Effect of *Rhazya stricta* extract on rat adiponectin gene and insulin resistance

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Abstract: *Rhazya stricta* plants have always played a major role in the treatment of human and animal diseases. The aim of this study was to study the effect of different doses of *Rhazya stricta* extract administered orally to rats, treatment period, effect on adiponectin protein, insulin resistance and finally its effect on exon 3 of adiponectin gene. Oral administration of aqueous leaves extracts of *Rhazya stricta* evoked fluctuations in adiponectin levels during eighteen weeks period of treatment. Serum adiponectin levels showed a significant increase after 2 and 4 weeks of treatments. Also a highly significant increase in adiponectin level, compared with the control group, was detected in rats treated with 0.125 gm/ml and 0.150 gm/ml after eighteen weeks of treatment. Insulin resistance is an important risk factor for type II diabetes mellitus and cardiovascular disease. Therefore, we performed HOMA-IR to check the degree of insulin resistance in rats. The results showed an inverse highly significant correlation between adiponectin levels and insulin resistance degrees after two weeks of treatment with *Rhazya stricta*. Studies published to date indicate that polymorphisms at the adiponectin gene (exon 3) are indeed predictors of circulating adiponectin levels. However, our results showed a significance increase in adiponectin levels, we did not detect any rare mutation in this locus using CSGE technique. The effects of *Rhazya stricta* extract on the increase of adiponectin levels concentrations could be promising issue (after avoiding its possible mutagenic effects) in treating diabetes, carbohydrate metabolism, hypertension, as well as inflammatory conditions.
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1-Introduction

Rhazya stricta plants have always played a major role in the treatment of human and animal diseases. The effect of an alkaloidal isolated from *Rhazya stricta* leaves (rhazimine) on arachidonic acid metabolism in human blood was described by Saeed *et al*. (1993). The alkaloid has been shown to be a dual and selective inhibitor of platelet activating factor (PAF)-induced platelet aggregation and arachidonic acid metabolism. It was concluded that these effects might provide additional beneficial anti-inflammatory and anti-PAF effects by comparison with classical non-steroidal anti-inflammatory drugs.

In addition, the lyophilized extract of *Rhazya stricta* was shown to relax isolated intestinal muscles of rats (Tanira *et al*., 1996a). The plant may have potential as an antispasmodic drug. This seems to corroborate the folk medicinal use of the plant in certain localities.

The effect of the lyophilized extract of *Rhazya stricta*, on some indices of the antioxidant status, was studied in rats (Ali *et al*., 2000a). It was found that the high doses were effective in significantly increasing the hepatic and renal concentrations of reduced glutathione (GSH) and ascorbic acid, and significantly reducing the degree of lipid peroxidation. The low doses were ineffective in significantly altering these indices.

A lyophilized extract of *Rhazya stricta* leaves was found to be of low toxicity in rats. In this species, the oral lethal dose (LD_{50}) has been estimated to be 16 g/kg in rats and 2.36 g/kg in mice. Subchronic treatment of rats with *Rhazya stricta* extract at doses of 0.5-2.0 g/kg/day did not significantly affect the body weights, food, water intake, urine, and faecal output during 28 days of treatment (Tanira *et al*., 1996b). Subchronic treatment also had no significant effect on haematological and plasma biochemical constituents. However, Adam, (1998) dosed Saudi Arabian sheep with powdered *Rhazya stricta* leaves in water and reported that the higher doses produced body-weight depression, bloating, diarrhea, dyspnoea and weakness of the hind limbs. Histologically, there were entero-hepatonephropaty, pulmonary congestion, haemorrhage and emphysema.

An intravenous dose of 80 mg/kg of *Rhazya stricta* extract administered to dogs, produced intense salivation, and rigor, followed by respiratory failure, convulsions and death within 15 minutes (Siddiqui and Bukhari, 1972).

Several studies were performed by Baeshin's team to assess the mutagenic potential of *Rhazya stricta* (Decne) leaf extract: It was first evaluated by using the Saccharomyces cerevisiae auxotrophic mutant test (Baeshin *et al*., 2005) then on Aspergillus terreus (Baeshin *et al*., 2009a). Also, the cytogenetic status and DNA integrity of human lymphocytes were studied after exposure to an aqueous extract of *Rhazya stricta* leaves in Vitro (Baeshin *et al*., 2009b) and its effect on plant cells were also studied on Allium cepa root tip meristem (Baeshin *et al*., 2009c). The results of this battery of tests indicated a mutagenic potential of *Rhazya stricta* Leaf extract on different organisms and tissues.

Subsequently, the biochemical parameters such as Blood Lipid Profile Concentrations, Liver Enzyme Activities and Kidney Functions in Rats were the subjects for detecting the effect of this extract in these respects and results indicated that, aqueous extract of the *R. stricta* leaves significantly decreased concentrations of TGs, LDL-c, cholesterol, uric acid and creatinin, but increased concentration of HDL-c. It triggered all these activities without affecting liver enzyme activities or kidney functions. These findings may have a positive impact on the cardiovascular patients and may provide a new therapeutic strategy to reduce hypertriglyceridemia. (Baeshin *et al*., 2010)

As far as we are aware, the effects of *Rhazya stricta* on adiponectin concentrations have not been identified yet. However, from the literature reviewed, there are similarities in the effects of *Rhazya stricta* and adiponectin on diabetes, carbohydrate metabolism, hypertension, as well as inflammatory conditions. The leaves of *Rhazya stricta* have been used, among other aliments, for the treatment of diabetes mellitus. Diabetic patients in the Arabian Gulf region commonly use water extracts of the leaves (Ali *et al*., 2000b). Tanira *et al*. (1996b) reported that acute oral treatment with the extract at a dose of 4 g/kg produced a significant and short-lived increase in plasma insulin concentration, accompanied by a significant reduction in plasma glucose concentration. On the other hand, animal studies have demonstrated that adiponectin reduces hyperglycemia in different models of obesity/diabetes mellitus (Yamauchi *et al*., 2001; Berg *et al*., 2001). In addition, multiple animal and human studies have shown a correlation between adiponectin levels and insulin sensitivity (Berg *et al*., 2001; Combs *et al*., 2002). One of the most interesting findings is that, a decline in adiponectin levels seems to identify insulin resistance before the development of diabetes (Oh *et al*., 2007).

In many studies, relatively large doses of the plant extract were used to determine the pharmacological and toxicological actions (Tanira *et al*., 1996b and Adam *et al*., 2002). Therefore, it was necessary to study the effects of this plant using doses, almost near that is used by humans in the folk medicine.

2.Material and Methods

The experimental work was conducted at the biochemistry lab of King Abdul-Aziz University Hospital (KAUH) and at King Fahd Medical Research Centre (KFMRC), Jeddah, Saudi Arabia.

2.1. Materials

2.1.1 *Rhazya stricta* plant

It was collected from the nearby areas of Jeddah, KSA. The leaves were washed, shade-dried and ground to a fine powder with a blender and the resulting powder were diluted in distilled water.

2.1.2.Experimental design (Samples)

Fifty five locally bred adult male Wistar rats initially weighing 150- 200 gm were used. They were housed in groups of five animals at a temperature of 22 °C under a 12 h dark- light cycle. They were fed standard pelleted diet (Grain Soils and Flourmills Organization Jeddah, KSA) and drinking water.

The animals were divided into four groups: Group 1 (n= 10) was the control and was dosed orally by gavage with distilled water (0.5 ml). Group 2 , 3 and 4 (n= 15), were treated orally by gavage with 0.5 ml *Rhazya stricta* extract at single doses of 0.1 gm/ml, 0.125 gm/ml and 0.150 gm/ml respectively for 18 week.

Blood samples were obtained from rats (after an overnight fast) by penetrating the retro-orbital plexus with a glass capillary tube or Pasteur pipette. Blood samples were collected after one, 2, 4, 8, 12 and 18 weeks from all treated and control groups.

2.2.Methods

2.2.1.Preparation of plasma:

Few drops of rat's whole blood were collected in an EDTA tube. The blood was centrifuged for 10 minutes at 3000 rpm. Plasma was separated carefully in a number of ependroof tubes and then stored at-80˚C.

2.2.2.Preparation of serum:

Blood was collected in a plain tube, allowed to stand to clot for one hour at room temperature and centrifuged for 10 minutes at 3000 rpm. After centrifugation, serum was separated carefully in a number of Ependrof tubes and then stored at -80˚C.

2.2.3.Determination of adiponectin levels:

Rat adiponectin kit was used in an enzyme-linked immune-sorbent assay (ELISA) for quantitative determination of adiponecin in rat serum. The intensity of color was measured at 450 nm in a plate reader.

2.2.4.Determination of insulin levels:

Rat insulin ELISA kit was purchased from Linco research and was used as recommended by the manufacturer.

2.2.5.Determination of glucose levels

Glucose was determined using enzymatic methods on automated chemical analyzer (Dimension R Clinical Chemistry System, USA). The glucose kit was used as recommended by the manufacturer.

2.2.6.Assessment of Insulin Resistance:

According to Matthews *et al*. (1985), insulin resistance can be estimated from fasting blood glucose and insulin concentrations. In the present study, degree of insulin resistance was measured by Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), which was calculated with the formula: Fasting serum insulin (µ U/ml) x fasting plasma glucose (m mol/l)/ 22.5. High HOMA-IR scores denote low insulin sensitivity (insulin resistance).

2.2.7.DNA extraction:

Few drops of rat's whole blood were collected in tubes containing EDTA and genomic DNA was extracted using QIA amp DNA mini kit according to manufacturer's instructions. DNA Samples were obtained from both treated and control rats.

2.2.8.Primer design and PCR amplification:

PCR primers were designed (for exon 3 of adiponectin gene) with the computer program Primer 3.0 (Rozen and Skaletsky, 2000) and were synthesized commercially (Tib Molbiol Syntheselabor Berlin, Germany). The forward primer sequence is 5'-taa ggg tga ccc agg aga tg-3' and the reverse primer sequence is 5'-gcg gag act agg gag tgc tt-3'.

The PCR mixtures were prepared using ready to use HotStar TaqTM Master Mix as recommended by the manufacturer (Qiagen, Germany). The PCR program consisted of initial activation step for 15 minute at 95°C, followed by 35 cycles at 94°C for 30 seconds, 54°C for 1 minutes, and 72°C for 1 minute, and a final extension at 72°C for 10 minutes.

Few µl of PCR products were electrophoresed on 0.8 % agarose gel in 1X TBE buffer.. The relative size of the PCR products were determined using 100–1500 bp DNA ladder (Qiagen, Inc. USA). A visual image was obtained using Gel Documentation System (UVP Products Ltd, Cambridge, UK).

2.2.9.Conformational sensitive gel electrophoresis (CSGE):

To generate heteroduplexes for analysis by CSGE, 5µl of both control and treated PCR product (*Rhazya stricta* extract, 0.150 gm/ml) were mixed and incubated at 68°C for 30 minutes. Prior to electrophoresis, 4 µl of each PCR product was mixed with 4 µl of 20% ethylene glycol/30% formamide containing 0.025% (wt/ vol) each of Xylene Cyanol FF and Bromphenol Blue. Samples were separated by electrophoresis on a standard DNA sequencing gel apparatus [1-mm-thick gel prepared with 10% polyacrylamide made from 40% stock of acrylamide containing 99:1 acrylamide to bis-acrolylpiperazine (BAP)] using 0.5 ×TTE (Tris base, Taurine, EDTA) as the electrode buffer. The gel was pre-electrophoresed at 400V for 45 min and the samples were electrophoresed at 750 volts for 16 hr at room temperature.

After electrophoresis, one glass plate was removed and the gel on the second glass plate was stained with 1µg/ml ethidium bromide for 10 min followed by destaining in distilled water for 10 min. The bands were visualized with a UV light and the gel was then photographed under standard conditions.

3.Results

3..1 Relation between adiponection and insulin levels

Oral administration of aqueous leaves extracts of *Rhazya stricta* evoked fluctuations in adiponectin levels during eighteen weeks period of treatment with *Rhazya stricta* leaves (Table 1). Serum adiponectin levels were significantly ($p < 0.05$) lower (15.77±3.84 µg/ml) than control after one week of treatment in rats receiving 0.150 gm/ml *Rhazya stricta* aqueous extracts, On the other hand, serum adiponectin levels showed a significant increase ($p < 0.05$) after 2 and 4 weeks of treatments. This increase was clear in rats of group 4 receiving 0.150 gm/ml *Rhazya stricta* (33.40±8.38) and after 4 weeks of treatment in group 2 receiving 0.1 gm/ml *Rhazya stricta* (33.89± 5.46). Also a significant ($p < 0.01$) increase in adiponectin level, compared with the control group, was clear in rats of group 3 (20.77± 7.03 µg/ml) after eighteen weeks of treatment with *Rhazya stricta*. A highly significant ($p < 0.001$) increases in adiponectin concentration was recorded with the same dose in group 4 (24.06± 4.65 µg/ml).

Table 1: Relation between adiponection and insulin levels

Week	Variable	Group 1 Control 10 rats	Group 2 (0.1gm/ml) 15 rats	Group 3 (0.125gm/ml) 15 rats	Group 4 (0.150gm/ml) 15 rats	p-value
01	Insulin (µU/ml)	22.42±18.08	18.35±15.97	21.81±20.77	46.68±24.82	<0.01[(c)]
	HOMA-IR (µU/ml.mmol/l)	3.93±2.42	4.37±4.16	4.95±4.94	9.68±5.23	<0.01[(c)]
	Adiponectin (µg/ml)	19.01±2.76	18.60±2.00	17.46±2.94	15.77±3.84	<0.05[(c)]
02	Insulin (µU/ml)	4.62±2.50	14.81±9.66	14.92±21.01	9.53±9.42	NS
	HOMA-IR (µU/ml.mmol/l)	0.92±0.48	3.70±2.82	3.41±5.16	2.15±2.52	<0.05[(a)]
	Adiponectin (µg/ml)	27.7±7.35	24.12±4.80	24.11±5.75	33.40±8.38	<0.05[(c)]
04	Insulin (µU/ml)	8.76± 8.71	9.03± 8.13	19.24± 14.60	20.70± 12.72	<0. 05[(b,c)]
	HOMA-IR (µU/ml.mmol/l)	1.62± 1.96	1.95± 1.96	4.22± 4.31	5.50± 3.73	<0. 01[(c)]
	Adiponectin (µg/ml)	30.41± 8.96	33.89± 5.46	28.20± 3.33	27.58± 5.93	NS
08	Insulin (µU/ml)	29.70± 25	16.39± 9.96	12.83± 7.97	8.88± 3.54	<0.05[(a)] <0.01[(b)] <0.001[(c)]
	HOMA-IR (µU/ml.mmol/l)	8.30± 7.32	3.89± 29.55	2.56± 1.64	1.51± 0.65	<0.01[(a)] <0.001[(b,c)]
	Adiponectin (µg/ml)	22.85± 5.38	24.21± 4.78	23.53± 6.22	24.28± 5.26	NS
12	Insulin (µU/ml)	17.36± 18.72	12.76± 7.73	16.44± 20.83	15.86± 13.93	NS
	HOMA-IR (µU/ml.mmol/l)	4.71± 6.20	2.86± 2.08	3.99± 5.67	3.48± 3.25	NS
	Adiponectin (µg/ml)	19.62± 4.54	21.06± 4.21	18.40± 4.64	18.77± 4.58	NS
18	Insulin (µU/ml)	10.86± 8.53	7.82± 3.65	9.11± 5.95	8.52± 6.65	NS
	HOMA-IR (µU/ml.mmol/l)	2.29± 1.96	1.57± 0.83	1.84± 1.39	1.60± 1.41	NS
	Adiponectin (µg/ml)	14.61± 3.91	16.28± 2.92	20.77± 7.03	24.06± 4.65	<0.01[(b)] <0.001[(c)]

NS: Not significant
Values were represented as the mean ± SD.
(a: control vs. group 2, b: control vs. group 3,
c: control vs. group 4).
p- value ≤ 0.05 was used as a criterion of significance.
p- value ≤ 0.01and ≤ 0.001were used as a criterion of highly significance.
($p < 0.01$, r = -0.35).

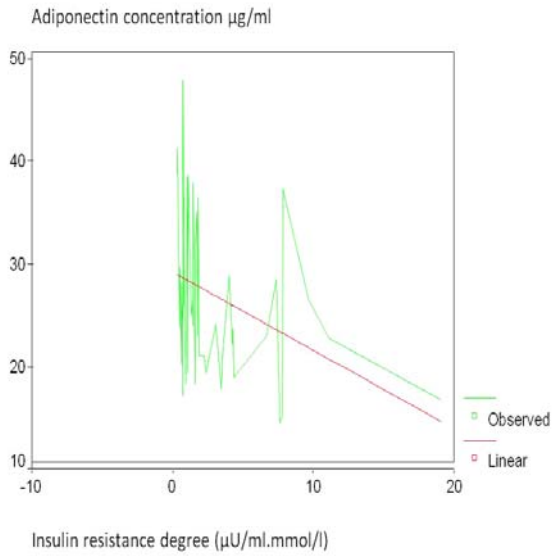

Figure 1: Correlation of adiponectin and insulin resistance degree after 2 weeks of treatment with *Rhazya stricta*

3.2.The Relationship between Adiponectin and Insulin Resistance:

Interestingly, analysis of data using Pearson correlation showed an inverse highly significant correlation between adiponectin levels and insulin resistance degrees after two weeks of treatment with *Rhazya stricta.* ($p < 0.01$, $r = -0.35$) as shown in figure 1.

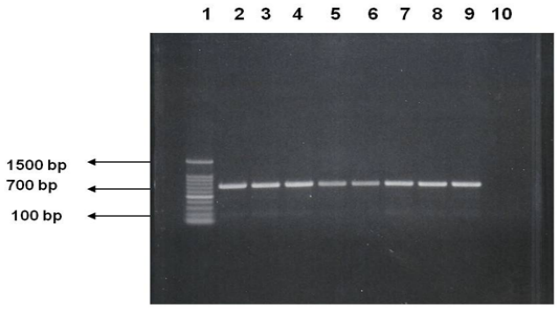

Figure 2: Gel electrophoresis image of PCR products separated on 0.8% agarose gel. Lane 1 is the DNA ladder, Lane 2-9 the 713-bp PCR products containing sequences for exon 3 (680-bp) of *Adipoq* gene. Lane 10 is the negative control (without DNA)

3.3.PCR and Gel Electrophoresis

The PCR amplification products were analyzed on 0.8% agarose gel. The electrophoresis of the PCR products is shown via an image of the ethidium bromide-stained agarose gel visualized under ultraviolet using Gel Documentation System (Figure 2). The figure shows PCR amplified bands of 713-bp containing sequences for exon 3 (680-bp) of the *Adipoq* gene.

3.4.Conformational Sensitive Gel electrophoresis (CSGE)

The PCR products were screened for mutations by conformation sensitive gel electrophoresis (CSGE). Figure 3 shows CSGE image of a 713-bp PCR product that contains the sequence for exon 3 of the *AdipoQ* gene. Lane 15 contains the control sample and Lane 1-14 contains PCR products from rats treated orally for 18 weeks with *Rhazya stricta* extracts mixed with PCR products from control rats after heteroduplexing. The CSGE analysis of the PCR products showed that there is no single-base mismatches (heteroduplexes) were detected by CSGE in a 713-bp PCR product that contains sequences for exon 3 of the *AdipoQ* gene. The results obtained showed that there is no mutation or polymorphism in exon 3 of AdipoQ genes.

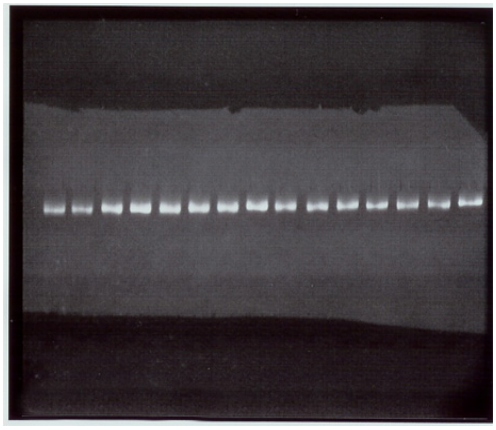

Figure 3: **CSGE image** of **a 713-bp PCR product that contains sequences for exon 3 of the *AdipoQ* gene. Fifteen lanes, lane 1-14 (from left to right) are PCR products from rats treated with the plant extract. Lane 15 is the control sample.**

4.DISCUSSION

Rhazya stricta is commonly used in folk medicine for the treatment of many diseases. In many studies, relatively large doses of the plant extract were used to determine the pharmacological and toxicological actions (Tanira *et al*., 1996b and Adam *et al*., 2002).

Recently, adiponectin has been identified as one of the adipocytokines with important metabolic

effects. It plays an important role in the development of insulin resistance and atherosclerosis. (Ekmecki and Ekmecki, 2006). It was important to realize that adipocytokine is circulating in concentrations exceeding the concentrations of any other known hormone, though still there is much uncertainty with regard to its primary physiological role especially in healthy subjects (Lihn *et al*., 2005).

The biochemical effects of *Rhazya stricta* aqueous leaves extract were examined in liver and kidney of rats by Baeshen *et al*. (2010). The same condition of the previous experiment was conducted in the present experiment to determine the effects of oral administration of aqueous leaves extract of *Rhazya stricta* on serum adiponectin concentrations and to study its molecular effects on exon 3 of adiponectin gene in rats. Also the relationship between serum adiponectin and insulin resistance after treatment with aqueous leaves extracts of *Rhazya stricta* in rats were estimated.

Oral administration of aqueous leaves extracts of *Rhazya stricta* evoked fluctuations in adiponectin levels during eighteen weeks period of treatment with *Rhazya stricta* leaves (Table 1). This increase in adiponectin concentration induced by the *Rhazya stricta* extract show the importance of using this plant as a medicinal treatment. However, the mode of modulating adiponectin concentration by *Rhazya stricta* is not clear till now.

Moreover, our results showed inverse significant correlations between adiponectin levels and serum insulin levels after week 1 and 2 of treatment with the plant extract. In agreement with our results, Wasim *et al*. (2006) reported an inverse significant correlation between adiponectin levels and insulin levels in a British South-Asian population with a high incidence of type 2 diabetes, cardiovascular disease, central obesity and metabolic syndrome.

Insulin resistance is an important risk factor for type II diabetes mellitus and cardiovascular disease (DeFronzo and Ferrannini, 1991). Therefore, we performed HOMA-IR to check the degree of insulin resistance in each rat. This method has been supported by other studies as a precise method for the assessment of insulin resistance (EL-Midaoui and de Champlain, 2002; Bonora, 2000).

The present work showed an inverse significant correlation between adiponectin and insulin resistance degrees in all treated rats after one week of treatment with *Rhazya stricta* leaves. Our finding is in consistent with previous studies that observed inverse correlation between adiponectin levels and insulin resistance (D'Anna et al., 2006; Farvid *et al*., 2006).

To our knowledge, this work is the first to study the effects of treatment with *Rhazya stricta* leaves on adipoQ gene (exon 3) and its protein (adiponectin). This study clearly shows that oral administration of *Rhazya stricta* leaves for eighteen weeks produced highly significant increases in adiponectin concentrations in treated rats especially on rats that were treated with the highest dose of the plant extract.

The adiponectin gene was shown to be associated with adiponectin levels in healthy Caucasians (Mackevics *et al*. 2006). It was reported that the presence of at least 1 non-synonymous mutation in exon3 showed evidence of association with adiponectin levels. (Cancello *et al*., 2004). The significance of the genetic variations in human adiponectin gene on its plasma concentrations and obesity were examined in Japanese subjects (Takahashi *et al*. 2000). Mutations in the adiponectin gene were screened by direct sequencing or restriction-fragment polymorphism. The levels of plasma adiponectin were determined by the enzyme-linked immunosorbent assay (ELISA). The results showed that, two nucleotide changes have been identfied in the adiponectin gene. G=T polymorphism in exon 2 was associated with neither plasma adiponectin concentrations nor the presence of obesity. A subject carrying missense mutation in exon 3 (R112C) showed markedly low plasma adiponectin concentration.

The frequency of adiponectin gene mutations in exon 3 of Polish origin patients with type 2 diabetes was 3.9%, while in the control group 0.98% and this difference was not statistically significant. It was also observed that adiponectin level is significantly lower in patients with C.331 TC mutation (Krętowski *et al*., 2005)

In summary, the studies published to date indicate that polymorphisms at the adiponectin locus (exon 3) are indeed predictors of circulating adiponectin levels, insulin sensitivity, and atherosclerosis, highlighting the pivotal role of this adipokine in the modulation of metabolism and atherogenesis (Menzaghi *et al*. 2007).

The technique of CSGE was developed for Detection of mutations in double-stranded DNA by gel electrophoresis (Ganguly and Prockop, 1995). Under the initially described conditions, no single-base mismatches (heteroduplexes) were detected by CSGE in a 713-bp PCR product that contains sequences for exon 3 of the AdipoQ gene. Since mean adiponectin levels were significantly higher ($p < 0.01$- $p < 0.001$) than the control group after 18 weeks of treatment with *Rhazya stricta* leaves extracts, and molecular analysis showed that no single base mismatches were

detected, these results indicated that no mutagenic action was practiced by *Rhazya stricta* extracts on exon- 3 of adipoQ gene . This does not exclude the possibility that it is mutagenic elsewhere in the genome of the rat since it was reported previously by Baeshin's team that it is mutagenic (Baeshin *et al*., 2005: 2009 a, b, c).

Therefore, a screening of the total genome of the rat for mutagenic action of *Rhazya stricta* liquid leaf extract will be revealing and this what it is being done in a current work in our laboratory. Furthermore a knockout mutation of the adipoQ gene will be more revealing in this respect and will be considered in future work. Adiponectin has drawn much attention because of its insulin-sensitizing and antiatherogenic actions, suggesting that genetic deficits in its production or action may contribute to insulin resistance and coronary artery disease (CAD) (Lacquemant *et al*., 2004). Diabetes is categorized into: Type 1- Insulin Dependent Diabetes Mellitus (IDDM) – which is an autoimmune destruction of Pancreatic β cells: Type 2: Non-insulin Dependent Diabetes Mellitus (NIDDM) – which is characterized by Insulin resistance in target tissues. The current focus of drugs discovery research in diabetes includes exploration of alternative medicines, discovery of new synthetic anti-diabetic agents as well as isolation of active compounds from plants which have been the source of traditional herbal medicines and have been documented and described for their anti-diabetic properties. A number of bioactive compounds have been isolated from plants which are potent α-glycosidase and /or α-amylase inhibitors and show good antidiabetic properties. The main phytochemicals with reported anti-diabetic activities were flavonoids, polyphenolic compounds, tannins, glycosides, alkaloids and terpenoids. A potent α-glycosidase inhibitor which helps in prevention of diabetes was isolated from water extract of leaves of mulberry trees (*Morus alba* L.) (Day, 2005). It is known that *Rhazya stricta* leaves extract contains the main phytochemicals with reported anti-diabetic activities such as: alkaloids, glycosides, flavonoides, tannins and triterpenes (Al-yahya *et al*., 1990: Badreldin *et al*., 2000: Szabó, 2008).

The possibility that some of the claimed therapeutic actions of *Rhazya stricta* extract may be due to immunomodulatory capacity. The alkaloidal fraction of *Rhazya stricta* significantly increased the production of IL-1 and TNF-α (Tanira *et al.,* 1998). On the other hand, it was reported that there is a highly significant correlation between IL-1 and TNF-α levels and adiponectin concentrations (Lihn *et al.,* 2003).

The effect of *Rhazya stricta* leaves extract on rat adiponectin level concentration could be a promising issue (after avoiding its possible mutagenic effects) in treating human diabetes, coronary artery disease, as well as inflammatory conditions. Future studies will be conducted for studying the mode of action of separated alkaloids of *Rhazya stricta* extract on whole adipo Q gene, adiponectin receptor loci and the identification of additional genes which might be involved in the regulation of serum adiponectin levels.

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