Rats were fed standard laboratory diet and given tap water. This study was approved by the Animal Care and Use Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia with the reference number being UPM/FPSK/PADS/BR-UUH/00442.

The experimental rats were randomly divided into 6 groups consisting of eight rats each:

Group I (NC): Normal control (gavaged distilled water)
Group II (DC): Diabetic control (gavaged distilled water)
Group III (D250): Diabetic (250 mg/kg b.w. ginger suspension in distilled water)
Group IV (D500): Diabetic (500 mg/kg b.w. ginger suspension in distilled water)
Group V (D750): Diabetic (750 mg/kg b.w. ginger suspension in distilled water)
Group VI (DG): Diabetic (10 mg/kg b.w glibenclamide in 15% DMSO)

Diabetic rats were fasted overnight and intraperitoneally injected with niconinamide (110 mg/kg b.w. dissolved in physiological saline), 15 min prior to intraperitoneal injection of STZ (65 mg/kg b.w. dissolved freshly in prepared citrate buffer 0.1 M, pH4.5) (Madkor *et al.*, 2011). To confirm diabetes induction in rats, glucose level was checked after 72 h of injection using Accu-chek strips three times, and those with fasting blood glucose of 11.1 mmol/L and higher were considered diabetic and uniformly included as diabetic rats in this study(Liang, 2004).

Following 6 weeks of treatment, control and treated rats were fasted overnight, blood samples were collected via posterior vena cava for biochemical tests. Blood samples were collected in tubes containing EDTA/sodium fluoride and lithium heparin for estimation of lipid profile and plasma glucose. Cytokines were assessed by the collection of serums in plain tubes. Animals were sacrificed by a high dose of chloroform, and spleens were isolated based on

a standard procedure presented by Millar *et al.*(1993). They were transferred to different petri dishes in 2 ml of RPMI media.

Biochemical analysis

Body weight of rats was assessed weekly using digital analytical balance-Scaltec ® SBA51 weighing scale. Blood glucose was measured using commercial diagnostic kits (Randox Laboratories Limited, UK) with Selectra XL chemical analyser (Vital Scientific, Netherlands) on days 0, 14, 21, 28 and 42 of the treatment, and the results were expressed as mmol/L. Components of lipid profiles consisting of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglycerides (TG) were assessed based on the methods of Knight, Anderson & Rawle (1972), McGowan *et al.* (1983), Allain (1974), and Grove *et al.* (1979) on the last day of the study. Results were expressed as mmol/L and mg/L, respectively. Calculation of atherogenic indices followed that of Madkor *et al.* (2011):

Atherogenic index (1) = total cholesterol/ HDL-cholesterol,
Atherogenic index (2) = LDL-cholesterol/ HDL-cholesterol.

Interleukin-6 (IL-6), tumor necrosis factor-α(TNF-α) and C-reactive protein (CRP) were measured by sandwich enzyme linked immune-sorbent assay (Sandwich ELISA) using the methods of previous studies (Erhardt *et al.* 2004; Godoy *et at*. 1996; Hele *et al*. 1991).

Immunological tests

To perform proliferation assay, $1x10^6$ splenocytes from each individual rat were placed in each well of 96-well plates. Cells were stimulated with two common mitogens (PHA and LPS) respectively at two concentrations (1 and 5 μg/ml) and left to incubate for 72 h. Cultures were pulsed with 0.5 μCi of 3H-thymidine at 24 h of incubation and later harvested onto glass filter mats via a 96-well plate automated cell harvester (Harvester Mach III M, TOMTEC). The filter mat was dried using an oven (120^{o}C) for 10 min before adding 5 ml scintillation fluid. The filter mat was then sealed and fitted into a scintillation cassette for radioactive measurement using the luminescent Microbeta counter (Wallac) (John *et al.*, 2011). Results were expressed as counts per minute (CPM).

To determine the expression level of cell surface markers of splenocytes, immune-phenotyping assay was performed using flow cytometery analysis (Krutzik *et al*. 2005). From each rat, a total of $1x10^6$ cells were assessed for the expressions of CD25-FITC, CD4-PE, CD3-APC, CD45RA-FITC, and CD161a-PE. The percentage of total T, B and NK cells was measured by a commercially available TBNK cocktail. LSR Fortessa II flow cytometer was used to acquire the samples, and the data were analysed using FACS Diva software. The relevant isotype antibody controls were used in parallel with all measurements to set negative gating(International Conference on Harmonisation, 2006).

Statistical analysis

Data were analysed by one-way ANOVA followed by Post Hoc LSD test using SPSS-20.0 and expressed as the mean values ± S.E.M for 8 rats in each group. The relationship between different variables was first analysed using Pearson product moment correlation, and following the identification of a relationship, multiple regression analysis was used to quantify the impact of measured variables on the dependent target variable. The values were considered statistically significant when $p<0.05$.

RESULTS

Following induction of diabetes (in baseline tests), there were no significant differences in metabolic factors and inflammatory cytokines among healthy and diabetic control rats except for fasting blood glucose. Throughout the experiment, the body

weight of rats in treatment groups showed rising trends, but still no significant changes were observed compared with the baseline test. However, comparing the final body weight of treatment groups with the diabetic control group indicated a significant decrease in the groups of D500, D750 and glibenclamide ($p<0.01$). D250 had higher body weight than the diabetic control group on day 42 of treatment ($p>0.05$) (Table 1). Meanwhile, fasting blood glucose of ginger-treated rats did not significantly change compared with the baseline; however, comparing final fasting blood glucose of treated rats with the diabetic control rats showed a significant decline in D250 ($p<0.001$), D500 ($p<0.05$) and glibenclamide ($p<0.001$). Feeding a high dose of ginger (750 mg/kg b.w) showed no benefit on elevated fasting blood glucose (Table 1). Regarding changes in lipid profile, ginger could regulate elevated triglyceride in D250 ($p<0.001$), but no significant alterations were found in other groups. Surprisingly, ginger treatment did not have any positive effect on the levels of HDL-c; rather, it even caused a significant decrease in D500 ($p<0.01$). Therefore, administration of ginger rhizome seems to increase atherogenic indices dose-dependently. Among pro-inflammatory cytokine, both ginger and glibenclamide were powerful inhibitors of elevated level of TNF-α($p<0.05$). Neither ginger nor glibenclamide seems to have any regulatory effects on levels of CRP and IL-6 (Table 2).

Proliferation of splenocyte

Results of the proliferation assay are presented in Figure 1. In an experiment with 1 μg/ml of mitogen, T cells from diabetic control rats were significantly rejected to activate in response to both PHA and LPS when compared with normal rats ($p<0.05$). However, PHA stimulation induced a significant proliferation with 250 and 500 mg/kg b.w. of ginger ($p<0.001$). Although, LPS did not induce considerable proliferation among the test groups, except for a significant rise at D250 ($p<0.001$). Administration of glibenclamide did not significantly change lymphocyte proliferation compared with the diabetic control group (Figure 1a).

In relation to the stimulation of spleen cell with 5 μg/ml of mitogens (Figure 1b), once the level of mitogens increased, diabetic rats displayed greater proliferation to both LPS and PHA resulting in no significant differences between normal and diabetic control rats. Among treatment groups, only PHA stimulation induced a significant proliferation in all groups including glibenclamide ($p<0.001$). In this assay, since all groups were stimulated by LPS, no significant differences were observed among the treated groups when compared with the diabetic control group.

Flow cytometer analysis

The effects of ginger treatment on lymphocyte sub-population in diabetic rats were analysed by flow cytometry and the results are presented in Table 3. Comparing data from normal and diabetic control groups did not represent any significant differences. Nonetheless, ginger in doses of 500 and 750 mg/kg significantly governed the percentage of regulatory T cells (27.3% and 26.2% respectively, $p<0.05$) compared with the diabetic control group, while glibenclamide did not alter the percentage of CD4+CD25+ cells. The percentage of B cells, presented by the marker of CD45ra+ , was significantly improved in the D250 group ($p<0.05$). Although, B cells of the other treatment groups increased compared with the diabetic control group, the rise was not statistically significant ($p>0.05$).

Relationship between different variables of metabolic indexes and immunological factors

The relationship of various factors of metabolic indexes and immunological factors

Table 1. Body weight and fasting blood glucose changes through 6 weeks of supplementation with ginger in controls and diabetic rats

	Normal control		*Diabetic control*		*Diabetic 250*		*Diabetic 500*		*Diabetic 750*		*Diabetic Glibenclamide*	
	BW	*FBS*	*BW*	*FBS*	*BW*	*FBS*	*BW*	*FBS*	*BW*	*FBS*	*BW*	*FBS*
Day 0	258± 16	4.8±0.3	249±15	16.7±2.5	244±16	18.2±2.5	251±16	15.6±1.7	254±16	16.4±1.3	246±16	18.2±2.6
Day 7	280±8.5	-	259±12	-	247±21	-	269±9	-	168±8	-	263±16	-
Day 14	293±10	4.5±0.3[c]	255±13	18.2±2.5	254±21	14.9±2.5	276±19	16±1.6	254±12	16.8±1.3	248±21	6.6±1.8[c]
Day 21	313±12	-	295±11	-	281±25	-	262±16	-	268±18	-	269±22	-
Day 28	312±13	4.4±0.09[c]	294±14	26.1±0.6	311±24	16.8±0.8[c]	266±20	18.5±1.5[c]	279±18	23.6±1.6	272±23	10.1±1.4[c]
Day 35	322±13	-	299±14	-	324±26	-	279±19	-	273±17	-	275±26	-
Day 42	345±24	4.3±0.09[c]	309±23	20.9±0.6	325±28	14.6±0.8[c]	278±23[b]	18.3±1.2[a]	276±24[b]	21.2±1.7	279±22[b]	8.5±1.8[c]

Notes: BW: body weight (g); FBS: fasting blood sugar (mmol/l). Values are presented as means with their standard errors. Mean values were significantly different from the diabetic control group: [a] $p<0{\cdot}05$, [b] $p<0{\cdot}01$, [c] $p<0{\cdot}001$.

Table 2. Levels of metabolic indexes and proinflammatory cytokines in controls and diabetic rats treated with ginger for 42 days

	Normal control		*Diabetic control*		*Diabetic 250*		*Diabetic 500*		*Diabetic 750*		*Diabetic Glibenclamide*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Total Cholesterol (mmol/L)	1.01	0.05	1.20	0.09	1.08	0.16	1.04	0.08	1.18	0.06	1.35	0.05
Triglyceride (mmol/L)	0.48	0.06	1.03	0.06	0.35[c]	0.11	0.97	0.08	0.87	0.16	1.10	0.17
LDL-c (mmol/L)	0.24	0.03	0.32	0.02	0.32	0.02	0.23[b]	0.04	0.20	0.02	0.24[b]	0.05
HDL-c (mmol/L)	0.66	0.04	0.75	0.06	0.70	0.09	0.57[b]	0.05	0.61	0.01	0.81	0.02
Atherogenic Index 1	1.57	0.07	1.61	0.03	1.53	0.05	1.90[a]	0.19	1.91	0.07	1.66	0.04
Atherogenic Index 2	0.38	0.07	0.44	0.03	0.32	0.05	0.37	0.07	0.39	0.09	0.21a	0.05
CRP (mg/l)	2.28	0.20	2.13	0.18	2.86	0.19	2.19	0.21	2.57	0.58	2.35	0.25
IL-6 (pg/ml)	219.09	9.44	202.93	6.25	219.37	13.42	208.40	7.53	198.33	9.12	198.27	9.40
TNF-α(pg/ml)	279.16	15.59	291.97	13.59	241.66[a]	8.44	247.71[a]	5.90	234.91[b]	5.41	220.45[c]	10.32

Notes: CRP: C-reactive protein, IL-6: interleukin-6; TNF-α: tumor necrosis factor-α. Values are presented as means with their standard errors. Mean values were significantly different from the diabetic control group: [a] $p<0{\cdot}05$, [b] $p<0{\cdot}01$, [c] $p<0{\cdot}001$.

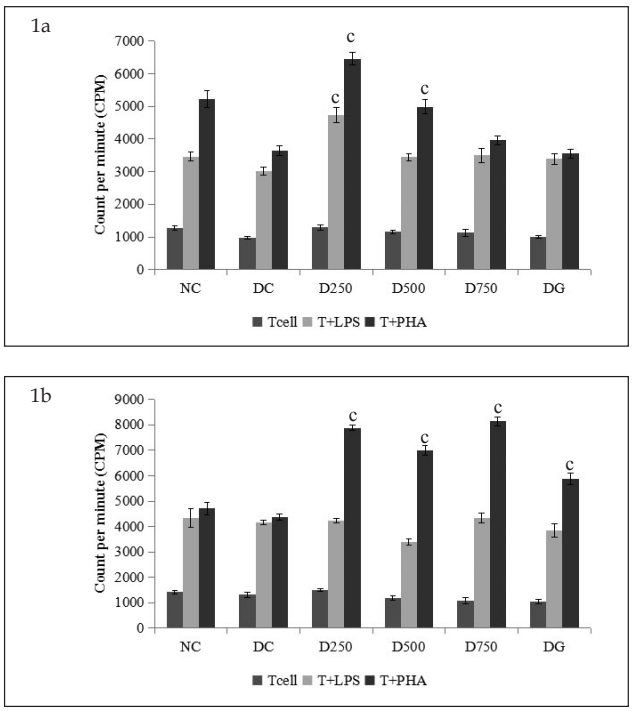

Figure 1. Assessment of Splenocytes Proliferation using 1 (a) and 5µg/ml (b) PHA and LPS.
Notes: N: normal; D: diabetic; C:control; G: glibenclamide.
Splenocytes were cultured for 48 h using 1µg/ml of PHA and LPS. The results are expressed as means with their standard errors. [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ were significantly different from the diabetic control group.

Table 3. Flowcytometry analysis of lymphocyte subsets in rats` splenocytes

	NC		DC		D250		D500		D750		Glibenclamide	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Regulatory T cells (CD4+CD25+)	4.77	0.2	6.18	0.53	5.73	0.76	7.87a	0.62	7.80a	0.43	5.56	0.46
Helper T Cell (CD3+CD4+)	20.14	3.2	23.15	1.8	17.26	1.21	26.42	2.33	28.70	4.42	28.64	2.48
B cell (CD45r[a]+)	13.6	1.8	12.82	1.9	30.67[a]	2.59	18.43	1.29	16.84	1.6	21.76	1.8
T cell (CD3+)	50.84	6.9	49.18	4.07	13.90	1.14	20.25	1.94	15.16	1.60	17.74	1.25
Natural killer cell (CD161[a]+)	18.62	1.2	20.8	4.2	20.33	2.92	16.77	2.46	16.84	2.62	22.32	2.23

Notes: Rats` splenocytes were isolated and immune-phenotyped to determine the various lymphocyte subsets using flow cytometry. Values are presented as means with their standard errors. Mean values were significantly different from the diabetic control group: [a] $p<0.05$.

on day 42 of the study showed that the fasting blood glucose of the rats had significant positive association with level of CD4+CD25+(r=0.45, p<0.01). The regression results suggest that approximately 18% of total variation in CD4+CD25+ can be explained by a change in blood glucose level. Proliferation of lymphocytes were increased following body weight gain in the sense of un-stimulation and PHA-stimulation (5 μg/ml); on the other hand, lymphocytes with no stimulation and PHA stimulation (1 μg/ml) were inversely correlated with blood glucose changes (p<0.01).

DISCUSSION

In this study, streptozotocin-nicotinamide induced diabetic rats were used as an experimental model of diabetes. As proposed by Masiello *et al.* (1998) earlier, this model is characterised by almost a 40% reduction in β-cell mass, which resulted in moderate and stable hyperglycaemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion and shared a number of features with human type 2 diabetes mellitus. Injection of nicotinamide and streptozotocin produced changes in fasting blood glucose, lipid profile and imbalances in the immune system. Ginger supplementation in lower concentrations improved body weight, fasting blood glucose, LDL-c and triglyceride. Such effects were accompanied by a reduction in the level of TNF-α. On the contrary, ginger supplementation did not change levels of CRP and IL-6. Furthermore, ginger undesirably decreased levels of HDL-c and elevated levels of atherogenic indices. In relation to immunological changes, ginger was capable of enhancing the lost activation of splenocytes to mitogens which occurred due to diabetes. Moreover, low concentration of ginger increased the percentages of B cells (CD45ra+). The highest intake of ginger was attributed to increasing levels of regulatory T cells. Glibenclamide, similar to low doses of ginger, regulated TNF-α and improved lipid profile, but showed no significant alteration in immunological tests. Rather, it led to an enhancement of splenocytes proliferation in response to high concentrations of PHA. Overall, the immunological effects of glibenclamide have not been fully addressed in previous studies, necessitating more research on the effects of this common anti-diabetic drug on the immune system.

The results of changes in the body weight were related to the food intake of the experimental rats. In the current work, ginger-treated groups consumed less pellets than the diabetic control group which represented an enhancement of the satiety feeling of ginger rhizome (Mansour *et al.,* 2012). The dry ginger is believed to increase thermogenic effects of food, an effective factor in weight management (Eldershaw *et al*., 1992). Regulatory effects of lower concentrations of ginger in fasting blood glucose are consistent with previous studies (Abdulrazaq *et al*. 2012 in ref list**;** Al-Amin *et al.*, 2006; Madkor *et al.* 2011; Nirmala *et al.,* 2012). But, for the first time, the current study did not find ginger to be efficient at a high dose of 750 mg/kg. In this regard, previous studies on toxicity effects of ginger reveal that the toxic effect of ginger only appeared at high doses (2000 mg/kg and above) with signs of mortalities and abnormalities under general conditions (Rong *et al.*, 2009). The ineffectiveness of high concentrations of ginger may relate to the presence of aldose reductase inhibitors in its volatile part (Giannoukakis, 2006) rather than its toxic effect. A subsequent test on the triglyceride level similarly indicated the benefit of ginger at the lowest dose. The data on lipid profile revealed no benefits of ginger consumption on HDL-c that is consistent with the findings of Madkor *et al.*(2011). Hence, the alteration of atherogenic indexes is mostly relevant to the decrease in HDL-c among treated groups. Earlier work demonstrated the anti-inflammatory effect of ginger by the presence of

6-gingerol and 6-shogaol (Ojewole, 2006); likewise, this study also indicated the ability of ginger to modulate elevated levels of TNF-α. Reportedly, 6-gingerol inhibits COX-2 expression and acts by blocking the activation of p38 MAPK and NF-κB (Kim *et al*., 2005); meanwhile, 6-shogaol inhibits the TNFα-mediated down-regulation of adiponectin expression via PPARγ transactivation (Isa *et al*., 2008).

Oral administration of ginger was capable of promoting reduced lymphocyte proliferation due to diabetes. Markedly, ginger showed the best enhancement effect in the lower doses of supplementation even in low concentrations of mitogen. Previously, Zhou, Deng & Xie (2006) investigated the effects of the volatile oil of ginger and showed it inhibited T lymphocyte proliferation (p<0.01) and decreased the number of total T lymphocytes in mice. The inconsistency of the findings of the two studies can be explained by the lack of any gingerol and shogaol compounds in the oil of ginger. As mentioned earlier, ginger at high doses of intake suppresses immune responses via increasing levels of CD4+CD25+ regulatory T cell, and the correlation test from our results suggest a suppressing capacity of CD4+CD25+regulatory T cells on B cell activation. The involved mechanism and probably B cell death following increasing CD4+CD25+ regulatory T cell are mediated by a granzyme-dependent and partially perforin-dependent pathway(Zhao *et al*., 2006). Perforin/granzyme-induced apoptosis is the main pathway used by cytotoxic lymphocytes to eliminate virus-infected or transformed cells (Trapani & Smyth 2002). The benefits of a low dose of ginger were clearly evident in B cell changes; level of CD45ra+ increased by 58% with 250 mg/kg of ginger.

CONCLUSION

Overall, the results presented here show that oral administration of low concentrations of crude extract of ginger possess significant hypoglycemic effects. It could effectively modulate induced inflammation, and had the ability to enhance immune response by improving the level of B cells and not increasing regulatory T cells. The immunological effects of ginger rhizome and efficiency of a low dosage of ginger in this study serve as a promising guideline on the treatment of diabetes and its associated complications. However, further studies may be needed to determine the cut-off dosage point for ginger efficacy in hyperglycemia and related immunologic conditions.

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