Role Of Grape Seed Extract As Anti Hyperglycemia And Antioxidant In Experimental Diabetic Rats

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Abstract:

The anti hyperglycemia and antioxidant effect of an aqueous extract of grape seed used in popular medicine in many countries of the world was studied in rats with streptozotocin-induced diabetes. Oral administration of grape seed extract (20 mg/250 g rat body weight) for 30 days resulted in a significant in glucose, GPT, GOT reduction, ALP and ACP. The extract also causes a significant increase in reduced glutathione, in the liver and kidneys of rats with streptozotocin-induced diabetes. These results clearly show the antihyperglycemic and antioxidant property of grape seed extract.

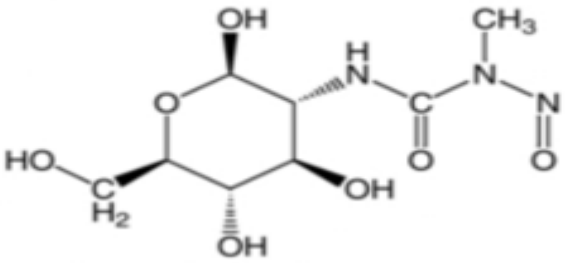
Introduction:

Diabetes mellitus type 2 is associated with increased oxidative stress; free radicals, lipid peroxides, and oxidation of low-density lipoproteins (LDL) have been suggested to play a role in the increased risk of cardiovascular disease associated with diabetes mellitus type 2 (1). In diabetes, impaired glucose metabolism may lead to an increase in hydroxyl radical production; free radicals may also be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids (2). The free radicals produced may react with polyunsaturated fatty acids in cell membranes, leading to lipid peroxidation (3). The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavenger systems (4 and 5). The levels of these defense mechanisms are altered in diabetes, and therefore, the ineffective scavenging of free radicals plays a crucial role in determining tissue injury (5) Grape seed is commonly used as a prehension with grape fruit or as extra free seed mass (consumption in Asian countries as popular drugs) (6). Various plants have been extensively

used in medicine in the world as a result of good goal obtained with less harmful side effect that may be induced by chemical drugs and the viability of these plants with cheap price; Most of these plants are used for the treatment of acute and chronic diseased are known by spirituals and religious recommendations or by fix scientific researches results (7). (8) showed that the aqueous extract of grape seed possessed anti hyperglycemic activity. Grape seed was also reported to contain nearly 50 mg of falconoid per 100 g (9). (10,11 and12)results showed that the aqueous extract of grape seed have proven the insulin-stimulatory by existing ß-cells in diabetic rats. (13,14 and 15) are fixed in their studies the efficacy of grape seed extract in the treatment of common vascular conditions such as heart disease, stroke, reduced peripheral circulation, macular degeneration, varicose veins, increased the repair of the degeneration of haematopoietic tissue and hemorrhoids. Grape seed extract is one of the few antioxidants capable of crossing the blood-brain barrier - a selectively permeable guard which prevents harmful substances from reaching the brain; The ramification of this phenomenon is that the beneficial aspects of this powerful antioxidant can be benefited by the brain and central

Nervous system (16). The action of oligomeric proanthocyanidin (OPC), the active ingredient of grape seed extract, serves to increase blood flow to all areas of the body. In particular, OPCs have the ability to improve microcirculation by strengthening delicate capillary walls, This results in increased circulation to peripheral areas of the body served by fine blood vessels, such as the liver, testis, spleen Pancreas and eyes (17). Streptozotocin (Streptozocin, STZ, Zanosar) is a naturally occurring chemical that is particularly toxic to the insulin-producing beta cells of the pancreas. In mammals, it is used in medicine for treating certain cancers of the Islets of Langerhans and used in medical research to produce an animal model of diabetes (18). Streptozotocin is aglucosamine- nitrosourea compound, as with other alkylating agents in the A nitrosourea class, it is toxic to cells by causing damage to the DNA, though other mechanisms may also contribute. Streptozotocin is similar enough to glucose to be transported into the cell by the glucose transport protein GLUT2 but is not recognized by the other glucose transporters. This explains its relative toxicity to beta cells, since these cells have relatively high levels of GLUT2 (19).

Streptozotocin was originally identified in the late 1950s as an antibiotic (19). The drug was discovered in a strain of the soil microbe Streptomyces achromogenes by scientists at the drug company Upjohn (now part of Pfizer) in Kalamazoo, Michigan (20). Streptozotocin Character: C.A. Sregistuy: 18883-66-4. Molecular formula: C8H15N3O7. Molecular weight: 265.221 g/mol. Half life: 5–15 minutes (21).



Materials and methods

1- Animals:

Male Rattus norvegicus rats weighing 200–250 g were used in this study. They were reared in the animal house of the College. The animals were fed ad libitum with a normal laboratory pellet diet and water

2- Chemicals and reagent kits:

All chemicals and reagent kits used in this experiment were purchased from traditional pharmacies and drug stores.

3- Plant material:

Grape seed extract was purchased by isolating the seed from grapes and homogenizing it using a machine grinder. A total of 250 g of dried grape seed extract was extracted from 5 kg of fresh grape fruit. The extract was evaporated to dryness in an oven at 60 °C for 40 minutes.

The extract was suspended in distilled water and used in this study in concentrations as recommended.

4- Induction of experimental diabetes:

A freshly prepared solution of streptozotocin 50 mg/ml in citrate buffer, pH 4.5, was injected intraperitoneally in a volume of 0.3 ml containing 15 mg streptozotocin/rat (using an insulin syringe) (22).

After 48 h of streptozotocin administration, rats with moderate diabetes having glucose urea and hyperglycemia (i.e. with a blood glucose of 200-280 mg/dL) were taken for the experiment

5- Experimental procedure:

In the experiment, a total of 36 rats (24 diabetic surviving rats, 12 normal rats) were used. The rats were divided into three groups of 12 rats each: G.1 normal rats; G.2 diabetic rats given grape seed extract suspension twice daily for 30 days (20 mg dissolved in 1 ml D.W/250 g rat body weight) administered orally with a modified syringe daily for 30 days; and G.3, diabetic control. Blood samples were collected for glucose estimation before induction of diabetes and treated with grape seed extract suspended from the heart at a volume of 1 ml from each rat every week at the end of the experimental period. After 3 days of diabetes induction, 3 rats from each group were killed by decapitation for histological studies to confirm the effect of Streptozotocin as a diabetic gene; then at the end of the experiment period; After 30 days, all rats in all groups were killed to collect pancreas gland specimens for histological studies using the protocol described by (23). Also, the liver and kidneys were dissected out, washed with cold saline, weighed, and prepared for glutathione estimation.

6-Analytical methods:

Total blood glucose was estimated by the enzymatic method (24). Glutamic oxaloacetic transaminase (25). Glutamic pyruvic transaminase (25). Acid phosphatase activity and alkaline phosphatase activity (26). Glutathione was estimated in liver and kidney tissue by the Ellman method (27).

7-Statistical analysis

The data for various biochemical parameters were analyzed using ANOVA and LSD tests (28).

Results:

1-Total blood glucose (mg/dl): Table (1) demonstrates the

level of blood glucose in normal and experimental animals. There was a significant elevation in blood glucose in diabetic rats compared to control rats. Administration of grape seed extract suspended significantly decreased the level of blood glucose in treated diabetic rats compared to untreated diabetic rats

2-Glutamic oxaloacetic transaminase (GPT) IU: Table (2) shows the activity of (GPT) in the blood of normal and experimental animals. There was a significant elevation in

Activity of (GPT) enzyme during diabetes compared to the corresponding control group. Administration of grape seed extract suspended significantly decreased the level of this enzyme in rats with streptozotocin-induced diabetes.

3-Glutamic pyruvic transaminase (GOT) IU: Table (3) shows the activity of (GOT) in the blood of normal and experimental animals. There was a significant increase in the activity of (GOT) enzyme during diabetes compared to the corresponding control group. Administration of grape seed extract suspended significantly decreased the level of this enzyme in rats with streptozotocin-induced diabetes.

4- Acid phosphatase activity (ACP) KAU/dl & Alkaline phosphatase activity (ALP) KAU/dl:

Tables 4 and 5 show the activity of ACP and ALP in the blood of normal and experimental animals. There was a significant elevation in the activity of ACP enzyme during diabetes compared to the corresponding control group. Administration of grape seed extract suspended significantly decreased the level of this enzyme in rats with streptozotocin-induced diabetes.

at 5%

5- Glutathione mg/100g tissue: Table (5) shows the content of reduced glutathione in tissues of liver and kidney in normal and experimental groups. There was a significant decrease in the concentration of reduced glutathione in these tissues during diabetes compared to the corresponding control groups. Administration of grape seed extract suspended increased the content of glutathione in the liver and kidneys of diabetic rats.

Discussion:

The most important risk factors for diabetes development are viability of free radicals; among these are the oxidizing activity leading to changes by direct injury to DNA, and increased free radical penetration through the tissue, and perhaps by increased mucosa permeability associated with free radical's activity. Elevation of total blood glucose, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, acid phosphatase activity, alkaline phosphatase activity, and decreased glutathione are characteristic features of chronic diabetes. Tissue antioxidant status is suggested to be an important factor in the development of diabetic complications (28). Any high levels of harmful free radicals in the blood or in vital and sensitive organs such as the brain, pancreas, liver, and kidneys, leading to increased cellular infiltration and cell damage (29). Glucose is the major source of energy in the body tissue; The level of glucose in the blood is present in equilibrium between body tissue storage and glucose shift to blood stream under body hormone control (29). The defect in the production of insulin by the pancreas, insufficient liver synthesis of important metabolic enzymes, and kidney failure led to

The body cannot utilize glucose; as a result, the level of glucose circulating in the blood is high and the level of glucose absorbed by the body tissues is low (30). Perhaps more than most diseases, diabetes mellitus is associated with diet; It is a chronic disorder of carbohydrate metabolism that over time increases the risk of kidney disease, atherosclerosis, blindness, and neuropathy (loss of nerve function) (30). Serum glutamic-pyruvic transaminase (GPT) and glutamic pyruvic transaminase (GOT) are enzymes that reversibly exchange amino and keto groups on alpha carbon positions of serum organic acids; this enzyme is prevalent in heart, liver, muscle, and kidney tissue; its elevation in serum can be used for differential diagnosis involving these organs and indicate the integral stress (31). Acid phosphatase (ACP) is a phosphatase, a type of enzyme, used to free attached phosphate groups from other molecules during digestion; it is stored in lysosomes and functions when these fuse with endosomes, which are acidified while they function; therefore, it has an acid pH optimum (32). Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups in the 5- and 3- positions from many types of molecules, including

nucleotides, proteins, and alkaloids; the process of removing the phosphate group is called dephosphorylation (33). Different forms of acid phosphatase and alkaline phosphatase are found in different organs, and their serum levels are used as a diagnostic for disease in the corresponding organs (34). Acid-/Alkaline Balance is a dualistic model representing the two opposite abnormalities of pH control; Failure to maintain normal pH may be associated with one or more of seven causative factors such as water/electrolyte imbalance, anaerobic/dysaerobic imbalance, glucogenic/ketogenic imbalance, sympathetic/parasympathetic imbalance, endocrine insufficiencies (kidney, adrenal, testosterone, estrogen, progesterone, thyroid, posterior pituitary parathyroid), chronic dietary imbalance with respect to the acid/alkaline character of foods, and respiratory dysfunction (34). In truth, excess alkalinity is just as harmful as excess acidity; to clear the confusion, all physiological systems are maintained in homeostasis by a negative feedback mechanism that operates in a dualistic manner; dualistic means that for every normal condition (normal pH, normal body temperature, normal gastric secretion, etc.), there are two.

Abnormally high and abnormally low (34). Glutathione is the most important bimolecular against chemically induced toxicity and is found in organs with high metabolic activity (35). The increased susceptibility of the tissues of diabetic animals to free radicals may be due to the observed increased concentration of glutathione; the decrease in the glutathione level represents increased utilization due to oxidative stress (35). The results in this study show the role of grape seed extract in improving the levels of glucose, GPT, GOT, ALP, ACP, and glutathione in body tissues of rats exposed to harmful and toxic substances that produced free radicals such as streptozotocin, all of these results agree with (8, 10, 11, 12, and 16). The results of protecting the tissues from highly reactive hydroxyl radicals are due to an increase in the capacity of detoxification through enhanced scavenging of harmful radicals. In conclusion, the present investigation shows that grape seed extract possesses an antioxidant activity that may contribute to its protective scavenger action and to enhancing the cellular antioxidant defense. The conclusions of most studies that fixed the grape seed extract contain dozens of different phytocompounds

that have different effects on different areas of the human body, these phytocompounds are quercetin, resveratrol, proanthocyanidins and anthocyanins all of these phytocompounds are classified as polyphones.

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دور مستخلص بذور العنب كمضاد لارتفاع سكر الدم ومضاد للأكسدة في الجرذان المستحدث فيها السكري مختبرياً

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الخلاصة:

التأثيرات المضادة لارتفاع سكر الدم وعوامل الأكسدة لمستخلص المائي لبذور العنب المستخدم كعلاج شعبي في العيد من أقطار العالم تمت دراستها على الجرذان المستحدث فيها السكري بعقار الستربتوزوتوسين. جرعت الجرذان مستخلص بذور العنب فمويا بجرعة (٢٠ ملغ\٢٥٠ غم من وزن الجرذ) لمدة ٣٠ يوم، بينت النتائج انخفاض معنوي في سكر الدم وخمائر (GPT,GOT,ALP,ACP) كذلك سبب المستخلص زيادة معنوية في كلوتوثايونين المختزل في أنسجة الكبد والكلية في الجرذان المستحدث فيها السكري بعقار الستربتوزوتوسين . هذه النتائج أوضحت خواص مستخلص بذور العنب كضاد لارتفاع سكر الدم والأكسدة في الجسم .