Study the action of pycnogenol on glucose and lipid profile in type -2- diabetic patients

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Summary :-

Pycnogenol *pinus pinaster Ait* (a water extract of polyphenolic compounds) was extracted from Iraqi pine bark and analyzed using high performance liquid chromotography (HPLC) coupled to ultra violet UV detection that was recorded at (254 nm).

Pycnogenol action on some biochemical parameters (glucose , total cholesterol (TC) , triacylglycerol (TG) , high density lipoproteins (HDL) ,low density lipoproteins (LDL) , very low density lipoproteins (VLDL))level was determined in (30) type -2- diabetic patients who treated with capsules (500 mg) of pycnogenol three times daily to examine the reactive role of pycnogenol against oxidative stress.The above biochemical parameters were measured in plasma before treatment with pycnogenol and after (1 week) , (2 weeks) and (3 weeks) of treatment with this polyphenolic extract.

Our results have shown that glucose level was increased relatively for diabetic patients compared with control group ($p<0.001$) but after treatment with pycnogenol , glucose level would be decreased relatively ($p<0.001$) after (1 week) , ($p<0.001$) after (2 weeks) and ($p<0.05$) after (3 weeks) of treatment with pycnogenol.Total cholesterol (TC) level increased relatively for diabetic patients compared with control group ($p<0.001$) , but after treatment with this reactive extract (TC) level decreased relatively ($p<0.001$) after (1 week) , ($p<0.001$) after (2 weeks) and ($p<0.01$) after (3 weeks) of treatment with pycnogenol.Triacylglycerol (TG) level was increased relatively for diabetic patients compared with control group ($p<0.001$) , but after treatment with this antioxidant extract (TG) level decreased relatively ($p<0.001$) after (1 week) , ($p<0.001$) after (2 weeks) and ($p<0.01$) after (3 weeks) of treatment with pycnogenol.High density lipoproteins (HDL) level was decreased relatively for diabetic patients compared with control group ($p<0.001$) , but after treatment with pycnogenol (HDL) level increased relatively ($p<0.01$) after (1 week) , ($p<0.01$) after (2 weeks) and ($p<0.05$) after (3 weeks) of treatment with pycnogenol.Low density lipoproteins (LDL) level increased relatively for diabetic patients compared with control group ($p<0.001$) , but after treatment

with pycnogenol , (LDL) level decreased relatively ($p<0.001$) after (1 week) , ($p<0.001$) after (2 weeks) and ($p<0.01$) after (3 weeks) of treatment with pycnogenol. Very low density lipoproteins (VLDL) level increased relatively for diabetic patients compared with control group ($p<0.001$) , but after treatment with pycnogenol , (VLDL) level decreased relatively ($p<0.001$) after (1 week) , ($p<0.001$) after (2 weeks) and ($p<0.01$) after (3 weeks) of treatment with pycnogenol.

Introduction :-

Pycnogenol (PYC) is a super antioxidant extract of pine bark .It mainly consists of a high reactive polyphenolic compounds called procyanidins. Additionally pycnogenol contains monomeric flavonoids such as catechin , taxifolin , and various phenolic acids like ferulic acid..[1,2]

The role of pycnogenol as exceptional free radical scavenger is just beginning to emerge and the protective potential of pycnogenol is impressive to say the least. It is only a matter of time before scientific data support to the fact that the family of nutrients is far more effective in its antioxidant capacity than previously assumed.[1,2,3]

A number of pharmacological function award pycnogenol a prominent role for helping diabetic people to cope with their various health problems. [2,4]

Pycnogenol appears to facilitate cellular uptake of glucose as it does not affect insulin levels. [1,3]

Pycnopenol protects against oxidative stress in several cell systems by doubling the intracellular synthesis of anti- oxidative enzymes and by acting as a potent scavenger of free radicals that cause different diseases such as diabetes mellitus and its complications like atherosclerosis and heart disease. This group of compounds is like other polyphenols acts on special mechanism to decrease oxidative stress. This mechanism can be characterized by decreasing oxygen concentration in living cells, intercepting singlet oxygen and preventing first-chain initiation by scavenging initial redicals such as $OH^{\cdot}$. [4,5]

The procyanidins contained in pycnogenol may well become the most important nutritional breakthrough of the 21st century. [1,2]

Patients and Methods :-

- **Patients and materials:**

Ethanol (80%) was used for pycnogenol extraction and methanol (30%) was used for high performance liquid chromatography (HPLC) analysis. Iraqi pine bark was used for pycnogenol extraction.

- **Extraction and analysis of Pycnogenol.**

Pine bark (100 gm) was subjected to a series of extraction steps involved (100 ml) of ethanol.[5] The extraction was achieved using saxholet and then liquid evaporator was performed by rotator evaporator and lypholizer.Extraction percent was (75%).A very fine brownish colored , water soluble powder was identified by high performance liquid chromatography (HPLC 2010A / Shimdzo

/ Japan) coupled to ultra violet (UV)detection. The optimized conditions of HPLC for analysis were as follows:- The analytical column (octadecyl / silan column ODSc18) (12.5 cm long × 4mm i.d. and 5 µm particle diameter) , methanol (30%) as the mobile phase.[6] Ultra violet (UV) detection was recorded at (254 nm) ,column temperature (25C) and injection volume is (20µL). The resulted polyphenolic compounds are :- procyanidin B1 , procyanidin B2 , catechin , procyanidin C2 , taxifolin and ferulic acid.the extraction and analysis were performed in minister of sciences and technology / center of chemicals researches .

- **Patients Groups:-**

Blood Samples were collected from (30) type -2- diabetic patients with age in range of (40-65) years.These samples were collected from patients in the national center of diabetic treatment and researches in Mustansirya University in Baghdad cooperated with a specific laboratory called al –benok laboratory. This group compared with control group which consists of (thirty) healthy subject with age in range of (40-65) years.

- **Pycnogenol samples:-**

Pycnogenol was in a form of capsules (500 mg). These capsules were given to the patients three time daily and parameters were measured after (1 week), (2 weeks) and (3 weeks) of treatment.

- **Determination of glucose level in plasma.**[7]

Glucose level was determined in the sample by an enzymatic colorimeter test using glucose oxidase (GOD) and peroxidas (POD). The absorbance was recorded at (λ max =500 nm).

- **Determination of total cholesterol (TC) level in plasma.** [8]

An enzymatic procedure was used in the presence of peroxidase , cholesterol oxidase and cholesterol esterase. The detection was recorded at (λ max = 510nm).

- **Determination of triacyglycerol (TG) level in plasma.** [9]

A enzymatic procedure was used in the presence of lipase, glycerol kinase , glycerol -3- phosphate oxidase , peroxidase. The absorbance was recorded at (λ max=510 nm).

- **Determination of High density lipoproteins (HDL) level in plasma.**[10]

HDL was measured using Precipitation method. phosphotungestic acid and magnesium ion were used. The Absorbance was recorded at (λ max = 510 nm).

- **Determination of low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels in plasma.** [11,12]

LDL and VLDL levels were determined using using the equations below :-

VLDL = TG / 5

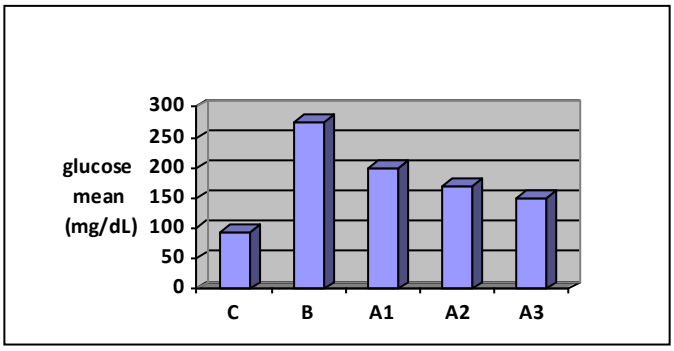
LDL = TC – (HDL + VLDL)

- **Statistical analysis:-**

Statistical significance between means values of biochemical parameters (glucose , Total chplesterol(TC) , triacylglycerol (TG) , high density lipoproteins (HDL) ,low density lipoproteins (LDL) , very low density lipoproteins (VLDL)) was evaluated by the student t.test in the text and tables. This statistical method was performed to determine the significance of the means between the control group and patients 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 :-



(Figure 1) :- Pycnogenol action on fasting plasma glucose level where :-

C:- control group**.**

B :- Patients group before treatment with Pycnogenol.

A_1 :- Patients group after(1 week) of treatment with pycnogenol.

A_2 :- Patients group after(2 weeks) of treatment with pycnogenol. .

A3:- Patients group after(3 weeks) of treatment with pycnogenol.

(Table 1):-The effect of pycnogenol on the level of glucose in patients group in comparison with control group.

Subject	No.	M ± S.D	t.test
control	**30**	**95±12.4**	
B	**30**	**275±36.2**	**P<0.001**
A1	**30**	**200±18.3**	**P<0.001**
A2	**30**	**170±10.2**	**P<0.001**
A3	**30**	**150±7.5**	**P<0.05**

M :- Glucose mean (mg/dL)

No. :- number of patients.

S.D:- standard deviation.

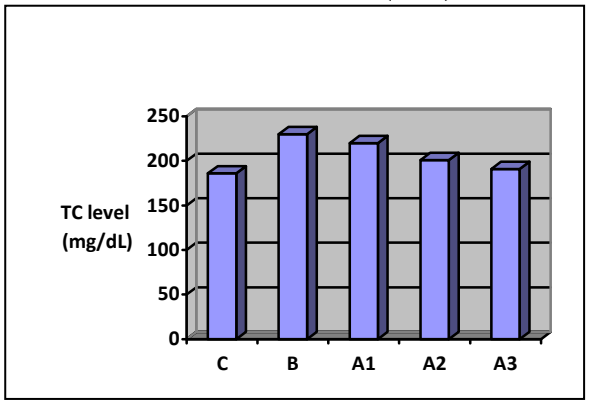
(Figure 1) and (table 1) represent the important action of pycnogenol extract on glucose level in type-2-diabetic patients. The plasma glucose in group B was increased ($p<0.001$) compared with control group (figure 1) and (table 1). This increasing could be explained as insufficient production of insulin from beta cells (insulin regulates glucose metabolism) or because of inability of body cells to use glucose ideally (insulin resistance) , so glucose level would be elevated in the plasma. [13,14]

After treatment with pycnogenol , glucose level was decreased relatively .($p<0.001$) was noted between control group and A1 group. ($p<0.001$) was noted between control group and A2 group and ($p<0.05$) was noted between control group and A3 group. Despite the significant variation between B group and A3 group, glucose level in A3 group (after three weeks of treatment with pycnogenol) could not be reached the normal value. This decreasing could be explained as pycnogenol is a reactive antioxidant able to protect against oxidative stress in several cell systems. [9]

In diabetic patients, the exess amount of glucose could be converted to toxic compounds such as hydrogen peroxide that impair reactive molecules in living cells (lipid peroxidation) . [13,14]

Pycnogenol doubles the intracellular synthesis of antioxidant enzymes such as paraoxonase (PON) and kinases by acting as a potent scavenger of free radicals. [1,2]

2-Pycnogenol action on total cholesterol (TC) level.



(Figure 2) :- Pycnogenol action on total cholesterol (TC) level where:-
C:- control group.
B:- Patients group before treatment with Pycnogenol.
A_1:- Patients group after(1 week) of treatment with pycnogenol.
A2:- Patients group after (2 weeks) of treatment with pycnogenol.
A3:-Patients group after (3 weeks) of treatment with pycnogenol.

(Table 2):-The effect of pycnogenol on total cholesterol (TC) in patients group in comparison with control group.

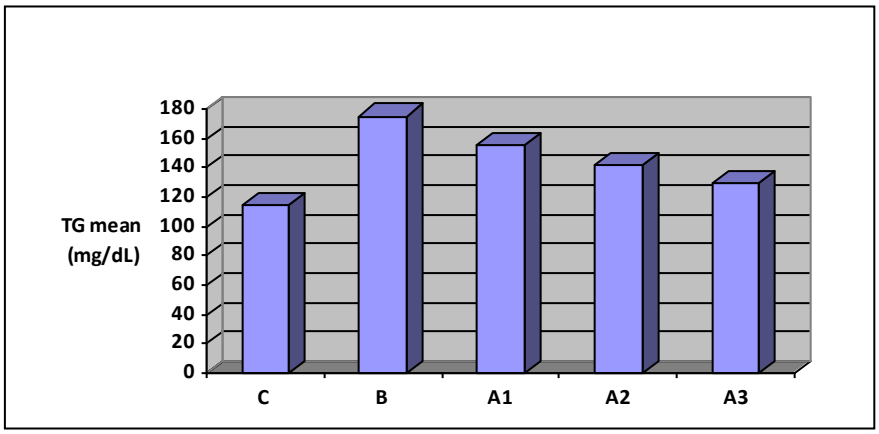
subject	No.	M± S.D	t.test
control	30	186±9.5	
B	30	230±27.2	P<0.001
A1	30	220±14.8	P<0.001
A2	30	201±8.3	P<0.001
A3	30	191±4.2	P<0.01

M:- TC mean mg/dL
No. :- number of patients.
S.D:- standard deviation.

(Figure 2) and (table 2) represent the reactive antioxidant effect of pycnogenol extract on total cholesterol (TC) level in type-2- diabetic patients. The plasma (TC) in group B was increased (p<0.001) compared with control group (figure 2) and (table 2) because storaged lipids are hydrolysed to be used as an energy source instead of glucose , so lipids level will be increased in plasma. (15,16)

After treatment with pycnogenol ,Total cholesterol (TC) level was decreased relatively .(p<0.001) was noted between control group and A1 group. (p<0.001) was noted between control group and A2 group and (p<0.01) was noted between control group and A3 group. This decreasing (after treatment with pycnogenol) are explained as pycnogenol activates enzymes that regulates lipid metabolism and inhibit lipid peroxidation such as kinases and in the same time pycnogenol inhibit enzymes that activate lipid peroxidation such as xanthine oxidase (XO). (15,16)

3-Pycnogenol action on triacylglycerol (TG) level.



(Figure 3) :- Pycnogenol action on triacylglycerol (TG) level where :-
C:- control group.
B:- Patients group before treatment with Pycnogenol.
A_1:- Patients group after(1 week) of treatment with pycnogenol.
A2- Patients group after (2 weeks) of treatment with pycnogenol.
A3:-Patients group after (3 weeks) of treatment with pycnogenol.

(Table 3):-The effect of pycnogenol on triacylglycerol (TG) in patients groups in comparison with control group.

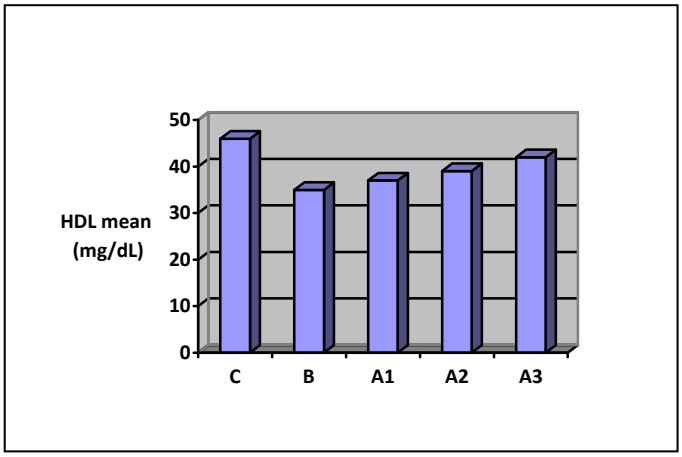
subject	No.	M±S.D	t.test
control	30	115±26	
B	30	175±35	P<0.001
A1	30	155±26	P<0.001
A2	30	142±12	P<0.001
A3	30	130±8.5	P<0.01

M:- TG mean mg/dL
No. :- number of patients
S.D:- standard deviation

(Figure 3) and (table 3) explain the plasma antioxidant properties of pycnogenol extract on triacylglycerol (TG) level in type-2- diabetic patients. The plasma (TG) in group B was increased ($p<0.001$) compared with control group (figure 3) and (table 3) because of body dependence on lipids hydrolysis from tissues as an energy source instead of glucose. [16]

After treatment with pycnogenol ,Triacylglycerol (TG) level was decreased relatively .($p<0.001$) was noted between B control group and A1 group. ($p<0.001$) was noted between control group and A2 group and ($p<0.01$) was noted between Bcontrolgroup and A3 group.AThis decreasing are explained as pycnogenol activates many enzymes that depress oxidative stress and inhibit enzymes that cause lipid peroxidation. [16,17]

4-Pycnogenol action on high density lipoproteins level (HDL) level.



(Figure 4) :- Pycnogenol action on high density lipoproteins level where :-
C:- control group.
B:- Patients group before treatment with Pycnogenol.
A_1:- Patients group after(1 week) of treatment with pycnogenol.
A2:- Patients group after (2 weeks) of treatment with pycnogenol.
A3:-Patients group after (3 weeks) of treatment with pycnogenol.

(Table 4):-The effect of pycnogenol on (HDL) in patients group in comparison with control group.

subject	No.	M± S.D	t.test
control	30	46±9.5	
B	30	35±27.2	P<0.001
A1	30	37±14.8	P<0.01
A2	30	39±8.3	P<0.01
A3	30	42±4.2	P<0.05

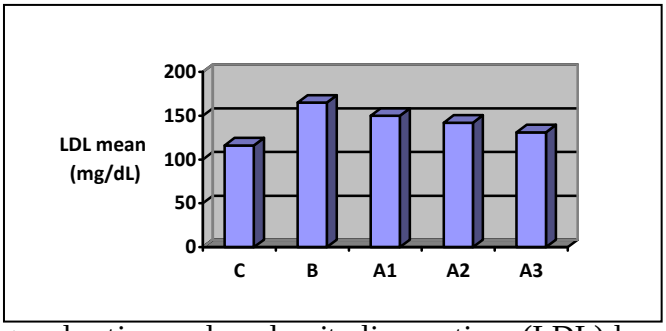
M:- HDL mean mg/dL
No. :- number of patients.
S.D:- standard deviation.

(Figure 4) and (table 4) represent the important role of pycnogenol on high density lipoproteins (HDL level in type-2- diabetic patients. The plasma (HDL) in group B was decreased ($p<0.001$) compared with control group (figure 4) and (table 4). This decreasing are explained as the increasing of malondialdehyde level for diabetic patients and decreasing of polyunsaturated fatty acids ratio that lead to increasing of cholesterol ester transfer protein (CETP) activity that transfer cholesterol ester from(HDL) to very low density lipoproteins (VLDL) , the result is free (HDL) that is filtrated from the kidney.[17]

After treatment with pycnogenol ,high density lipoproteins (HDL) level was increased relatively .($p<0.01$) was noted between control group and A1 group. ($p<0.01$) was noted between control group and A2 group and ($p<0.05$) was noted between control group and A3 group.

Despite the significant variation between A3 group and control group, HDL level in A3 group (after three weeks of treatment with pycnogenol) could not be reached the normal value. This increasing due to pycnogenol activates paraoxonase (PON) action. The last enzyme found in plasma associating with (HDL). Paraoxonase catalyze hydrolysis of lipid peroxides , cholesteryl linoleate hydroperoxides and organophosphates in oxidized HDL.[17]

5- Pycnogenol action on low density lipoprotiens level.



(Figure 5) :-Pycnogenol action on low density lipoprotiens (LDL) level where:-

C:- control group.
B:- Patients group before treatment with Pycnogenol.
A_1:- Patients group after(1 week) of treatment with pycnogenol.
A2:- Patients group after (2 weeks) of treatment with pycnogenol.
A3:- Patients group after (3 weeks) of treatment with pycnogenol.

(Table 5):-The effect of pycnogenol on (LDL) in patients group in comparison with control group.

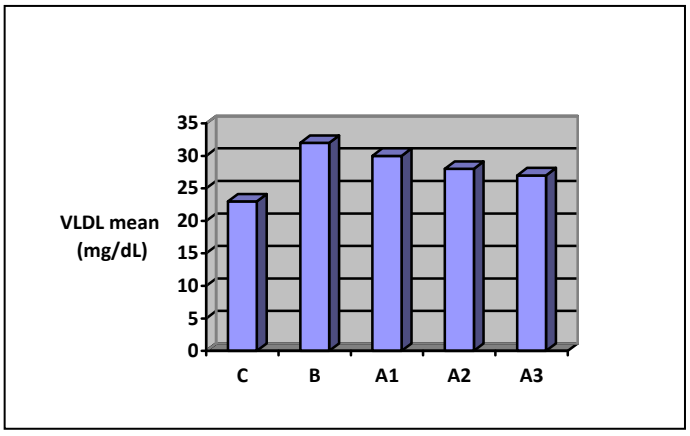
Subject	No.	M±S.D	t.test
control	30	116±12.1	
B	30	165±28.1	P<0.001
A1	30	150±18.6	P<0.001
A2	30	142±11.2	P<0.001
A3	30	130±11.2	P<0.01

M:- LDL mean (mg/dL).
No. :- number of patients.
S.D:- standard deviation

(Figure 5) and (table 5) represent the important effect of pycnogenol on low density lipoproteins (LDL) level in type-2- diabetic patients. The plasma (LDL) in group B was increased (p<0.001) compared with control group (figure 5) and (table 5). Because of the elevation of lipid peroxidation that causes an impairment of (LDL) receptors in cellular membranes (oxidative modification of (LDL) and precipitation in arterial walls. [18]

After treatment with pycnogenol ,low density lipoproteins (LDL) level was decreased relatively .(p<0.001) was noted between control group and A1 group. (p<0.001) was noted between control group and A2 group and (p<0.01) was noted between control group and A3 group. This decreasing are explained as pycnogenol is a reactive antioxidabt that depress oxidative stress maximally by activating enzymes that inhibit lipid peroxidation such as activated monoprotein kinase (AMP) and inhibit enzymes that activate oxidative stress such as phosphodiesterase. [19,20]

6- Pycnogenol action on very low density lipoprotiens level.



(Figure 6) :- Pycnogenol action on very low density lipoprotiens (VLDL) level where:-
C:- control group.
B:-Patients group before treatment with Pycnogenol.
A_1:- Patients group after(1 week) of treatment with pycnogenol.
A2:- Patients group after (2 weeks) of treatment with pycnogenol.
A3:- Patients group after (3 weeks) of treatment with pycnogenol.

(Table 6):-The effect of pycnogenol on (VLDL) in patients group in comparison with control group

Subject	No.	M±S.D	t.test
control	30	23±4.8	
B	30	32±7.9	P<0.001
A1	30	30±5.9	P<0.001
A2	30	28±3.1	P<0.001
A3	30	27±2.3	P<0.01

M:- VLDL mean (mg/dL)
No. :- number of patients.
S.D:- standard deviation

(Figure 6) and (table 6)) represent the reactive action of pycnogenol on very low density lipoproteins (VLDL) level in type-2- diabetic patients. The plasma (VLDL) in group B was increased (p<0.001) compared with control group (figure 6) and (table 6).

After treatment with pycnogenol , very low density lipoproteins (VLDL) level was decreased relatively .(p<0.001) was noted between control group and A1 group. (p<0.001) was noted between control group and A2 group and (p<0.01) was noted between control group and A3 group. Triacylglycerol (TG) is an important component (VLDL). So, the above results could be explained just like pycnogenol action on (TG) level.[16,17]

Conclusion :-

1- Glucose level decreases relatively after treatment with pycnogenol for type -2- diabetes patients. (glucose correlates possitively with oxidative stress).
2- Total cholesterol (TC) ,Triacylglycerol (TG) , Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels decreases relatively after treatment with Pycnogenol for type -2- diabetes patients.(These parameters correlates positively wih oxidative stress).
3- High density lipoproteins (HDL) level increases relatively after treatment with Pycnogenol for type -2- diabetes patients.(HDL correlates negatively with oxidative stress).

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دراسة تأثير البايسنوجينول على سكر الكلوكوز والدهون في المرضى المصابين بالنوع الثاني للسكري.

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قسم الكيمياء

الخلاصة :-

تم إستخلاص البايسنوجينول (المستخلص المائي المتكون من المركبات متعددة الفينول) من لحاء الصنوبر عراقي المنشأ وتم تحليله باستخدام كروموتوغرافيا السائل ذات الكفاءة العالية المترافقة مع طيف الاشعة فوق البنفسجية عند الطول الموجي (254) نانومتر.

إن تأثير البايسنوجينول على بعض المتغيرات الكيميائية الحياتية (الكلوكوز و الكوليستيرول الكلي و الكليسيريدات الثلاثية و البروتينات الدهنية عالية الكثافة و البروتينات الدهنية قليلة الكثافة و البروتينات الدهنية ضئيلة الكثافة) تم تعيينه في (30) مريضا مصابا بالسكري والذين تمت معاملتهم بعينات دوائية (500 ملغم) من البايسنوجينول ثلاث مرات يوميا لاختبار الدور الفعال للبايسنوجينول ضد شدة الاكسدة.إن المحددات الكيميائية أعلاه تم قياسها في البلازما قبل العلاج بالبايسنوجينول وبعد مرور (أسبوع) و(أسبوعين) و (ثلاثة أسابيع) من العلاج بهذا المستخلص متعدد الفينول.

إن نتائجنا أظهرت أن مستوى الكلوكوز يزداد نسبيا لدى مرضى السكري بالمقارنة مع مجموعة السيطرة ($p<0.001$) ولكن بعد المعاملة بالبايسنوجينول فإن مستوى الكلوكوز ينخفض نسبيا ($p<0.001$) بعد أسبوع و($p<0.001$) بعد أسبوعين و ($p<0.05$) بعد ثلاثة أسابيع من العلاج بالبايسنوجينول.

إن مستوى الكوليسترول الكلي يرتفع نسبيا لدى مرضى السكري بالمقارنة مع مجموعة السيطرة ($p<0.001$) ولكن بعد العلاج بهذا المركب الفعال فإن مستوى الكوليسترول الكلي ينخفض نسبيا ($p<0.001$) بعد أسبوع و($p<0.001$) بعد أسبوعين و ($p<0.01$) بعد ثلاثة أسابيع من العلاج .

إن مستوى الكليسيريدات الثلاثية يزداد نسبيا لدى المصابين بالسكري مقارنة مع مجموعة السيطرة ($p<0.001$) ولكن بعد المعاملة بهذا المستخلص المضاد للاكسدة فإن مستوى الكليسيريدات الثلاثية ينخفض نسبيا ($p<0.001$) بعد أسبوع و($p<0.001$) بعد أسبوعين و ($p<0.01$) بعد ثلاثة أسابيع من العلاج .

إن مستوى البروتينات الدهنية عالية الكثافة ينخفض نسبيا لدى المصابين بالسكري بالمقارنة مع مجموعة السيطرة ($p<0.001$)ولكن بعد العلاج بالبايسنوجينول فإن مستوى البروتينات الدهنية عالية الكثافة يزداد نسبيا ($p<0.01$) بعد أسبوع و($p<0.01$) بعد أسبوعين و ($p<0.05$) بعد ثلاثة أسابيع من العلاج بالبايسنوجينول.

إن مستوى البروتينات الدهنية قليلة الكثافة يزداد نسبيا لدى المصابين بالسكري بالمقارنة مع مجموعة السيطرة ($p<0.001$)ولكن بعد العلاج بمستخلص البايسنوجينول فإن مستوى البروتينات الدهنية قليلة الكثافة ينخفض نسبيا ($p<0.001$) بعد أسبوع و($p<0.001$) بعد أسبوعين و ($p<0.01$) بعد ثلاثة أسابيع من العلاج بالبايسنوجينول.

إن مستوى البروتينات الدهنية ضئيلة الكثافة يزداد نسبيا لدى المصابين بالسكري بالمقارنة مع مجموعة السيطرة ($p<0.001$)ولكن بعد العلاج بالبايسنوجينول فإن مستوى البروتينات الدهنية ضئيلة الكثافة ينخفض نسبيا ($p<0.001$) بعد أسبوع و($p<0.001$) بعد أسبوعين و ($p<0001$) بعد ثلاثة أسابيع من العلاج بالبايسنوجينول.