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HYPOGLYCEMIC EFFECT OF LUPIN ALKALOIDS AND/OR ROSIGLITAZONE IN PREGNANT DIABETIC RATS IN RELATION TO ATHEROGENIC INDEX

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ABSTRACT

Objective: This study aims to evaluate the changes in homeostasis model assessment of insulin resistance (HOMA- IR) and indices of atherogenic lipid profile parameters in pregnant diabetic rats after the administration of rosiglitazone and /or alkaloid lupin extract.

Methods: Blood glucose, hemoglobin content (Hb), lipid profile, insulin and glucagon concentrations were investigated on 20th day of gestation.

Results: The treated groups showed the improvement of HOMA-IR and The control of the hyperglycemic status. Also, there are an increase in insulin concentration, and a decrease in glucagon concentration in all treated groups, in comparison to, STZ group. Meanwhile, it showed alternation in the atherogenic profile in rosiglitazone treated groups, in comparison to, lupin treated groups. The correlations among (HOMA-IR and HDL), (AIP and LDL) and (glucose and Hb) were observed in our study at (r = 0.594, p< 0.01), (r = 0.690, p< 0.01), and (r = 0.811, p< 0.01) respectively.

Conclusion: The usage of lupin alkaloids to treat type 2 diabetes during the pregnancy is more favorable to avoid rosiglitazone side effects. More studies are needed to explore the effect of rosiglitazone on lipids and cardiovascular risk.

Keywords: Atherogenic index (AIP), Homeostasis model assessment of insulin resistance (HOMA-IR), Type 2 diabetes, Lupin alkaloids, rosiglitazone.

INTRODUCTION

Diabetes (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion and/ or insulin action. It can be classified into; type 1 (T1DM), type 2 (T2DM), gestational diabetes and other specific types of diabetes (1).

Diabetes is the most common pre-existing medical condition complicating pregnancy. In a retrospective study of 175,249 pregnancies, the prevalence of pregestational diabetes rose 1% from 1999 to 2005 (2, 3, 4). T2DM causes higher rates of maternal and fetal morbidity, because it constitutes an unfavorable environment for the fetal development (5, 6, 7). Maternal diabetes complications related to glycaemic control or diabetes complications are hypoglycaemia, diabetic ketoacidosis, gastropathy, retinopathy, nephropathy and coronary heart disease (8). Spontaneous abortion in poorly controlled diabetic women is thought to be secondary to hyperglycemia (9). In early gestation period, there are normally many metabolic changes lead to insulin resistance; the cells fail to respond to insulin (10). This can be reversed after delivery, but it is irreversible in T2DM. This due to the insulin resistance increases the demand for insulin secretion which leads to β cell malfunction, de-differentiation, and death (11, 12, 13). Atherogenic Index of Plasma (AIP) is used as a marker of plasma atherogenicity and quantifies the response to therapeutic intervention (14). The AIP is more effective than single lipid parameters in identifying atherogenic risk (15).

Insulin resistance is accompanied by atherogenic lipid profile. It interferes with lipid metabolism leading to atherosclerosis, hypertension, and cardiovascular disease (16). More common clinical measure of fasting glucose and insulin is (HOMA-IR). This model based on the theory of a feedback loop between β cells and the liver (17, 18). On the other hand, the (AIP) is used as a marker of plasma atherogenicity and quantifies the response to therapeutic intervention (14).A number of drugs exists to improve glycemic control that acts on the β-cells or peripheral tissues. Despite current treatments, pregnant women with either T1DM or T2DM are at increased risk of pregnancy complications (5, 19). Thiazolidinediones

(TZDs), including Rosiglitazone increase insulin sensitivity in muscles; these agents have a primary effect to alter gene regulation in adipose tissue through proliferator-activated receptor ɣ (PPAR ɣ) gene (20, 21).

Treatment with Rosiglitazone is associated with body weight gain and fluid retention which can progress to edema and congestive heart failure (22). So, it was withdrawn from the market in many countries and its use is restricted in the USA (23). The U. S. The food and Drug Administration (FDA) have determined that recent data for Rosiglitazone-containing drugs do not show an increased risk of heart attack compared to metformin and sulfonylurea. So FDA is requiring removal of the prescribing and dispensing restrictions for Rosiglitazone medicines that were put in 2010 (24). Traditional ethnic medicine has used extracts of medicinal plants to treat diabetes (25). *Lupinus* species and their derivates are good candidates to be used as hypoglycaemic agents (26). Lupin belongs to the *Leguminosae* (or *Fabaceae*) family, which includes over 450 species. Only four *Lupinus* species are cultivated, one of them is *Lupinus albus* (white lupin). There are more than 150 alkaloid lupin 150 lupin alkaloids are known at concentrations up to 6 % (27). Bitter lupin seeds have a hypolipidemic effect which may prevent the increase in lipid peroxidation, therefore depleting GSH in hypercholesterolemia. So, it can protect against coronary heart diseases and cardiovascular diseases (28).

Therefore, the present study aims to evaluate the changes in HOMA-IR and indices of atherogenic lipid profile parameters in pregnant diabetic rats after the administration of alkaloid lupin extracts and/or Rosiglitazone.

MATERIALS AND METHODS

Materials

Streptozotocin: Streptozotocin (STZ, 2-deoxy-2 (((methyl nitrosoamino) carbonyl) amino) -D-glucopyranose) is synthesized by streptomycetes achromogenes and it is used to induce non-insulin-dependent diabetes mellitus (29). The drug was purchased from (Sigma Aldrich, USA).

Rosiglitazone

Rosiglitazone (AVANDIA ®, AVA) is an antihyperglycemic agent (30). The drug was purchased from a local pharmacy. It was manufactured by Galaxo Wellcome Cairo, Egypt, each tablet 4 mg. Tablets were then dissolved in distilled water; the drug was given in a dose level of 0.58 mg/100g of body weight.

Extraction of the alkaloids from the seeds of Lupin

Powdered seeds (1 Kg) were defatted with petroleum ether, extracted with ethyl alcohol (70%) by cold percolation till exhaustion (3liters). The alcoholic extract obtained was evaporated under reduced pressure. The alcoholic extract (5 Kg) was dissolved in chloroform and passed on column (50x 3 cm) packed with alumina. The alkaloids were eluted with chloroform -methanol (3:2) till elution complete. The collected eluates were evaporated at temperatures not exceeding 45 °C, and the residue (1g) represents a fraction of total alkaloids (31) the daily dose 500 mg/kg of body weight (32).

Experimental animals

Eighty female albino (*rattus albus*) rats weighing from 140 to 200 g were used. Animals were obtained from the animal lab of the holding company for biological products & vaccines [VACSERA], Helwan, Egypt. The animals were kept under normal conditions through the whole experimental period. All animal treatments were conducted according to the Ethics Committee of the National Research Center and in accordance with the recommendations for the proper care and use of laboratory animals (NIH Publication No. 85-23, revised 1985) in accordance with international ethical considerations.

Induction of the diabetes

Rats that had undergone one week as an adjustment period were kept under fasting for a minimum of 12 h, and the intraperitoneal injection of sterptozotocin (STZ) diluted in 0.01M citrate buffer anhydrous (PH 4.5) was administered at 50 mg/kg of body weight. Blood collected from the tail vein was used to confirm the induction of diabetes, and the rats with fasting blood glucose levels (200 mg/dL) were used for further experiments (33).

Pregnancy

The estrus cycles were examined by vaginal smears, and at the right point mature virgin females were mated over night with male rats of proven fertility (two females with one male in each cage). The next morning, the mating was confirmed by the presence of a vaginal plug (34). Also, vaginal smears were examined for the presence of sperm. The day of detection of a vaginal plug or sperm-positive smear was designated Day 0.5 postcoitum (35).

Experimental design

The animals were divided into eight groups. Control (C): untreated pregnant female rats, Control lupin (Lupin): received orally lupin alkaloids extract 0.5 mg/kg during the gestation period, Control Rosiglitazone (Rosi): received orally Rosiglitazone 4mg/kg during the gestation period, Control buffer (Buffer): injected intraperitoneally (i. p.) with 0.01M citrate buffer anhydrous (PH 4.5), Diabetic control (STZ): female rats injected (i. p.) STZ 50 mg/kg of body weight dissolved in 0.01M citrate buffer anhydrous (PH 4.5), Diabetic Rosiglitazone (STZ+Rosi): received orally Rosiglitazone 4mg/kg during the gestation period, Diabetic lupin (STZ+Lupin): received orally lupin alkaloids extract 0.5 mg/kg during the gestation period, Diabetic lupin+ Rosiglitazone (STZ+ Rosi+ lupin): were received orally Rosiglitazone 4mg/kg during the 1st and 3rdtrimester, and lupin alkaloids extract 0.5 mg/kg during the 2nd trimester.

Blood sampling

Animals were anesthetized and scarified on the 20th day of gestation. Blood samples were collected by heart puncture. Serum sample was separated by centrifugation at 3000 r. p. m. for 15 minutes and then collected and stored in a deep freezer for the prospective biochemical analysis.

Biochemical analysis

Blood Hb was determined (Diamond diagnostics reagent kit) by Drabkin colorimetric method (36). Glucose was determined (Diamond Diagnostics reagent kit (37). Serum cholesterol level was measured (Spectrum Diagnostic kit) (38). Serum high density lipoprotein cholesterol (HDL cholesterol) level was measured (Spectrum diagnostic kit) (39). Serum triglycerides were measured by spectrum diagnostic kit (40). Serum insulin was determined (WKEA Med Supplies Corp Elisa kit) (41). Serum glucagon was determined (WKEA Med Supplies Corp Elisa Kit) (41). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from this equation (42): HOMA- IR= fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5.

Low density lipoprotein cholesterol (LDL-C) concentration was calculated using the Friedewald formula (43):

LDL-C (mg/dl) = [total cholesterol − HDL-C − triglyceride/5].

The (AIP), which negatively correlates well with LDL particle size, was calculated from this equation (44):

AIP = log [triglyceride (mg/dl)/HDL-C (mg/dl)].

Statistical analysis

Results were expressed as mean ± SE. Data was analyzed by one way ANOVA. The differences between means were tested at P < 0.05 by least significant test (LSD). In all statistical tests, the probability level (P <0.05) was considered significant. Spearman correlation coefficient was used to determine the relationship between different variables. All analysis was made by SPSS version 16.0 for windows (Statistical package for Social Science, Chicago, USA)

RESULTS

Rosiglitazone and/or lupin alkaloids effects on Glucose, Insulin, Glucagon, Hb concentrations and HOMA-IR in pregnant diabetic rats were represented in Table (1).

The plasma glucose concentration was increased significantly in the STZ group, in comparison to normal control group. There was a marked decrease in plasma glucose concentration in the treated diabetic groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi), when compared to the diabetic control.

The plasma insulin concentration was showed a marked decrease in STZ group from the normal control group. As a result of the treatment with rosiglitazone and/or lupin, the plasma insulin concentration was increased in all treated groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi), in comparison to STZ group. Insulin concentration in Lupin, Rosi, and buffer groups were significantly higher (p < 0.05) than STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi groups.

Glucagon concentration in STZ group was highly increased from that of the control group. But, it was not significant in Lupin, Rosi, and buffer groups. On the other hand, (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi groups) showed a significant decrease in glucagon concentration from that of the normal. There was a significant (p< 0.05) increase in the glucagon concentration between Lupin, Rosi, and buffer groups and STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi groups. Also, it was observed a significant (p < 0.05) change between STZ+ Rosi group and STZ+ lupin+ Rosi group.

HOMA-IR was significantly (p < 0.05) increased in diabetic group, in comparision to, the normal control value. The STZ group was significantly increased (p < 0.05) compared with diabetic treated groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi groups), and non diabetic treated groups (Lupin, Rosi, and buffer). Also, there was a significant (p < 0.05) decrease in the HOMA-IR in (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi) groups, compared with (Lupin, Rosi, and buffer) groups.

The HbA concentration was markedly decreased in STZ group from all treated groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi) and control groups (Lupin, Rosi, and buffer). While the treatment of the lupin and both of lupin and rosiglitazone caused a significant (p < 0.05) increase in the HbA concentration in comparison to STZ group.

But the treatment with rosiglitazone in STZ+ Rosi group didn't induce a significant (p < 0.05) amelioration of HbA concentration as in STZ+ lupin and STZ+ lupin +Rosi groups.

The changes in lipid profile (Total cholesterol, Triglycerides, HDL, and LDL) and the atherogenic index (AIP) between all experimental groups were represented as mean ± SE in Table (2).

The total cholesterol concentration was significantly (p< 0.05) increased in the STZ group from that of the control group.

There was no significant (p< 0.05) change between normal control group and both of lupin and buffer groups while, there was a significant (p< 0.05) increase in Rosi group and STZ + Rosi group from that of the normal.

While the treatment with lupin in STZ+ lupin group, and combination between rosiglitazone and lupin in STZ+ Lupin+ Rosi group caused a significant (p< 0.05) increase from normal control group.

Table 1: Effect of Rosi and /or Lupin in non-diabetic and diabetic pregnant rats

Groups Parameters	Non-diabetic group				Diabetic group			
	Normal control	Rosi	Lupin	Buffer	Diabetic control (STZ)	STZ + Rosi	STZ + lupin	STZ +Rosi+ Lupin
Glucose (mg/dl)	95.5 ± 1	96.3 ± 0.8 b	96.6 ± 0.5 b	96.2 ± 0.6 b	325 ± 3.4 a,b	97.4 ± 0.5 b	97.1 ± 0.7 b	98.3 ± 0.7 b
Insulin (mU/L)	51.8 ± 0.2	52 ± 0.2 b,d	52 ± 0.2 b,c	51.7 ± 0.2 b,e	18.9± 0.18 a,b	37.4 ± 0.1 a, b,c,d,e	37.9 ± 0.1 a, b,c,d,e	38 ± 0.1 a, b,c,d,e
Glucagon (Pg/ml)	50.1 ± 0.6	51.3 ± 1 b,d	51.2 ± 0.5 b,c	50.8 ± 1 b,e	75.6 ± 0.8 a,b	63 ± 0.5 a,b,c,d,e,f	61.8 ± 0.4 a,b,c,d,e	60.3 ± 0.4 a,b,c,d,e,f
HOMA-IR Score	12.8 ± 0.1	12.3 ± 0.08 b,d	12.4 ± 0.06 b,c	12.3 ± 0.08 b,e	15.21 ± 0.2 a,b	9± 0.07 a,b,c,d,e	9± 0.06 a,b,c,d,e	9± 0.1 a,b,c,d,e
Hb (mg/dl)	13.5 ± 0.1	10.9 ± 0.1 b	13.5 ± 0.1 b	13.2 ± 0.1 b	13.3 ± 0.2 a,b	11.4 ± 0.1 a,b	13.2 ± 0.1 b	13.2 ± 0.1 b

Data are represented as mean ±SE. Significant at p < 0.05. a, significant with the control. b, significant with the diabetic control. c, significant with the control lupin, d, significant with the control Rosiglitazone, e, significant with the control buffer, f, significant with the diabetic Rosiglitazone, g, significant with the diabetic lupin.

Table 2: Effect of Rosi and /or Lupin on lipid profile in non-diabetic and diabetic pregnant rats

Groups Parameter	Non-diabetic group				Diabetic group			
	Normal control	Rosi	Lupin	Buffer	Diabetic control (STZ)	STZ + Rosi	STZ + lupin	STZ +Rosi+ Lupin
Total cholesterol (mg/dl)	150.5 ± 1	173.8 ± 0.8 a,b,c,d	155.4 ± 1 b,c	152.6 ±2.1 b,d,e	256.1 ± 1.2 a,b	270.5± 2.2 a,b,c,d,e,f	156.4 ± 1.5 a,b,c,d,e,f,g	182.7 ± 0.9 a,b,c,d,e,f,g
Triglycerides (mg/dl)	86.5± 0.7	130.2 ± 1.1 a,b,d	86.7 ± 0.7 b,c	86.7 ± 0.6 b,c,d,e	185 ± 1.3 a,b	201.9 ± 1.9 a, b,c,d,e,f	122.6 ± 1.1 a, b,c,d,e,f,g	174.5 ± 1 a, b,c,d,e,f,g
HDL (mg/dl)	42.6 ± 0.2	35.9 ± 0.5 a,b,c,d	43.6 ± 0.5 b,c	42.1 ± 0.16 b,d,e	28.3 ± 0.3 a,b	35.7 ± 0.47 a,b,d,e,f -	50.9 ± 0.3 a,b,c,d,e,f,g	44 ± 0.3 a,b,c,d,e,f,g
LDL (mg/dl)	90.6 ± 1.1	111.8 ± 0.7 a,b,c,d	92.7 ± 1 b,c	93.2 ± 2 b,d,e	190.7 ± 1.1 a,b	194.4 ± 1.5 a,b,c,d,e,f	90 ± 1 b,c,d,f,g -	103.7 ± 0.7 a,b,c,d,e,f,g
Atherogenic index (AIP)	0.30 ± 0.003	0.55 ± 0.004 a,b,c,d	0.29± 0.006 b,c	0.30± 0.003 b,c,d,e	0.81 ± 0.006 a,b	0.74 ± 0.003 a,b,c,d,e,f	0.37 ± 0.002 a,b,c,d,e,f,g	0.59 ± 0.003 a,b,c,d,e,f,g

Data are represented as mean ±SE. Significant at p < 0.05. a, significant with the control. b, significant with the diabetic control. c, significant with the control lupin, d, significant with the control Rosiglitazone, e, significant with the control buffer, f, significant with the diabetic Rosiglitazone, g, significant with the diabetic lupin

There was a highly increase in triglycerides in STZ group from that of the normal control. The triglycerides concentrations in control groups (Lupin, Rosi, and buffer) were significantly (p< 0.05) lower than that of the treated groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi). The three treated groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi) were significant (p< 0.05) with each other.

There was a dramatic decrease in the HDL cholesterol concentration in the STZ group, from the normal value. There was a significant (p< 0.05) change between the control groups (Lupin, Rosi, and buffer)

with each other, and also did the treated groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi). The HDL cholesterol concentration was decreased from the control in STZ+ Rosi group; meanwhile it was increased in both STZ+ lupin and STZ+ lupin+ Rosi groups.

There was a tremendous increase in LDL concentration in the STZ group from the normal control, and it was significantly (p< 0.05) high when compared to all experimental groups except STZ+ Rosi group. The administration of lupin in STZ+ lupin group was significantly decreased the LDL concentrations. In both STZ+ Rosi

and STZ+ lupin+ Rosi groups LDL was increased from the normal value. Also, there were significances between the treated groups (STZ+ lupin, STZ+ Rosi, and STZ+ lupin+ Rosi).

The AIP was very highly significant (p< 0.05) in STZ group, and it was significantly higher from that of both STZ+ lupin and STZ+ lupin+ Rosi groups except in STZ+ Rosi group, it was significantly lowers. The AIP was increased in Rosi group from that of the normal control value. On the other hand, it was decreased in Lupin group.

Fig (1) represents a negative correlation between HOMA-IR and HDL at (r = 0.594, p< 0.01). In addition Fig (2) shows a negative correlation between blood plasma glucose and HbA at (r = 0.690, p< 0.01). While Fig (3) represents a positive correlation between AIP with LDL at (r = 0.811, p< 0.01).
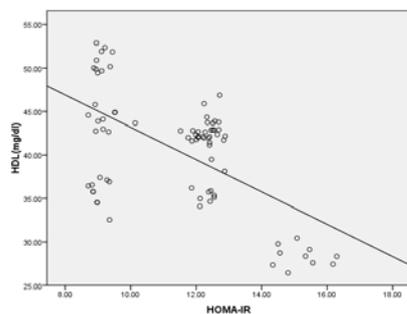
Fig. 1: The negative correlation between HOMA-IR and HDL (r = 0.594, p< 0.01)
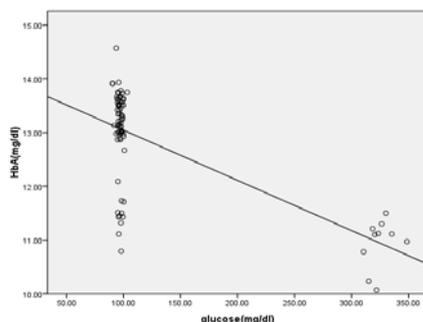
Fig. 2: The negative correlation between blood plasma glucose and HbA (r = 0.690, p< 0.01)
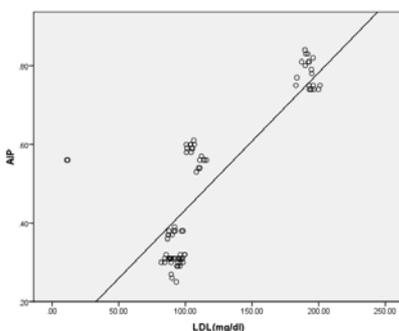
Fig. 3: The positive correlation between AIP and LDL (r = 0.811, p< 0.01)

DISCUSSION

Normal pregnancy is described as a 'diabetogenic state' due to progressive increase in insulin resistance thus; pre-existing diabetic pregnancies remain a challenging high risk group (35). Insulin

resistance is associated with increased risk for cardiovascular diseases (36).

So, this study explores the therapeutic effects of Rosiglitazone and / or alkaloid lupin extract, on pregnant diabetic rats during the gestation period. These effects are best reflected on the improvement of HOMA-IR and AIP.

We induced T2DM chemically by sterptozotocin before pregnancy; that causes damage to β cells and induces the metabolic changes associated with T2DM. In agreement with (47, 48, 49) The pregnant diabetic rats showed an elevation in blood plasma glucose, total cholesterol, triglycerides, and LDL and decreasing in both HDL and blood insulin levels. These results are in agreement with (47, 48, 49). A single injection of STZ results in selective β cell destruction (50). (51) explained the mode of action of STZ. When STZ metabolized inside the cell, NO is liberated and other reactive oxygen species. These cause fragmentation of β cell DNA and excite the other deleterious changes in the cells. Another study (52) gives an explanation to the elevation of LDL. It could be as a result of the overproduction of LDL by the liver or a defective in removal from the circulation or both secondary to insulin deficiency. Along with (14), High levels of triglycerides compete with glucose for the entrance in the cells causing elevation of insulin resistance. This result is in agreement with the present study that found an elevation in HOMA-IR within the diabetic group.

Rosiglitazone was developed to treat insulin resistance and hyperglycemia in T2DM patients (53). It modulates the key communication signals between fat and muscle. Specifically, by binding to peroxisome proliferator-activator- receptor-gamma nuclear receptors (PPARγ) (54). So, Consistent with (55, 56, 57), the treatment of pregnant diabetic rats with Rosiglitazone, in this study, was associated with the decrease of in blood glucose and, the increase of in insulin levels and the decrease in HOMA-IR. Also, the insulin sensitivity was improved as described by (58, 59, 60, 61). (62) proposed that TZDs increase skeletal muscle glucose uptake via modulation of humoral adipocytokines, (e. g., leptin, adiponectin, tumor necrosis factor-α, interleukin-6) that directly affect skeletal muscle insulin sensitivity and, to a lesser degree, reduce hepatic glucose production.

The diabetic lupin group showed improvement of HOMA-IR, this was in agreement with (63, 64, 65, 66) and (67) who stated that the different quinolizidine alkaloids, especially 2-thionosparteine, stimulate insulin secretion in a glucose-dependent manner. This effect can be due to the blockage of β cell plasma membrane K_{ATP}-sensitive channels. Also, 13-α-OH lupanine and 17-oxo-lupanine exert their secretagogue effect only in the presence of high glucose could be of additional value when considering these compounds as potential agents for the treatment of T2DM. Because, glycolysis process produces ATP, which block K^+ channels and induce exocytosis of insulin.

Glucagon blood level concentration was increased in the diabetic group and this was consistent with (68, 69), and (70) who reported the decrease of glucagon by in situ hybridization of the pancreas. And this was anticipated by (71) as a function of disorganization of islet architecture with reduction in β cell number, and a paradoxical rise in glucagon is seen in the postprandial period in T2DM, as opposed to the fall in non-diabetic persons.

In this study, the treatment of pregnant diabetic rats with Rosiglitazone decreases the glucagon level. This is in harmony with (72) who declared that Rosiglitazone repress glucagon gene through PPAR γ gene. PPARγ inhibits Pax6 transcriptional activity, resulting in inhibition of glucagon gene transcription. This cause the decrease in glucagon secretion and glucagon tissue levels in primary pancreatic islets. Also, (70) revealed the same results by in situ hybridization and immunohistochemistry.

The diabetic lupin group showed also, a decrease in glucagon level, and this was in agreement with (73). Thus, lupin alkaloids could help to the improvement of hyperglucagonemia in T2DM.

The Rosiglitazone treated diabetic group showed an increase in the total cholesterol, triglyceride, LDL and HDL these findings were in

agreement with (74, 75, 76, 77, 26, 43). Although, (78) declared that Rosiglitazone reduces the incidence of macrovascular complications in individuals with T2DM. The possible explanation of the elevation of HDL cholesterol was showed by (79). Who stated that the upregulation of the ATP-binding cassette transporter A1 (ABCA1) gene leads to apolipoprotein A1–mediated cholesterol efflux from macrophages, which results in an increase in HDL cholesterol. Another study by (80) who found that PPARγ have also been shown to inhibit cholesterol esterification in cholesterol-loaded macrophages. On the other hand, (81) declared that Rosiglitazone increases the expression of genes associated with hydrolysis of triglyceride-rich lipoproteins and fatty acid uptake and storage.

The Rosiglitazone control group showed an alternation in lipid profile and this was in consistent with (82) who stated that there is no clear mechanistic explanation for the effects of Rosiglitazone on lipid metabolism in non-diabetic patients.

The diabetic lupin treated group, in this study, showed overall improvement in lipid profile as there was a decrease in total cholesterol, triglycerides, LDL and an increase in HDL. These findings were in accordance with (83, 63, 84, 85). (86) found that induced hypercholesterolemia decreased by blue lupin. This is due to the reduction in micellular solublization of cholesterol, attenuated by elevation in bile acid reabsorption and phytosterols rich diet. (87) assumed that the upregulation of the mRNA of LDL receptors and CYP7A1. The CYP7A1 provides an important pathway to eliminate abundant cholesterol from the liver. Also, the upregulation of the apoA1 mRNA expression in the liver was the cause of the HDL elevation. (88) stated that wheat lupin consumption may have a protective effect against cardiovascular risk by improving the anti-atherogenic metabolic pathway of cholesterol. (89) declared that dietary lupin protein decreases serum triglycerides in rats through down regulation of sterol regulatory element-binding protein (SREBP)-1c, which regulates the expression of lipogenic enzymes in rats' livers.

In this study, the diabetic group showed an increase in atherogenic index (AIP) and this was in accordance with (43, 90). (91) proposed that high glucose condition decreases the expression of hepatic LRP1, which leads to the development of atherogenic dyslipidemia.

Our results showed decrease in atherogenic index in Rosiglitazone treated group these results as the same as obtained by (92) while (93) declared that the elevation of both LDL and triglycerides causes pro inflammatory, which cause atherosclerosis. Also, (80) explained that PPARγ stimulate CD36 gene that causes macrophage foam cell formation and the development of atherosclerosis in mice

The administration of an antidiabetic herb with a hypoglycaemic drug for the treatment of diabetes may pose for potential drug–herb interaction that enhanced effect and reduces the adverse effects of drugs (94). In this study, the combined therapy group showed a decrease in plasma glucose and glucagon and increase in insulin secretion, and HOMA-IR was improved due to the combined mechanisms of Rosiglitazone and alkaloid lupin. The STZ+ Rosi+ Lupin group showed overall hyperglycemic control as the STZ+rosi group and STZ+ Lupin group. However, the combined effect of them results in improvement of atherogenic index, in comparison with, STZ+ Rosi group. Lupin Alkaloids exert an antiatherogenic effect by reducing the cholesterol, triglycerides, and LDL. This group is unique, so the mechanism of action of Rosiglitazone and alkaloid lupin together is not yet studied. More researches should be devoted to drug – herb interaction. However, there is another study (95) used combinational therapy to reduce dislypedemia resulted from Rosiglitazone.

Our results showed negative correlation between HDL and HOMA-IR (r = 0.594, p< 0.01). This was in agreement with (96, 17). This means that elevation of HDL has an anti-atherogenic role, associated with the decrease in insulin resistance and this emphasis on the relation between insulin resistance and cardiovascular risk (97). There was also a positive correlation between AIP and LDL (r = 0.811, p< 0.01). The increase in atherogenic index is associated with the increase in LDL and vice versa. This is in consistent with (15) who stated that the increase in LDL increases cardiovascular risk.

This study showed a negative correlation between total Hb and blood glucose (at r = 0.690, p< 0.01). (98, 99) showed a positive correlation between plasma glucose and glycosylated haemoglobin (HbA1c). The possible explanation for the inverse correlation between total heamoglobin and blood glucose is shown by (100). Who stated that the excess glucose present in the blood reacts with haemoglobin to form HbA1c thus; they increase along with each other, and causing the decrease in the total haemoglobin.

In conclusion, this study showed that the treatment with Rosiglitazone and/or lupin alkaloids results in decreasing hyperglycemia in pregnant diabetic rats and improving HOMA-IR. While it reveals the increase of AIP in Rosiglitazone treated groups (either diabetic or non diabetic groups), in comparison with lupin alkaloids groups (diabetic and non diabetic). So, the current study recommend the usage of lupin alkaloids during the pregnancy to avoid the hazard effects of Rosiglitazone. Also, there is an urgent need for more studies to explore the effect of Rosiglitazone on lipids and cardiovascular risk.

CONFLICT OF INTEREST

The authors declared no conflict of interest

ABBREVIATIONS

AIP: Atherogenic index, DM: Diabetes mellitus, HOMA-IR: Homeostasis model assessment of insulin resistance, STZ: Sterptozotocin, T1DM: Type one diabetes, T2DM: Type two diabetes

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