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Biochemical role of ferulic acid in modulation and treatment of hyperglycemia associated with hyperlipidemia

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A B S T R A C T

Diabetes, when uncontrolled, causes dyslipidemia often followed by atherogenic abnormalities. The present study was aimed to determine the effect of ferulic acid (FA) in diabetes-induced dyslipidemia. Diabetes in male rats induced by streptozotocin and treated by ferulic acid (FA) significantly reduced the elevated blood glucose and lipid profile levels. Thirty two waster male rats divided into four equal groups of 8 rats. Group I (Control group): received no drugs, Group Π (Ferulic acid group): received (10mg/kg body weight/day) orally for 21 days. Group III (Streptozotocin (STZ) group): received intramuscular injection once by STZ (40mg/kg body weight). Group VI :(Streptozotocin+Ferulic acid group (STZ+FA)): received intramuscular injection once by STZ (40mg/kg body weight) and after 3 days received orally ferulic acid (10mg/kg body weight/day) daily. Treatment in groups for 21 days after diabetes induction blood samples and pancreatic tissue were collected at 22th day from treatment of ferulic acid. The obtained results showed that, STZ induced diabetes caused significant increase in blood glucose, liver enzymes (alanine aminotransferase(ALT), aspartate aminotransferase(AST)), lipid profile (cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein(LDL)), malondialdehyde (MDA) level, while showed a decrease in insulin, glucose-6-phosphate(G6PD), anti-oxidant activities (Superoxide dismutase(SOD), Catalase (CAT), Glutathione Peroxides (GPx) activity, and Glutathione (GSH) level. Ferulic acid able to ameliorate pancreatic damage induced by STZ through significant decrease in blood glucose, liver enzymes (ALT, AST), lipid profile(cholesterol, triglyceride, HDL, LDL), malondialdehyde (MDA) levels and significant increase in insulin,G6PD, anti-oxidant activities SOD, CAT, GPX activity, and GSH level.
Histological studies of pancreatic tissue showed atrophy in island of Langerhans cells ameliorated by ferulic acid treatment. These results suggest that, ferulic acid is effective treatment against diabetes type 1 induced by STZ by its anti-oxidant, anti-diabetic, anti-lipidemic and ameliorate liver enzyme activity.

Keywords: STZ, ferulic acid, Diabetes, biochemical analysis, Histological studies

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1. INTRODUCTION

Changes in human behavior and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes. The past two decades have seen an explosive increase in the number of people diagnosed with diabetes worldwide (Zimmet et al., 2002). Diabetics are at an increased risk of developing chronic complications related to ophthalmic, renal, neurological, cerebrovascular, cardiovascular and peripheral vascular diseases. Consequently people with diabetes are more likely than those without the disease to have cardiac arrest, stroke, amputation, kidney failure and blindness (Hirany et al., 2002). Typical

dyslipidemia associated with diabetes is highly atherogenic and plays a key role in pathogenesis of other complications. Therefore prevention of complications is a key issue because of the huge premature morbidity and mortality associated with the disease (Zimmet et al., 2000).The continuous use of synthetic drugs like sulphonly ureas and biguanides is known to produce serious side effects (Moller, 2001).There is a wealth of in vitro evidence for the powerful antioxidant properties of flavonoid components of the diet. However, few studies have examined the in vitro antioxidant potential of hydroxycinnamates, major constituents of fruits, some vegetables, beverages and grains (Bourne and Rice-Evan, 1998). Hence, we tested the natural antioxidant ferulic acid (3-methoxy 4- hydroxycinnamic acid), which is more bioavailable than other dietary flavonoids and monophenolics so far studied (Graf, 2000). Ferulic acid has a protective effect in liver toxicity induced by drugs and is used as an anti-inflammatory drug in Japanese oriental medicine (Wuet al., 1995). Ferulic acid is reported to terminate free radical chain reactions and reduce the risk of coronary artery diseases (Bourneet al., 2004). Also, ferulic acid reduces the level of cholesterol and triglyceride, (Trombino et al., 2004). Therefore, in our study we focused on the effects of ferulic acid on lipid levels in experimentally induced by diabetes.

2. MATERIALS AND METHODS

2.1. Experimental animals:

One hundred Thirty two adult male Wistar strain rats of body weight 150 - 200 g. Rats were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). Rats were housed at the animal house of the national Centre of Radiation Research and Technology (NCRRT) (Cairo, Egypt). Animals were cared in accordance with the standards outlined in the guide for the Care and Use of Laboratory Animals (DHHS publication 85-23). Rats received food and water adlibitum. Animals were randomly divided into 4 groups (n=8).

2.2. Induction of diabetes

Streptozotocin (STZ) powder manufactured by Sigma chemical Co. (St. Louis, USA) was used for induction of diabetes. After acclimatization for 7 to 10 days, rats were fasted for 12 hours. Intramuscular injection of streptozotocin (40 mg/kg body weight dissolved in citrate buffer, pH 4.0) once two days after STZ treatment, rats were considered diabetic after glucose level determination (Sri et al., 2003).

2.3. Experimental design

Rats were randomly divided into four main equal groups, 8 rats each, placed in individual cages and classified as follow:

Group I (control normal group): Rats received no drugs, served as control non-treated for all experimental groups.

Group II (Ferulic acid (FA) group): Rats received Ferulic acid (10 mg/kg body weight) orally for 21 days (Sri et al., 2003).

Group III (Streptozotocin (STZ) group): Rats were injected intramuscularly (40 mg /kg body weight) once at the first day of experiment by STZ (Sri et al., 2003).

Group IV (Streptozotocin+Ferulic acid (STZ+FA) group): Rats received cadmium chloride (4.4 mg/kg. body weight) and treated daily with melatonin (10 mg/kg body weight/orally).

Group V (Cadmium Chloride +Alpha-lipoic acid + melatonin treated group): Rats were intramuscular injected (40 mg /kg body weight) once at the first day of experiment by STZ and after 3 days were orally injected by FA (10mg/kg body weight/day) orally for 21th days.

2.4. Sampling:

Blood samples and tissue specimens (pancreatic tissues) were collected at the end of experiment on 22th day for all groups.

2.4.1. Blood samples:

Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental period in dry, clean, and screw capped tubes and serum were separated by centrifugation at 2500 r.p.m for 15 minutes .The clear serum was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze at -20oC until used for subsequent biochemical analysis. All sera were analyzed for Glucose, Insulin, Glucose-6-phosphate, Anti-oxidant analysis, Liver and kidney function determination.

2.4.2. Tissue specimens (pancreas tissue):

At the end of the experiment, rats of each group were sacrificed by cervical decapitation. The abdomen was opened and the pancreatic specimen were taken and fixed in 10 % neutral buffered formalin. Specimens were processed tell be in paraffin blocks. Section of 5 µ thickness were cut and stained by haematoxylin and eosin (Banchroft et al., 1996).

2.5. Biochemical analysis

Serum glucose, insulin, G6PD, liver enzymes (ALT, AST), kidney function (creatinine, urea), lipid profile (cholesterol, triglyceride, HDL, LDL), anti-oxidant activities, (MDA, SOD, CAT, GPX activity, and GSH level were analyzed according to the methods described by Trinder (Trinder, 1969). Rat insulin ELISA was measured using kit of Accu-bind ELISA micro wells, Monobind Inc., Lake Forest, CA 92630, USA (Eastham, 1985; Gerbitz, 1980).

2.6. Statistical Analysis

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Values of $P<0.05$ were considered to be significant (Daniel, 1991; Bailey, 1994**)**.

3. RESULTS

3.1. Effect of ferulic acid treatment on serum and pancreatic tissue parameters of streptozotocin-induced diabetes in rats

The obtained results in table (1),before treatment show that, a significant increase in serum glucose, liver enzymes (ALT, AST), lipid profile (cholesterol, triglyceride, HDL , LDL), MDA levels while showed a significant decrease in insulin, G6PD, anti-oxidant activities(SOD, CAT, GPx activity, and GSH level) when compared with control group. But, after treatment with ferulic acid show that, a significant decrease in serum glucose, liver enzymes (ALT, AST), lipid profile (cholesterol, triglyceride, HDL, DL), MDA levels while showed a significant increase in insulin, G6PD, anti-oxidant activities(SOD, CAT, GPx activity, and GSH level) when compared with control group.

3.2. Effect of ferulic acid treatment on pancreatic tissue of streptozotocin-induced diabetes in rats

In figure (1), (2), (3), (4) after injection of streptozotocin Show severity atrophy in island of Langerhans cells and after treatment with ferulic acid ameliorated Langerhans cells.

Table (1): Effect of ferulic acid treatment on serum and pancreatic tissue parameters of streptozotocin-induced diabetes in rats.

Parameters	*Group I*	*Group II*	*Group III*	*Group IV*
Glucose (mg/dl)	88.00± 6.04^{b}	86.1 ± 3.13^{b}	430.00± 7.9^{a}	90.00± 3.80^{b}
Insulin(mIU/ml)	8.63± 0.83^{b}	8.52± 0.31^{b}	1.18± 0.07^{a}	8.35± 0.11^{b}
G6PD(U/g Hb)	39.50± 3.25^{b}	39.00± 3.60^{b}	16.00± 3.80^{a}	38.50± 2.85^{b}
TC(mg/dl)	106.7± 4.47^{b}	104 ± 3.1^{b}	183± 2.55^{a}	105± 3.16^{b}
TG(mg/dl)	63.3± 2.7^{b}	63.1 ± 4.7^{b}	176.8± 3.5^{a}	64.3± 3.16^{b}
HDL mg/dl)	39.6± 3.37^{b}	39.1 ± 3.15^{b}	55.1± 4.1^{a}	39.5± 2.8^{b}
LDL(mg/dl)	54.4± 3.31^{b}	52.2 ± 1.5^{b}	92.5± 4.1^{a}	52.6± 2.94^{b}
GSH(mg/dl)	3.3± 0.25^{b}	3.4 ± 0.32^{b}	1.2± 0.15^{a}	3.5± 0.22^{b}
GPX(mU/ml)	5.6± 0.32^{b}	5.7 ± 0.25^{b}	3.1± 0.16^{a}	5.8± 0.32^{b}
MDA(nmol/L)	8.7± 0.25^{b}	8.9 ± 0.29^{b}	22.5± 1.12^{a}	8.2± 0.32^{b}
CAT(U/L)	618.00± 3.16^{b}	625.24± 4.25^{b}	316.48± 41.14^{a}	617.12± 2.47^{b}
SOD(U/ml)	25.00± 4.12^{b}	26.00± 2.54^{b}	12.00± 2.54^{a}	25.00± 2.91^{b}
ALT (U/L)	23.0± 2.55^{b}	22.0 ± 1.58^{b}	66.00± 4.7^{a}	25± 4.12b
AST (U/L)	55.0± 3.16^{b}	57.0 ± 2.54^{b}	61.00± 4.53^{a}	57.00± 2.55^{b}

a: significant compared with control group, b: significant compared with streptozotocin group, Data are presented as (Mean ± S.E). S.E = Standard error.Mean values with different superscript letters in the same row are significantly.

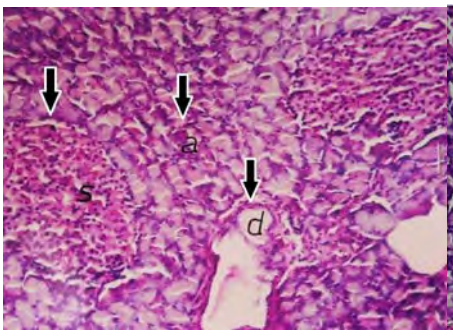

Fig.1

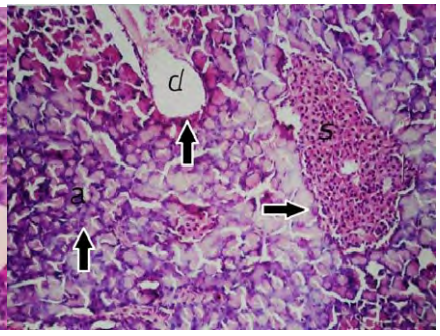

Fig.2

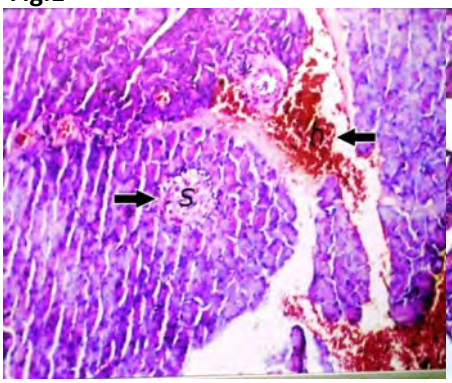

Fig.3

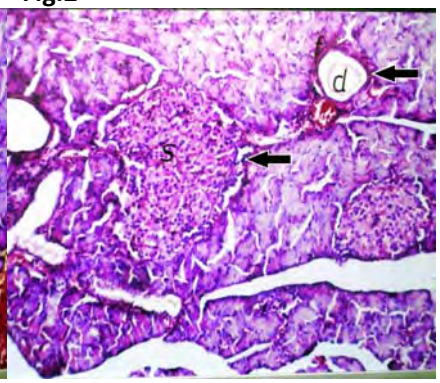

Fig.4

Fig. (1). Group of control rats :(negative control group) showing normal island of Langerhans cells (H&E x 40). (s) island of Langerhans cells (a) acini (d) duct. Fig. (2). Group of rats administrated ferulic acid: (positive control group): Pancreas of male rat (positive control group) showing normal histological structure of island of Langerhans cells (H&E x 40).Fig. (3).Group of experimentally induced diabetic rats by administration of streptzotocin: Pancreas of male rat (positive control group) showing atrophy in island of Langerhans cells(s) with focal hemorrhage (h) between the tubules (H&E x 40).(s) island of Langerhans cells(h) focal hemorrhage. Fig. (4) Group of experimentally diabetic rats treated with ferulic acid: Pancreas of male STZ rat treated with FA showing intact normal histological structure of island of Langerhans cells (s) (H&E x 40).

4. DISCUSSION

In this study, rats treated with STZ showed elevated levels of blood glucose when compared to normal rats. The elevation was due to the action of STZ, which creates an oxidative stress on the pancreas, producing single-strand breaks in pancreatic islet DNA. This ultimately leads to the impaired secretion of insulin, which paves the way for the decreased utilization of glucose by the tissues. Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids (Omamoto et al., 1981).

In normal conditions, insulin increases the receptor-mediated removal of LDL-cholesterol reduction in insulin level during diabetes causes hypercholesterolemia. Glycosylation of lipoproteins in long-term diabetes may also decrease cholesterol degradation and plays a chief role in the genesis of atherosclerosis of the vital arteries.

Hyperlipidemia is due to the defective removal of lipid profile, decreased lipoprotein lipase activity and overproduction of lipid profile during diabetes. Under normal conditions, insulin activates lipoprotein lipase (LPL) which hydrolyzes lipid. Elevation in the levels of lipid is also due to the enhanced esterification of free fatty acid, released in circulation. This leads to an increased risk of ischemic heart diseases. Enhanced lipolysis during diabetes also increases the release of glycerol and hence increases the synthesis of phospholipids. Thus, the levels of lipids were increased in diabetic rats.

Ferulic acid, which has been shown to have antioxidant properties (Bourneet al., 2004) helps to neutralize the free radicals produced by STZ in the pancreas and thereby decreases the toxicity of STZ. This may help the pancreatic beta cells to proliferate and secrete more insulin. The increased insulin secretion can cause increased utilization of glucose by extra hepatic tissues and thereby decreases the blood glucose level. Our dose dependent study showed that treatment with ferulic acid showed significant decrease in serum glucose, liver enzymes (ALT, AST), lipid profile (cholesterol, triglyceride, HDL, LDL), MDA levels while showed a significant increase in insulin, G6PD, anti-oxidant activities SOD, CAT , GPx activity, and GSH level). Our results were accordance with (Sri, et al., 2003) who found that ferulic acid decreased the levels of free fatty acid, triglyceride, cholesterol and phospholipids in plasma. Hypolipidemic effect of low-dose ferulic acid was found to be better than that of the high dose. The exact mechanism by which ferulic acid lowers lipid levels is not known, however, studies have shown , ferulic acid can decrease cholesterol level in blood and lower the incidence of coronary heart disease (Scavariello and Ardlano, 1998). Ferulic acid is a potent antioxidant and prevents LDL oxidation induced by copper ions; hence it facilitates the uptake and degradation of cholesterol by the liver (Bourne and Rice, 1997). In this context, finally ferulic acid can use as a treatment of diabetic type 1 and hyper-lipidemia.

It could be concluded that, ferulic acid because no side effect, safe therapeutic agent and we recommended consumption of ferulic acid as a potential efficient natural therapeutic against induced type I diabetes accompanied with hyper-lipidemia rats.

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الدور الكيميائى الحيوى لحمض الفريولكفي علاج وتعديل زيادة السكر المصاحبة لزيادة الدهون في الدم.

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الملخص العربي

يستخلص في هذه الدراسة تم تقييم التأثير الوقائي والعلاجيللفريوليك اسيد على التغيرات في مستويات السكر والدهون المرتفعة والمحدثة تجريبيا فى الجرذان، حيث تم حقن الفئران بمادة الاستربتوزوتوسينمما ادى الى زيادة فى نسبة الكوليستيرول والدهون الثلاثية فى الدم والتي تعتبر من الاثار الجانبية لمرض السكر، والسبب في ذلك ان مادة الاستربتوزوتوسين لها تاثير مدمر لخلايا جزر البنكرياسمما يؤدي الي ضعف في افراز هرمون الانسولين. في الظروف العادية الانسولينيساعد مستقبلات الدهون منخفضة الكثافة ان يخفض من مستوي الكولستيرول في الدم وفي حالة الفئران المصابة بالسكر لوحظ انخفاض في مستوى الانسولين في الدم مما يسبب ارتفاع في نسبة الكوليستيرول وهذا التدهور يلعب دور كبير في حدوث تصلب في الشرايين الحيوية. لاجراء هذه التجربةتم استخدام عدد 32 من ذكور الجرزان البيضاء اعمارهم تتراوح بين 12-16 اسبوع واوزانها من 150-200 جرام وقد قسمت الي اربع مجموعات متساوية اشتملت كل مجموعة علي 8 فئرانوتم توزيعها كالاتي: المجموعة الاولي: (المجموعة الضابطة): تم حقنها بمحلول فسيولوجي،المجموعة الثانية: (حمض الفيروليك اسيد): تماعطاء الفئران حمض الفيروليك اسيد عن طريق الفم بتركيز (10 مللي جرام/كيلوجرام)،المجموعة الثالثة: (المجموعة المحدث بها مرض ارتفاع السكر و الدهون) تم حقن الفئران (40 مللي جرام/كيلوجرام) بمادة الاستربتوزوتوسينمرة واحدة فقط لاحداث ارتفاع السكر والدهون.المجموعة الرابعة:(الفريوليك اسيدوالاستربتوزوتوسين): تم حقن الفئران بمادة الاستربتوزوتوسين (40 مللي جرام/كيلوجرام) مرة واحدة فقط لاحداث ارتفاع السكر والدهونوبعد مرور ثلاث ايام وقياس نسبة السكر تمحقن الفئران بحمض الفريوليك اسيد لمدة 21 يوم يومياً عن طريق الفم بجرعة مقدراها (10مللى جرام/ كيلوجرام). وقد تم تجميع عينات الدم والانسجه فى اليوم الثاني والعشرون من بدايه التجربه. و أسفرت نتائج التحليل البيوكيميائى عن وجودارتفاع معنوى فى مستويات كلا من سكر الدم وانزيمات الكبد (AST&ALT) ودهون الدم (الكولسترول والدهون الثلاثية ودهون مرتفعة الكثافة و دهون منخفضة الكثافة) و المالونالدهيد، من جهة اخرى اظهرت النتائج انخفاض في مستوي هرمون الانسولين وجلوكوز-6-فوسفات ومضادات الاكسدة مثل(سوبر اوكسيد ديسميوتيز ، الكتاليز ، الجلوتاثيون بيروكسيديز ، الجلوتاثيون). كما أن نتائج مجاميع الجرذان المصابهبمرض السكر وارتفاع الدهون التيتم وقايتهاوعلاجها بالفريوليك اسيدأظهرت انخفاض فيمستويات كلا من سكر الدم وانزيمات الكبد (ALTوAST) ودهون الدم (الكولستيرول و الدهون الثلاثية و دهون مرتفعة الكثافة و دهون منخفضة الكثافة) و المالونديالدهيد وارتفاع في مستويات كلا من هرمون الانسولين و جلوكوز-6-فوسفات و مضادات الاكسدة مثل(سوبر اوكسيد ديسميوتيز ، الكتاليز ، الجلوتاثيون بيروكسيديز ، الجلوتاثيون).وأوضحت الدراسة أن استخدام الفريوليك اسيد كعامل علاجيمضاد للأكسدة ومضاد للألتهابات له دور فعال في علاج تحسين خلاياالبنكرياس من التنكرز والتقرح المحدث تجريبيا فى الجرذان باستخدام مادة الاستريبتوزوتوسين وتحسين نسب القياسات البيوكيميائية فى الدم والأنسجة لما يقارب النسب الطبيعية.لذلك توصى الدراسة بضرورة استغلال تلك المزايا الهائلة للفريوليك اسيد كماده علاجيةومضادة للأكسدة والألتهابات و إدخاله كماده فعالة فى صناعة العقاقير الطبية المستخدمة فى العلاج من مرض السكر وارتفاع تركيز الدهون.