

The Prophylactic Role of Flavonoids of Black Grape (*Vitis vinifera* L.) on Some Biochemical Parameters in Adult females Rats Treated with Sodium Fluoride

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Abstract

Present study was aimed to investigate the protective role of flavonoid of black grape (*Vitis vinifera* L.) to contrast the damaging of 100ppm sodium fluoride (NaF) on some biochemical parameters (glucose, insulin, α -amylase and peroxy nitrate) in females rats. Twenty females rats were divided randomly into four groups and treated for (30) days as follows: control group (C) which were given tap water. First treated group (T1) was given 100ppm in drinking water, the second treated group (T2) was given flavonoids extract from black grape (300mg/kg) by oral intubation, third group (T3) was given 100 ppm of sodium fluoride plus 300mg/kg of flavonoids of black grape. Blood samples were collected after 30 days for measuring the following parameters: glucose, insulin, α -amylase and peroxy nitrate. The results illustrated a significant increase ($P < 0.05$) in blood glucose concentration of sodium fluoride treated group (T1) and sodium fluoride plus flavonoids (T3), concentration of insulin in sodium fluoride treated group, and concentration of peroxy nitrate radical in sodium fluoride treated group (T1) and sodium fluoride plus flavonoids (T3) groups as compared with control group. Significant depression ($P < 0.05$) in α -amylase activity in sodium fluoride treated group (T1) and sodium fluoride plus flavonoids (T3) as compared with other treated groups was also recorded. From the outcomes of this study documented seems that treatment with flavonoids of black grape (*Vitis vinifera*) at concentration of 300mg/kg B.W has the ability to correct the adverse effect of sodium fluoride in females rats. However, there is no significant differences in group treated with flavonoids as compared with control. Histological sections of liver illustrated clear impact of group treated with NaF, manifested by necrosis of hepatic cells with infiltration of inflammatory cells while animals treated with flavonoids of black grape plus NaF revealed slight necrosis area and inflammatory cells in liver. However, there are no abnormal changes in animal treated with flavonoids of black grape. In inference, the outcomes of this study documented the ameliorative effect of flavonoids of black grape apposite the noxious effect of NaF on some biochemical parameters that effect on liver function.

Key words: sodium fluoride, flavonoids, insulin, peroxy nitrate, black grape.

الدور الوقائي لفلافونويدات العنب الاسود في بعض المعايير الكيموحيوية في ذكور الجرذان المعاملة بفلوريدات الصوديوم

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الخلاصة:

هدفت الدراسة الحالية الى توضيح تأثير فلافونويدات العنب الاسود (300ملغم/كغم) وفلوريدات الصوديوم (100 جزء من المليون/لكل حيوان) في بعض المعايير الكيموحيوية (مثل سكر الدم وانزيم الاميليز وهرمون الانسولين والبيروكسي نايتريت) في ذكور الجرذان المعاملة ب فلوريدات الصوديوم. عشرون جردي تم تقسيمهم عشوائيا الى اربع مجاميع وعوملت لمدة ثلاثون يوما وكما يلي: مجموعة سيطرة اعطيت ماء عادي. المجموعة المعالجة الاولى (T1) اعطيت فلوريدات الصوديوم في ماء الشرب، المجموعة المعالجة الثانية (T2) اعطيت مستخلص لفلافونويدات العنب الاسود فمويا، المجموعة المعالجة الثالثة (T3) اعطيت فلوريدات الصوديوم مع فلافونويدات العنب الاسود. جمعت عينات الدم بعد 30 يوم لقياس المعايير التالية: الكلوكوز، الانسولين، الفا - الاميليز وبيروكسي نايتريت. اوضحت النتائج ارتفاع معنوي ($p < 0.05$) في تركيز سكر الدم في مصلى حيوانات المجموعة المعاملة بفلوريدات الصوديوم (T1) والمجموعة المعاملة بفلوريد الصوديوم مع فلافونويدات العنب الاسود (T3)، وكذلك اوضحت النتائج ارتفاع معنوي في تركيز هرمون الانسولين في مصلى الحيوانات المعاملة بفلوريدات الصوديوم وكذلك في تركيز جذر البيروكسي نايتريت في مصولى حيوانات المجموعة المعاملة بفلوريدات الصوديوم فقط (T1) والمجموعة المعاملة بفلوريدات الصوديوم مع الفلافونويدات (T3) كمقارنة مع مجموعة السيطرة. وسجلت الدراسة انخفاض معنوي في فعالية انزيم الاميليز في مصولى الحيوانات المعاملة بفلوريدات الصوديوم والفلافونويدات معا (T3) كمقارنة مع المجاميع الاخرى. مع ذلك لا توجد اي اختلاف معنوي في المجموعة المعاملة بالفلافونويدات فقط مقارنة مع مجموعة السيطرة. وكننتيجة لهذه الدراسة فقد تم اثبات بان المعالجة بفلافونويدات العنب الاسود (*Vitis vinifera*) وبتركيز 300ملغم/كغم لها القدرة على تصحيح تأثير اضرار فلوريدات الصوديوم في ذكور الجرذان. اظهرت الفحوصات المرضية النسجية للكبد تأثيرا واضحا في المجموعة المعاملة بفلوريدات الصوديوم حيث لوحظ تنخر في الخلايا الكبدية مع ارتشاح للخلايا الالتهابية، وعندما جرعت حيوانات التجربة بفلافونويدات الزبيب الاسود مع فلوريدات الصوديوم لوحظ تنخر اقل وارتشاح طفيف للخلايا الالتهابية في الكبد. وفي النهاية اثبتت هذه الدراسة التأثير المفيد لفلافونويدات الزبيب الاسود ضد التأثير الضار لفلوريد الصوديوم في بعض المعايير الكيموحيوية وتأثيراتها في الكبد في اناث الجرذان البالغة.

Introduction

Vitis vinifera L. (black grape) belonging to family *Vitaceae* which is contain a large important components like protein, fat, carbohydrates, vitamins like A, C, B1, B2 & B3, also contain iron, calcium, phosphate and potassium (1,2). Accumulating evidence suggest that consumption of grapes and grape products can positively influence risk factors associated with cardiovascular health, cancer, neurodegenerative disease and age-related cognitive decline. These effects are often attributed to the antioxidant activity and function of flavonoid compounds found in grapes as well as other actions such as increasing nitric oxide production. The well-established health effects of grapes on cardio vascular disease risk,

mainly on endothelial function, LDL, oxidation, progression of atherosclerosis, and reduction oxidation stress, have been clearly identified. Emerging research has also demonstrated that grapes have beneficial effects on other chronic degenerative disease such as cancer, Alzheimers disease, age-related cognitive decline, and diabetes (3,4). Sodium fluoride (NaF) is used in fluoridating municipal water supplies, resulting in chronic exposure of millions of people worldwide. Fluoride is a very abundant chemical element on the surface of the earth and is recognized as an element that may produce chronic alterations in human when they exposed to it. Fluorosis (intoxication by fluoride) is caused by acute or chronic intake of fluoride and

is chemically characterized in human by means of alterations in dental cavities, as well as changes in the musculo-skeletal & nervous systems (5,6) Sodium fluoride is also effect on hyperactivation and Ca^{+2} signaling pathway of sperm in mice (7). Most acute poisonings in humans caused by flavonoids as described in literature, are associated with suicidal or accidental deglutition of fluoride-containing preparations, for example, insecticides, most often, poisoning resulted from the intake of sodium fluoride, sodium fluosilicate. In dismissing the occurrence of allergic reactions to fluoride, the Newzealand report(8), and Australian(9). Other reports are equally conflicting. (5) found in rabbits that 2-4 weeks exposure to 10 and 50mg NaF/kg B.W. led to hypoplasia of bone marrow, markedly decrease total nucleated cell counts and anemia.

Materials & Methods

Twenty female's albino wistar rats (200-250gm) were used in this study. Animals in all groups of the experiment were housed in plastic cages in conditional room (20-25⁰C) in the animal house of department of physiology and pharmacology at the college of veterinary medicine – university of Baghdad .Animals had free access to water and standard pellet diet along the experiment(30 days). The rats were randomly divided into four groups (5 rats/group) and were handled as follows: Group C (-ve): animals in this group were administered tap water and served as control; Group T1: animals were administered sodium fluoride 100ppm/for each animal (10)in drinking water ;group T2:animals in this group were administered flavonoids of black grape (*Vitis vinifera* L.)300mg/kg.B.W(11)by

orally gavage; group T3:animals were administered 100ppm of sodium fluoride plus 300mg/kg B.W. of flavonoids of black grape (*Vitis vinifera* L.) according to the method of (12)modified by (13)was used(acid hydrolysis was carried out by 2N hydrochloric acid, ethyl acetate and petroleum ether as organic solvent) .The rats were sacrificed after using ketamine(87mg/kg) and xylazine(13mg/kg) as anasethsia and blood samples collected for evaluation the following parameter like insulin was measured by analyzer/ELIZA (TOSOH/Japan) ,glucose,amylase enzyme were measured by using a kit (Linear /Spain) and peroxy nitrate was measured according to (14) . Statistical analysis show differences between experimental groups were evaluated using one-way analysis of variance (ANOVA) specific group differences were determined using least significant differences (LSD) for all analysis, $P<0.05$ was considered to be significant, according to (15).

Results & Discussion

The effects of and 100ppm of sodium fluoride (T1)and 100ppm of sodium fluoride plus flavonoids of black grape (*Vitis vinera* L.) (T2) on blood glucose is clarified in table(1),showed significant increase in this portion ($P<0.05$) which may be attributed to toxicity of sodium fluoride and its effect on liver function(16,17)as well as it is effect on charbohydrates balance (18,19,20) ,intoxication by NaF affect metabolic process including blood glucose concentration in animals & human who lives in zones of fluorosis (21,17),treatment of animals with black grape-flavonoids alleviate the hyperglycemic effect of NaF due to resveratol&quercetin compound which

prevent oxidative injury (22) ,and modulate glucose haemostatic(23,24).

Table (1): Effect of flavonoids of black grape fruit (*Vitis vinifera L.*)at dose 300mg/kg and 100ppm of sodium fluoride on the blood glucose concentration mg/dl in male rats.

Groups	Blood glucose conc. mg/dl(mean+SE)
Control C	140.00 +5.14 B
Sodium fluoride (T1)	158.28+3.58 A
Flavonoids (T2)	140.90+1.71 B
NaF+flavonoid (T3)	150.67+0.26 A
LSD	7.7

Values expressed as means±SE. (n=5/group)

Capital letters denote between group differences (P≤ 0.05)

Table (2),pointed to significant (P<0.05)increase erum of insulin concentration in sodium fluoride group(T1) ,which may due to toxic effect of fluorosis on tissue (19)causing or increase in blood glucose concentration in this group that cause increase insulin secretion to maintance the blood concentration in normal values.Grape seed extract ,a well-known dietary supplement (25) with its proanthocyanidins,posses insulinomimetic properties (26)by stimulating glucose uptake in insulin-sensitive cell lines and decrease hyperglycemia,streptozotocin-diabetic rats(27).

Table (2): Effect of flavonoids of black grape fruit (*Vitis vinifera L.*)at dose 300mg/kg and 100ppm of sodium fluoride on the Insulin activity μU/ml in male rats.

Groups	Concentration of Insulin μU/ml
Control C	7.232500 ±0.5607194 B
Sodium fluoride T1	14.56250±0.483242 A
Flavonoids T2	7.24500±0.356031 B
NaF+flavonoid T3	7.58250±±0.346804 B
LSD	1.2

Values expressed as means±SE. (n=5/group)

Capital letters denote between group differences (P<0.05)

Table,3 clarified a significant decrease ($P<0.05$)in α -amylase activity in both sodium fluoride group and sodium fluoride plus flavonoid as comparing with control which may be attributed to inhibition of enzymes involved in cellular metabolism by fluoride mainly those in glycolytic pathway(28), also there is a slight significant decrease in animals treated with flavonoids of black grape as comparing to T1 and T3 ,this may be due to the inhibitory activity of grape against intestinal alpha-glucosidase and pancreatic α -amylase, resulting in delayed carbohydrates digestion(29).

Table (3): Effect of flavonoids of black grape fruit(*Vitis vinifera L.*)at dose 300mg/kg and 100ppm of sodium fluoride on the α -amylase activity U/L ($\Delta A/\text{min} \times 3591$) in male rats.

Groups	Activity of α -amylase U/L ($\Delta A/\text{min} \times 3591$)
Control C	2028.91± 349.66 A
Sodium fluoride T1	500.34± 74.21 B
Flavonoids T2	1773.95±278.54 A
NaF+flavonoid T3	680.49 ± 57.65 B
LSD	546

Values expressed as means±SE. (n=5/group)

Capital letters denote between group differences ($P< 0.05$)

Sodium fluoride alone causes a significant increment in concentration of peroxy nitrate radical as shown in table (4) comparing to sodium fluoride +flavonoids group and others groups ,this may be due to injury of fluoride which was caused damage for tissue,with disturbances of the antioxidant system,and causes lipid peroxidation and oxidative stress(30,31).

Table(4):Effect of flavonoids of black grape fruit(*Vitis vinifera L.*)at dose 300mg/kg and 100ppm of sodium fluoride on the peroxy nitrate activity M/L in male rats.

Groups	Concentration of Peroxy nitrate M/L Mean±SE
Control c	36.67± 0.81 C
Sodium fluoride T1	58.71± 2.60 A
Flavonoids T2	34.64±1.05 C
NaF+flavonoid T3	49.14±5.21 B
LSD	6.9

Values expressed as means±SE. (n=5/group)

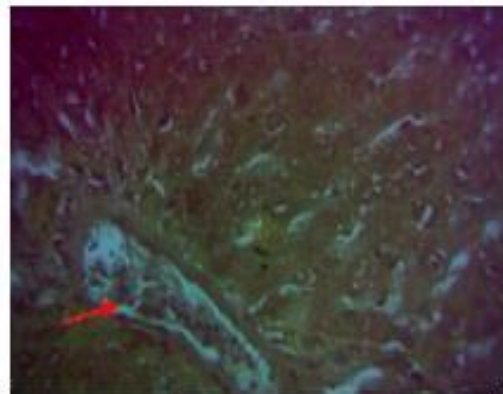
Capital letters denote between group differences (P<0.05)

Histopathological changes in liver tissue: According to control (**Fig.1**),the microscopic examination of rats livers received 100ppm/animal of sodium fluoride show congested central vein with infiltration of inflammatory cells surrounded with necrotic cells (**Fig.2**)and absence of sinusoids and vaculated of hepatocytes (**Fig.3**)and thrombosis (**Fig.4**). This may be due to effect of NaF that causes oxidative stress which is one of the important mechanisms of toxic effect of

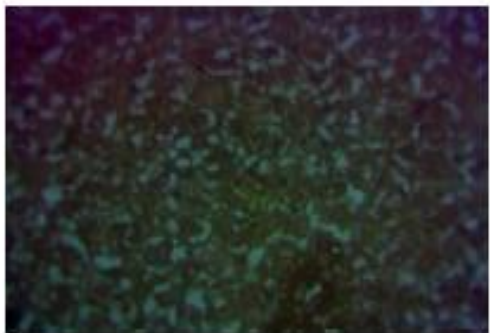
fluoride(**32,31,33**).The histopathological examination of liver rats received 100 ppm/animal plus 300mg/kg showed dilated of central vein with slight number of inflammatory cells and necrotic area (**Fig.5**)and fibrosis of necrotic cells (**Fig.6**), this result may be due to presence of flavonoids like resveratrol in grapes (**34,35,36 and 10**).Other (**Fig.7 and 8**) showed there are no abnormal changes in liver tissue .



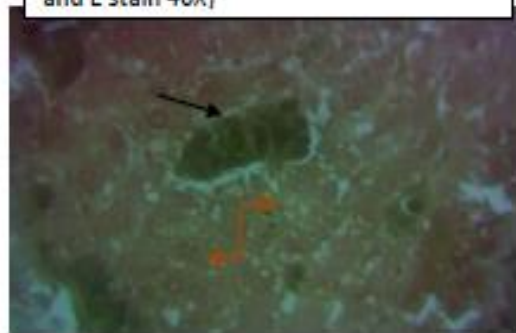
Figure, 1: Histological section (control group) in the rat liver at 30 days post treated with tap water (H and E stain 40X)



Figure, 2: Histopathological section in the rat liver at day 30 post treated with sodium fluoride shows congested central vein filled with inflammatory cells → surrounded with necrotic hepatocytes(H and E stain 40X)



Figure, 3: Histopathological section in the rat liver at day 30 post treated with sodium fluoride shows absence of sinusoids and vacuolated hepatocytes(H and E stain 40X)



Figure, 4: Histopathological section in the rat liver at day 30 post treated with sodium fluoride shows thrombosis → with necrotic area → (H and E stain 40X)

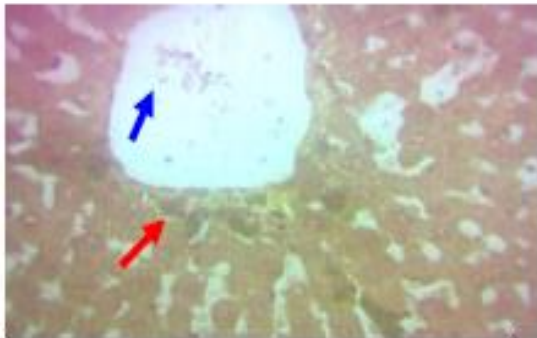


Figure5,;Histopathological section in the rat liver at day 30 post treated with sodium fluoride plus flavonoids of black grape shows dilated central vein surrounded by necrotic hepatocytes → and infiltrated with slight number of inflammatory cells → (H and E stain 40X)

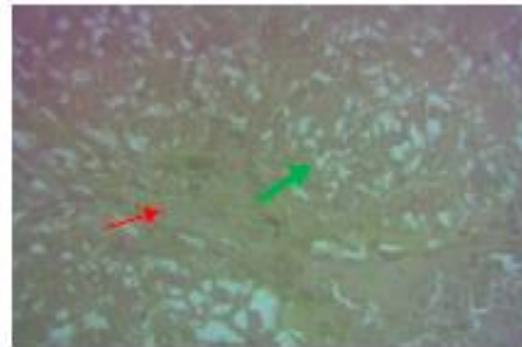
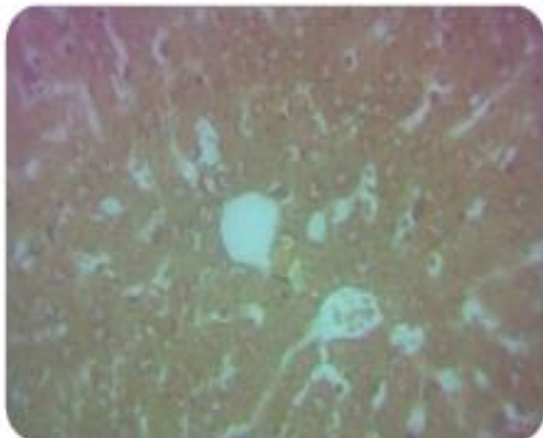
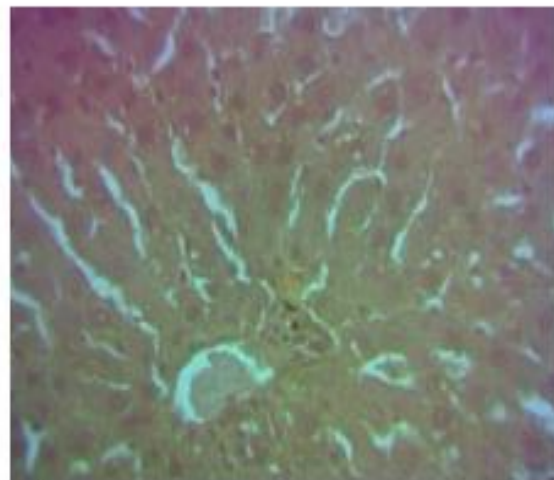


Figure6,;Histopathological section in the rat liver at day 30 post treated with sodium fluoride plus flavonoids of black grape shows vacuolated hepatocytes → with necrotic cells → (H and E stain 40X)



Figure,7:Histopathological section in the rat liver at day 30 post treated with flavonoids of black grape ,there is no abnormal changes (H and E stain 40X)



Figure,8:Histopathological section in the rat liver at day 30 post treated with flavonoids of black grape ,there is no abnormal changes (H and E stain 40X)

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