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Efficiency Beet Root *(Beta vulgaris, L.)* Juice for Improving Status of Nephropathy Diabetic Rats

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Abstract

Consumption of beet root juice is associated with numerous health benefits, attributed to its high content of bioactive compounds and its antioxidant capacity. Beet root is a rich source of phytochemical compounds, that includes betalain, ascorbic acid, carotenoids, flavonoids and polyphenols. The current study was performed to assess the effects of beet root juice (BJ) on gentamicin induced nephropathy in diabetic rats. Rats were randomly divided into two main groups. The first, served as a negative control (6 rats) fed basal diet and the second group (Nephropathy Diabetic rats) fed basal diet and received a daily intraperitoneally injection of gentamicin (GM) 85 mg / kg body weight for 8 days. Then, given a single dose via intraperitoneal injection of 65 mg / kg body weight of streptozotocin (STZ). This group was reclassified divided into four groups(6 rats /each) , the first group was positive control, the second, third -and fourth groups were administrated a daily oral dose 5, 10 and 15 µl / g BW, respectively of beet root juice for 60 days . After the end of the experimental period, blood samples were collected for the biochemical analysis as blood and urine glucose, insulin, kidney functions, lipid profile and antioxidants of kidney tissues were performed. Results showed that nephropathy diabetic rats had elevation in blood and urine glucose levels, lipid profile, kidney functions (urea. creatinine, uric acid and blood urea nitrogen) in the serum and MDA levels with reduction in the activity of GSH.px, SOD and CAT in kidney tissues, serum total protein, albumin, globulin and insulin levels. However, ingestion of BJ significantly reduced the levels of blood and urine glucose, lipid profile and improved the level of insulin, kidney functions and mitigated renal dysfunction and structural damages by decreasing oxidative stress in kidney tissues. Meanwhile, giving rats with 15µl/g BW of beet root juice showed the best effects through improving the previous changes to near the normal levels. In conclusion, BJ had hypoglycemic effects and can attenuate GM-induced nephropathy.

Keywords: Beet root juice, betalain, gentamicin, hypoglycemic, nephropathy diabetic rats.

Introduction

Diabetes mellitus (DM) is rapidly becoming one of the main health issues among humans in the 21st century and the number of patients is steadily increasing globally, both in the developed and developing countries. Diabetes mellitus is a heterogeneous metabolic disorder characterized by common feature of chronic hyperglycemia with disturbance in the metabolism of carbohydrates, fat and protein. Moreover, the impaired metabolism leads to the progression and aggravation of oxidative stress through several mechanisms, such as glucose autoxidation, protein glycation leading to the development of secondary diabetic complications such as nephropathy, retinopathy, neuropathy, macro and microvascular damages **(WHO, 2016).** The diabetic kidney disease (Diabetic nephropathy) is a progressive kidney disease caused by damage to the capillaries in the kidneys' glomeruli. It is characterized by nephrotic syndrome and diffuse scarring of the glomeruli. It is due to longstanding diabetes mellitus, and is a prime reason for dialysis in many developed countries **(Kittell, 2012)**. Diabetic Nephropathy (DN) is a microangiopathic manifestation of diabetes occurring in about 30% of patients and it is the most common cause of end-stage kidney disease (**Mora- Fernandez *et al*., 2014; Ding and Choi, 2015)** which may require hemodialysis or even kidney transplantation. It is associated with an increased risk of death in general, particularly from cardiovascular disease **(Fink, *et al*., 2012; Pálsson and Patel, 2014).** Fruits and vegetables are an important part of the human diet over the world **(Cherfi *et al*., 2015)**. Optimal fruits and vegetables consumption has been recognized as one of the cornerstones of a healthy diet for decades. Fruits and vegetables provide key nutrients essential to promoting and maintaining health (**Hromi-Fiedler *et al*., 2016).** Drinking vegetables juice is one of the ways to increase a dose of essential microelements, vitamins and other antioxidants as well as fiber delivered to the organism **(Groele, 2010).**So, the diets is rich in fruits and vegetables reduce chronic disease risk **(Hromi-Fiedler *et al*., 2016)**

Beet root cultivated in many countries of the world. The edible portion of beet root is the root. It is a low cost plant that has widespread use in many traditional dishes. Beet root is a rich source of polyphenols and antioxidants. It contains also other valuable bioactive compounds, making its consumption of highly beneficial to a human body **(Sawicki**

***et al*., 2016)**. The roots of beet have long been used in traditional Arab medicine to treat a wide variety of diseases. The therapeutic use of beet root includes its anti-inflammatory, anticarcinogenic, hepato protective activities, antitumor, carminative, emmenagogue, hemostatic and renal protective properties and is a potential herb used in cardiovascular conditions, hypertensive and wound healing benefits **(Slavov *et al*., 2013).** Also, beet root has antioxidant and antidiabetic properties in addition to its protective effect against hyperglycemia-induced tissue damage **(Sacan and Yanardag, 2010; Gezginci-Oktayoglu *et al*., 2014)**. Beet root is one of the plants used as an alternative hypoglycemic medication by diabetic people. Beet root juice is also used as a popular folk remedy for the treatment of liver and kidney diseases as well as for the stimulation of immune and haematopoietic systems (**Moreno *et al*., (2008).** So, utilization of beet root as an ingredient in different food products imparts beneficial effects on human health and provides opportunity for development of different functional foods. Therefore, this study aimed to evaluate the effects of beet root *(Beta vulgaris, L.)* juice to improve diabetic and renal parameters in nephropathy diabetic rats.

MATERIALS AND METHODS

MATERIALS

Fresh beet root *(Beta vulgaris, L.)* was purchased from the local market of (Quwaysina City, Menoufia Governorate, Egypt). Gentamicin (GM) sulfate was obtained from Memphis Company, For Pharm. & Chem. Ind. Cairo –A.R.E. Streptozotocin (STZ) was obtained from Sigma – Aldrich Inc. (St. Louis, Mo, USA) and used for inducing diabetes mellitus in rats. Kits for estimating biochemical analysis were purchased from Alkan Medical Company, El-Doky, Giza, Egypt. Malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH.Px) and superoxide dismutase (SOD) activity Kits were obtained from Biodiagnostic Company, El-Doky, Giza, Egypt.

Thirty adult male albino rats, Sprague Drawly strain, weighing (180 ± 5g) were obtained from Medical Insects Research Institute, El-Doky, Giza, Egypt. Rats were housed individually in wire cages under the normal laboratory conditions in Biological Laboratory, Faculty of Home Economics, Menoufia University.

Methods

Preparation of beet root juice

The fresh beet root was cleaned under running tap water to remove dust and soil residues and squeezed without adding water in a Braun blender (Type 4294, 4293, 4292.Germany) at a maximum speed, for 15 minute. The pure juice was stored at -18 ºC until used.

Induction of experimental rats for nephropathy and diabetes

Nephropathy was induced in rats via intraperitoneally injection daily of gentamicin (GM) 85 mg / kg body weight for 8 days as described by **Jeyanthi and Subramanian (2009)**. Nephropathy rats were given a single dose via intraperitoneal injection of 65 mg / kg body weight of streptozotocin (STZ) dissolved in a freshly prepared 0.01 M citrate buffer (PH 4.5) according to **Yanardag *et al*., (2003).** Diabetes was identified by polydipsia, poly-uria (visual observations) and measuring fasting blood glucose level after 72 h of injection of STZ. Rats with a fasting blood glucose level above 200 mg/dl were considered diabetic and were used in this study.

Experimental Design

Rats were housed individually in well aerated cages under hygienic laboratory condition and fed basal diet for one week for adaptation according to AIN -93 guidelines **(Reeves *et al*., 1993)**. The basal diet comprised of casein (120g/kg), corn starch (677g/kg), cellulose (50g/kg), corn oil (100g/kg), mineral mixture (40g/kg), vitamins mixture (10g/kg) and DL- methionine 3.0g/kg). Thirty rats were randomly divided into two main groups, first main group: Negative control (n=6), rats were fed on the basal diet only and the second main group: Nephropathy diabetic rats (n=24). This group was divided into four groups (6 rats each): the first group was positive control rats (untreated), the second, third and fourth group were administration of beet root juice for 60 days at doses of (5, 10 and 15 µl / g BW) orally by gavages, respectively. At the end of the experimental duration, rats were anesthetized with diethyl ether after fasting for 12h, and blood samples were collected. The blood samples were placed in dry clean centrifuge tubes and left to clot for 1-2 h at room temperature. Serum was then removed by centrifuging at 1500 g for 10 min. The clear supernatant serum was kept at – 20 °C until analysis. Kidneys were taken and washed in saline solution until all blood was removed. The kidney samples were stored at – 20°C until analysis

Chemical Analysis

The total phenols, total flavonoids and β.carotene contents of beet root juice were determined according to the methods of **Kaškonienė *et al.*, (2009), Franke *et al.*, (2004) & Nagata and Yamashita (1992)),** respectively. Ascorbic acid and betalain were determined according to the methods described by **Mazumdar and Majumder, (2003)** and **Casteller *et al.*, (2003),** respectively. Antioxidant activity of beet root juice was determined by 2, 2 diphenyl-1-picrylhydrazyl (DPPH) according to **Yang *et al.*, (2006)**.

Biochemical Analysis

Serum glucose level was estimated according to **Rojas *et al*., (1999)**. Glucose was measured in urine according to **Hugget and Nixon (1957).** While, Serum insulin level was assayed with a Rat Insulin ELISA kits. Insulin sensitivity from the final fasting insulin and glucose values was estimated by the Homeostasis Model Assessment of insulin resistance (HOMA-IR) according to the following formula: [fasting glucose (mM) × fasting insulin (mUI/L)] / 22.5 **(Cordero-Herrera *et al*., 2015).** Urea, creatinine and uric acid levels were estimated in serum using commercial kits according to **Trinder (1969); Tietz (1986)** and **Fossati *et al*., (1980),** respectively. Serum total protein was determined by the method of **Doumas (1975).** Albumin and globulin levels were estimated spectrophotometrically using commercial kits according to **Tietz (1994).** BUN is more easily measured than urea and is used as an index of blood urea level **(Philip, 1994)**. Blood Urea Nitrogen (BUN) = 28 / 60 × serum Urea in mg / dl. Cholesterol was determined according to **Allain (1974).** Triglycerides and high density lipoprotein (HDL–c) were determined according to **Fossati and Prencipe, (1982)** and **Burstein *et al*., (1980),** respectively. Low density lipoprotein (LDL–c) and very low density lipoprotein (VLDL–c) were calculated according to the methods of **Lee and Nieman, (1996)** as follows: **VLDL–c (mg /dl) = Triglycerides / 5. LDL–c (mg /dl) = Total cholesterol - (HDL–c + VLDL–c).** Malondialdehyde (MDA) was estimated in kidney tissues according to **Lefevre *et al*., (1998).** Catalase (CAT), Glutathione peroxidase (GSH.Px) and Superoxide dismutase (SOD) activities were assayed using the methods described by **Aebi (1984); Necheles *et al*., (1968)** and **Masayasu and Hiroshi (1979),** respectively.

Statistical Analysis

Data were analyzed using a statistical analysis system SPSS for windows (Version 20.0) and recorded as means ± standard deviation (SD). Analysis of variance among groups was performed using one – way ANOVA test followed by Duncans's multiple range tests at a significance level of $P \leq 0.05$ **(Armitage and Berry, 1987).**

Results And Discussion

Total phenols, flavonoids, β.carotene, ascorbic acid, betalain and antioxidant activity of beet root juice are shown in Table (1). Data indicated that beet root juice had total phenols (635.9 mg / 100 ml), flavonoids (59.2 mg / 100 ml), β.carotene (23.6 mg / 100 ml), ascorbic acid (4.33 mg / 100 ml), betalain (382.9 mg / 100 ml) and antioxidant activity (81.95 %). **Fidelis *et al.*, (2017)** found that beet root juice had a high amount of total phenols (1169 mg GAE/L), flavonoids (925 mg catechin equivalent/L), ascorbic acid (325 mg equivalent/L) and pigments (854 mg/L). Also, **Guldiken *et al.*, (2016)** they reported that beet root contains highly active pigments betalains, ascorbic acid (**Clifford *et al.*, 2015)**, carotenoids (**Ninfali and Angelino, 2013)**, polyphenols, flavonoids and saponins **(Lidder and Webb, 2013).** Moreover, **Vasconcellos *et al.*, (2016)** showed that antioxidant activity of beet root juice was 80.48% .This antioxidant activity of beet root may be attributed to its high content of phenolic compounds and betalain.

Table (1): Total phenol, flavonoids, β.carotene, ascorbic acid, betalain and antioxidant activity of beet root juice

Parameter	Beet Root Juice
Total phenols (mg gallic acid/100ml)	635.9 ± 3.04
Flavonoid (mg catechin/100 ml)	59.2 ± 2.12
β.carotene (mg/100ml)	23.6 ± 1.06
Ascorbic acid (mg/100ml)	4.33 ± 1.02
Betalain (mg/100ml)	382.9 ± 2.56
Antioxidant activity (%)	81.95

Each value in the table is the mean ± standard deviation of three replicates.

Results in Table (2) revealed the effect of beet root juice on glucose in blood and urine and insulin of nephropathy diabetic rats. At the beginning of experimental period (0^{th} day), nephropathy diabetic rats had significantly ($p \leq 0.05$) high blood glucose level as compared with the normal control rats. During hyperglycemia, the body tries to recompense normal glucose homeostasis by secreting higher amounts of insulin, which caused pancreatic β-cell dysfunction, decreased β-cell mass, and insulin deficiency **(Toschi *et al.*, 2002 and Nichols and Remedi 2012).** At 30th and 60th day of experimental period, it was observed that there were a significant ($p \leq 0.05$) decrease in blood glucose level in nephropathy diabetic rats administrated with beet root juice (5, 10 and 15 µl /g BW) compared to positive control rats by increasing the period. This improvement in glucose levels is due to the betalain pigment in beet root, where it has antidiabetic and antioxidant effects. These results consent with those obtained by **Indumathi *et al.*, (2017)** who found that administration of natural pigment betanin reduced the blood glucose level in wistar rats, whereas betanin improved the glucose utilization by the peripheral tissues either by promoting the absorption of glucose and metabolism by increasing insulin secretion. Also, **Gezginici- Oktayoglu *et al.*, (2014)** reported that administration of chard aqueous extract to streptozotocin-induced diabetic rats decline the fasting blood glucose and increased glycogen levels in liver.

Moreover, there were a significant ($P \leq 0.05$) differences in blood glucose levels among nephropathy diabetic rats administrated with 5, 10 and 15 µl /g BW of beet root juice on the day 30th and 60th of the experiment. However, the highest reduction in blood glucose levels was noted in nephropathy diabetic rats administrated with 15 µl /g BW / day of beet root juice for 60 days. The hypoglycemic effect of beet root juice may be due to its content of flavonoids and β-carotene. These results are compatible with the findings of **Bahdorane *et al.*, (2013)** who revealed that the consumption of flavonoids-rich foods may reduce the risk of diabetes. Furthermore, **Murthy and Manchali (2012)** showed that red beet has anti-diabetic activity which may be attributed to its content of fiber, flavonoids, betalains, pectin and other phytochemicals compounds. The dietary ingredients and the bioactive compounds can prevent diabetes by increases in insulin sensitivity, as well as the antioxidant capacity of bioactive compounds in red beet play a vital role.

On the other hand, glucose was not detected in the urine of normal rats, while nephropathy diabetic rats (positive control rats) were highly glycosuric. But nephropathy diabetic rats administrated with beet root juice (5, 10 and 15 µl /g BW) had a significant ($P \leq 0.05$) reduction in the levels of urine glucose on the day 30th and 60th of experiment compared to positive control rats. At 60th day of the experiment, administration of

5, 10 and 15 µl /g BW of beet root juice resulted in a significant ($P \leq 0.05$) reduction in urine glucose level by 30.51, 58.62 and 79.31%, respectively in comparison with the 0^{th} day. Also, administrated with15 µl /g BW of beet root juice was more effective in lowering urine glucose levels by 79.31% as compared with other concentrations of beet root juice. These improvement effects of beet root juice on urine glucose levels may be related to its high content of flavonoids and saponin. **Ishikawa *et al*., (2007)** showed that beet root juice had an anti-hyperglycemic effects in type 2 diabetes. This is consistent with the results of **Kameswara *et al*., (2003)** who reported that beet root extract is effective in controlling hyperglycemia in STZ diabetic rats. The anti-hyperglycemic effects and prominent improvement may be attributed to flavonoids and saponin contents of beet root. Moreover, **Shetty *et al*., (2004)** found that quercetin, the flavone present in red beet root has anti-diabetic activity, administration of 1 g/kg of quercetin to STZ-induced diabetic rats up to 6 weeks resulted in reduction in blood sugar levels and sugar excretion in urine.

The results in the same table showed that there were a significant decrease ($P \leq 0.05$) in serum insulin levels in nephropathy diabetic rats when compared with normal rats. **Poretsky (2010)** found the reduction of endogenous insulin secretions due to pancreatic destruction. This might be attributed to a decrease in the kidney and liver functions. Such combination is due to inefficient metabolism and excretion. This suggests that insulin clearance due to liver degradation and kidney elimination, was reduced, as the liver and kidneys are the major organs for insulin turnover in blood. On the other side, no significant ($P > 0.05$) differences were observed in serum insulin levels between nephropathy diabetic rats administrated with 5 µl/g BW of beet root juice and positive control rats on 30^{th} day of experimental period, while nephropathy diabetic rats administrated with 10 and 15 µl/g BW of beet root juice had significant elevation ($p \leq 0.05$) in serum insulin levels as compared to positive control rats.

Moreover, the results revealed a significant increase ($p \leq 0.05$) in serum insulin levels in nephropathy diabetic rats after feeding them with 5, 10 and 15 µl/g BW of beet root juice in 60^{th} day of experimental period, whereas serum insulin levels was increased by 46, 90 and 170 %, respectively for the 0^{th} day of experiment. Administrated diabetic rats with betanin (20 mg/ kg BW) significantly ($p \leq 0.05$) improved the levels of insulin as compared with untreated diabetic rats as reported by **Indumathi *et al*., (2017)**. Also, **Panda and Kar, (2007)** observed that the anti diabetic properties of red beet flavonoids resulted in increased pancreatic insulin. Treatment with red beet did not influence the plasma insulin level, but decreased glycemia by 22% while, red beet extracts significantly decreased glycemia and serum insulin levels by 2.5 fold, suggesting the anti-diabetic synergistic activity. Beet root juice contains many bioactive components (betaxanthins, betanins, flavonoids, phenolic acids) and the potential beneficial effects of these compounds on glycaemia may be due to a reduced digestion and absorption of glucose, stimulation of insulin release or alterations in insulin sensitivity **(Kujala *et al*., (2002) and Wootton-Beard *et al*., (2014).**

Furthermore, nephropathy diabetic rats administrated with 15 µl/g BW of beet root juice had the highest insulin sensitivity index value than rats administrated with 5 and 10 µl/g BW of beet root juice ($P \leq 0.05$). This increase in insulin sensitivity may be related to the polyphenols and flavonoids. Similar results were obtained by **Matsui *et al*., (2007)** who showed that Polyphenols and related compounds reduced both postprandial hyperglycaemia and prevent reactive hyperinsulinaemia by reducing the digestion, absorption and transport of glucose. Meanwhile, **Wootton-Beard *et al*., (2014)** observed that the bioactive components of beet root juice and its high total antioxidant capacity have been described to influence on the glycaemic response, either by direct inhibition of glucose uptake or by indirect action affecting insulin sensitivity. Moreover, **Bolkent *et al*., (2000)** reported that beet root extract increased insulin synthesis, while blood glucose levels were reduced.

Table (2): Effect of beet root juice on glucose in blood and urine, and insulin of nephropathy diabetic rats

Groups / Parameters	Normal Control	Nephropathy Diabetic Rats			
		Positive Control	Beet Root Juice		
			5 µl/g B.W	10 µl/g B.W	15 µl/g B.W
Blood Glucose (mg/dl)					
0^{th} Day	$97^{Ab}\pm2.16$	$231^{Aa}\pm0.96$	$230^{Aa}\pm2.82$	$229^{Aa}\pm1.63$	$229^{Aa}\pm1.93$
30^{th} Day	$97^{Ae}\pm0.82$	$231^{Aa}\pm0.96$	$220^{Bb}\pm1.96$	$207^{Bc}\pm1.23$	$194^{Bd}\pm1.38$
60^{th} Day	$97^{Ae}\pm1.63$	$231^{Aa}\pm0.96$	$171^{Cb}\pm3.42$	$140^{Cc}\pm2.45$	$105^{Cd}\pm2.65$
% Lowering of Blood Glucose level	0.0	0.0	25.65	38.86	54.15
Urine Glucose (mmol/L)					
0^{th} Day	-	$59^{Aa}\pm1.86$	$59^{Aa}\pm1.41$	$58^{Aa}\pm1.63$	$58^{Aa}\pm2.58$
30^{th} Day	-	$59^{Aa}\pm1.86$	$47^{Bb}\pm2.94$	$30^{Bc}\pm2.16$	$20^{Bd}\pm2.66$
60^{th} Day	-	$59^{Aa}\pm1.86$	$41^{Cb}\pm2.82$	$24^{Cc}\pm2.10$	$12^{Cd}\pm1.12$
% Lowering of Urine Glucose level	-	0.0	30.51	58.62	79.31
Serum Insulin (ng/mg)					
0^{th} Day	$3^{Aa}\pm0.21$	$0.50^{Ab}\pm0.03$	$0.50^{Bb}\pm0.01$	$0.50^{Cb}\pm0.02$	$0.50^{Cb}\pm0.01$
30^{th} Day	$3^{Aa}\pm0.21$	$0.50^{Ad}\pm0.01$	$0.52^{Bd}\pm0.02$	$0.71^{Bc}\pm0.02$	$0.92^{Bb}\pm0.09$
60^{th} Day	$3^{Aa}\pm0.21$	$0.50^{Ae}\pm0.03$	$0.73^{Ad}\pm0.04$	$0.95^{Ac}\pm0.13$	$1.35^{Ab}\pm0.14$
% Change of Serum Insulin	0.0	0.0	46	90	170
Insulin sensitivity index (HOMA-IR)	$29.95^{a}\pm2.48$	$11.89^{c}\pm0.56$	$12.76^{bc}\pm2.20$	$13.69^{bc}\pm1.83$	$14.58^{b}\pm1.30$

Data are expressed as mean ± SD. Values within a same row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one way ANOVA followed by Duncan's multiple range test (a > b > c > d > e), while capital letters referred to statistical differences among experimental periods, HOMA-IR.

Effect of beet root juice on kidney functions of nephropathy diabetic rats are presented in Table (3). In nephropathy diabetic rats with high glucose, noticeable changes were seen in kidney functions as compared to normal rats and consequently serum levels of urea, creatinine, blood urea nitrogen (BUN) and uric acid were significantly increased ($P \leq 0.05$) in nephropathy diabetic rats with reducing in the levels of total protein, albumin and globulin when compared to those of the normal control rats. This happens as a result of the accumulation of gentamicin (GM) in the renal tubular caused renal dysfunction. These results are in accordance with **Ullah *et al*., (2013)** who found that GM induced a significant increase in the serum level of urea, creatinine and uric acid as compared to control indicating renal dysfunction. As the serum urea, creatinine and uric acid are the final metabolites of purine which may change the glomerular filtration rate and lead to increasing their levels in serum referring renal damage. Also**, Indumathi *et al*., (2017)** observed that there were elevation in the kidney parameters such as urea, creatinine and uric acid in STZ induced diabetic rats.

No significant ($P>0.05$) differences was found in creatinine, BUN, uric acid, albumin and globulin between nephropathy diabetic rats administrated with 5 µl/g BW beet root juice and positive control rats. However, the levels of urea, creatinine, BUN and uric acid in serum were significantly ($P\leq0.05$) decreased in nephropathy diabetic rats administrated with 10 and 15 µl/g BW of beet root juice as compared with positive control rats. While, nephropathy diabetic rats administrated with 15 µl/g BW beet root juice had the highest reduction ($p\leq0.05$) in the levels of urea, creatinine, BUN and uric acid. Moreover, the levels of total protein, albumin and globulin in serum were significantly ($P\leq0.05$) elevated in nephropathy diabetic rats administrated with15 µl/g BW of beet root juice by 77.45, 64.81 and 94.5%, respectively compared with positive control rats. The positive effect of beet root juice against gentamicin induced kidney toxicity may be attributed to its antioxidant and anti-inflammatory. This finding is compatible with the results of **Safa *et al*., (2010); Khan *et al*., (2011) and El- Gamal *et al*., (2014)** they showed that an administration of beet root extract with gentamicin resulted in significant decline in serum levels of urea, creatinine and uric acid and a significant elevation in serum total protein suggested that beet root juice has potent effects against GM-induced nephrotoxicity, that may improving renal function. **Hassan *et al*., (2018)** reported that red beet root attenuates renal dysfunction and structural damage through the reduction levels of serum kidney markers and oxidative stress in kidneys. Also, **Indumathi *et al*., (2017)** found that betanin pigment present in red beet root (20 mg/kg BW) decreased the levels of urea, creatinine and uric acid in STZ induced diabetic rats, recovered renal function, which is due to improve glycemic control thereby it elicits the renoprotective nature of betanin. Moreover, **Christiana *et al*., (2005) and V´ali *et al*., (2007)** they showed that beet root juice is a popular flok remedy for the treatment of kidney diseases. These results confirm the use of beet root juice in Arab traditional medicine for the therapy of renal disorders **(El Gamal *et al*., 2014).**

Table (3): Effect of beet root juice on kidney functions of nephropathy diabetic rats

Groups / Parameter	Normal Control	Nephropathy Diabetic Rats			
		Positive Control	Beet Root Juice		
			5µl /g B.W	10µl /g B.W	15µl /g B.W
Urea (mg/dl)	23^{e}±0.82	51^{a}±2.22	44^{b}±0.96	35^{c}±1.83	28^{d}±1.83
Creatinine (mg/dl)	0.20^{d}±0.03	0.93^{a}±0.29	0.82^{a}±0.02	0.54^{b}±0.05	0.33^{c}±0.01
BUN (mg/dl)	10.73^{d}±0.82	23.80^{a}±3.32	20.53^{a}±0.82	16.33^{b}±1.82	13.07^{c}±1.81
Uric Acid (mg/dl)	1.84^{d}±0.25	4^{a}±0.08	3.68^{a}±0.22	2.7^{b}±0.29	2.14^{c}±0.17
Total Protein (mg/dl)	10.53^{a}±1.88	4.7^{e}±0.08	5.4^{d}±0.29	6.68^{c}±0.45	8.34^{b}±0.26
Albumin (mg/dl)	5.53^{a}±1.73	2.7^{d}±0.29	3.1^{d}±0.09	3.8^{c}±0.35	4.45^{b}±0.13
Globulin (mg/dl)	5^{a}±1.87	2^{c}±0.41	2.3^{c}±0.22	2.88^{b}±0.29	3.89^{ab}±0.64

Data are expressed as mean ± SD. Values within a same row having different superscripts are significantly different ($p \leq 0.05$); **BUN:** Blood Urea Nitrogen.

Data presented in Table (4) reflect the effect of beet root juice on serum lipid profile in nephropathy diabetic rats. Nephropathy diabetic rats had higher ($P \leq 0.05$) levels of serum cholesterol, triglyceride (TG), low density lipoprotein cholesterol (LDL c), very low density lipoprotein cholesterol (VLDL c) and lower high density lipoprotein cholesterol (HDL-c) level than normal control rats. The obtained results agree with those obtained by **Rashid and Khan, (2017)** who reported that treatment with gentamicin (80 mg/kg BW) caused elevation in the levels of total cholesterol, triglycerides and LDL c in serum of rats as compared with normal control. **Aly *et al*., (2015)** found that the levels of serum and plasma cholesterol, TG, LDL c and VLDL c increased in diabetic rats.

On the other hand, there were no significant (P>0.05) differences in the levels of cholesterol and LDL-c among nephropathy diabetic rats administrated with 5 µl/g BW of beet root juice and the positive control rats while that, the levels of TG and VLDL-c were lower ($P \leq 0.05$) in nephropathy diabetic rats administrated with 5 µl/g BW of beet root juice than positive control rats. However, a significant reduction in the levels of cholesterol, TG, LDL-c and VLDL-c were observed in nephropathy diabetic rats administrated with beet root juice (10 and 15 µl/g BW) while, HDL-c had an opposite trend compared to positive control rats. These results agree with those reported by **Joris and Mensink (2013); Al-Yahya *et al.*, (2015) and Raish *et al.*, (2019)** who showed that administration of beet root juice resulted in significantly supported HDL c with reduced cholesterol, TG, LDL c and VLDL c levels in rats.

As the results, indicated that administration of 15 µl/g BW of beet root juice was more effective ($p \leq 0.05$) in reducing the levels of cholesterol, TG, LDL c and VLDL c and increasing HDL c as compared to other concentrations. This improvement of serum lipid profile in rats administrated with different concentrations of beet root juice may be related to its content of flavonoids, whereas higher dietary flavonoids intake was associated with improving lipid profile **(Li *et al.*, 2013)**. Moreover, **Al Dosari *et al.*, (2011)** who showed that administration of beet root extract at doses of 250 and 500 mg/kg BW for 70 consecutive days led to significant decrease in cholesterol and triglycerides levels with increase in HDL C level of hypercholesterolemic rats. Also, **Guldiken *et al.*, (2016)** reported that the nitrates present in beet root have capability to reduce the bad cholesterol, oxidized LDL cholesterol.

Table (4): Effect of beet root juice on serum lipid profile of nephropathy diabetic rats

Groups / Parameter	Normal Control	Nephropathy Diabetic Rats			
		Positive Control	Beet Root Juice		
			5 µl/g B.W	10 µl/g B.W	15 µl/g B.W
Cholesterol (mg/dl)	38[d]±2.44	71[a]±1.41	69[a]±2.16	51[b]±2.71	43[c]±2.16
Triglyceride (mg/dl)	22[e]±1.29	43[a]±2.94	40[b]±1.41	33[c]±1.41	29[d]±0.82
HDL c (mg/dl)	28[a]±1.29	14[d]±1.83	16[d]±0.82	19[c]±1.47	23[b]±1.43
LDL c (mg/dl)	5.6[d]±1.89	48.4[a]±2.55	45[a]±2.35	25.4[b]±3.88	14.2[c]±1.73
VLDL c (mg/dl)	4.4[e]±0.26	8.6[a]±0.59	8[b]±0.43	6.6[c]±0.28	5.8[d]±0.16

Data are expressed as mean ± SD. Values within a same row having different superscripts are significantly different ($p \leq 0.05$); **HDL c:** high density lipoprotein cholesterol, **LDL c:** low density lipoprotein cholesterol, **VLDL c:** very low density lipoprotein cholesterol.

Effect of beet root juice on antioxidant parameters in kidney of nephropathy diabetic rats are recorded in Table (5). The results indicated that positive control rats showed significantly decreases ($P \leq 0.05$) in catalase (CAT), glutathione peroxidase (GSH.px) and superoxide dismutase (SOD) activities and higher level of malonaldehyde (MDA) when compared to normal control rats. The increase in MDA levels and the decrease in CAT, GSH.px and SOD activities may be related to the action of oxidative stress resulting from hyperglycemia and gentamicin. These results are in the same trend of **Hassan *et al*., (2018)** who reported that gentamicin treatment induced oxidative stress in rats kidneys as evidenced by a reduction in the activities of renal antioxidant enzymes CAT, GSH.px and SOD with elevation MDA level. **Eliza *et al*., (2010)** found that STZ elevate the levels of lipid peroxides and reduce the antioxidant enzymes activity of diabetic rats.

Data in the same table showed a significant increase ($P \leq 0.05$) in the activities of CAT, GSH.px, SOD and decline MDA levels in rats administrated with (10 and 15 µl/g BW) of beet root juice. However, the levels of MDA and the activities of CAT, GSH.px and SOD in nephropathy diabetic rats administrated with 5 µl/g BW of beet root juice did not differ from positive control rats ($p > 0.05$). The improvement in the antioxidants activities may be due to potent antioxidant capacity of beet root juice which alleviates kidney dysfunction and structural damages through decreasing oxidative stress and inflammation in kidney tissues. The obtained results corroborate the findings of **Raish *et al*., (2019)** who reported that oral administration of beet root juice for 28 days at doses of 150 and 300 mg/kg BW caused an increase in the activities of CAT, SOD and decreased MDA levels. As well as, **Netzel *et al*., (2005)** indicated that betalains and other phenolic compounds presente in red beet decrease oxidative damage of lipids and improve antioxidant status in humans.

Moreover, nephropathy diabetic rats administrated with 15 µl/g BW of beet root juice had significantly ($P \leq 0.05$) high CAT, GSH.px and SOD compering to nephropathy diabetic rats administrated with10 µl/g BW of beet root juice whereas, the level of MDA was lower ($p \leq 0.05$). All of these results may be due to bioactive polyphenol compounds in beet root juice, which play a vital role against lipid peroxidation. This is consistent with the results of **Georgiev *et al*., (2010) and El- Gamal *et al*., (2014)** showed that beet root juice diminishes the elevation in MDA level and restored the renal endogenous antioxidant CAT, GSH.px and SOD levels. Furthermore, **Kujawska *et al*., (2009)** found that administration of rats with 8 ml/kg/day of red beet juice for 4 weeks led to protect them from oxidative stress and related damage by reducing the lipid peroxidation and increasing the antioxidant enzymes activities (catalase, glutathione peroxidase and SOD). Red beet extracts were rich in flavonoids and saponins, which are known to have strong antioxidant potential and free radical scavenging properties **(Sacan and Yanardag, 2010; Al-Dosari *et al*., 2011).**

From the above results, it could be concluded that the beet root juice could be a promising therapeutic agent in alleviating gentamicin induced nephropathy in diabetic rats. So, the present work data recommend that beet root in everyday diet would be beneficial.

Table (5): Effect of beet root juice on antioxidant parameters of kidney of nephropathy diabetic rats

Groups / Parameter	Normal Control	Nephropathy Diabetic Rats			
		Positive Control	Beet Root Juice		
			5 µl/g B.W	10 µl/g B.W	15 µl/g B.W
MDA (nmol/mg. tit)	$9^{d}\pm1.59$	$29.93^{a}\pm4.14$	$27.2^{ab}\pm1.64$	$20^{b}\pm1.29$	$12.5^{c}\pm1.63$
CAT (ng/mg. tit)	$34.25^{a}\pm3.77$	$14^{d}\pm1.41$	$16.25^{cd}\pm1.71$	$18.5^{c}\pm1.30$	$29^{b}\pm0.82$
GSH.px. (ng/mg. tit)	$41.6^{a}\pm1.66$	$15.18^{d}\pm3.76$	$18^{cd}\pm1.41$	$21^{c}\pm1.09$	$34.75^{b}\pm1.71$
SOD (U/L. tit)	$40.7^{a}\pm2.88$	$9.2^{d}\pm2.18$	$12.5^{cd}\pm2.08$	$15.9^{c}\pm0.87$	$33.4^{b}\pm4.11$

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); **MDA:** malonaldehyde, **CAT:** catalase, **GSH.Px:** glutathione peroxidase, **SOD:** superoxide dismutase.

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كفاءة عصير البنجر لتحسين حالة الفئران المصابة بإعتلال الكلي والسكري

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الملخص العربى

يرتبط تناول عصير البنجر بالعديد من الفوائد الصحية والتى ترجع لمحتواه العالى من المركبات الفعالة ونشاطه المضاد للأكسدة. حيث تعد جذور البنجر مصدر غني بالمركبات الكيميائية الفعالة والتي تشمل البيتاليين ، حمض الأسكوربيك ، الكاروتينات ،الفلافونويدات و البوليفينول. وقد أجريت هذه الدراسة لتقييم تأثير عصير جذور البنجر علي الفئران المصابة بكلا من إعتلال الكلي الناجم عن الجينتاميسين والسكري. قسمت الفئران عشوائيا إلي مجموعتين رئيسيتين ، المجموعة الأولى كانت بمثابة المجموعة الضابطة السالبة (6 فئران) والتى تغذت على الوجبة الأساسية ، و المجموعه الثانية (الفئران المصابة بإعتلال الكلي والسكري) والتى تغذت على الوجبة الأساسية و تم حقنها بالجنتاميسين بجرعة يومية مقدارها85 ملجم / كجم من وزن الجسم لمدة 8 أيام متتالية. ثم اعطاءها عن طريق الحقن داخل البريتون جرعة واحدة من الأستربتوزوتوسين (STZ) مقدارها 65 ملجم / كجم من وزن الجسم . وقسمت هذه المجموعة إلى أربع مجموعات (6 فئران بكل منها). المجموعة الأولى كانت المجموعة الضابطة الموجبة ، المجموعة الثانية والثالثة والرابعة أعطيت جرعة يومية عن طريق الفم مقدارها 5 ، 10 و 15 ميكرولتر / جرام من وزن الجسم علي التوالي من عصير البنجر لمدة 60 يوم. بعد انتهاء فترة التجربة ، تم تجميع عينات الدم لإجراء التحاليل البيوكيميائية مثل سكر الدم والبول ، الانسولين ، وظائف الكلي ، صورة دهون الدم ومضادات الأكسدة بالكلي. وقد أشارت النتائج إلى أن الفئران المصابة باعتلال الكلى والسكرى لديها ارتفاع في مستوي سكر الدم و البول ، دهون الدم ،وظائف الكلي (اليوريا, الكرياتينين, اليورك أسيد و نيتروجين يوريا الدم) و المانوالدهيد مع إنخفاض بنشاط الجلوتاثيون بيروكسيديز ، السوبر أوكسيد ديسميدتيز والكتاليز بـأنسجة الكلي و البروتين الكلي و الألبيومين والجلوبيولين والأنسولين في السيرم. في حين أن تناول الفئران لعصير البنجر أدي إلي إنخفاض معنوي في مستويات سكر الدم والبول ، دهون الدم مع تحسن مستويات الأنسولين و وظائف الكلي و خفف من الخلل الحادث والتلف بالكلى من خلال خفض الجهد التأكسدي بأنسجة الكلي, في حين أن تناول الفئران 15 ميكرولتر / جرام من وزن الجسم من عصير البنجر أعطى أفضل التأثيرات في تحسين التغيرات السابقة لتقارب المعدلات الطبيعية. وقد خلصت الدراسة إلي أن عصير البنجر لديه تأثيرات مخفضه للسكر كما يمكنه أن يخفف إعتلال الكلي الناجم عن الجينتاميسن.

الكلمات الكشافة : عصيرالبنجر– البيتاليين– الجينتاميسن – إنخفاض سكر الدم –الفئران المصابة بإعتلال الكلي والسكري.