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Full Length Article

Propolis restored adiponectin level in type 2 diabetes through PPAR γ activation



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ABSTRACT

Adipose tissue regulates insulin sensitivity via the circulating adipocytokines, leptin, resistin and adiponectin. Hypoadiponectinemia contributes to the development of obesity and related disorders such as diabetes, hyperlipidemia, and cardiovascular diseases. In this study, we investigated the effects of Brazilian propolis on adiponectin levels in type 2 diabetes mellitus (T2DM), the mechanism of signaling pathway was explored as well. T2DM was induced in male Wistar rats using high fat diet and low dose of streptozotocin (STZ, 35 mg/kg, i.p.). Propolis was administered by oral tubes. Peroxisome proliferator activated receptor gamma (PPAR γ) levels in sub abdominal adipose tissue, serum levels of adiponectin, tumor necrosis factor- α (TNF- α) and insulin were detected by Enzyme Linked Immunosorbent Assay (ELISA). Malondialdehyde (MDA) and reduced glutathione (GSH) in sub abdominal adipose tissue, fasting plasma glucose, plasma triglycerides and total cholesterol levels were measured by colorimetric method. Results showed that Brazilian propolis ameliorated hypoadiponectinemia in T2DM rats and relieved high glucose-induced adiponectin decrease. The signaling pathway analysis indicated that PPAR γ regulation was involved. In conclusion, Brazilian propolis could have beneficial effect in T2DM by increasing tissue PPAR γ levels, restoring serum adiponectin levels, enhancing insulin sensitivity and subsequently, attenuating elevated glucose level.

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1. Introduction

It is undeniable to say that there are more than 194 million people with diabetes worldwide [1,2]. Diabetes mellitus is characterized by high blood glucose levels and is associated with devastating and life-threatening complications that affect

various body organs, such as blood vessels, eyes, kidneys and nerves [3,4]. Among different types of diabetes mellitus, Type 2 account for 90% of diagnosed patients. Type 2 diabetes mellitus (T2DM) is a metabolic syndrome, which is characterized by both fat accumulation and impairment in insulin action, insulin production, or both; a condition called insulin resistance. Insulin resistance leads to the development of

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hyperglycemia. Such harmful hyperglycemia produced tissue damaging glucotoxicity, which is the major cause of diabetic complications [5]. In addition, abnormal metabolism of accumulated fat in adipose tissues can cause lipotoxicity, which can further exacerbate diabetic complications [6].

The management of T2DM entails lifestyle modification and/or pharmaceutical treatment such as insulin, biguanides, sulfonylureas, and alpha glucosidase inhibitors. However, these anti-diabetic medications are far from being satisfactory because of limited efficacy and many undesirable side effects [7]. As a consequence, T2DM is still an incurable disease with poor quality of life, high morbidity and mortality. Thereby, the social and economic burdens of this disease pose an urgent need for development of novel therapeutic strategies for treatment with satisfactory efficacy and no adverse effects [8].

Adipose tissue secretes many proteins and hormones such as adipocytokines, resistin, leptin, and adiponectin to control insulin sensitivity [9]. Adiponectin is a protein hormone secreted into the blood stream in average 0.01% of total plasma proteins. The important role of adiponectin comes from modulation of glucose and lipid metabolism in insulin sensitive tissue [10,11]. Accumulating evidence showed that hypoadiponectinemia played a key role in the pathogenesis of obesity and related diseases [12,13]. Furthermore, adiponectin administration to obese or diabetic mice can reduce body weight and blood glucose levels while enhancing insulin sensitivity [14,15]. Based on these data, adiponectin was conceived to be a novel therapy target for obesity and insulin resistance [16].

Various factors are involved in regulation of adiponectin expression. These factors include, peroxisome proliferator-activated receptor (PPAR- γ), CCAAT-enhancer-binding protein (C/EBP) α , Kruppel-like factor 7 (KLF7), and sterol regulatory element binding protein-1c (SREBP-1c). Among these factors, PPAR γ is recognized as the master regulator of gene transcription and plasma concentrations of adiponectin [9]. PPAR γ binds directly to a functional PPAR-responsive element (PPRE) in adiponectin promoter, leading to enhancement of adiponectin gene transcription [17]. Indeed, adiponectin was believed to be a marker for activity of PPAR γ [18].

Propolis (Brazilian) is a sticky resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources. Its color varies depending on its botanical source, the most common being dark brown [19]. The chemical compositions of propolis are mainly flavonoids, aromatic acids and esters, aldehydes and ketones, fatty acids and esters, terpenes, steroids, amino acids, polysaccharides, hydrocarbon, alcohol, hydroxybenzene and other compounds [20]. Brazilian propolis composed mainly of phenolic compounds artemillin C. Besides, it was reported to contain 3-prenyl-4-hydroxycinnamic, p-coumaric, caffeic acid, and caffeoylquinic acids, cinnamic acids and the flavonoids pinobanksin and kaempferol [21]. Brazilian propolis has been reported to possess various biological activities including antioxidant, anti-microbial, liver protective, immunoregulatory, anti-inflammatory, and anticancer effects [22]. In addition, it was reported to have hypoglycemic and hypolipidemic effects. Further, propolis was also demonstrated to control metabolic disorders in diabetic rats and to accelerate the tissue regeneration and repair of damaged pancreatic cell [23].

A recent study demonstrated that Brazilian propolis restored obesity-induced down regulation of adiponectin expression. In view of this recent claim, we investigated the effect of Brazilian propolis on adiponectin levels in T2DM induced experimentally in rats [24]. The signaling pathway mechanism was explored along with the regulatory roles of PPAR γ .

2. Materials and methods

2.1. Ethics statement

Experimental design and animal handling were according to the guidelines of the Ethical Committee of the Faculty of Pharmacy, Mansoura University, for Animal Use.

2.2. Animals

Male Sprague Dawley rats (160–180 mg) were housed in a certified animal care at a constant temperature (22 °C) under a 12-hour light–dark cycle, and were provided with standard rat food and water.

2.3. Experimental design

The rats were randomly divided into 3 groups with two dietary regimens. **Group1** (control group) was fed certified standard chow; **Group2** (diabetic untreated group) was fed high fat (HF) diet. **Group3** (diabetic group treated by Brazilian propolis) was fed HF diet for an initial period of 2 weeks without treatment. HF diet consists of (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) [25]. After the 2 weeks of dietary manipulation, the rats from group 2 and 3 were injected intraperitoneally (i.p.) with low dose of STZ (Sigma-Aldrich Co, St Louis, MO) (35 mg kg⁻¹) after overnight fasting [26]. The rats with blood glucose levels ≥ 250 mg/dl were considered diabetic and selected for further studies. The rats were allowed to continue to feed on their respective diets until the end of the study [27]. Type 2 diabetic rats in group 3 were treated with propolis (in aqueous solution, 0.6 g/kg), by oral tube for 21 days. The dose used for propolis in this study was in the range used in other studies applied for the same animal species [28].

At the end of the study, the rats were fasted overnight, and then sacrificed. Blood samples were collected via puncture of retro-orbital venous plexus using heparinized capillary hematocrit tubes. Blood was centrifuged at 3000 rpm for 5 minutes, and then plasma and serum samples were separated for determination of the biochemical parameters. Rats' sub abdominal adipose tissues were isolated, weighed and then homogenized in a 10-fold volume of ice-cold sodium potassium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl. The homogenates were centrifuged at 3000 rpm at 4 °C for 10 minutes and immediately used for determination of oxidative stress or stored at –80 °C until used.

2.4. Assessment of biochemical parameters

Fasting plasma glucose concentrations were determined using the glucose oxidase method, and Triglyceride (TG) and total

cholesterol (TC) were assayed using calorimetric kits purchased from Biodiagnostic Company (Egypt, Cairo), according to manufacturer's instructions.

2.5. Assessment of oxidative stress

Malondialdehyde (MDA) and reduced glutathione (GSH) were estimated in sub abdominal adipose tissue using commercial kits from Biodiagnostic Company (Egypt, Cairo), according to manufacturer's instructions.

2.6. Enzyme-linked immunosorbent assay (ELISA)

ELISA technique was used to assess serum adiponectin concentration, serum tumor necrosis factor- α (TNF- α), circulating insulin and PPAR γ concentration in sub abdominal adipose tissue according to manufacturer's instructions. The kits were purchased from MyBioSource Company (5520 Hubner Rd, San Diego, CA 92105, United States).

2.7. Statistical analysis

Results are expressed as means \pm SEM of 6 animals, and differences between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey's post hoc test. The level of statistical significance was taken at $P \leq 0.05$. Statistical analysis of the experimental data was performed using the statistical package SPSS as the definitive analyzer of drug effects.

3. Results

3.1. Effect of Brazilian propolis on body Weight and Sub abdominal adipose tissue weight

As shown in Fig. 1A, B, diabetes induction resulted in significant decrease in body weight by 25.25% and marked reduction in sub abdominal adipose tissue weight by 43.49% without affecting the food intake compared to control group. However, propolis treatment increased body weight by 1.05 folds and sub abdominal adipose tissue weight by 1.07 folds without affecting the food intake compared to diabetic group.

3.2. Effect of Brazilian propolis on fasting blood glucose and fasting insulin levels

Fasting blood glucose and fasting serum insulin levels (M \pm SE) in diabetic and control groups are illustrated in Fig. 2A, B. Fasting

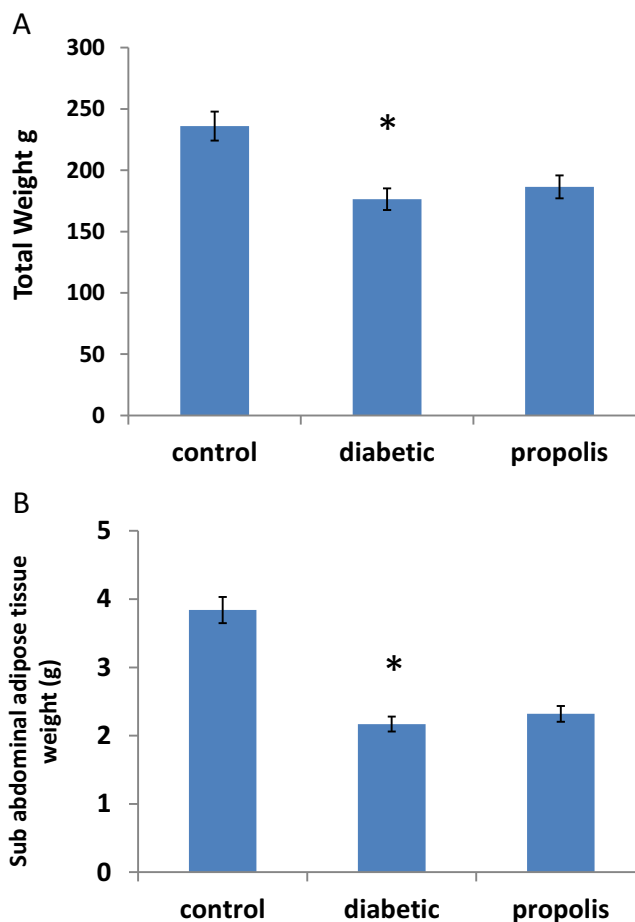


Fig. 1 – Effects of Brazilian propolis treatment on (A) total weight and (B) sub abdominal adipose tissue weight. *Significant compared to control group, $p < 0.05$.

blood glucose increased 4.48 fold in diabetic group when compared to control group. Besides, fasting serum insulin increased 2.05 folds in diabetic group when compared to control group. Propolis treatment reduced fasting blood glucose level by 69.52% and reduced fasting insulin level by 50% compared to diabetic group.

3.3. Effect of Brazilian propolis on lipid profile

The effects of propolis on lipid profile in rats are given in Table 1. The results showed that propolis treatment significantly increased the serum levels of high density lipoprotein 3.99 fold

Table 1 – Effect of Brazilian propolis treatment on the level of total cholesterol, total lipids, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, Triglyceride (TG) in rats with type 2 induced diabetes mellitus (Mean \pm SE).

GROUP	Triglyceride mg/dl	Total cholesterol mg/dl	HDL-cholesterol mg/dl	LDL-cholesterol mg/dl	VLDL-cholesterol mg/dl	Total lipids mg/dl
Control group	102.2 \pm 9.22	137 \pm 4.6	65.4 \pm 4	51 \pm 5.85	20.4 \pm 1.91	254 \pm 16
Diabetic group	366.4 \pm 14.47*	238 \pm 8.25*	26.28 \pm 7.3*	110.8 \pm 4.9*	73.2 \pm 2.85*	1344 \pm 196*
Propolis group	145.4 \pm 22.54#	182.4 \pm 12.6#	104.8 \pm 15.5#	57.4 \pm 4.97#	28.8 \pm 4.55#	457.2 \pm 63#

* Significant compared to control group, $p < 0.05$.

Significant compared to diabetic group, $p < 0.05$.

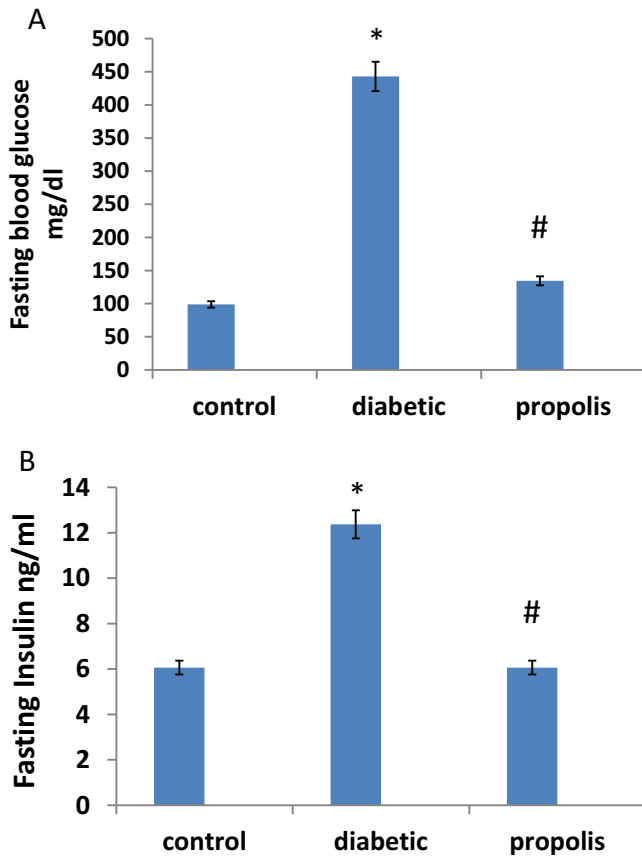


Fig. 2 – Effect of Brazilian propolis treatment on (A) fasting blood glucose and (B) fasting insulin levels. *Significant compared to control group, $p < 0.05$. #Significant compared to diabetic group, $p < 0.05$.

compared to diabetic group. In parallel, propolis treatment markedly decreased low density lipoprotein, total cholesterol, triglyceride, total lipid and very low density lipoprotein compared to diabetic group ($P < 0.05$).

3.4. Effects of Brazilian propolis on oxidative stress

Lipid peroxides in sub abdominal adipose tissue were measured as MDA. Results showed that MDA increased 4.78 fold in diabetic group when compared to control group. However, Propolis treatment reduced MDA by 58.5% compared to diabetic group, Fig. 3A. On the other hand, diabetes significantly reduced GSH levels in sub abdominal adipose tissue of diabetic rats by 31.3% compared to control group. Propolis treatment restored GSH levels in sub abdominal adipose tissue of treated group compared to diabetic group ($P < 0.05$), Fig. 3B.

3.5. Effect of Brazilian propolis treatment on TNF- α

As illustrated in Fig. 4, serum TNF- α level increased 5.89 fold in diabetic group when compared to control group. However, propolis treatment reduced TNF- α by 59.78% compared to diabetic group.

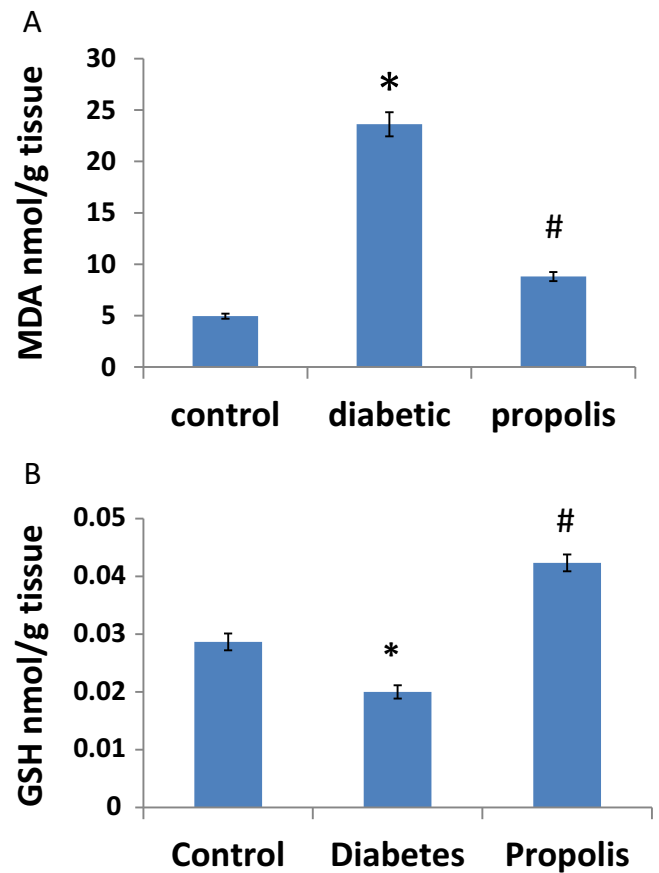


Fig. 3 – Effect of Brazilian propolis treatment on (A) MDA in sub abdominal adipose tissue and (B) GSH in sub abdominal adipose tissue. *Significant compared to control group, $p < 0.05$. #Significant compared to diabetic group, $p < 0.05$.

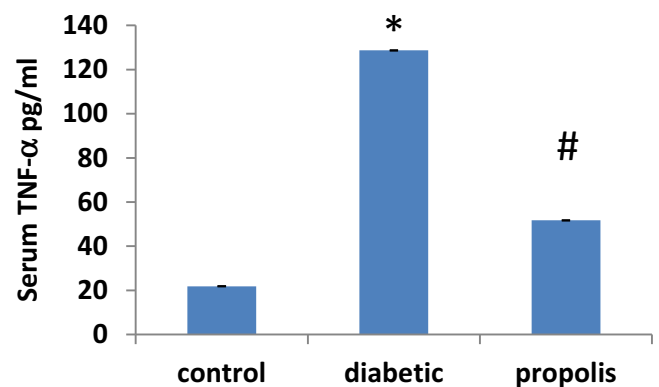


Fig. 4 – Effect of Brazilian propolis treatment on serum TNF- α . *Significant compared to control group, $p < 0.05$. #Significant compared to diabetic group, $p < 0.05$.

3.6. Effect of Brazilian propolis treatment on adiponectin and PPAR γ concentration

As illustrated in Fig. 5A, B, serum adiponectin decreased by 63.01% in the diabetic group when compared to control group.

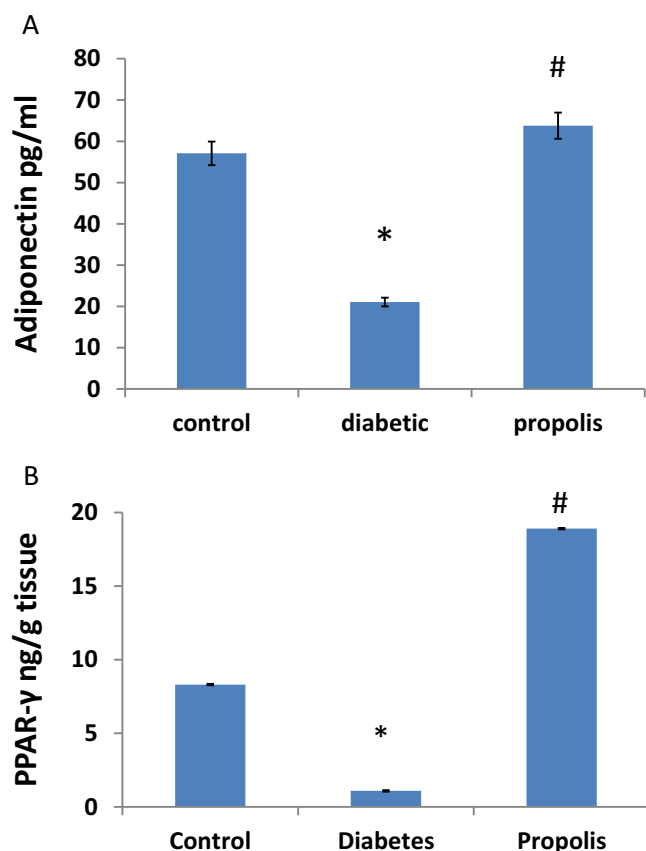


Fig. 5 – Effect of Brazilian propolis treatment on serum adiponectin and PPAR γ in sub abdominal adipose tissue. *Significant compared to control group, $p < 0.05$. #Significant compared to diabetic group, $p < 0.05$.

In addition, sub abdominal adipose tissue (PPAR- γ) levels decreased by 87.71% in diabetic group when compared to control group. On the other hand, propolis treatment markedly increased the serum levels of adiponectin 3 folds and increased the concentration of PPAR γ 1.9 fold compared to diabetic group.

3.7. Correlation analysis of studied parameters

Results illustrated in Fig. 6 showed that fasting blood glucose level negatively correlated with serum adiponectin ($r = -0.3$, $p < 0.05$) and PPAR- γ levels ($r = -0.7$, $p < 0.05$). In addition, serum adiponectin positively correlated with PPAR- γ ($r = 0.677$, $p < 0.05$) and HDL-cholesterol ($r = 0.77$, $p < 0.05$) and GSH ($r = 0.869$, $p < 0.05$). Moreover, serum adiponectin correlated negatively with insulin ($r = -0.765$, $p < 0.05$), total lipids ($r = -0.86$, $p < 0.05$), MDA ($r = -0.82$, $p < 0.05$).

4. Discussion

The results of the present investigation confirmed earlier reports that propolis treatment could almost control the hyperglycemia in the STZ-induced diabetic rat model [29]. The glycemic control achieved by Brazilian propolis treatment could be due

to increasing adiponectin levels, up-regulation of PPAR γ levels and enhancing insulin sensitivity.

At the first steps, diabetes induction by STZ caused rapid reduction in body weight. This was in agreement with previous reports [30]. The body weight loss in diabetic rats could be explained by many reasons, including dehydration as well as excessive fats and proteins catabolism [31], which ultimately leads to muscle wasting [32]. On the contrary, propolis treated rats showed non significant increase in body weight compared to control group, which could be attributed to better control of hyperglycemic state compared to the untreated diabetic group.

Several studies have documented the association between diabetes mellitus and abnormalities in lipid metabolism [33]. Dyslipidemia is believed to be a major risk factor for development of various diabetic complications. Diabetes-associated dyslipidemia resulted from excessive production of free fatty acids along with abnormal lipoprotein metabolism. Hence, diabetes mellitus is associated with an increase in TG and LDL, and decrease in HDL [34]. Similarly, the results of our investigation revealed disturbance in lipid metabolism in diabetic untreated rats. These effects were attenuated by propolis treatment. Of note, our findings provide ample support to the notion that propolis preparations could modulate lipid metabolism [35].

Insulin resistance is considered as a hallmark of T2DM. Accumulated fat in different body cells disturb their response to insulin, leading to insulin resistance and elevated blood glucose levels [36]. Previous studies have convincingly showed that propolis treatment decreased insulin resistance in obese diabetic rats [37]. In addition, propolis was found to enhance translocation of glucose transporter 4 and glucose uptake in mouse myocyte cell lines, as well as in the ICR mouse strain [38]. In confirmation with these reports, our results demonstrated that propolis markedly reduced fasting plasma glucose level in treated rats compared to untreated diabetic groups. This suggested that propolis could be a beneficial anti-hyperglycemic agent in T2DM.

As already noted, lipid peroxidation plays a significant role among oxidative defects that damages β cells in T2DM [39]. Convincing evidence has established a link between oxidative stress and insulin resistance. Increased free radical levels have deleterious effects on β cells, including decreased insulin secretion in response to glucose, impaired gene expression and cell death, leading ultimately to hyperglycemia and diabetes [40]. Moreover, Elevated free radical concentrations stimulate various signaling pathways that lead eventually to degradation of insulin receptors [41]. Therefore, targeting oxidative stress could be a potential therapeutic approach in T2DM. In the present study, diabetic treated rats showed significantly lower MDA levels and restored GSH levels nearing normal control values. This finding is consistent with the earlier report that propolis caused the partial restoration of β -cell function, possibly by an antioxidant defense mechanism [42]. Therefore, the protective mechanism of propolis against HF-induced diabetic changes could be attributed to its potent anti-oxidative properties.

In addition to oxidative stress, inflammation is considered an important pathogenic factor in the development of insulin resistance in T2DM. Oxidative stress and endoplasmic reticulum stress stimulate inflammatory signaling in T2DM.

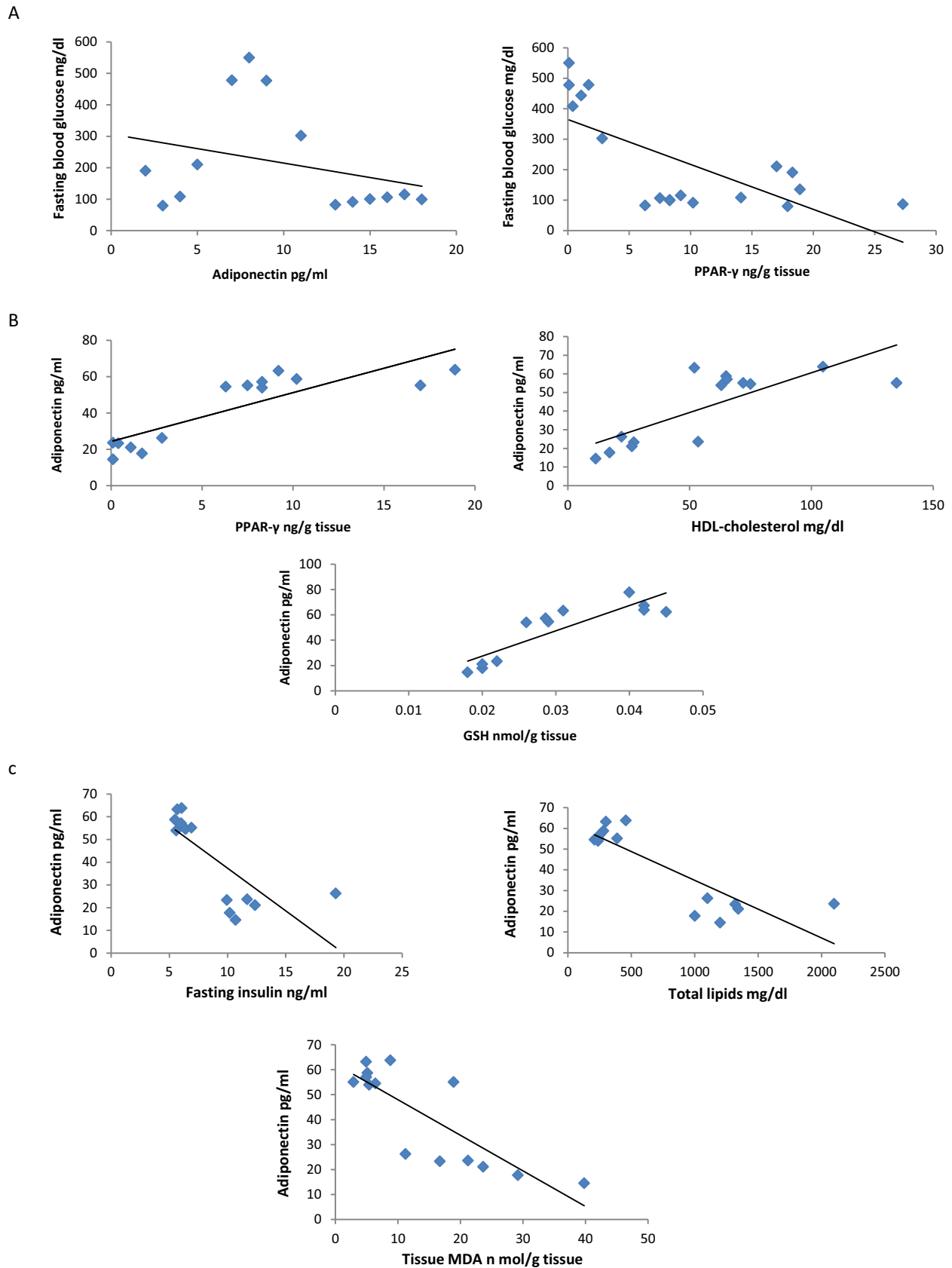


Fig. 6 – (A) Negative correlation between fasting blood glucose level and serum adiponectin ($r = -0.3$, $p < 0.05$) as well as PPAR γ levels ($r = -0.7$, $p < 0.05$). (B) Positive correlation between adiponectin level and PPAR γ ($r = 0.677$, $p < 0.05$), HDL-cholesterol level ($r = 0.77$, $p < 0.05$) and GSH level ($r = 0.869$, $p < 0.05$). (C) Negative correlation between adiponectin level and serum insulin ($r = -0.765$, $p < 0.05$), serum total lipids ($r = -0.86$, $p < 0.05$) and tissue MDA levels ($r = -0.82$, $p < 0.05$).

Inflammatory stimuli, in turn activate multiple serine/threonine kinases that inhibit insulin signaling [43]. Specifically, TNF- α was strongly linked to insulin resistance and diabetes. TNF- α increases free fatty acids production, interferes with insulin receptor signaling, decreased insulin sensitivity and inhibit adiponectin synthesis [44]. Our results showed that diabetes markedly increased serum TNF- α compared to control group. Propolis treatment reduced TNF- α level in treated diabetic rat. These results are in agreement with other studies that reported anti-inflammatory properties of propolis [45].

Adipose tissue is an endocrine organ that plays a crucial role in pathophysiology of T2DM [46]. Adiponectin is defined as anti-diabetic hormone secreted by adipose tissue. Adiponectin was shown to be associated with various metabolic disorders, including obesity, insulin resistance, and obesity related cardiovascular and fatty liver diseases [47]. Moreover, adiponectin production was reported to be negatively correlated with accumulated visceral fat [48]. Further, reduced adiponectin levels were observed in obesity [49] and knocking out adiponectin resulted in severe insulin resistance and diabetes [50]. Similarly, our results showed reduced levels of adiponectin in diabetic rats. Interestingly, adiponectin levels in our study were inversely correlated to the levels of blood glucose, insulin, total lipids and MDA. On the other hand, a high adiponectin level is found to be a consistent indicator of lower risk of T2DM because of its anti-diabetic and anti-atherogenic effects [51]. In line with this study, our results revealed that propolis restored reduced adiponectin levels in treated diabetic rats compared to untreated diabetic rats.

This finding led us to study how propolis may lead to down regulation of adiponectin expression. Gene expression of adiponectin is mainly regulated by nuclear transcriptor PPAR γ . PPAR γ is known to regulate adipocyte differentiation and to control the transcription of many adipocyte-specific genes. Research demonstrated that PPAR γ agonists increased the circulating adiponectin in high fructose fed rat model [52]. Hence, adiponectin expression was believed to be a pertinent target for PPAR γ agonists. In addition, epidemiological study proved that PPAR γ gene polymorphism would reduce the serum adiponectin levels [53]. In the present investigation, decreased protein concentration of PPAR γ and adiponectin was observed in diabetic rats. These adverse changes were counter regulated by propolis treatment. These results were further confirmed by the observed significant positive correlation between PPAR γ and adiponectin levels, suggesting PPAR γ activation as a possible pathway involved in propolis protective effect. Moreover, PPAR γ activation was found to attenuate insulin resistance by elevating the number of mature adipocytes, increasing glucose disposal rate and decreasing circulating free fatty acids levels [54]. In this context, our study revealed that both tissue PPAR γ and serum adiponectin levels negatively correlated with fasting blood glucose level.

5. Conclusion

In conclusion, this study demonstrated that Brazilian propolis can reverse changes evoked by T2DM induced experimentally in rats, possibly by combating oxidative stress, activating PPAR γ ,

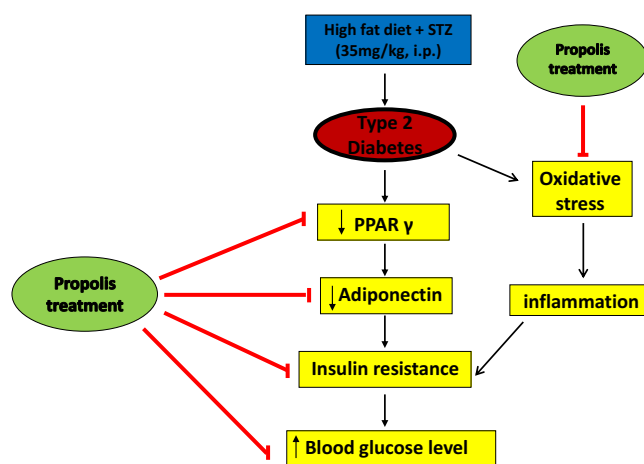


Fig. 7 – Proposed mechanism of action for Brazilian propolis in abrogating Type 2 diabetes- induced changes in high fat diet fed rats.

elevating adiponectin levels and reducing insulin resistance, Fig. 7. This ability of Brazilian propolis to target various pathways involved in T2D makes it a promising therapy for management of T2D.

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