High fat diet induced insulin resistance and elevated retinol binding protein 4 in female rats; treatment and protection with *Berberis vulgaris* extract and vitamin A

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Abstract: This research was conducted to investigate two main aims; the first aim was to find if there is a relationship between insulin resistance (IR) and retinol binding protein 4 (RBP4). The second aim was to use *berberis vulgaris* extract and vitamin A as protective and/or curative agents against insulin resistance. IR was developed by feeding the female rats a high fat diet (HFD) for six weeks then treating or protecting them with *b. vulgaris* extract (0.2 g/Kg body weight) or vitamin A (12.8µg/Kg/day) for two weeks. *Results:* HFD intake elevated insulin level and RBP4 expression that associated with hyperglycemia and hyperlipidemia. Co-administration of vitamin A and *B. vulgaris* extracts reduced blood glucose level, insulin, body weight and RBP4 expression before, during and after HFD. Furthermore, vitamin A reduced the blood glucose, triglycerides (TG) and cholesterol levels. IR syndrome associated with the RBP 4 alteration that gives high indication about the role of RBP4 expression in the IR progression and development. Furthermore, the treatment with vitamin A and/or *b. vulgaris* alleviated the IR syndrome through the action on RBP4 and Insulin secretion. On the other hand, vitamin A must be avoided for the predisposed IR and prediabetic patients.

Keywords: Insulin resistance, retinol binding protein 4, *Berberis vulgaris*, vitamin A, obesity, diabetes.

INTRODUCTION

The emergence of the metabolic syndrome with frightful consequences to the health of humans, worldwide, took the scientists attention in the new millennium. The metabolic syndrome or diabesity (Astrup and Finer, 2000) describes the diabetes incidence elevation combining with obesity which resulting of human behavior, nutritional availability, and the adoption of more sedentary lifestyles alterations (Basciano *et al*., 2005). Chronic obese state is implicated in a variety of disease such as diabetes, cardiovascular disease, fertilization problems, neurology-cal disorders and certain cancers (Waldron, 2007). Insulin resistance (IR) accounts as the main important factor in diabetes pathogenesity because it precedes the onset of type 2 diabetes (Stumvoll and Haring, 2001).

Type 2 diabetes

Accounts as healthy and economically serious problem faced the worldwide as it is faster growing and more costly disorders than any other disease. Female is more vulnerable to diabetes incidence than male where the male: female incidence of diabetes is 0.67 (Ekoe *et al*., 2001).

Retinol binding protein 4 (RBP4)

Produces from liver and adipose tissue and it is encoded by chromosome 10q23-q24. It impairs insulin sensitivity throughout the body. Therefore, RBP4 is classified as fat derived peptides, which modulate glucose homeostasis (Yang *et al*., 2005). As RBP4 is highly expressed in visceral adipose tissue, it is accounted as intra-abdominal fat mass marker as well as diabetes and cardiovascular disease progression predictors (Maria *et al*., 2008). Chenhui and Jiahua (2008) stated that plasma RBP4 is correlated with the magnitude of IR and age. Also, it is positively associated with serum triglyceride, systolic blood pressure and other components of metabolic syndrome.

After long term administration, approximately, all oral hypoglycemic agents have failed in diabetes treatment. Therefore, the need of new long acting term oral medications has become an urgent necessity for control of blood glucose in patient with type 2 diabetes mellitus (Jun *et al*., 2008). The metabolic risk factors treatment is based on combination therapy of antihypertensive agents, hypoglycemic drugs and lipid lowering drugs. Unfortunately, there are several problems raised from these complicated therapeutic regimens due to polypharmaceutical problems of adverse effects, drug-drug interaction, failure of adherence and medication errors (Wei-Jia *et al*., 2009).

Since 3,000 years, *Berberis vulgaris* as well as other berberine (BER) containing plants are medicinally used in approximately all-traditional medicine (Timothy *et al*.,

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1997). Barberry is recommended as food additive and its juice is described to cure cholecytitis (Khosrokhavar *et al*., 2010). Phytochemical analysis of root or stem bark extract of *B. vulgaris* demonstrated the presence of protoberberines and bisbenzyl-isoquinoline alkaloids (berbamine, tetrandrine and chondocurine) for which anti-inflammatory and immuno-suppressive activities have also been well established (Golzarand *et al*., 2008). All barberry parts have been medically reported, because they can be used as tonic, antimicrobial, antiemetic antipyretic, antipruritic, antioxidant, anti-inflammatory, hypotensive, antiarrhythmic, sedative, antinociceptive, anticholinergic and cholagogue actions. Furthermore, barberry has been used in the treatment of some cases combined with jaundice like cholecystitis, cholelithiasis, gall stones and dysentery, and in the treatment of infection with leishmaniasis and malaria (Khosrokhavar *et al*., 2010).

As sulphonureas or metformin actions, BER dministration was reduced blood glucose level in diabetic patients even at fasting or postprandial blood glucose detection (Yin *et al*., 2008). Furthermore, BER administration reduced weight gain and enhanced insulin sensitivity. At animal level, BER intake successfully decreased body weight, fasting blood sugar, postprandial blood sugar, fasting insulin and homeostasis model assessment (HOMA IR) in obese rats fed on high fat diet (Yin *et al*., 2004). Ko *et al*., (2005) stated that BER administration prevented fatty liver incidence through decreasing serum triglycerides, and triglyceride deposition in liver and muscle.

Vitamin A is an essential nutrient that is required for normal growth, epithelial differentiation, fetal development, vertebrate morphogenesis, spermatogenesis, night vision and a variety of other functions. It also has an immune-regulatory role (Marc, 2004). Several investigators have shown that DM2 patients have higher serum vitamin A concentration than normal subjects (Anna *et al*., 1997). Sasaki *et al*. (1995) and Anna *et al*. (1997) have shown that there is no correlation between serum vitamin A concentration and hypertriglyceridemia in diabetic patients.

This research was conducted to investigate two main aims; the first aim was to find if there is a relationship between insulin resistance and retinol binding protein 4 (RBP4). The second aim was to protect from and/or treat IR by using natural product (berberis and vitamin A).

MATERIALS AND METHODS

Materials

Berberis were collected from the fields and authenticated by Prof. Salma Eldareir, Botany Department, Alexandria University, Egypt. Firstly the intact plant was removed from the soil (faculty herbarium), roots were washed more than one time and the plant was firmly pressed between paper towels. Then the plant roots were separated, packed within sealable plastic bags and kept out of direct sunlight.

Trichloroacetic acid (TCA), thiobarbituric acid (TBA), Sulfosalsalyic acid, reduced glutathione (GSH) and 5,5′-dithiobis 2-nitrobenzoic acid (DTNB), were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Organic solvents; ethanol 95%, methanol and petroleum ether of HPLC-grade were brought from Merck (USA). Kits of cholesterol and HDL were purchased from Human (Germany), Glucose and Triglycerides were bought from Spinreact (Spain), ELISA kit of insulin and RBP4 were purchased DRG (USA) and Assaypro (USA) respectively. All other chemicals and reagents were of analytical grade.

Animals

Female rats were obtained from department of biochemistry, Faculty of Medicine, University of Tanta. The rats aged (8 - 10) weeks old and weighted (80-110 g) were used in this study. The animals were housed (4 rats/ cage) and they were allowed free access to pelleted food and tap water for one week before treatment. All animals were kept under conventional conditions of temperature, humidity and light. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals, National Institutes of Health; NIH (Institute of Laboratory Animal Resources, 1996).

Berberis vulgaris crude extract preparation

The dried powdery roots of berberis were exhaustively defatted with petroleum ether. The dried residue was dried in air then extracted with ethanol for 8 hours using Soxhlet apparatus. The ethanolic extract was concentrated to minimum volume using rotary evaporator (BÜCHI, Switzerland) then lyophilized (DISHI, DS-FD-SH10, Xi'an Heb Biotechnology Co, China) to obtain a powder barberry crude extract (El Sayed *et al.,* 2011).

Diet

The prepared high fat diet (HFD) was contained 20 g of fat /100 g of diet (19 g butter oil and 1 g of soybean oil to provide essential fatty acids) and provided 19.34 KJ/g of diet, including 7.74 KJ/g as fat. On the other hand, the low fat (LFD) diet contained 3 g of butter oil and 1 g of soybean oil/ 100 g of diet provided 16.12 KJ/g of diet, including 1.29 KJ as fat. Because the emphasis in these experiments was on dietary fat, the amount of protein and all of the essential minerals and vitamins required for rats 30 per KJ for HFD and LFD diets (table 1).

Animal treatment

Animals were divided into six groups (each of 8 rats), Group 1 (Control group), rats of this group were fed LFD, Group 2 fed HFD only, Group 3 fed HFD then orally given berberis crude extract powder dissolved in water (0.2 g/ Kg body weight) for six weeks, Group 4 fed HFD

then orally given vitamin A dissolved in corn oil (12.8µg/ Kg/day) for six weeks, Group 5 fed HFD for six weeks then orally treated with berberis crude extract powder dissolved in water (0.2 g/Kg body weight) for another 2 weeks and Group 6 fed HFD for six weeks then orally treated with 12.8µg/Kg/ day of vitamin A dissolved in corn oil for another 2 weeks. All animals were fasted for 12 hours before scarification. After complete anesthesia, the abdominal cavity was rapidly opened following the median line of the abdomen. Blood was collected, and then centrifuged at 3000 rpm for 10 min, to get serum from all groups. The obtained sera were kept at -20°C until analyzed.

Table 1: High fat diet and low fat diet compositions.

Component	High fat diet (g%)	Low fat diet (g%)
Casein	19.61	17.32
Corn starch	36.24	56.39
Sucrose	10.75	12.37
Cellulose	7	6.18
Butter oil	19	3
Soybean	1	1
Vitamin mix	1.27	1.24
L–cystine	0.25	0.22
Acetyl choline	0.27	0.3
Cholesterol	0.59	----

Liver tissues were removed quickly and washed in cold saline, then cut into small pieces. 1/gm of liver pieces was homogenized in 9 ml phosphate buffer (0.1 M, pH 7.4) to prepare a liver homogenate. The liver homogenate was centrifuged at 3000 rpm and the clear supernatant was collected to perform the RBP4, TBARS and GSH assays.

The standardized methods for determination of serum glucose level (Hjelm and De Verdier, 1963), serum cholesterol level (Watson, 1960), serum triglyceride level (Fossati, 1982), serum HDL cholesterol level (Grove, 1979), serum LDL level (Friedewald *et al*., 1972), liver thiobarbituric acid-reactive substance in liver (Tappel and Zalkin, 1959), liver GSH (Jollow *et al.*, 1978), liver retinol binding protein-4 (RBP4) level (Bernard *et al*., 1982), blood insulin level (Jerome and Barbara, 1973) and HOMA IR (Turner *et al.,* 1979) were carried out.

STATISTICAL ANALYSIS

Data were analyzed by one-way analysis of variance (ANOVA) using Primer of Biostatistics (Version 5) software program. Significance of means ± SD was detected among groups by using multiple comparisons Student-Newman-keuls test at $p<0.05$.

RESULTS

table 2 showed that the feeding on HFD for 6 weeks increased the serum lipid profile in the rats than that of control levels where the cholesterol, TG and LDL levels were significantly increased by 68.1, 91.22 and 153% over control level, while the HDL level was decreased by 36.85% than that of control group, at $p<0.05$. The co-administration of *berberis* crude extract or vitamin A before and after HFD intake prevented diet adverse effects as the HDL and LDL levels were kept in control levels while the cholesterol and TG levels were markedly decreased than that of HFD rats group but they were still slightly higher than normal level , at $p<0.05$. Furthermore, HFD intake for 6 weeks increased the serum TBARS level that was accompanied with high GSH level than those of control levels, $p<0.05$. The protection or the treatment with berberis crude extract or vitamin A maintained TBARS in the normal level. On the other hand, co-administration of *berberis* crude extract normalized GSH level, while vitamin A co-administration failed to normalized GSH level, at $p <0.05$.

There was a marked significant increase (20.75%) in the body weight (BW) of the HFD rats when compared to control group. The body weight of protected group and treated group with *berberis* was significantly lower than that of HFD (13.58 and 11.18%, respectively), as shown in table 3, at $p<0.05$. On the other hand, the protection and treatment with vitamin A showed BW similar to HFD level.

Fasting serum insulin and glucose levels in the study animals were also measured. Untreated rats fed with HFD for 6 weeks demonstrated a significant increase of fasting serum insulin and glucose levels by 98.93% and 274.8 %, respectively, as compared with normal control (table 3), at $p<0.05$. Protection of animals with *berberis* crude extract significantly reduced the serum insulin level and glucose level than those of HDF group. Moreover, the treatment of animals with *berberis* crude extract normalized both of the serum insulin and glucose levels, $p<0.05$. On the other hand, the vitamin A protection failed to maintain the blood glucose and insulin levels but the treatment with it successfully normalized these levels. The same pattern was shown for HOMA IR which elevated in case of HFD intake and protected group with vitamin A. HOMA IR was normalized in case of protection with *berberis* crude extract and treatment with both *berberis* crude extract and vitamin A. Moreover, The HFD intake was increased the level of RBP4 three times more than control level as showed in table 3. The co-administration with *berberis* crude extract and vitamin A as a protective or curative agents prevented this positively liver RBP4 increment, at $p<0.05$.

DISCUSSION

It is reported that HDF intake induced insulin resistance through its increased muscle TG content, visceral fat accumulation in the whole body and muscle insulin

Table 2: Effect of berberis crude extract on lipid profile during the prevention and treatment of IR-induced-experimental animal

	Control 1	High fat diet 2	HFD+ Berberis crude extract 3	Berberis crude extract treatment 4	HFD+ Vitamin A 5	Vitamin A treatment 6
Cholesterol	168.7±7.8	283.6±24.4*	188.1±9.1*‡	190±8.9*‡	179.3±7.1*‡	183.8±10.6*‡
Triglyceride	125.4±20	239.8±23.2*	160.8±14.7*‡	161.5±15.2*‡	168.6±13*‡	151.5±11.3*‡
HDL	66.2±10	41.8±7.5*	63.2±7.7‡	49.1±5.9*	61±7.1‡	61.4±7.5‡
LDL	77.9±11.4	197.1±17.1*	82.6±4.9‡	97.6±17.6*‡	85.6±5.4‡	84.4±7.6‡
TBARS	3.96±0.29	4.9±0.35*	4.01±0.41‡	4.1±0.18‡	4.1±0.12‡	4.2±0.42‡
GSH	0.177±0.01	0.198±0.01*	0.178±0.01‡	0.181±0.016‡	0.193±0.013*	0.193±0.008*

Table 3: Effect of berberis crude extract on body weight, fasting glucose and insulin level, HOMA IR and RBP4 during the prevention and treatment of IR-induced- experimental animal.

	Control 1	High fat diet 2	HFD+ Berberis crude extract 3	Berberis crude extract treatment 4	HFD+ Vitamin A 5	Vitamin A treatment 6
Weight	172.5±25.3	208.3±14.7*	180±25.8‡	185±15.1‡	202.5±10.8*	202±8.3*
Fasting Glucose	63.9±13.9	239.5±19.6*	66.8±6.1‡	90.1±6.9‡*	279.1±22.7‡*	69±10.2‡
Fasting insulin	9.4±1.6	18.7±3.1*	10.4±1.3‡	9.9±2‡	13.6±2.1*‡	9.5±0.9‡
HOMA- IR	1.67± 0.17	11.05± 2.01*	1.7± 0.15‡	2.2±0.26‡	9.24±0.72*	1.68±0.18‡
RBP4	193.5±49.7	570±90.8*	252.5±81.3‡	251.6±81.6‡	323±88.3‡*	226±70.4‡

1: Six healthy rats were fed low fat diet for six weeks.
2: Six rats were fed high fat diet for six weeks.
3: Eight rats were fed high fat diet and given water suspension of 0.2 g/Kg/day berberis crude extract orally for six weeks.
4: Six rats were fed high fat diet for six weeks then were treated with water suspension of 0.2 g/Kg/day berberis crude extract orally for 2 weeks.
5: Six rats were fed high fat diet and given vitamin A in corn oil 12.5µg/Kg/day orally for six weeks.
6: Six rats were fed high fat diet for six weeks then were treated with vitamin A dissolve in corn oil 12.5 5µg/Kg/day orally for two weeks.
* There is significant difference with control, at $P<0.05$.
‡ There is significant difference with High Fat diet, at $P<0.05$.

resistance which all lead to hyperlipedemia and hyperinsulinemia (Jong-Yong *et al*., 2000; Ji *et al*., 2011). In agreement our data proved that HFD intake increased the body weight, lead to hyperinsulinemia associated with hyperglycemia and hyperlipedemia.

There are many mechanisms by which glucose and excess fat can cause distortions in the transfer of glucose and insulin action that developing T2DM as follows; excess fat intake causes IR in muscle and adipose tissue that stimulates gluconeogenesis and resulting in excess glucose supply which by timing has worsened to peripheral IR (So-Young *et al.,* 2001). It is well known that when hyperglycemia took place this is accompanied with the increase in cholesterol, LDL, TG and fall of HDL (Sharma *et al.,* 2003) that may be contributed to the development of coronary artery disease (Li- Qin *et al*., 2006). In the present study, hepatica and serum TBARS level was increased in the HFD group. It is reported that, hyperglycemia and hypertriglyceridemia evoke the hepatic lipid peroxidation due to β-oxidation overload that is characterized by high TBARS level (Shanmugam *et al*., 2009).

Adipokinases affect the whole body insulin sensitivity through its action in glucose and lipid metabolism (Barbara *et al*., 2007) therefore; their secretion is changed specifically in IR and obese patients (Beverly *et al*., 2010). RBP4 which regulates adipocytes glucose intake with cellular insulin sensitivity, contributes to systemic IR (Chenhui and Jiahua, 2008).

Our data demonstrated that, rats fed on HFD had hyperglycemia, high RBP4 level, these findings emphasized the direct correlation between RBP4 and hyperglycemia incidence. RBP4 was linked with hepatocytes IR, which increased hepatic glucose output, and aggravated the dyslipidemia (Haiya *et al*., 2008). Therefore, RBP4 could be accounted as a key player in diabetes pathogenesis. In support to this thought, Yang *et al*. (2005) found that there was a correlation between serum RBP4 levels elevation and obesity and T2DM incidence.

RBP4 inhibits the phosphorylation of insulin receptor substrate 1 and the activation of phosphoinositide 3-kinase, while increases phosophoenolpyruvate carboxy kinase activity which stimulates hepatocytes' glucose biosynthesis (Jun- Bin *et al*., 2010). Furthermore, RBP4 induces the expression of the gluconeogenic enzyme phosphoenolpyruvate carboxy kinase in the liver (Yang *et al*., 2005). Moreover, Timothy *et al*. (2006) stated that

high RBP4 serum level down regulates GLUT4 expression in adipocyte which in turn contributes to systemic IR development or worsened.

Additional, lipid metabolism also altered the RBP4 expression where Yang *et al.,* (2005) proved that the serum level of free fatty acid diminished in both RBP4 knockout and RBP4 heterozygous mice. In our study, we found that hyperglycemic rats had hypertriglyceridemia, which is in agreement with Haiya *et al*. (2008) whom stated that RBP4 plays a crucial role in lipid metabolism that achieving through study the correlation between RBP4 and IR related factors (TG and HDL). Lipid accumulation plays an important role in inducing IR in skeletal muscle and liver (Giorgio, 2006). It is well known that, insulin inhibits fat mobilization; therefore, it has been thought that obese adipocytes enlargement may be arisen from resistant to the antilipolytic effect of insulin, which resulted in the increased reflux of free fatty acid into blood (Gijs, 2008). Our data showed a strong positive correlation between RBP4 and TG and a negative correlation between RBP4 and HDL. That could be resulted from the direct effect of RBP4 on hepatocytes lipogenesis through the overproduction of TG in liver and release vLDL into blood plasma (Gijs, 2008).

Regardless of IR related disorders were discovered since ancient times, it takes the scientists attention of the present time due to the high-accompanying morbidity and mortality incidence. Therefore, the finding of a cheap and effective treatment strategy with low side effects and risk incidence against IR is one of the most serious topics in IR research area. We observed that *berberis* crude extract showed a protective and/or curative capacity with markedly hypoglycemic, hypolipidemic, and antioxidant properties against IR incidence and progression.

In our previous published data we approved that the activity of extract could be due to the presence of tannins, saponins, flavonoids, alkaloids and steroids components (El Sayed *et al*., 2011). Berberine, the main *berberis* crude extract active constituent, activates the adenosine monophosphate activated protein kinase (AMPK) which reduces IR Through enhancement of adipocytes' Glucose uptake. BRB also increases the expression of hepatocytes insulin receptor and improves cellular glucose consumption (Kong *et al*., 2009). Furthermore, BRB increases GLUT4 translocation in adipocytes (Lee *et al*., 2006).

The berberis crude extract hypolipidemic effect that observed in our treatments could be returned to the presence of berberine that reported to activate extracellular signal regulated kinase pathway which in turn; increases the LDL receptor (LDLR) expression at the posttranscriptional level (Kong *et al*., 2009). Moreover, BRB reduces hepatocytes lipid synthesis through AMPK activation and decreases PPARα mRNA and protein expression, which emphasis the hypotriglyceridemic effect of BRB (Wei-Jia *et al*., 2009).

The pathogenesis of T2DM and its complication is linked with free radicals production that induced tissue damage. Vitamin A acts as a potent antioxidant preventing the free radicals adverse effects over lipid membrane (Marc, 2004). In our study, treatment of HFD group with vitamin A reduced the blood glucose, TG and cholesterol levels as shown in tables 2 and 3.

The functions of RBP4 are hepatic vitamin A stores mobilization and retinol deliverable to peripheral tissue (Beverly *et al*., 2010). RBP4 concentration is positively and significantly correlated with total vitamin A intake. Recently, it was found that high vitamin A intake increased the expression of RBP4 to transport, store and metabolize this vitamin (Jun-Bin *et al*., 2010).

Our results showed that vitamin A as a protective or curative agent was able to decrease the increment in RBP4 liver level. Unfortunately, our results show that vitamin A has no protective role against IR or T2DM incidence. It has been suggested that high insulin level increases serum vitamin A concentration by decreasing hepatocytes vitamin A stores (Anna *et al*., 1997). Furthermore, it is possible that higher TG concentration was contributed to the differences in vitamin A levels. Multiple regression analysis showed that serum TG was associated independently with serum vitamin A levels (Anna *et al*., 1997).

CONCLUSION

HFD intake leads to IR syndrome and T2DM progression that associated with the alteration in RBP4 with high indication about the role of RBP4 in the IR progression and development. Also, we concluded that the treatment with vitamin A and/or *berberis* crude extract as a natural product can alleviate the IR syndrome and act as hypoglycemic and Hypolipidemic through their action on RBP4 and Insulin secretion. On the other hand, vitamin A must be avoided for the predisposed individual to IR and prediabetic patients. Otherwise, berberine had a powerful protection role.

Authors' contribution

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; GD and TH conducted the experiments, EM and GD wrote the manuscript.

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