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The Significance of Vasoactive Intestinal Peptide in the Treatment of *Schistosoma mansoni*-Infected Diabetic Mice

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Abstract: The effect of Vasoactive Intestinal Peptide (VIP) on Insulin Dependent Diabetes Mellitus (IDDM) and schistosomiasis together in combination has not been previously investigated. To assess its efficacy in such condition, VIP was administered to *Schistosoma mansoni*-infected streptozotocin-induced diabetic (ID) mice at a dose level of 41.6 ng kg^{-1} b.wt., 3 times/week, for 8 consecutive weeks starting from the 1st week of infection. The administration of VIP to ID mice induced a potential amelioration of serum glucose, insulin and C-peptide levels indicating the insulinogenic effect of this peptide. VIP also produced a significant decrease of hepatic granuloma volume and worm fecundity in the ID mice without affecting worm burden. The granuloma volume was found to be lower in the ID mice as compared to that of the infected non-diabetic ones. VIP administration produced marked decreases of the elevated liver collagen, serum carbohydrate antigen (CA.19.9) and liver alpha fetoprotein (AFP) content of ID mice as well as it succeeded, at least partially, to alleviate the altered liver enzyme activities. It also successfully increased the anti-inflammatory cytokine, IL-10 and decreased the elevated pro-inflammatory chemokines, IL-12 and TNF-α level in the serum of ID mice. These changes in cytokines explain the decrease in hepatic granuloma volume and reflect the anti-inflammatory effects of VIP. The increased oxidative stress markers and perturbed antioxidant defense system were profoundly improved in the ID mice treated with VIP. In conclusion, the VIP may have anti-hyperglycemic and insulinotropic effects, decrease liver and intestinal egg count and ameliorate liver pathologic deteriorations via its immunomodulatory effects on cytokines released from macrophages and T helper cells in addition to its improvement effect on the antioxidant defense system of the infected diabetic mice.

Key words: Diabetes mellitus, *Schistosoma mansoni*, vasoactive intestinal peptide

INTRODUCTION

Insulin-Dependent Diabetes Mellitus (IDDM) or type 1 diabetes, characterized by absolute insulin deficiency, is associated with cellular infiltration and an inflammatory response in the islets of Langerhans (Nielsen *et al*., 1999). Cellular components of this infiltration include monocytes, macrophages and CD^{4+} and CD^{8+} T-cells (Mandrup-Poulsen, 1996). The balance between T-helper (Th)-1 type and T-helper (Th)-2 is crucial in the pathogenesis of IDDM; thus, a bias toward the proinflammatory Th-1 cytokines promotes insulitis and IDDM (Amirshahrokhi *et al*., 2008). Released cytokines, interleukin 1β, Tumor Necrosis Factor (TNF)-α and interferon (IFN)-γ, exert cytotoxic effects specifically on β-cells in the islets of Langerhans, in part via., the induction of free radicals production (Kubisch *et al*., 1997). Th2 anti-inflammatory cytokines, IL-4 and IL-10, have

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been found to suppress insulitis, β-cell damage and IDDM in Non-Obese Diabetic (NOD) mice (Cameron *et al*., 1997) and in Multiple-Low-Dose Streptozotocin (MLDS)-induced diabetic mice (Amirshahrokhi *et al*., 2008).

Schistosomiasis remains to be one of the most prevalent parasitic infections and has significant public health consequences (Engels *et al*., 2002). Similarly to IDDM, the regions of inflammation in schistosomiasis, granulomas, contain macrophages and $CD4^+$ and $CD8^+$ T-cells in addition to eosinophils (Caldas *et al*., 2008). Both Th1 and Th2 cytokines can regulate granuloma formation. Initially, the host develops a Th1 response, which is characterized by the production of IL-1, IFN-γ, TNF-α and nitric oxide (De Jesus *et al*., 2002; Caldas *et al*., 2008). However, as the infection progresses, this Th1 response gradually succumbs to a dominant Th2 response induced by the parasite eggs' antigens and is characterized by the secretion of IL-4, IL-5, IL-10 and IL-13 (Grzych *et al*., 1991; Sher *et al*., 1991) resulting in diminished inflammation (Araujo *et al*., 2004). IDDM has been described to affect schistosomiasis in laboratory animals by different authors. Several experiments performed by Mahmoud *et al.* (1975, 1976, 1979) revealed a smaller size of granulomas in the chemically-induced and genetic diabetic mice infected with *S. mansoni*. Moreover, Hulstijn *et al*. (2001) found lower faecal egg excretion in STZ-induced diabetic mice infected with *S. mansoni*.

Vasoactive Intestinal Peptide (VIP) is a neuropeptide synthesized by immune cells that can modulate several immune aspects, including the function of cells involved in the inflammatory response (Metwali *et al*., 2002). It was reported that VIP has several functions: (1) Promotes Th2 differentiation and inhibits Th1 responses, (2) inhibits *in vitro* and *in vivo* production of the proinflammatory cytokines TNF-α, IL-2, IL-6, IL-12, IFN-γ and of Nitric Oxide (NO) and (3) stimulates the production of the anti-inflammatory cytokine IL-10 (Xin and Sriram, 1998; Delgado *et al*., 1999a-d, 2002). Granulomas in murine schistosomiasis mansoni contain VIP (Weinstock and Blum, 1990) and contain VIP-responsive lymphocytes (Weinstock *et al*., 1991). Administration of VIP to *S. mansoni*-infected mice reduces liver pathology induced by schistosome eggs, inhibits IL-12 and TNF-α production and stimulates IL-10 production (Allam, 2007). In addition to the immunoregulatory role in schistosomiasis, VIP was found to evoke profound increases in insulin secretion from the pancreas of diabetic rats (Adeghate *et al*., 2001) and mice (Ahmed, 2009).

Pathogenesis of auto-immune diseases, including IDDM, mediated by T cells involves the type I immune response with production of IFN-γ and TNF-α. However, egg antigens of *S. mansoni* infection induce a strong Th2-type cytokine response with increased production of IL-10 and prevent the onset of type 1 (autoimmune) diabetes (Zaccone *et al*., 2003). Thus, schistosomiasis may affect the induction of the immune-mediated diabetes mellitus and vice versa (El-Wakil *et al*., 2002). Thus, this study was designed to evaluate the significance of VIP in the treatment of diabetic mice infected with *S. mansoni*.

MATERIALS AND METHODS

Animals

Male 8-week old CD1 albino mice were purchased from the Schistosome Biological Supply Centre, Theodore Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt. Mice were maintained under suitable living conditions in the animal house (Zoology Department, Faculty of Science, Beni-Suef University, Egypt) at controlled temperature (20-25°C) and natural daily 12 h light-dark cycles throughout the experiment. They were supplied with food pellets, which contain at least 25.4% protein and tap water *ad libitum*. They were also kept under observation for 1 week before the onset of the experiment to exclude any intercurrent infection and for proper acclimatization. All animal procedures follow the animal research bioethics of SBSC, TBRI and are in accordance with the recommendations for the proper care and use of laboratory animals (CCAC, 1993).

Induction of Insulin-Dependent Diabetes Mellitus

Experimental diabetes mellitus was induced in overnight fasted CD1 albino mice by intraperitoneal injection of streptozotocin (STZ) (Sigma Chemical Company, USA) at dose level of 150 mg kg^{-1} b.wt. dissolved in citrate buffer (pH 4.5) (Miyamoto *et al*., 2008). Ten days after STZ injection, blood samples were obtained from the lateral tail vein of mice that had been deprived of food for 10-12 h and after 2 h post-oral glucose loading (3 g kg^{-1} b.wt.). Mice having 2 h serum glucose concentration that ranged from 180 to 300 mg dL^{-1} were considered mild diabetics and were included in the experiment.

Parasite and Infection

Schistosoma mansoni (John Bruce Egyptian strain) cercariae were obtained from infected *Biomphalaria alexandrina* snails (TBRI) as previously described by Schubert (1948). Normal and STZ-diabetic mice were infected with 40 live cercariae for each individual according to Stirewalt and Bronson (1955) and Bruce and Radke (1971).

Experimental Design

Synthetic porcine VIP (Sigma Chemical Company, USA) was reconstituted in sterile 0.9% saline-0.1% gelatin containing 0.5% albumin as described by Karlsson and Ahren (1990). The peptide was divided into aliquots and immediately stored at -70°C until use. Infected Diabetic Treated (IDT) mice were injected intraperitoneally with VIP at a dose of 41.6 ng kg^{-1} b.wt. 3 injections/week for 8 consecutive weeks, starting from the first week of infection; the total dose at the end of the experiment was 1 μg kg^{-1} b.wt. (Allam, 2007; Ahmed, 2009). The other groups [naive (N), infected only (I) and Infected Diabetic (ID) mice] were given the same amount of the vehicle at the same time as treated groups.

Parasitological Analysis

At the 8th week post-infection, worm burdens were estimated by portal perfusion according to Duvall and DeWitt (1967). The numbers of *S. mansoni* eggs in the perfused liver and in the intestine were estimated after alkali digestion as described by Cheever (1968).

Blood and Tissue Sampling

Sera were collected from the clotted blood samples after centrifugation at 3000 rpm for 15 min, then divided into aliquots and stored at -70°C until use. The liver from each mouse was excised immediately after perfusion and rinsed with ice-cold saline solution. The ventral median lobe of the liver was fixed in 10% neutral bnffered formalin for preparation of hematoxylin and eosin-stained sections. Another portion was homogenized by Teflon homogenizer (Glas-Col, USA) in 0.9% saline (0.5 g tissue/5 mL saline), centrifuged at 3000 rpm for 15 min and the supernatant was decanted and stored at -70°C until use for different biochemical estimations.

Histopathological Analysis

The diameters of granulomas surrounding schistosome eggs were measured in the hematoxylin and eosin-stained liver sections by using an ocular micrometer (Carl Zeiss, Thornwood, NY, USA). Twenty granulomas per mouse were measured and the largest diameter and that perpendicular to it were averaged (Lichtenberg, 1962). The volume (mm^3) of each granuloma was calculated assuming a spherical shape using the following formula: Volume of sphere = $3\pi R^3$ (the radius R was obtained by dividing the main diameter of the lesion by two) and the mean volume for each group was calculated (Mahmoud and Warren, 1974).

Immunological Analysis

Cytokines level was measured by using sandwich Enzyme-Linked Immunosorbent Assay (ELISA) in the Virology Research Laboratory (Faculty of Veterinary, Beni-Suef University, Egypt). Cytokine concentrations (pg mL^{-1}) for mouse IL-10, IL-12 +p40 and TNF-α were determined with commercially available reagents and ELISA kits purchased from BioSource International (Camarillo, California, USA). Cytokine concentrations were determined using a standard curve obtained from the known concentration of cytokine standards included in each assay plate according to manufacture's instructions.

Biochemical Investigations

All biochemical determinations were performed in the Zoology Department Research Laboratory (Faculty of Science, Beni-Suef University, Egypt) except for those mentioned elsewhere. Hnmalyzer 2000 Chemistry Analyzer (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) was used for spectrophotometric and colorimetric determinations.

Serum glucose concentration (mg dL^{-1}) was determined according to the enzymatic method described by Siest and Schielef (1981) using reagent kits obtained from BioMerieux Chemical Company (France). Serum insulin (μIU mL^{-1}) and C-peptide (pmol L^{-1}) concentrations were assayed in the Radioactive Isotopes Unit, National Research Centre (Dokki, Cairo, Egypt) using radioimmunoassay kits supplied by Diagnostic Products Corporation (Los Angeles, USA) according to the methods of Marschner *et al*. (1974) and Bonser and Garcia-Webb (1984), respectively.

Hepatic alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (mU/100 mg tissue) in the homogenate supernatant were estimated according to the kinetic method of Bergmyeyer *et al*. (1978), using reagent kits purchased from Spinreact Company (Spain). Hepatic γ-glutamyl transferase (γ-GT) activity (mU g^{-1} tissue) was determined according to the kinetic method of Young (1990) and Tietz (1991) using reagent kits obtained from BioSystems (Spain). Liver LDH activity (mU/100 mg tissue) was determined according to the method described by Bühl and Jackson (1978), using reagent kits purchased from Stanbio Laboratories (Texas, USA).

The amount of collagen (μg/100 mg tissue) in liver samples was determined from its hydroxyproline content by the method of Neuman and Logan (1950a, b). Briefly, the defatted and dried samples were hydrolyzed by autoclaving in 2 mL of 6 N HCl at 50 pounds pressure. The protein hydrolysates were used for the determination of hydroxyproline and the percentage of collagen of a tissue sample was calculated by using the 7.46 factor of Neuman and Logan (1950a) and Baykal-Erkilic *et al*. (1995). Meanwhile, a standard curve was constructed in order to determine the reproducibility of the assay.

The levels of hepatic reduced glutathione (GSH) (nmol/100 mg tissue), total thiol (nmol/100 mg tissue) and lipid peroxidation products (nmol MDA/100 mg tissue) were determined by the methods of Bentler *et al*. (1963), Koster *et al*. (1986) and Preuss *et al*. (1998), respectively, using reagents prepared in the laboratory. Liver catalase ($k.10^2$) level was determined according to the technique of Cohen *et al*. (1970) using reagents prepared in the laboratory. Results of catalase activity were expressed in terms of the first-order reaction rate constant (k) as indicated by Cohen *et al*. (1970). Heme peroxidases (myeloperoxidase and eosinophil peroxidase) activity (U g^{-1} tissue) in the liver of naive and infected diabetic mice and infected diabetic mice treated with VIP was assayed according to the methods of Chance and Maehley (1955) and Kar and Mishra (1976) with some modifications, using pyrogallol as a substrate (Conyers and Kidwell, 1991; Joseph *et al*., 1992) in the presence of H_2O_2. To 25 μL of supernatant, 2 mL phosphate buffer solution (pH 6.8), 100 μL 1.4% pyrogallol and 100 μL 1.1 mmole H_2O_2 were added. After exactly 5 min, the density of colour of formed purpurogallin was measured against the blank by recording absorbance at 420 nm. The enzyme activity (in units) for each sample was obtained from the standard curve performed by using different dilutions of heme peroxidase (EC.1.11.1.7) (Sigma-Aldrich Company, USA).

Serum CA 19.9 concentration (U mL^{-1}) was estimated by an immunoradiometric assay kit (Coat-A-Count GI-MA IRMA, DPC, USA) according to the method of Frebourg *et al*. (1988). α-Fetoprotien (AFP) concentration (U g^{-1} tissue) in liver homogenate supernatant was determined by a radioimmunoassay (RIA) kit (Double antibody kit, DPC, USA) according to the methods of Waldmann and Mclntire (1974) and Krikpatrick *et al*. (1977). Both CA19.9 and AFP concentrations were measured in the Radioactive Isotopes Unit, National Research Center (Dokki, Cairo, Egypt).

Statistical Analysis

The data were analyzed using one way Analysis of Variance (ANOVA) followed by LSD analysis to compare various groups with each other using PC-STAT program, University of Georgia, USA (Rao *et al*., 1985). The results were expressed as Mean±SE and values of $p>0.05$ were considered statistically insignificant, while those of $p<0.05$ and $p<0.01$ were considered statistically significant and highly significant respectively. F-probability for each variable expressed the general effect between groups.

RESULTS

Worm Burden

As shown in Fig. 1, there was no significant ($p>0.05$) difference in worm burden of the different infected groups.

Granuloma Volume

Granuloma volume was measured in hematoxylin and eosin-stained liver sections of 8 weeks I, ID and IDT mice. The data of the present study showed that, VIP treatment of ID mice highly significantly reduced ($p<0.01$) hepatic granuloma volume by 84.7 and 60.2% as compared with I and ID mice, respectively. Also, ID mice showed 61.6% significant ($p<0.01$) reduction in hepatic granuloma volume compared to I mice (Fig. 1). One-way ANOVA indicated that the effect between groups was very highly significant ($p<0.001$) throughout the experiment.

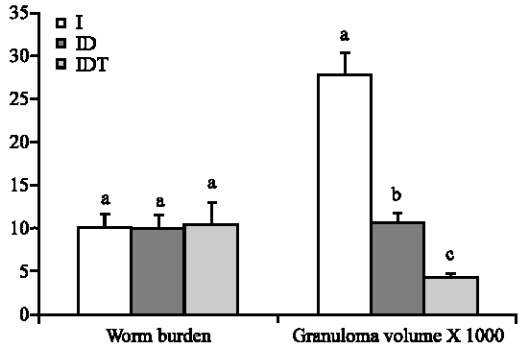

Fig. 1: Effect of VIP treatment on worm burden (number of worms) and hepatic granuloma volume (mm^3) of *S. mansoni*-infected (I), infected diabetic (ID) and infected diabetic VIP treated (IDT) mice. Values represented as the Mean±SE of ten mice. Columns not sharing common superscript(s) denote significant differences. F-probability: $p>0.05$ for worm burden and $p<0.01$ for granuloma volume

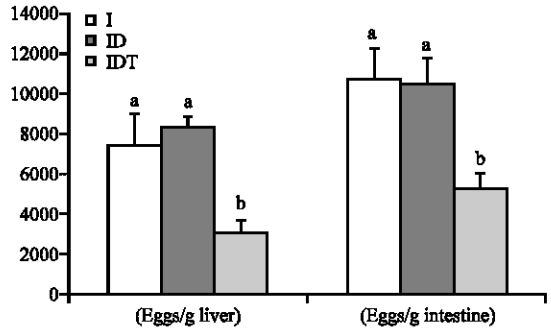

Fig. 2: Effect of VIP treatment on tissue egg load of infected (I), infected diabetic (ID) and infected diabetic VIP treated (IDT) mice. Values represented as the Mean±SE of ten mice. Columns not sharing common superscripts denote significant differences. F-probabilitiy: $p<0.05$

Liver and Intestine Egg Load

After the perfusion had been completed, about 0.5 g of both liver and intestine for each animal was digested in 5 mL 5% KOH overnight at 37°C. Eggs were counted in triplicates in liver and intestine homogenates for each mouse by using MacMaster counting slide and mean eggs per gram liver and intestine was calculated. VIP treatment of infected diabetic mice led to significant decrease ($p<0.05$) in the liver egg load which was 58.6 and 63.5 % as compared with infected non-diabetic and infected diabetic mice, respectively. Similarly, the intestine egg load of IDT mice showed 51.2 and 50% significant ($p<0.05$) reduction as compared to I and ID mice, respectively (Fig. 2). With regards to one-way ANOVA, it was found that the general effect between groups on both liver and intestine egg counts was only significant ($p<0.05$) throughout the experiment.

Level of Cytokines

Serum levels of IL-10, IL-12 and TNF-α were quantified by using sandwich ELISA. As depicted in Fig. 3, IL-10 level of IDT mice showed about 9- and 2-folds significant ($p<0.01$) increase compared to those of N and ID mice, respectively, at 8 weeks post-infection and treatment. Also, ID mice showed about 5-fold significant ($p<0.01$) increase in IL-10 level compared to N mice. However, IL-12 and TNF-α levels of ID mice were significantly increased as compared to N group. The administration of VIP to ID mice induced a profound decrease ($p<0.01$) of the elevated IL-12 and TNF-α levels (Fig. 3). With regards one-way ANOVA, it was found that the effect between groups on serum IL-12 and IL-10 was highly significant ($p<0.01$), while the effect on TNF-α was only significant ($p<0.05$) throughout the experiment.

Biochemical Results

The serum glucose concentration, at fasting state and after 2 h of oral glucose loading, was tremendously increased ($p<0.01$) in schistosoma-infected diabetic mice. The administration of VIP 3 times/week for 8 consecutive weeks to the ID mice induced a potential amelioration of the hyperglycemia. Fasting serum insulin and C-peptide levels, on the other hand, were remarkably decreased ($p<0.01$) in the infected diabetic mice. The administration of VIP profoundly ameliorated ($p<0.01$) the lowered levels of these parameters. One-way ANOVA depicted that the general effect between groups on serum glucose, insulin and C-peptide levels was very highly significant ($p<0.001$) throughout the experiment (Table 1).

With the exception of liver LDH activity, which showed a significant increase, the other tested liver enzyme activities were significantly diminished in the ID mice. The altered levels of LDH and

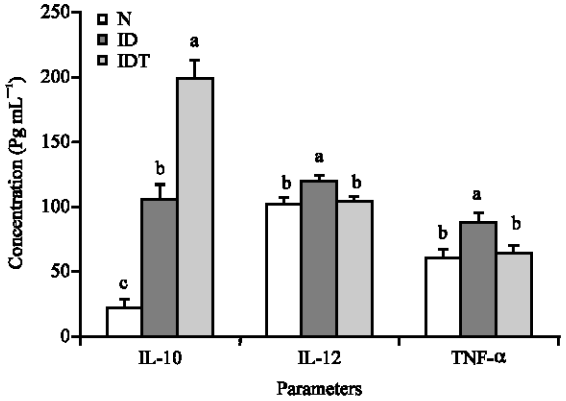

Fig. 3: Effect of VIP treatment on IL-10, IL-12 and TNF-α serum levels of infected diabetic (ID) and infected diabetic VIP treated (IDT) mice as compared to normal (N). Values represented as the Mean±SE of 8 mice. Columns not sharing common superscripts denote significant differences: F-probability: $p<0.05$ for TNF-α and $p<0.01$ for IL-10 and IL-12 levels

Table 1: Effect of VIP administration on serum glucose, insulin and C-peptide levels of *S. mansoni*-infected diabetic mice

	Parameters			
	Glucose (mg dL^{-1})			
Groups	Fasting	2 h	Insulin (μIU mL^{-1})	C-peptide (pmol L^{-1})
N	57.88±4.65^{bc}	106.33±2.61^{c}	22.08±1.08^{a}	279.17±2-01^{a}
ID	94.10±1.89^{a}	294.42±20.69^{a}	17.00±0.37^{b}	166.67±4.21^{c}
IDT	66.18±6.03^{bc}	138.69±7.46^{b}	24.00±0.55^{a}	175.83±1.90^{b}
F-prob.	$p<0.001$		$p<0.001$	$p<0.001$
LSD at the 5% level	27.704		2.205	8.772
LSD at the 1% level	37.310		3.050	12.310

Data are expressed as Mean±SE. Number of animals in each group is ten. Mean values, which share the same superscript symbol(s) are not significantly different

Table 2: Effect of VIP administration on liver enzyme activities of *S. mansoni*-infected diabetic mice

	Parameters			
	ALT	AST	LDH	γ-GT
Groups	(mU/100 mg tissue)			(mU g^{-1} tissue)
N	374.79±29.27^{a}	230.88±8.75^{a}	45.82±2.25^{b}	63.89±4.91^{a}
ID	202.33±2.07^{b}	166.12±7.45^{b}	64.77±2.72^{a}	50.14±2.64^{b}
IDT	222.00±31.45^{b}	182.83±18.56^{b}	50.80±4.59^{b}	66.37±4.62^{a}
F-prob.	$p<0.001$	$p<0.01$	$p<0.01$	$p<0.05$
LSD at the 5% level	74.842	37.989	10.077	12.601
LSD at the 1% level	103.500	52.535	13.936	17.427

Data are expressed as Mean±SE. Number of animals in each group is ten. Mean values, which share the same superscript symbol(s) are not significantly different

γ-GT activities were normalized by VIP treatment. One-way ANOVA depicted that the general effect between groups on the liver enzymes was at least significant throughout the experiment (Table 2).

The liver collagen, serum CA 19.9 and liver α-fetoprotein levels were highly significantly elevated ($p<0.01$) in the ID mice. The VIP administration normalized the elevated level of liver collagen, while it significantly decreased serum CA 19.9 and liver α-fetoprotein levels below the normal one. One-way ANOVA depicted that while general effect between groups on liver collagen was highly significant ($p<0.01$), the effect on serum CA 19.9 and α-FP was very highly significant ($p<0.001$) throughout the experiment (Table 3).

Table 3: Effect of VIP administration on liver collagen and AFP and serum CA19.9 levels of *S. mansoni*-infected diabetic mice

	Parameters		
Groups	Liver collagen (μg/100 mg tissue)	Serum CA19.9 (U mL^{-1})	Liver AFP (U g^{-1} tissue)
N	4.20±0.09^{b}	6.28±0.15^{b}	7.90±0.05^{a}
ID	7.18±0.87^{a}	8.00±0.26^{a}	9.50±0.92^{a}
IDT	4.83±0.52^{b}	4.78±1.21^{c}	3.80±0.09^{b}
F-prob.	p<0.01	p<0.001	p<0.001
LSD at the 5% level	1.776	0.518	1.610
LSD at the 1% level	2.456	0.713	2.231

Data are expressed as Mean±SE. Number of animals in each group is ten. Mean values, which share the same superscript symbol(s) are not significantly different

Table 4: Effect of VIP administration on various liver oxidative stress markers of *S. mansoni*-infected diabetic mice

	Parameters				
Groups	Total thiol (umol/100 mg)	GSH (nmol/100 mg)	LPO (nmol MDA/100 mg)	Peroxidase (U g^{-1})	Catalase (k.10^{2})
N	187.28±23.57^{a}	40.47±0.59^{a}	48.33±5.32^{b}	26.61±2.22^{c}	54.68±3.41^{a}
ID	63.52±8.77^{b}	23.10±1.68^{b}	60.80±1.72^{a}	61.77±3.48^{a}	23.36±1.97^{c}
IDT	78.69±6.73^{b}	37.10±3.02^{a}	47.45±1.90^{b}	40.64±3.88^{b}	40.56±6.47^{b}
F-prob.	p<0.001	p<0.001	p<0.05	p<0.001	p<0.001
LSD at the 5% level	45.300	6.094	10.275	9.857	13.175
LSD at the 1% level	62.640	8.428	14.209	13.631	18.220

Data are expressed as Mean±SE. Number of animals in each group is ten. Mean values, which share the same superscript symbol(s) are not significantly different

Regarding the oxidative stress and antioxidant defense system (Table 4), liver total thiol, glutathione and catalase levels were tremendously decreased in the ID mice as compared to normal ones while the liver peroxidation and peroxidase activity were vigorously increased. The administration of VIP induced a detectable amelioration to various extents. The effect of VIP on liver total thiol content was non-significant, while its effect on other tested oxidative stress and antioxidant markers was at least significant. One-way ANOVA revealed that the effect between groups on liver lipid peroxidation was only significant ($p<0.05$), while the effect on the liver total thiol, glutathione, peroxidase and catalase levels was very highly significant ($p<0.001$) between the groups throughout the experiment.

DISCUSSION

Several recent reviews have recapitulated previous numerous studies demonstrating the involvement of VIP immune functions in the control of homeostasis and health. The widespread knowledge of the anti-inflammatory function of VIP has led investigators to suggest the use of VIP for the treatment of several inflammatory and autoimmune diseases including type I diabetes mellitus (Delgado *et al*., 2002, 2004; Abad *et al*., 2006; Gomariz *et al*., 2001, 2006, 2007; Gonzalez-Rey *et al*., 2007). IDDM is associated with cellular infiltration consisting of monocytes/macrophages and T-cells subsets ($CD4^{+}$ and $CD8^{+}$) and an inflammatory response in the islets of Langerhans (Nielsen *et al*., 1999). Similarly, schistosomiasis exhibited cellular filtration (eosinophils, macophages, $CD4^{+}$ and $CD8^{+}$ T cells) and an inflammation around the schistosome eggs entrapped in the liver. There are numerous studies showing that proinflammatory Th1 cytokines IL-1β, IFN-γ, TNF-α and NO are critically involved in the pathogenesis of IDDM and schistosomiasis (Rabinovitch, 1998; Caldas *et al*., 2008). The preponderance of the anti-inflammatory Th2 cytokines IL-4 and IL-10 in both diseases counteracts Th1 cytokine effects and may prevent liver damage or β-cells destruction (Allam, 2007; Amirshahrokhi *et al*., 2008). Thus, the two diseases at different stages may affect each other. Based

on these findings, this study highlights this interaction by following the progression of each disease in the presence of the other. In addition, the effect of VIP on the two diseases together in combination as an animal model is assessed.

The data of the present study showed that the administration of VIP to ID mice significantly reduced serum level of pro-inflammatory cytokines, TNF-α and IL-12. These results are in agreement with the previous reports which stated that VIP inhibits the transcription (Delgado and Ganea, 1999) and production of pro-inflammatory cytokine IL-12 from macrophages (Xin and Sriram, 1998; Gomariz *et al*., 2000) and from Th1-cells (Wang *et al*., 1999) as well as it reduces the serum level of IL-12 in I mice (Allam, 2007). Delgado *et al*. (1998, 1999d, 1999e) also reported that VIP inhibits TNF-α production *in vitro* and *in vivo* from LPS-stimulated macrophages through the binding to VIP/pituitary adenylate cyclase-activating peptide (VPAC) receptor 1 that leads to an activation of the adenylate cyclase system and a reduction of the nuclear factor-κB (NF-κB) binding. Increase in cAMP as a result of the adenylate cyclase activation has been demonstrated to inhibit the expression of a variety of T-cell functions in schistosomiasis (Weinstock *et al*., 1991). In a earlier study, Allam (2007) showed that serum TNF-α level of I mice was potentially reduced with VIP treatment. On the other hand, the IDT mice exhibited a significant increase in the level of serum IL-10 as compared with N and ID mice. This finding confirmed the previous reports which have shown that VIP stimulates *in vitro* and *in vivo* production of the anti-inflammatory cytokine IL-10 (Delgado *et al*., 1999b; Gomariz *et al*., 2000; Allam, 2007; Larocca *et al*., 2007). It has been shown that in addition to increasing IL-10 production from Th2 cells, VIP treatment can increase production of regulatory T cells, which produce high levels of IL-10 (Chorny *et al*., 2005; Delgado *et al*., 2005; Reinke and Fabry, 2006). The stimulation of IL-10 production by VIP administration attenuates the production of pro-inflammatory cytokines including TNF-α and NO (Nicoletti *et al*., 1997). Consequently, VIP strongly reduces inflammatory response and exerts its anti-inflammatory function in several ways: (1) Direct inhibition of pro-inflammatory cytokine production (TNF-α, IL-6 and IL-12); (2) up-regulation of IL-10 production (a potent anti-inflammatory cytokine); (3) inhibition of expression and release of pro-inflammatory chemokines from activated macrophages and microglia; (4) inhibition of B7.1/B7.2 expression in activated macrophages and dendritic cells and subsequent inhibition of their stimulatory activity for antigen-specific T cells and (5) inhibition of Th1 responses (reduction in both the amounts of Th1 cytokines and the number of cytokine-producing Th1 cells) (Delgado *et al*., 2004).

In the current study, the serum glucose concentration at fasting state and after 2 h of oral glucose loading was profoundly elevated in the *S. mansoni*-infected diabetic mice as compared to the normal group. This change was associated with a significant deficiency of circulating insulin and C-peptide level, which was due to the deleterious damage of β-cells in the islets of Langerhans in the pancreas after streptozotocin (STZ) injection in these animals. Several mechanisms were reported by various authors to explain the action of streptozotocin to induce β-cells damage and in turn to decrease blood insulin and C-peptide levels. These mechanisms include: an increase in the oxidative stress and production of reactive oxygen and nitrogen species, an induction of DNA strand breaks, a depletion of NAD, an inhibition of ATP synthesis and/or a perturbed immune regulation (Okamoto, 1985; Pusztai *et al*., 1996; Amirshahrokhi *et al*., 2008). Moreover, Ahmed (2009) suggested that TNF-α, which was remarkably increased in STZ diabetic mice, may have a role in the β-cell destruction. Pehuet-Figoni *et al*. (1992) suggested that TNF-α could play a fundamental role in the progressive autoimmune destruction of β-cells in IDDM. Kaneto *et al*. (1995) stated that in the presence of TNF-α, large amounts of NO were produced and DNA cleavage occurred more noticeably in the islets of Langerhans. The NO-induced DNA cleavage is considered as an important step in the destruction and dysfunction of pancreatic β-cells induced by inflammatory stimulation or treatment with STZ (Kaneto *et al*., 1995). Also, Lnkic *et al*. (1998) and Amirshahrokhi *et al*. (2008) reported that multiple-low-dose of STZ diabetes, a model of IDDM, is induced by Th1 lymphocytes that secrete soluble

effector molecules that activate the macrophages and promote the destruction of β-cells possibly by both nitric oxide and non-nitric oxide - mediated mechanisms. In addition to the diabetogenic action of STZ, it was postulated that chronic and persistent elevated levels of glucose induce the rate of β-cell damage and apoptosis (Butler *et al*., 2003; Mellado-Gil and Aguilar-Diosdado, 2004; Cnop *et al*., 2005). The administration of VIP to diabetic mice just after infection for 8 consecutive weeks, in the present study, induced potential amelioration of the elevated serum glucose and the lowered serum insulin and C-peptide levels. These results are in accordance with those of Adeghate *et al*. (2001) and Ahmed (2009), who found that VIP improved the glycemic state and evoked an increase of insulin secretion associated with the increase in the number of β-cells in STZ-induced diabetic rats and mice. The parallel increase of serum C-peptide, a β-cell function marker, with serum insulin level, in the present study, support that VIP is an insulinotropic agent in the infected diabetic mice. It is relevant here to mention that the effect of VIP in the pancreatic islets to increase insulin secretion seems to occur *via* adenylate cyclase signaling pathway (Winzell and Ahren, 2007). The activation of adenylate cyclase leads to an elevation of cyclic adenosine monophosphate (cAMP) which in turn activates protein kinase A (PKA) to promote insulin secretion *via* several different pathways (Winzell and Ahren, 2007).

It is astonishing in the present study that the granuloma volume was greatly decreased in the *S. mansoni*-infected diabetic mice as compared with the infected non-diabetic ones although the worm burden and liver and intestine egg count were not significantly altered between these two groups. These changes agree with those of Mahmoud *et al*. (1975, 1976) and Mahmoud (1979), who reported that IDDM affects cell mediated response around schistosome eggs. These authors found that the diabetic infected animals revealed markedly suppressed lesions in granulomas similar to those in animals treated with powerful immunosuppressive agents. Also, MacCuish *et al*. (1974) found that lymphocytes from poorly controlled diabetics, in contrast to those from well-controlled patients have been found to respond poorly to phytohemoagglutinin stimulation. Since lymphocytes play a key role in cell-mediated immunologic responses, the alteration in the function of these cells in diabetes may in part explain the impairment in granuloma formation. Kazura *et al*. (1979) found that lymphoid cells of STZ and diabetic db/db mice have a reduced capacity to produce/secrete the lymphokine Eosinophil Stimulator Promoter (ESP) in response to soluble egg antigens of *S. mansoni*. In accordance with the present study, Hulstijn *et al*. (2001) depicted that the total amount of eggs in the intestine and the number of worms recovered was not affected in *S. mansoni*-infected diabetic mice in comparison with the infected non-diabetic ones. However, the data of the present study showed that the treatment of ID mice with VIP led to a significant decrease in liver and intestine egg loads. This result is in accordance with pervious studies that showed *S. mansoni*-infected mice treated with VIP had low tissue egg loads (Osman *et al*., 1997; Allam, 2007).

The present study indicated that the ID mice administered VIP showed a marked decrease in the granuloma size in comparison to the infected diabetic control. According to Weinstock *et al*. (1991), the granulomas around schistosome ova have eosinophils that produce VIP and T-lymphocytes that have VIP receptors. Therefore, it is possible that VIP may participate in the immunomodulation in the granulomas. It was postulated that VIP suppressed T lymphocytes proliferation in the granulomas by decreasing IL-2 and IL-4 production (Metwali *et al*., 1993; Sun and Ganea, 1993; Tang *et al*., 1995). Indeed, both IL-2 and IL-4 play a role in granuloma formation (Wynn *et al*., 1993; Cheever *et al*., 1994; Brunet *et al*., 1998). Also, the present study demonstrated that ID mice treated with VIP showed a high level of IL-10 and low level of TNF-α. Both cytokines play an important role in granuloma formation. Mice treated with IL-10 have smaller granulomas (Flores-Villanueva *et al*., 1996), whereas IL-10-deficient mice make larger granulomas during the acute period of disease (Wynn *et al*., 1998). In contrast, TNF-α deficient mice are unable to form granulomas and the treatment of such mice with TNF-α, restore the capacity of such mice to form discrete granulomas around parasite eggs

(Amiri *et al*., 1992). Moreover, treatment of immunocompetent mice with anti-TNF-α serum resulted in reduced granuloma size (Joseph and Boros, 1993). So, VIP could reduce the granuloma size through up-regulation of IL-10 and down-modulation of TNF-α production (Allam, 2007).

With regards to the enzymatic changes in the liver, the current data indicated that liver ALT, AST and γ-GT activities were remarkably depleted in the *S. mansoni*-infected diabetic mice, while LDH activity was increased. Concomitant with these results, Awadalla *et al*. (1975), Allam and Ahmed (2005) and Allam (2007) noticed that liver ALT, AST and γ-GT activities were profoundly reduced in the *S. mansoni*-infected mice. In addition, Mansour *et al*. (1982) and Mahmoud *et al*. (2002) found that the level of these enzymes was elevated in serum of the parasite-infected mice. However, Abdel Reheim *et al*. (2007) and Ahmed (2009) revealed that liver ALT, AST and LDH activities were increased in the diabetic rats and mice due to greater need to the gluconeogenic substrates in such condition. Thus, based on these findings, it can be suggested that the depletion of liver activities of ALT, AST and γ-GT in the present study may be attributed to the increased leakage of these enzymes from necrotic tissue and the replacement of normal liver tissue by granulomatous lesions which develop around the entrapped schistosome eggs as well as due to the increased permeability as a result of anoxia and irritation by toxic metabolic products of worms in infected diabetic mice (Ahmed and Mostafa, 2003; Allam, 2007). Overall, in our opinion, the changes of these liver enzyme activities in the ID mice may be the net result of the effects of STZ diabetes and schistosomiasis together. The administration of VIP to the ID mice, in the current study, induced a detectable amelioration of these altered liver enzyme activities. This alleviation could be attributed to a reduction in the granuloma size and number as well as the decrease in the damage of hepatocytes in the infected diabetic treated group as compared to infected diabetic control. VIP may protect hepatocytes from the destructive inflammatory response induced by schistosome eggs via inhibition of pro-inflammatory, as well as, stimulation of anti-inflammatory cytokines. The data of the present study support this suggestion since it indicates that the elevated pro-inflammatory cytokine TNF-α level in serum was significantly decreased while the anti-inflammatory Th2 cytokine IL-10 was potentially increased in the infected diabetic mice administered VIP as compared to the infected diabetic control. The decrease in the size and number of granulomas in the liver as a result of VIP treatment of infected diabetic mice was associated with nearly normalization of the elevated liver collagen content. This indicates that VIP may protect the liver of infected diabetic mice from fibrosis and cirrhosis. VIP may reduce collagen deposition by direct effect on hepatic stellate cells similar to somatostatin, which has been shown to modulate collagen I and III synthesis in activated hepatic stellate cells during schistosomiasis (Chatterjee *et al*., 2004; Allam, 2007). It was also reported by Kmiec (2001) that VIP may attenuate the conversion of quiescent stellate cells into moyfibroblast-like cells that play a key role in the development of inflammatory fibrotic response.

Tumor markers CA 19.9 and AFP levels were detectably increased in the ID mice and were decreased as a result of VIP administration to values below normal levels. These results are in accordance with those of Allam (2007). Elevated serum CA 19.9 level is associated with gastrointestinal cancer, colorectal cancer and gastric ulceration (Fouad and Khalaf, 1994; Chan and Sell, 1996), while elevated AFP level is associated with hepatocellular carcinoma, massive hepatic necrosis, hepatitis and liver cirrhosis (Noeman *et al*., 1994; Abelev, 2001). The amelioration of these parameters after VIP administration may be secondary to the improvement effect on liver and intestinal eggs count and the decrease in liver inflammation, necrosis and granuloma size as indicated in this current study.

Schistosoma mansoni infection not ouly triggers the production of reactive oxygen species in the mouse liver but also leads to an alteration in antioxidant defenses (Abdallahi *et al*., 1999; Pascal *et al*., 2000; La Flamme *et al*., 2001). This finding raises the possibility that oxidative stress may be a contributor of *S. mansoni*-associated pathology (Pascal *et al*., 2000). On the other hand, considerable clinical and experimental evidence now exists suggesting the involvement of free radicals-mediated

oxidative processes in the pathogenesis of diabetes mellitus as well as its complications (Ahmed, 2003; Jain *et al*., 2007). These findings agree well with the results of the present study that revealed a profound depletion of the liver total thiol, glutathione, catalase levels while lipid peroxidation and peroxidase activity were elevated in the infected diabetic mice as compared to normal. The free-radical formation was evoked by the pro-inflammatory and inflammatory cytokines (La Flamme *et al*., 2001; Tabatabaie *et al*., 2003) such as IL-1β and TNF-α which was significantly increased in the infected diabetic mice of our study. Also, Reactive Oxygen Species (ROS) are considered to be a strong stimulus for the release of cytokines (Vassilakopoulos *et al*., 2003). The heme peroxidase detected in the liver of naive mice of the present study may be attributed to its activity in Kupffer cells and endothelial cells lining the hepatic sinusoids (Stöhr *et al*., 1978; Pino and Bankston, 1979; Brown *et al*., 2001; Tafazoli and O'Brien, 2005). Nonetheless, this enzyme is not found in hepatocytes as indicated by Abdallahi *et al*. (1999). It is also relevant to mention that the peroxidase, assayed in this study using pyrogallol as a substrate, in normal and infected mice, may be myeloperoxidase and eosinophil peroxidase (heme peroxidases) rather than glutathione peroxidase which has selenium in its active site, is highly specific for glutathione and can not oxidize any other substrate (Shigeoka *et al*., 1991). The marked increase of peroxidase activity in the liver of *S. mansoni*-infected mice may be the result of the release of eosinophil peroxidase from eosinophil granulocytes and myeloperoxidase from mononuclear phagocytes in the region of granulomas around parasite eggs in addition to the stimulation of Kupffer cells (Weiss *et al*., 1983; Anderson *et al*., 1997; Abdallahi *et al*., 1999; Gharib *et al*., 1999). The myeloperoxidase and eosinophil peroxidase oxidize halides (I^-, Br^-, Cl^-) using H_2O_2 generating hypohalous acids. This reaction leads to the production of long-lived oxidant species (Weiss *et al*., 1983; Kazura *et al*., 1985; Anderson *et al*., 1997; Abdallahi *et al*., 1999) in vicinity of the parasite eggs. The administration of VIP to the infected diabetic mice, in the present study, induced a remarkable amelioration of the tested liver oxidative stress markers and antioxidant defense markers. This alleviation in oxidative stress markers in VIP-treated infected diabetic mice may be secondary to the improvement in the hyperglycemic and metabolic states, at one hand and a decrease in the inflammatory cytokine TNF-α as well as an increase of anti-inflammatory cytokines as IL-10, on the other hand. In accordance with this suggestion, Larocca *et al*. (2007) stated that VIP inhibits nitric oxide through an increase of IL-10 level. Also, Ahmed (2009) found that VIP improved oxidative stress concomitant with both improvement in the glycemic state and great increase of serum IL-10.

In conclusion, the VIP may have anti-hyperglycemic and anti-schistosomal effects in *Schistosoma mansoni*-infected diabetic albino mice. These ameliorative effects may be mediated via insulinotropic, immunomodulatory (by increasing anti-inflammatory and decreasing pro-inflammatory cytokines) and antioxidant effects of VIP. However, further studies are required to assess the efficacy and safety of VIP in *S. mansoni*-infected diabetic human beings.

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