Study the effect of polyphenols extracted from Iraqi grape seeds on glucose , MDA levels and GST activity in streptozotocin (STZ) induced diabetic mice.

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Abstract:

1-Objective:- Polyphenols are biochemical compounds with antioxidant activity against differences diseases related to Lipid peroxidation such as diabetes mellitus. Polyphenols distributed widely in medical plants, the aim of the study is to extract and analyze some polyphenolic compounds from grape seeds and examine their effects on (STZ) induced diabetic mice.

2-Methods:- In the present study , a group of polyphenols has been extracted from Iraqi grape seeds by ethanol and the extract has been analyzed by using High Performance Liquid Chromatography (HPLC) coupled to ultra violet (UV) detection. Five fractions were eluted from the column : procyanidin B1, gallic acid , quercetin , catechin and epicatechin. The detection was recorded at (280 nm). The reactive action of the above polyphenols on glucose, and malondialdehyde (MDA) levels and glutathione – S – transferase (GST) activity was tested in (30) streptozotocin (STZ) induced diabetic mice which treated with extracted grape seeds polyphenols to examine the antioxidant effect of these compounds . Grape seeds polyphenols action on the above parameters was determined before treatment and after (1 week), (2 weeks) and (3 weeks) of treatment with this reactive extract.

3-Results:- The data have shown that glucose levels were increased in (30) diabetic mice (after injection with streptozotocin) compared with control group ($p<0.001$) but after treatment with grape seeds polyphenols , glucose levels were decreased relatively ($p<0.001$) after (1 week), ($p<0.001$) after (2 weeks) and ($p<0.01$) after (3 weeks) of treatment with these polyphenolic compounds. MDA levels were increased for diabetic mice (after injection with streptozotocin) compared with control group ($p<0.001$) but after treatment with grape seeds polyphenols , MDA levels were decreased relatively ($p<0.01$) after (1 week) ,($p<0.01$) after (2 weeks) and ($p<0.05$) after (3 weeks) of treatment. GST activity was increased in diabetic mice (after injection with streptozotocin) compared with control group ($p<0.001$) but after treatment with grape seeds polyphenols , GST activity was decreased relatively ($p<0.01$) after (1 week), ($p<0.01$) after (2 weeks) an ($p<0.05$) after (3 weeks)of treatment with these antioxidant compounds.

4-Conclusion:- Our data indicated that glucose , MDA levels and GST activity correlated positively with oxidative stress related to diabetes mellitus.

Key words : polyphenols , Iraqi grape seeds , MDA , GST.

Introduction:-

A great deal of agriculture by products is composed of plant tissues rich in phytochemicals , that could have valuable chemical and biological

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properties , thus there is an interest in the isolation and study of natural fruits. [1]

Grape seeds are one of the richest source of reactive compounds called polyphenols which have a high biochemical , pharmacological and chemoprotective properties against free radicals that cause different diseases which are related to lipid peroxidation such as diabetes mellitus. Polyphenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolities that found in plants. [1,2,3]

The presence of flavonoids monomers, dimmers and trimers have been extensively reported. Catechin , epicatechin and epicatechin-3-gallate are monomers. These monomers can easily condenses into (oligomeric procyanidins). The dimeric procyanidins are often referred as B-series and the trimers procyanidins as C-series. [1,2,3]

Polyphenols in grape seeds are essentially all flavonoids. Quercetin , is also a flavonoid prominent in grape seeds that has been epidemiologically associated with protection against lipid peroxidation. In addition to flavonoids, grape seeds contain some phenolic acids such as gallic acid and ferulic acid. [3,4,5]

Materials and methods:

1- Chemicals and mateials:

Ethanol (80%) was used for polyphenols extraction from grape seeds , Folin ciocaltea reagent was used for polyphenols measurements and methanol (30%) was used for High Performance Liquid Chromatography (HPLC) analysis. (10 gm) of Iraqi white grape seeds was used after drying and milling
while (0.01 gm) of grape seeds polyphenols were used as a standard.

2- Extraction of polyphenols from grape seeds. [1]

(10 gm) of dried , milled grape seeds have been mixed with (50 mL) of ethanol (80%) for (3 hours) at room temperature. The extraction was performed using suxolet and then by rotatory evaporator and lypholyzer . The concentration of polyphenols in the extraction was (70%). The last extract contains high levels of polyphenols . Total polyphenolic content was measured with Folin ciocalteau reagent.

3- High performance liquid chromatography (HPLC) analysis. [6]
Analysis of grape seeds polyphenols has been performed by high performance liquid chromatography (HPLC) using (HPLC 2010 A Shimadzo / Japan). The optimized conditions for (HPLC) analysis were:- The chromotographic column (octadecyl silan column ODSc18) (12.5 cm long × 4mm i.d. and 5 µm particle diameter) , the mobile phase was methanol (30%). Ultra violet (UV) detection has been recorded at the maximum wave length (280 nm) . Column temperature was (25°C) and injection volume is (20 µL). The extraction and HPLC analysis has performed in the department of chemical researches/ minister of sciences and technology.

4- Animal groups:.

Thirty (30) mice of (20-25) gm weight with age in range of (16-18) weeks were kept at room temperature and allowed free access to reactive diets and water cooperative with post graduate students in a specific laboratory in college of education (Ibn al–Haitham), the animals were injected with (150mg/kg) streptozotocin (STZ). (glucose and MDA levels in serum) and (GST in erythrocytes) were measured after (1 week) (A1) , (2 weeks) (A2) and (3 weeks) (A3) of treatment with (30 mg /mL) grape seeds polyphenols.

5- Control group (C):

Thirty (30) healthy mice (20-25) gm weight were selected with age in range of (16-18) weeks. They were not suffering from any disease may interference with the study measurements.

6- Determination of glucose levels.[7]

Glucose levels have been determined by an enzymatic colorimeter test using glucose oxidase (GOD) and peroxidase (POD). The detection was recorded at (500 nm).

7- Determination of MDA levels.[8]

Malondialdehyde (MDA) is an end product for lipid peroxidation. Its determination based on formation of colored complex upon reaction with thiobarbutyric acid. The detection was recorded at (500 nm).

8- Determination of GST activity.[9]

Glutathione –S- transferase (GST) activity has been measured by using glutathione as a substrate which conjugated with 1- chloro – 2,4 – dinitrophenyl benzene to form 1- chloro – 2,4 – dinitrophenyl glutathione . The absorbance was recorded at (340 nm).

9- Statistical analysis:

Statistical significance between mean values of (glucose and MDA) and activity values of GST have been evaluated by students t. test. This statistical method was performed to determine the significance between diabetic and control mice groups.

- Probability less than 0.05 ($P<0.05$) was considered to be significant.
- Probability less than 0.01($P<0.01$) was considered to be high significant.

- Probability less than 0.001($P<0.001$) was considered to be very high significant.

Results and discussion:

1- Identification of polyphenols extracted from grape seeds.

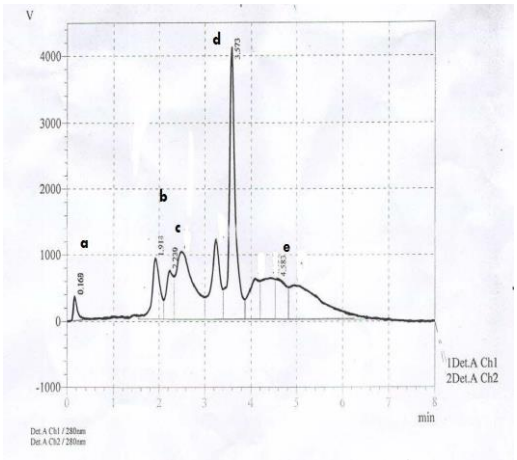
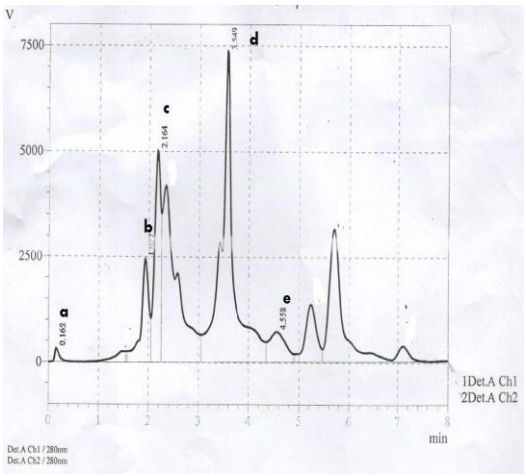


Fig.(1):- HPLC chromatogram of standard grape seeds polyphenols.
a:-procyanidin B1. d:-catechin.
b:- gallic acid. c:- quercetin.
e:- epicatechin.



Fig(2):- HPLC chromatogram of polyphenols extracted from grape seeds By ethanol.

Polyphenols have been extracted from grape seeds. The analysis and the identification were confirmed by. high performance liquid chromatography (HPLC) coupled to ultra violet (UV) detection .Fig(2) shows the chromatogram for these polyphenols. By comparsion retention times of polyphenols in the extract (figure 2) and the standard sample fig(1) , we

noticed that (peak a) was identified as procyanidin B1, (peak b) was identified as gallic acid, (peak c) was identified as quercetin , (peak d) was identified as catechin and (peak e) was identified as epicatechin.The retention times of these extract polyphenols are in a good agreement with those observed in standard (reference) sample . Ethanol and methanol were used because of high polarity to form hydrogen bonds with (-OH) groups in polyphenols. Grape seeds polyphenols were found to have a maximum absorbance (280 nm). [5,6,7]

The folin ciocalteu method, also called gallic acid equivalence method, which is the newest and the most commonly used method, uses gallic acid as an arbitrary reference standard. Gallic acid is a phenolic compound containing two hydroxyl (-OH) groups that react with chemical reagents to produce a blue color, the intensity of which is measured by colorimeter. The hydroxyl groups of the phenolic compounds in grape seeds extract also react with the same chemical reagent to produce a blue color when compared to the colorimeter index of a known quantity of gallic acid , can be used to determine the amount of polyphenols present in grape seeds extract. Significant variation are noted between fig (1) and fig (2). In fact, The amount and types of compounds presented in a particular grape seeds can vary and greatly influenced by the extraction process, as well as the source and variety of seeds.[1]

2-Grape seeds polyphenols action on glucose levels.

Fig(3) and table(1) represent the antioxidant properties of polyphenols extracted from grape seeds on glucose level in streptozotocin (STZ) induced diabetic mice .Glucose level in group B was increased ,very high significant differences ($p<0.001$) have been observed between control group and B group (fig 3) and (table 1). STZ is a specific β-cell toxin and can be used to induce hyperglycemia in rats and mice. It is taken up by pancreatic β-cells via a glucose transporter ($GLUT_2$) and causes alkylation of deoxyribonucleic acid (DNA). DNA damage induces activation of poly adenosine diphosphate (ADP)-ribosylation, a process that is more important for the diabetogenicity of STZ than DNA damage itself. Poly ADP- ribosylation leads to the depletion of cellular nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate (ATP). Enhanced ATP depphosphorylation after STZ treatment supplies a substrate of xanthine oxidase (XOD), resulting in the formation of superoxide radicals consequently, hydrogen peroxide and hydroxyl radicals that initiate (LPO) are also generated.[10]

After treatment with polyphenols , glucose levels have been decreased relatively .Very high significant differences($p<0.001$) were observed between control group and A1 group. And also very high significant differences ($p<0.001$) were observed between control group and A2 group while high significant differences ($p<0.01$) were observed between control group and A3 group.Flavonoids like other polyphenols are antioxidants which have a high biochemical activity, these compounds play important roles in inhibition enzymes that lead to increase glucose in blood like glucose-6- phosphatase (G-6-P) and phosphoenol pyruvate carboxy kinase (PEPCK). In addition to that catechins specially have an important action that seemed to be similar to insulin action and about 20 times potent on erythrocyte membrane Ca^{+2} – ATP ase in type -2- diabetes.Any defect in the last enzyme causes many complications related to diabetes like

cardiomyopathy and retinopathy.And in the same time flavonoids activate enzymes that inhibit (LPO) and regulate lipid metabolism such as paraoxonase (PON) and kinases. [11,12]

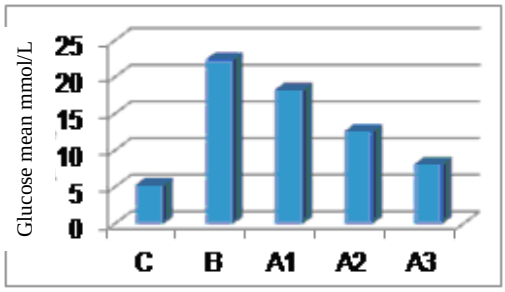


Fig (3):- The effect of polyphenols extracted from grape seeds on glucose levels in streptozotocin (STZ) induced diabetic mice.

Table (1): The effect of grape seeds polyphenols on glucose levels in studied groups.

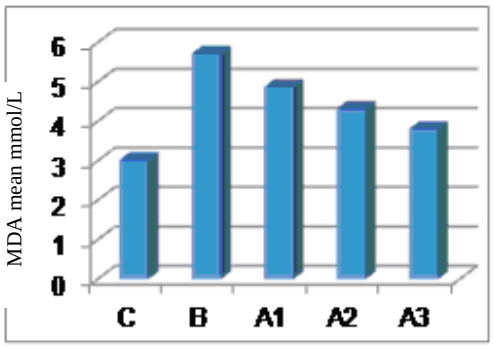
Subject	No.	Glucose mean mmol/L	±S.D	t.test
Control	30	5.33	0.23	
B t=(0 week)	30	22.40	0.80	P<0.001
A1 t=(1 week)	30	18.32	1.10	P<0.001
A2t=(2 weeks)	30	12.67	0.93	P<0.001
A3t=(3 weeks)	30	8.14	2.20	P<0.01

No.:number of animals
S.D : standard diviation.

3-Grape seeds polyphenols action on malondialdehyde (MDA) levels.

Fig(4) and table (2) explain the reactive role of polyphenols extracted from grape seeds on malondialdehyde (MDA) level in streptozotocin (STZ) induced diabetic mice .MDA level in group B was increased ,very high significant differences ($p<0.001$) were observed between control group and B group. fig(4) and table(2). MDA is a secondary product to lipid peroxidation, therefore. It is increased in group B because of elevation of lipid peroxidation (LPO) level.[13,14]

After treatment with polyphenols , MDA levels have been decreased relatively High significant differences ($p<0.01$) have been observed between control group and A1 group, and the same differences ($p<0.01$) have been also observed between control group and A2 group while significant differences ($p<0.05$) have been observed between control group and A3 group.Polyphenols role was explained as these compounds are reactive antioxidants, therefore they act against any factor related to (LPO). In other words, polyphenols activate the enzymes which inhibit (LPO) like kinases and in that time polyphenols inhibit the enzymes that activate (LPO) such as phosphodiesterase (PDE). The result is low level of (LPO) and its products like MDA [13,14].



Fig(4):- The effect of polyphenols extracted from grape seeds on malondialdehyde (MDA) levels in streptozotocin (STZ) induced diabetic mice.

Table (2):- The effect of grape seeds polyphenols on MDA levels in studied groups.

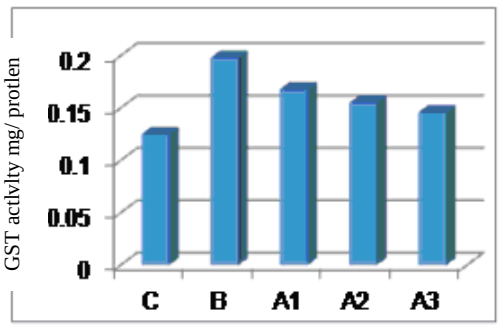
Subject	No.	MDA mean mmol/L	±S.D	t.test
Control	30	3.02	0.21	
B(t=0week)	30	5.72	0.77	P<0.001
A1(t=1week)	30	4.88	0.16	P<0.01
A2(t=2weeks)	30	4.32	0.35	P<0.01
A3(t=3weeks)	30	3.81	0.15	P<0.05

4-Grape seeds polyphenols action on glutathione-s- transferase (GST) activity.

Fig(5) and table (3) represent the important effect of polyphenols extracted from grape seeds on

glutathione-S- transferase (GST) activity in streptozotocin (STZ) induced diabetic mice .GST activity in group B was increased, very high significant differences ($p<0.001$) were observed between control group and B group fig (5) and table (3). This increasing was due to the elevation of free radicals and reactive oxygen species in streptozotocin (STZ) induced diabetic mice , therefore GST activity must be increased to scavenge these unfavorable compounds which initiate lipid peroxidation. [9,15]

After treatment with polyphenols , GST activity was decreased relatively .High significant differences ($p<0.01$) were observed between control group and A1 group. significant differences ($p<0.05$) were observed between A3 group and control group. the decreasing in GST activity happens because polyphenols, like other antioxidants , scavenge free radicals , therefore GST activity which is responsible for scavenging of free radicals will be evenly decreased. [9,15]



Fig(5):- The effect of polyphenols extracted from grape seeds on glutathione-S-transferase (GST) in streptozotocin (STZ) induced diabetic mice.

Table (3):- The effect of grape seeds polyphenols on GST activity in studied groups.

Subject	No.	GST activity mg/protien	±S.D	t.test
Control	30	0.125	0.21	
Bt=(0week)	30	0.198	0.77	P<0.001
A1t=(1week)	30	0.168	0.16	P<0.01
A2t=(2weeks)	30	0.155	0.35	P<0.01
A3t=(3weeks)	30	0.146	0.15	P<0.05

Conclusion:-

1- Iraqi grape seeds contains reactive antioxidant polyphenolic compounds:- (procyanidin B1 , gallic acid , quercetin , catechin and epicatechin).

2- Glucose levels , MDA levels and GST activity for STZ- induced diabetic mice decrease relatively after treatment with grape seeds polyphenols

3- Glucose, MDA levels and GST activity correlates positively with oxidative stress.

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دراسة تأثير المركبات متعددة الفينول المستخلصة من بذور العنب عراقية المنشأ على مستويات الكلوكوز و المالونداي ألديهايد وفعالية الكلوتاثايون-أس- ترانسفيريز في الفئران المصابة بالسكري المحث بالستربتوزوتوسين.

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الخلاصة:

1-الهدف أو الغاية:- المركبات متعددة الفينول هي مركبات كيميائية حيوية ذات فعالية مضادة للأكسدة ضد أمراض مختلفة مرتبطة بالأكسدة الفوقية للدهون مثل مرض السكري. إن هذه المركبات تنتشر بنطاق واسع في النباتات الطبية لذا فالغرض من هذه الدراسة هو إستخلاص و فصل البعض من هذه المركبات من بذور العنب وإختبار تأثيرها على الفئران المصابين بالسكري المحث بالستربتوزوتوسين .

2-طريقة العمل:- في هذه الدراسة تم إستخلاص مجموعة من المركبات متعددة الفينول من بذور العنب عراقي المنشأ بإستعمال الإيثانول ،وتم تحليل المستخلص بإستعمال كروموتوغرافيا السائل ذات الكفاءة العالية المترافقة مع طيف الاشعة فوق البنفسجية. وتم فصل خمسة مركبات من العامود :- البروسياندينB1 و حامض الكاليك و الكوارسيتين والكاتيشين والإيبيكاتيشين عند الطول الموجي (280 نانوميتر). إن التأثير الفعال للمركبات متعددة الفينول أعلاه على مستويات الكلوكوز والمالونداي ألديهايد و الكلوتأايون-أس-ترانسفيريز تم إختباره في ثلاثين فأرا من الفئران المصابين بالسكري المحث بالستربتوزوتوسين والتي عوملت بمتعدد فينولات بذور العنب لإختبار التأثير المضاد للأكسدة لهذه المركبات ذات الفعالية العالية. إن تأثير متعدد فينولات بذور العنب على المحددات أعلاه تم قياسه قبل العلاج وبعد (اسبوع) و(اسبوعين) و(ثلاثة اسابيع) من العلاج بهذا المستخلص متعدد الفينول.

3-النتائج:-لقد أظهرت النتائج أن مستوى الكلوكوز يرتفع نسبيا لدى الفئران المصابة بالسكري بعد الحقن بالستربتوزوتوسين ($p<0.001$)بالمقارنة مع المجموعة الضابطة ولكن بعد المعاملة بمتعدد فينولات بذور العنب فإن مستوى الكلوكوز ينخفض نسبيا ($p<0.001$) بعد أسبوع و ($p<0.001$) بعد أسبوعين و ($p<0.01$) بعد ثلاثة أسابيع من العلاج بهذه المركبات الفعالة. إن مستوى المالونداي ألديهايد يرتفع نسبيا لدى الفئران المصابة بالسكري بعد الحقن بالستربتوزوتوسين ($p<0.001$)بالمقارنة مع المجموعة الضابطة ولكن بعد المعاملة بمتعدد فينولات بذور العنب فإن مستوى المالونداي ألديهايد ينخفض نسبيا ($p<0.01$)بعد أسبوع و ($p<0.01$) بعد أسبوعين و ($p<0.05$) بعد ثلاثة أسابيع من العلاج . إن فعالية ترانسفيريز الكلوتاثايون – أس- ترانسفيريز تزداد في حالة الفئران المصابة بالسكري (بعد الحقن بالستربتوزوتوسين) ($p<0.001$) بالمقارنة مع المجموعة الضابطة ولكن بعد المعاملة بمتعدد فينولات بذور العنب فإن فعالية الكلوتاثايون-أس- تنخفض نسبيا ($p<0.01$)بعد أسبوع و ($p<0.01$) بعد أسبوعين و ($p<0.05$) بعد ثلاثة أسابيع من العلاج بهذه المركبات المضادة للأكسدة.

4-الإستنتاج:-إن نتائجنا أشارت إلى أن مستويات كل من الكلوكوز و المالونداي ألديهايد وفعالية الكلوكوز أس ترانسفيريز تتناسب طرديا مع شدة الأكسدة المرتبطة بمرض السكري.