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## The efficiency of red grapes and beetroot juice as nutrients for improving blood glucose levels, renal function, liver enzymes, and immunity factors

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### ABSTRACT

This investigation was to evaluate the biological effects of red grape and beetroot juice nutrients on glucose level, renal function, liver enzymes, and immune status using rats as an animal model. Results revealed that rat diets supplemented with 5% red grape juice and 10% beetroot juice significantly  $P < 0.05$  decreased the serum glucose levels from 150.3 mg/dL to 85.5 mg/dL. However, we recorded a significant decrease in serum urea, creatinine, and uric acid concentrations as compared to the positive control group. Rats supplemented with 10% food compositions of both red grape and beetroot juice had a significant  $P < 0.05$  decrease in both ALT and AST from 85.3 U/L and 155.3 U/L to 25.3 U/L and 59.6 U/L, respectively. On the other hand, (5% red grape/beetroot juice), increased both cellular immunity phagocytes and lymphocyte cells by (76,1.31 and 90,1.78 ) respectively, without any histopathological change in the kidney suggesting that the selected experimental diet is safe.

**Keywords:** Red grape, Beetroot, Nutrients, Glucose level, Immunity, Renal function

### المخلص:

تهدف هذه الدراسة لتقييم التأثير البيولوجي والوقائي لعصير العنب الأحمر (RGJ) وعصير البنجر (BRJ) على الحالة المناعية، وظائف الكبد، ووظائف الكلى، مستوى الجلوكوز ومستوى الدهون في الفئران المصابة بمرض السكر. تم استخدام اثنين وأربعين من الفئران البيضاء الذكور (١٩٠-٢٠٠ جم) وقسمت الى المجموعة الاولى كمجموعة ضابطة سالبة. وتم إحداث مرض السكر في باقى المجموعات، المجموعة الثانية تم تغذيتها على الوجبة القياسية فقط. اما المجموعات الثالثة والرابعة والخامسة والسادسة والسابعة تم تغذيتها على وجبات غذائية

مدعمة بما يلي: ٥% و ١٠% عصير العنب الاحمر، ٥% و ١٠% عصير ثمار البنجر و ٥% من كلا من (عصير العنب الاحمر + عصير البنجر) على التوالي لمدة 6 اسابيع. وفي نهاية فترة التجربة تم سحب عينات دم لقياس مستويات دلالات الحالة المناعية (المناعة الخلطية والمناعة الخلوية)، سكر الدم، وظائف الكبد، كولستيرول الدم والدهون الثلاثية وتم قياس وظائف الكلى. أوضحت النتائج أن الفئران التي تم تغذيتها على عصير العنب بنسبة ٥% وعصير البنجر ١٠% كان لديها انخفاض معنوي عند مستوى الدلالة ( $P \leq 0.05$ ) في مستويات مصل الدم من الجلوكوز من (١٥٠ ملجم الى ٨٥.٥ ملجم)، الكرياتينين، اليوريا، حمض اليوريك، AST، ALT. وعلى الجانب الاخر كانت هناك زيادة كبيرة في مستويات المناعة الخلوية والمناعة الخلطية. نستنتج أن عصير العنب الأحمر وعصير البنجر يمكن أن تحمي من مرض السكري من النوع الثاني وتزيد من مؤشرات المناعة، ويحسن حساسية الأنسولين، ويزيد من مستويات NO3 في المصل والأنسجة، ويقلل من اليوريا، والإجهاد التأكسدي، والخلل الكلوي. لذلك يوصى تناول عصير العنب الأحمر والبنجر لما له من فائدة للمرضى الذين يعانون من مرض السكري واختلال وظائف الكلى.

**الكلمات المفتاحية:** العنب الأحمر، البنجر مرض السكري، مؤشرات المناعة، وظيفة الكبد، الدم الجلوكوز، الدهون.

## 1. INTRODUCTION

A diet rich in fruits and vegetables has been linked to several health benefits, which have sparked interest in what are known as “functional foods” and their application in health and disease. Meanwhile, fruits and vegetables are beneficial for people of all ages and essential for good health. Among these fruits is the red grape, which is rich in antioxidants, vitamin C, and certain polyphenols beneficial to cellular immunity **Magrone et al.,(2020)**. Beetroot consumption has been linked to favorable physiological effects that may enhance clinical outcomes for a number of diseases, including dementia, type 2 diabetes, hypertension, and atherosclerosis, according to recent studies on dementia (**Presley et al., 2015 ; Vanhatalo et al., 2010 ; Bailey et al., 2009 and Webb and Patel, 2008** ). Numerous treatment interventions have focused on lowering blood pressure and blood sugar levels, and multiple trials have demonstrated the benefits of beetroot juice supplementation administered immediately (**Jajja et al., 2014; Hobbs et al., 2013 and Hobbs et al., 2014** ). Beetroot is an herbaceous biennial plant with yellow or red bulbs. consider one of the food sources rich in antioxidants and vitamin C

(Clifford and Howatson, 2015). Beetroot has been identified in several studies as one of the best food sources of nitrates and antioxidants Wootton et al .,( 2014)and Fu,(2020) . Beets have an impressive nutritional profile. They're low in calories yet high in valuable vitamins and minerals, (100-gram) serving of boiled beetroot contains 44 calories, 1.7 gramme protein, and 10 gramme carbs Wootton et al .,( 2014). Grape had a significant effect on lipid levels, decreasing total and low-density lipoprotein cholesterol and triacylglycerol levels. Systolic and diastolic blood pressure were also lowered by grape supplementation (Graham and Kemp, 2016).

The flavonoid content of red grapes may be attributed to their glucose level Ghorbani et al.,(2019). A person may benefit from consuming red grapes because they contain high levels of anthocyanins and resveratrol Rasines-Perea and Teissedre, (2017) 'which have been shown to prevent LDL oxidation, oxidative stress, dyslipidemia, and inflammation Haghghatdoost et al., (2020). Based on the above criteria, our study group sought to determine how fresh beetroot juice and red grape juice affected on the blood glucose level, renal function, lipid profile, and immune indicators .

## 2. MATERIALS AND METHODS:

### Fruits and vegetables:

Both fresh fully ripe red grapes (*Vitis vinifera L.*) and beetroots (*Beta vulgaris L., Chenopodiaceae*) were purchased from local stores in the Al-Baha region of Saudi Arabia. To make the juice, the red grape and beetroot were chopped into small pieces, rinsed with water, and then blended using an electric blender .The chemical composition was determined using the methods described by Kemmerer et al .(2004) Table( 1)

### Chemical composition of beetroot and red grape juice:

Beetroot and red grape juice contained the chemical composition shown in Table (1). The chemical composition was determined using the methods described in (Kemmerer et al ., 2004).

### Rats:

We obtained the rats from the Ministry of Health and Population in Helwan, Egypt's Laboratory Animal Colony. Forty-two adult male rats of Sprague Dawley strain, weighing between  $190 \pm 10$ g were used in this study.

### Basal diet:

- Basal diet was prepared according to the method of Reeves et al .,(1993). The composition included 10% carbohydrates, 4.7% fat

(corn oil), 1% vitamin combination, 2% choline chloride, 3.5% salt mixture, 5% fibers, and 20% protein (casein). 100% corn starch made up the remaining portion.

- **Chemicals and Kits:**

All chemicals and biochemical analysis kits were obtained from Gamma Trade Company for Pharmaceuticals and Chemicals, Dokki, Egypt.

**Diabetic mellitus induction:**

Diabetes has been induced by a single subcutaneous injection of alloxan dissolved in sterile normal saline at a dose of 120 mg/kg body weight according to the method described by (Buko et al., 1996). Non-diabetic control rats have been injected with an equivalent amount of slain solution. Diabetic rats were then permitted to drink water with a 10% glucose solution for the next 24 hours to prevent hypoglycemia. Seventy-two hours after injection with Alloxan, the diabetic rats have been confirmed by measuring the 4-hr fasting blood glucose level from the tail vein. Animals with a blood glucose level above 250 mg/dL were considered diabetic and included in the experiment.

**Experimental design:**

Forty-two males of Sprague-Dawley strain weighting (190 ± 10) were used in this experiment. All rats were kept in an animal home for one week to allow them to acclimate. The conditions included a room temperature of 24 ± 2 °C, a relative humidity of 50–55%, and 12-hour light/dark cycles. All animals were divided into seven equal groups (n = 6 rats) at random following the one-week acclimation phase.

- Control (negative control) group was fed on the basal diet only.
- diabetic control (positive control) group was fed on the basal diet only.
- Group A1: The diabetic group was fed a basal diet supplemented with 5% red grape juice.
- Group A2: The diabetic group was fed a basal diet supplemented with 10% red grape juice.
- Group B1: The diabetic group was fed a basal diet supplemented with 5% beetroot juice.
- Group B2: The diabetic group was fed a basal diet supplemented with 10% beetroot juice.
- Group C: The diabetic group was fed a basal diet supplemented with 5% of both red grape and beetroot juice were given to the investigation group during the six-week trial period, while the control (positive and negative) group drank tap water.

### **Blood collection and serum separation:**

At the end of the experiment the animals were starved for 24 hours, after which they were given the appropriate volumes of anesthetic agents (a dose of 80 mg/kg of ketamine and 10 mg/kg of xylazine) administered by intraperitoneal injection in the lower left quadrant of each mouse under manual restraint , and a blood sample was taken from the corner of each eye. Using a capillary tube) (**Borchard et al., 1990** ).Blood samples were taken within tube tests devoid of anticoagulant volume (4–5 mL), then the serum was separated by a centrifuge, and the serum was kept at a temperature of -20 degrees Celsius until the analyses were performed and 3 mL of the blood samples were transferred in ethylene diamine tetra-acetic acid (EDTA) tubes for immunological evaluation .

### **Biochemical analysis:**

Determination of blood glucose was carried out by **Siest et al., (1981)**. Determination of AST and ALT was carried out according to the method of **Bergmeyer et al., (1972)**. Serum total cholesterol (TC), high-density lipoprotein (HDL-c), and low-density lipoprotein (LDL-c) were determined according to Weichselbaum (1946). Triglycerides (TG) was determined according to **Larsen , (1972 )**.Creatinine concentration was determined according to **Carawy, ( 1955)** and uric acid was estimated by **Fossati et al., (1980)**.

### **Immunological study:**

Evaluation of the effectiveness of humoral and cellular immunity was done at AL-Baha University's Faculty of Sciences and Arts (Buljurshi). In accordance with the biuret reaction method (**Weichselbaum, 1946**). The total serum proteins were ascertained. Serum protein fractions can be quantitatively estimated using the method outlined by **Woldehiwet and Rowan ( 1990)** :total lymphocyte count, cellular immune response, viability cell count, and phagocytosis, as reported in **Laemmlli (1979)**.

### **3-Histopathology**

After the kidney's tissue samples were removed, they were cleaned of blood using regular saline solution and then put in 10% neutral buffered formalin. Following xylol clearing, the specimens were embedded in paraffin, sectioned at a thickness of 4-6 microns, and stained with hematoxylin and eosin (H&E) stain for histological analysis in accordance with (**Carleton, 1979**). Egypt's Cairo University's Faculty of Veterinary Medicine conducted the histology analysis.

#### 4-Statistical analysis

SPSS for Windows, version 20 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA) was used to analyze all of the data that were collected. The collected data was shown as mean +/- standard error (SE). The Analysis of Variance (ANOVA) test has been employed to ascertain the significances between various groups. If P-values were ( $P < 0.05$ ), then all differences were deemed significant **Snedecor and Cochran (1986)** .

#### 5.RESULT:

Nutrients and bioactive compounds of beetroot and red grape juice as shown in table (1), 100 mL of beetroot juice and grape juice contained 13- 0.84 g carbohydrates, 0.1-1.61 g proteins, 0.1- 1.61g total dietary fiber and 13 – 0.84g. The analysis of minerals content revealed that, juice contained the highest level of potassium (7250.00 - 1603,0 mg/100 g) ,Total phenolic compound (255- 5698.67) and Total flavonoid content ( 126 - 103.34).

#### Serum glucose level

In this study, the effect of red grape and beetroot juice feeding on three groups of adult male rats of the Sprague Dawley strain (A, B, and C) was studied. Our results showed a significant improvement in blood glucose levels . The best result for the decrease in serum glucose, 85.55 mg/dL, was obtained for the feeding diabetic rats on a diet containing 10% beetroot juice, which is very close to the negative control, 83 mg/dL. 10% of red grape juice feeding is enough to decrease the level of serum glucose to 104.67 mg/dL, which is almost like that obtained from the mixed juice composition in Group C, 5%, compared to the glucose level control. (Table 2).

**Table(1 ) The chemical composition of red grapes and beetroots**

Compound	Beetroot (mg)	red grape (mg)
<b>Water (g)</b>	87.58	84.51
<b>Protein (g)</b>	0.1	1.61
<b>Total fats (g)</b>	1.61	0.1
<b>Carbohydrate (g)</b>	13	0.84
<b>Fiber (g)</b>	13	0.84
<b>Sugars (g)</b>	11.76	6.76
<b>Calcium (mg)</b>	16	11
<b>Iron (mg)</b>	0.8	0.25
<b>Magnesium (mg)</b>	23	106.0
<b>Phosphorus (mg)</b>	40	14
<b>Potassium (mg)</b>	7250.00	1603,0
<b>Vitamin C (mg)</b>	68	81

Total phenolic compound (mg/L)	255	5698.67
Total flavonoid content (mg/L)	126	103.34

### Kidney function profile

Results in Table 3 declared that all groups fed a diet enriched with beetroot juice (5% and 10%) had a significant decrease in serum urea, creatinine, and uric acid concentrations as compared to the positive control group. The best group had 5% BRj (B1) ( $0.78 \pm .025$ ,  $24.67 \pm .41$  and  $2.57 \pm .44$ ) to compared to the positive control group

**Table (2) Effect of red grapes (RGj) and beetroot juice (BRj) on serum glucose level (mg/dL) in hyperglycemic rats.**

Animal groups	Nutrient composition%	Blood Glucose (mg/dL)
A	RGj (A1) RGj (A2)	5% 10%
		$131.3 \pm .0452$ $104.67 \pm .041^*$
B	BRj (B1) RGj (B2)	5% 10%
		$120.3 \pm .0358$ $85.55 \pm .024^*$
C	RGj/BRj	5% $106.2 \pm .0190^{**}$
Negative control	--	$83 \pm .04^*$
Positive control	--	$150.3 \pm .902$

(\* the values mean significant differences at  $(P < 0.05)$ )

(\*\* values mean significant differences at  $(P < 0.01)$ )

**Table (3): Effect of red grapes and beetroot juice on kidneys function in hyperglycemic rats**

Animal groups	Nutrient composition%	Creatinine mg/dL	Urea mg/dL	Uric acid mg/dL	
A	RGj (A1) RGj (A2)	5%	$0.92 \pm .0241$	$43.67 \pm .050$	$2.24 \pm .02^*$
		10%	$0.81 \pm .025^*$	$24 \pm .02^*$	$1.82 \pm .024$
B	BRj (B1) RGj (B2)	5%	$0.78 \pm .025^*$	$24.67 \pm .41$	$2.57 \pm .44$
		10%	$0.81 \pm .024$	$30.67 \pm .251$	$1.22 \pm .024^*$
C	RGj/BRj	5%	$0.98 \pm .29$	$28.3 \pm .045^*$	$2.18 \pm .024^*$



Negative control	--	0.81 ± .03*	25 ± .19	1.29 ± .20
Positive control	--	1.4 ± 0.04	45 ± .2	2.52 ± .065

(\* the values mean significant differences at (P < 0.05)

(\*\* values mean significant differences at (P < 0.01)

### lipid profile

Results showed significant improvements in serum lipids fractions (TG, TC, HDL, LDL, VLDL) of hyperglycemic rats treated with the two levels from beetroot Juice , red grape Juice and the combination of them. It could be noticed from (Tables 4) that fasting serum TC, TG, LDL and VLDL were increased, while the level of HDL decreased. In all groups fed on diet supplemented with beetroot juice and red grape at some levels had a significant decrease of the concentrations of TC, TG, LDL and VLDL as compared to the positive control group

**Table (4): Effect of red grapes and beetroot juice on serum lipids profile in hyperglycemic rats**

Animal groups	Nutrient composition%	Cholesterol	Triglyceride	HDL	LDL	VLDL
A	RGj (A1) 5%	115.3 ± 0.041	63 ± .02	61.8 ± 0.1	61.8 ± 0.1	12.8 ± 0.03*
	RGj (A2) 10%	100.6 ± 0.051	74 ± .5	45.2 ± 0.2	45.2 ± 0.2	14.6 ± 0.03
B	BRj (B1) 5%	89.67 ± 0.012**	71 ± .3*	30.97 ± 0.1	30.97 ± 0.01**	14.4 ± 0.021
C	RGj (B2) 10%	98.7 ± 0.05	62 ± .4*	42.3 ± 0.041*	44.0 ± 0.1	12.4 ± 0.03*
	RGj/BRj 5%	100.7 ± 0.6	95 ± 0.9	43.3 ± 0.41	38.8 ± 0.041*	19.2 ± 0.02*
Negative control	--	120.3 ± .62	96 ± 0.9	48.4 ± .3	48.4 ± 0.3	19.2 ± .02*
Positive control	--	145.5 ± .051*	103 ± .04*	87.4 ± 0.4	87.4 ± 0.4	20.4 ± .03*

(\* the values mean significant differences at (P < 0.05)

(\*\* values mean significant differences at (P < 0.01)

### Liver function

Table 5 data demonstrated that, in comparison to the negative control group, the positive control group's ALT and AST liver enzymes activity significantly increased. Administration of grape juice, beetroot juice and their combination significantly ( $P < 0.05$ ) decreased serum levels of ALT as compared to the positive control. The group treated with 10% grape juice showed the best mean ALT results, while the group treated with 10% beetroot juice had the best AST results compared to the negative control group, with values of  $25.3 \pm 0.041$  and  $59.6 \pm 0.03$  versus  $25 \pm 0.9$  and  $59.3 \pm 0.042$ , respectively.

**Table (5): Effect of red rats grape juice and beetroot juice on liver enzymes in hyperglycemic**

Animal groups	Nutrient composition%	ALT U/L	AST U/L
A	RGj (A1) 5%	$25 \pm 0.9$	$62.3 \pm 0.041^*$
	RGj (A2) 10%	$25.3 \pm 0.041^*$	$72.6 \pm 0.041$
B	BRj (B1) 5%	$36.3 \pm 0.061$	$76.3 \pm 0.061$
	RGj (B2) 10%	$24.67 \pm 0.071$	$59.6 \pm 0.03^*$
C	RGj/BRj 5%	$26.3 \pm 0.04^*$	$61.33 \pm 0.24$
Negative control	--	$25 \pm 0.9$	$59.3 \pm 0.042$
Positive control	--	$85.3 \pm 0.091$	$155.3 \pm 00.052$

(\* the values mean significant differences at ( $P < 0.05$ ))

(\*\* values mean significant differences at ( $P < 0.01$ ))

### Immunity indicators

The cellular immunity of rats, including phagocytes and lymphocytes, was affected by feeding them grape and beetroot juice, as shown in Table 6. Notably, alpha, beta, and gamma globulins were also observed, which are indicators of a healthy immune system. Based on the absolute values of phagocytes, regardless of statistical significance, the descending order is 5% and 10% beetroot juice, followed by 5% grape juice for lymphocytes.

**Table (6): Effect of red grapes and beetroot juice on cellular and humeral immunity indicators**

Animal groups	Nutrient composition%	Phagocytes	Lymphocytes	$\alpha$ -Globulin	$\beta$ -Globulin	$\gamma$ -Globulin
A	RGj (A1) 5%	75 ± 9	1.41 ± .21	1.12 ± .08	1.21 ± .012	1.89 ± .05*
	RGj (A2) 10%	76 ± 8	1.31 ± .031*	1.28 ± 0.02*	1.13 ± .01**	1.55 ± .03*
B	BRj (B1) 5%	82 ± 17	1.50 ± .08	1.21 ± .08	1.44 ± .07	2.51 ± 0.2
	RGj (B2) 10%	88 ± 9	1.83 ± .018**	1.36 ± .013*	1.43 ± .08	2.28 ± .08
C	RGj/BRj 5%	90 ± 21	1.78 ± .01	1.39 ± 0.18	1.18 ± .013**	2.1 ± .06
Negative control	--	72 ± 8	1.21 ± .13	1.28 ± .014*	1.41 ± 0.01**	1.95 ± .04*
Positive control	--	65 ± 5	0.81 ± .02	1.10 ± .027	1.21 ± 0.02*	1.57 ± .091

(\* the values mean significant differences at (P < 0.05)

(\*\* values mean significant differences at (P < 0.01)

Histopathology investigations of the kidneys' tissues of rats in the negative control group normally showed parenchyma (Figure 1a). On the other hand, the kidneys of rats in the positive control revealed congestion of renal blood vessels and the presence of eosinophilic proteinaceous casts in the lumen of renal tubules (Figure 1b). However, the kidneys of rats from groups A, B, and C revealed the presence of eosinophilic proteinaceous cast in the lumen of some renal tubules (Figures b, c, and e). Apparent normal renal parenchyma was noticed in the kidneys of rats from group C (Figure. 1f). However, kidneys of rats from the positive control showed atrophy of glomerular tufts and protein cast in the lumen of some renal tubules (Figure 1g). Histopathological changes of the kidney in the case of experimental diets were in limited lesions without any proliferation.

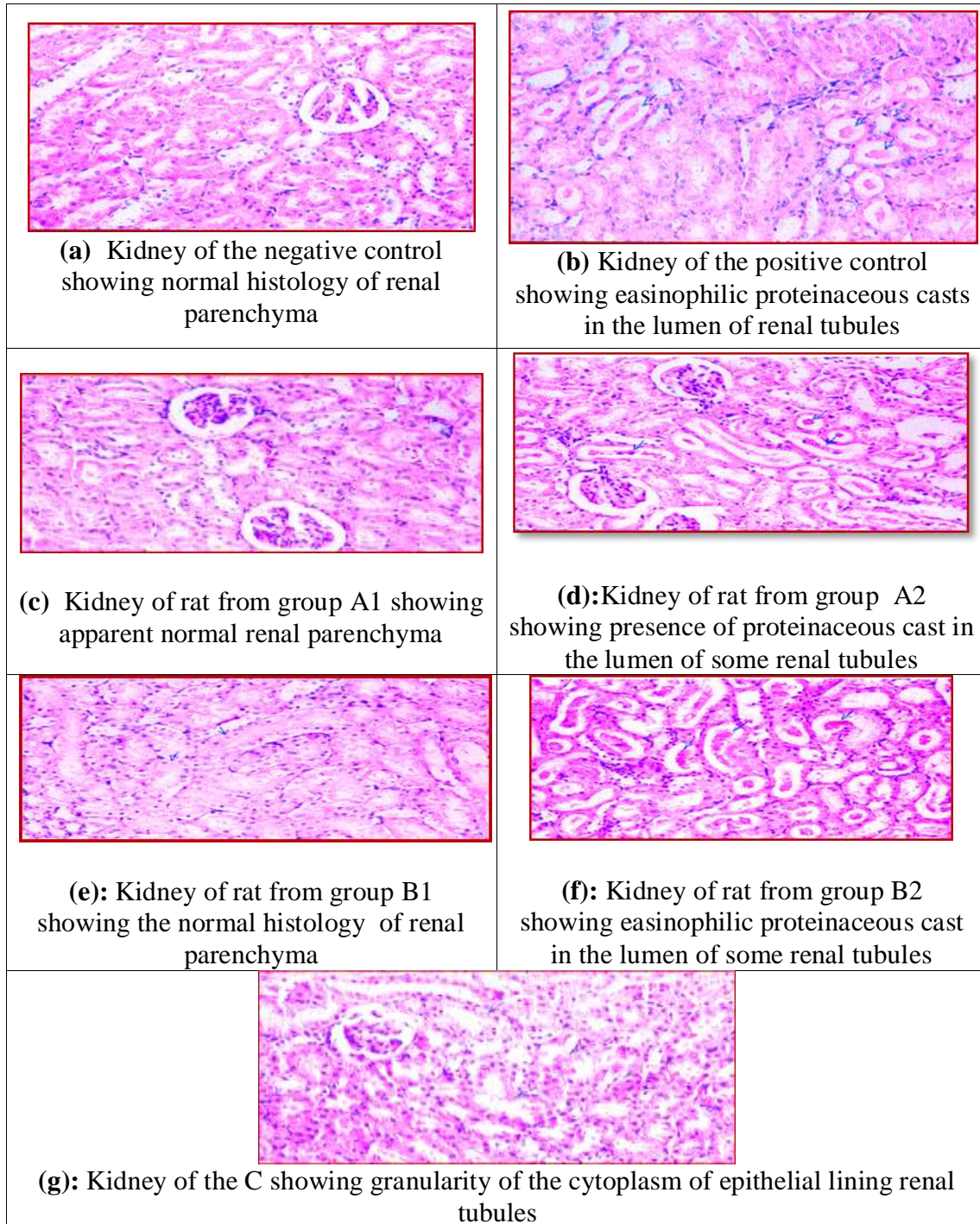


Fig.(1a-g) The effects of different nutrient compositions on the histopathological rat's kidney

## 6.DISCUSSION

The purpose of this research was to offer therapeutic uses of red grape juice and beetroot juice on metabolic processes. Naturally occurring fruits and root vegetables that are high in phytochemicals and bioactive compounds are red grapes and beetroot (*Beta vulgaris*). They

have been shown to have positive effects on a number of pathologic and clinical outcomes. From table (1) groups that have been fed mixed juices had a significant decrease in serum glucose level when compared to the positive control group, Our data showed that all groups fed on grape juice and beetroot juice had a significant decrease in concentrations of TC, TG, LDL and VLDL) as compared to the positive control group table (2).

The present study results showed significant improvements in serum urea, creatinine and uric acid levels. These findings were in agreement with those of ( **Wahba , 2016** ) who revealed oxidative stress and decline in NO as markers that are specific to renal tissue, the NO<sub>3</sub> of red grapes and beetroot in this study could stimulate NO formation in tissues and serum. Similarly, the presence of beneficial substances like betacyanins and betaxanthin decreased serum urea and creatinine contents

Oral administration of fresh red grape fruit and beetroot juice increased humoral immunity and cellular immunity responses in rats by increasing phagocytes and lymphocytes. . Our results were in the same line with those reported by **Kroll et al.,(2018)**. That was demonstrated because beetroot contains high amounts of nitrates in its juice can raise blood oxygenation levels, Betelains, saponins, polyphenols, flavonoids, and other biologically active substances are found in beetroot, along with a variety of minerals like potassium, sodium, phosphorus, calcium, magnesium, copper, iron, zinc, and manganese. A good source of the red pigments called betalains is red beetroot. This result was similar to the results of (**Shaw et al ., 2016**) . Our results were in the same line with those reported by (**Klewicka et al ., 2012**) who demonstrated that because beetroot contains high amounts of nitrates in its juice can raise blood oxygenation levels, which can help the immune system fight off many pathogens. Additionally, grape juice and beetroot juice have a 5% protective effect and can strengthen the immune response of T lymphocytes, monocytes, and immunity cells, which can help break down and eradicate cancerous tumors . The previous authors showed the effect of grape fruit and beetroot juice as well as apple peel increased cellular immunity responses. By blocking pro-inflammatory pathways and reducing the pro-inflammatory phase by upregulating Arg1 gene expression, the accumulation of alternatively activated (M2) macrophages promotes tissue healing (**Shalaby et al., 2023and Al-Zahrany,2016**) Beetroot juice has also been shown to reduce inflammation induced by Toll-like receptor 4 agonists in macrophage cells. This result, in agreement with many studies (**Butt et al ., 2016** ) revealed that betelains, saponins, polyphenols, flavonoids, and other biologically active

substances are found in beetroot, along with a range of minerals, including manganese, copper, iron, zinc, sodium, potassium, and phosphorus in calcium, magnesium.

A good source of the red pigments called betalains is red beetroot. Yellow betaxanthins and reddish-violet betacyanins are the two primary components of betalains. The results of this study indicated that drinking combination of red grape and beetroot juice can improve the nutritional value and increase the immune system.

## 7. CONCLUSION

In conclusion, red grapes, beetroot juice, and their mixture, when given to rats with high blood sugar levels, reduce blood glucose levels by increasing nitric oxide metabolites without the need for insulin. They also improve renal function, lipid profiles, and enhance both cellular and humoral immunity, without causing any histopathological changes in the kidneys, indicating that the selected experimental diet is safe.

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