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Effects of Grape Seeds Oil on the Antioxidants of Alloxan Induced Diabetes in Rats.

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Abstract :

This study was examined the effect of grape seed oil on glutathione, malondialdehyde, superoxide dismutase, ceruloplasmin and peroxynitrite radical in normal and alloxan-induced diabetic albino rat males (100 mg/kg body weight). Twenty four rats (12-14) weeks age were selected and randomly divided into four groups each with six replicates, These are: The normal control group, Diabetic group, Diabetic group and treated with grape seed oil and a healthy group receiving grape seed oil. The results of study showed that in the experimentally induced diabetes mellitus rats, oxidative stress caused a significant increase ($P<0.05$) in malondialdehyde and peroxynitrite radical levels compared with normal control group, while it caused a significantly decrease of glutathione and ceruloplasmin concentrations compared with the normal control. The treatment of alloxan-induced diabetic rats by grape seed oil administration revealed significant decrease in malondialdehyde and peroxynitrite radical levels in comparison with the diabetic control group, while the treatment with grape seed oil caused a significant increase in glutathione and ceruloplasmin. Treating the normal albino rats by grape seed oil showed significant increase ($P<0.05$) of glutathione and ceruloplasmin concentration and significant decrease ($P<0.05$) of malondialdehyde and peroxynitrite radical concentration compared with the normal control group. This study conclude that grape seed oil play an important role in treating diabetes mellitus complications and removal of the free radicals and elevating antioxidant levels.

المستخلص

الهدف من هذا البحث هو دراسة تأثيرات زيت بذور العنب على مستويات الكلوتاتايون والمالونالديهيد والسوبر اوكسيد دسميونيز والسيربيولوبلازمين وجذر البيروكسينايتريت في جردان تجارب سليمة و طبيعية وفي جردان تجارب قد استحدثت فيها داء السكري بعقار الالوكسان. ادخل عدد 24 من جردان التجارب في البحث وقسموا الى اربع مجاميع كل مجموعة تحتوي على ستة جردان. كانت المجموعة الاولى هي مجموعة ضابطة تحتوي على جردان سليمة وطبيعية اما المجموعة الثانية فهي المجموعة التي فيها جردان استحدثت فيهم داء السكري وكانت المجموعة الثالثة محتوية على جردان مستحدثت فيهم داء السكري ايضا وتم اعطاؤهم زيت بذور العنب كعلاج اما المجموعة الرابعة فكانت جردان تجارب سليمة وطبيعية وتم اعطاؤها زيت بذور العنب. اظهرت النتائج بأن مستويات كل من المالونالديهيد والبيروكسينايتريت قد ارتفعت بطريقة ملحوظة احصائيا بينما انخفضت مستويات الكلوتاتايون والسيربيولوبلازمين بمغزى احصائي ايضا في مجموعة داء السكري مقارنة بالمجموعة الضابطة السليمة. بعد ان تم اعطاء زيت بذور العنب لجردان التجارب المستحدثت فيها داء السكري لوحظ ان هناك انخفاض ذو معنى احصائي في مستويات المالونالديهيد والبيروكسينايتريت مقارنة بمستوياتهم في المجموعة الضابطة بالاضافة الى حصول زيادة

ذات مغزى احصائي في مستويات الكلوتاتايون والسيربولوبلازمين وحصلت نفس التغييرات في المجموعة الرابعة من جردان التجارب الطبيعية والسليمة عند اعطائها زيت بذور العنب لنفس الفترة ونفس الجرعة. بينت الدراسة ان زيت بذور العنب يلعب دورا اساسيا في علاج تأثيرات داء السكري ومضاعفاته ويقلل اويزيل الجذور الحرة ويرفع مستويات مضادات الأوكسدة.

Introduction

Diabetes mellitus is a wide-reaching spread and its prevalence is created by both environmental and genetic factors⁽¹⁾. It is a metabolic complaint of several etiologies depicted by chronic hyperglycemia with disruptions of fat, carbohydrate and protein metabolism arising from weakness in insulin action or secretion⁽²⁾. Diabetes (type I) is a persistent autoimmune illness caused by selective damage of pancreatic B-cells usually leading to absolute insulin deficiency⁽³⁾. Tsai et al concludes that, persistent fasting hyperglycemia may be a transition state between normal glucose tolerance and impaired glucose tolerance in the progression toward type-2-diabetes⁽⁴⁾. Epidemiologic documents confirm the recommendation for diabetes screening begin at of 45 years age in patient with no risk factors, on the other hand begin earlier and more frequently in patient with risk factors and be repeated every three years⁽⁵⁾. Several findings suggest that oxidative stress decline insulin mediated glucose uptake observed in non-insulin dependents diabetes mellitus, which is associated with impairment of insulin signaling and reduced glucose transporter type 4 exposure. Glucose transport in muscle cells increased via lipoic acid by accelerating translocation of glucose transporter type 4 from plasma membrane to the internal pools⁽⁴⁾. The onset of diabetes mellitus is commonly acute which happens in a period of days to weeks, about 95 % of people with type 1-diabetes acquire the disease before 25 years age with an equal prevalence in both genders⁽⁶⁾. The

quality and quantity of the reactive species (ROS) are influenced by exogenous influences such as stress, radiation and food. Care should be taken when assessing free radicals causative role. The adverse influences are established in several diseases but damage caused by free radicals is possibly involved in the pathogenesis of diabetes mellitus and B-cell damages⁽⁷⁾. Egyptians and Greeks have been reported the therapeutic and nutritional value of grapes for thousands of years. The grape leaves were applied to prevent inflammation, bleeding and hemorrhoid pain. Dried grapes or raisins were employed for thirst and constipation while the immature grapes were utilized to relieve sore throats⁽⁸⁾. Grape seeds extract has a number of very useful components such as proteins, fats, carbohydrates, and polyphenols. The polyphenols make up 8%-9% of the components of the seed, depending on the quality of the grapes. The polyphenols of the seeds are found in the bioflavonoids category known for making the color of the fruit⁽⁹⁾. Grape seeds oil has revealed numerous valuable pharmacological effects for instance its chemo-protective action against oxidative stress & reactive oxygen species in addition to anti-inflammatory, anti-bacterial, and anti-cancer effect⁽¹⁰⁾. The grape seed oil contains linoleic acid (75%), oleic acid (15%), palmitic acid (6%), stearic acid (3%) and linolenic acid (1%)⁽⁸⁾.

Aims of the study

This study was achieved to know the effect of grape seed oil on the antioxidant in normal and alloxan-induced diabetic albino male rats.

Materials and methods

Experimental design: Twenty four albino male rats *Rattus norvegicus* were used in this study. They were purchased from the center of Infertility and Embryology Research, Baghdad, Iraq. Rats were kept in the animal house at pharmacology department of Samarra drugs industry (SDI), in wire-meshed stainless steel cages. The environment in the animal house was controlled in which the temperature was maintained at (20-24C°), light schedule of 12:12 hours light: dark cycle with good ventilation. Animal house was provided with air vacuum, so air was changed daily during the maximum day temperature. The Rats were fed commercial pellet diet, deionized water were given freely. Care was taken to avoid unnecessary stress, such as noise, cage crowding, and smell. The animals were kept in this environment for one week before starting the experiment for acclimatization. The study was carried out during the period from March 2016 to July 2016. Diabetes mellitus was induced in (12) of these 24 rats experimentally by injected them subcutaneously with Alloxan for (30) days at a dose of (100mg/kg body weight)⁽¹¹⁾.

Animals were grouped into (4) groups:

Group1: consists of (6) healthy rats receiving only distilled water orally.

Group2: consists of (6) diabetic induced rats not receiving any treatment.

Group3: consists of (6) diabetic induced rats receiving grape seeds oil at a dose (4 ml/kg) orally⁽¹²⁾.

Group 4: consists of (6) healthy and normal rats receiving grape seeds oil at a dose (4 ml/kg) orally.

Chemicals and plants:

1. Alloxan (Coin brook, England - 0158 Alloxan puriss)100mg/kg body weight⁽¹¹⁾.
2. Grape seeds oil at a dose (4 ml/kg)⁽¹²⁾.

Blood Sampling

The blood was collected from each rat into a clean centrifuge tube immediately after sacrificing. The blood was centrifuged to discrete the serum for fifteen minutes. The separated serum was stored at (-20C°) for subsequent determination of biochemical parameters such as glucose, superoxide dismutase, glutathione, ceruloplasmin, malondialdehyde (MDA) and peroxynitrite (ONOO-).

Statistical Analysis

All data were analyzed using the statistical package for social sciences for windows (SPSS) and Microsoft Excel XP system. ANOVA test were used to assess the significance of variability between groups. Means of data were compared using Duncan's Multiple Range test⁽¹³⁾.

Results

The statistical analysis of the results demonstrated a significant (P< 0.05)

increase of glucose level, MDA and ONOO⁻, while there was a significant decrease of SOD, GSH, CP in group 2 compared with group 1 and group 4. while, the level of

glucose, MDA and ONOO⁻ was significantly (P< 0.05) reduced in group 4 and increase in SOD, GSH, CP levels compared with group 2. Table(1).

Table (1): Means ± standard deviations of the Glucose, Malondialdehyde (MDA), Peroxynitrite (ONOO⁻), Glutathione (GSH), Superoxide dismutase (SOD) & Ceruloplasmin (CP) in different groups of adult male rats.

Variable Group	Glucose (mg/dl)	MDA (μMOL/L)	ONOO ⁻ (μMOL/L)	GSH (μMOL/L)	SOD (U/L)	CP (μMOL/L)
Group 1	102±0.437 c	2.820±0.375 b	45.040±0.097 d	12.005±0.232 a	0.740±0.060 a	324.600±1.126 a
Group 2	297±0.873a	5.773±0.483 a	87.727±0.375 a	4.948±0.184 d	0.293±0.050 d	173.530±1.478 d
Group 3	119±0.586 b	2.895±0.354 b	70.072±0.346 c	9.323±0.179 c	0.613±0.081 b	266.830±0.863 c
Group 4	97±0.795 c	2.810±0.138 b	72.188±0.171 b	9.972±0.251 b	0.597±0.093 c	284.726±0.392 b

Different latters: significant (P< 0.05) - Similar latters: not significant.

Discussion

The alloxan induced diabetic state in the present study was verified by elevation the level of glucose, malondialdehyde (MDA) and peroxynitrite (ONOO⁻) when compared with the control males rats. Subcutaneous injection of alloxan results in generation of

free radicals which cause breaking of DNA strands of beta-cells. Alloxan has also been shown to inactivate calcium and calmodulin-dependent protein kinase, the activity of this enzyme was related to insulin secretion⁽¹⁴⁾. The immediate effect of

alloxan is the elevation of the blood glucose⁽¹⁵⁾. This elevation can be prevented by simultaneous treatment with insulin⁽¹⁴⁾. Prevention of the hyperglycemic phase does not prevent damage of the β cells, however, shortly after the initial hyperglycemic episode, there is a rapid drop of blood glucose to hypoglycemic levels, as a results of insulin released by the damaged β cells⁽¹⁶⁾. Many researches confirmed that a variation of plant extracts lower the glucose level effectively in alloxan induced diabetic state⁽¹⁷⁾. A few side effects associated with the use of insulin (hypoglycemia) and oral hypoglycemic agents prompted us to search new bioactive principles from antidiabetic plants used in traditional medicine⁽¹⁸⁾. The increased lipid peroxidation in diabetes, may be due to the ineffective antioxidant system as found in the present study. The increased lipid peroxidation causes lipid hydroperoxides formation and cause inhibition of membrane bounded enzymes and membrane structure destruction⁽¹⁶⁾. The lipid peroxides accumulation result in alteration in the cell function and membrane permeability⁽¹⁹⁾. The substantial decline seen in superoxide dismutase (SOD), glutathione (GSH), ceruloplasmin(CP) with the significant elevation found in malondialdehyde (MDA) and peroxynitrite (ONOO-), in the diabetic rats corroborate this reports. The data of the present study showed that the fall of glutathione, ceruloplasmin and superoxide dismutase levels of diabetic group suggests that there was a toxic effects of oxidative stress in the

diabetic rats. This in agree with Galletto *et al.*,⁽¹⁶⁾ who reported that, cytotoxic effects produced by oxidative stress seem to be mediated by a disconcertion of thiol homeostasis and intracellular free calcium. The oxidative stress induce reduction of glutathione, cellular soluble and vulnerable sulphhydryl groups⁽¹⁹⁾. The results of this study showed that grape seeds oil normalizes glycaemia in alloxan-treated rats. On the other hand, there is no side effects mentioned on the effect of grape seed oil in experimental diabetes mellitus rats. It is concluded that the flavonoids protecting beta cells from the oxidative stress by chelatory properties of the flavonoids in grape seeds oil⁽²⁰⁾. The present study also revealed that grape seeds oil groups had decreased (MDA) and (ONOO-) levels and increased superoxide dismutase, glutathione and ceruloplasmin levels not surprising since grape seeds oil are one of the richest resources of antioxidant⁽¹⁹⁾. The result of this study confirmed that grape seeds oil is effectual in regulating the antioxidant protection mechanism and antidiabetic effect suggest that grape seeds oil may have protective effect in alloxan induced diabetic state in experimental rats.

Conclusion

Grape seeds oil play an important role against certain aspects of the diabetes mellitus and its complications via removal of the free radicals and increment of antioxidants.

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