Egyptian Journal of Nutrition
Official journal of the Egyptian Nutrition Society

Print ISSN: 1687-1235
Online ISSN: 2090-2514
Vol. (38) No. (2) (2023)
https://ejn.journals.ekb.eg/

The Effect of Red Onion Peel and Fortified Bread with These Peels on Kidney Function, Liver Enzymes, and Antioxidant Enzymes in Diabetic Rats

Prof. Dr. Sonia Saleh E l- Marasy, Prof. Dr. Ashraf Abd El-Aziz Abd El - megeid Doaa Abd El - Badie Mohamed Mousa Mandor

Nutrition and Food Science Dept. Faculty of Home Economics Helwan University

ABSTRACT

The study aims to investigate the effects of diets containing two levels of red onion peels and bread fortified with these peels on nutrition status, some biochemical analysis, and histopathological examination of the kidney in rats suffering from diabetes. Forty-eight male albino rats were used in this study. The rats were divided into two main groups, as follows: **The first main group (12 rats)** was divided into two subgroups, as follows: **Subgroup (1):** fed on a basal diet (as a negative control group). **Subgroup (2):** fed on a diet containing 250 g of unfortified bread per kg of diet (as a control negative group, also). **The second main group (36 rats)** was injected with alloxan to induce hyperglycemia. The rats in the second main group were divided into six equal subgroups as follows: **Subgroup (1) was** fed a diet containing 250 g of unfortified bread per kg of diet (as a positive control group). Subgroups 2 and 3 were fed diets containing the best two samples of bread estimated by the sensory evaluation (250 g fortified bread with 10% and 15% red onion peels per kg diet, respectively). **Subgroup (4)** was fed a basal diet (as a positive control group also). **Subgroups 5 and 6** were fed diets containing the amounts of red onion peels present in 250 g fortified bread with a 10% and 15% red onion peels/kg diet, respectively. The results indicated that alloxan increased kidney weight, glucose in serum, kidney functions, liver enzymes, while this treatments decreased feed intake and antioxidant enzymes in the liver. Treating diabetic rats with diets containing fortified bread with two levels of red onion peels ("ROP") (10 and 15%) and also the peels used in preparing this bread improved all of these parameters and the histopathological changes in the kidney as compared to non-treated diabetic rats. Conclusion: Red onion peels and bread fortified with these peels can be used to reduce the side effects of diabetes.

Keywords: Diabetes, Red onion peels, Fortified Bread, Rats.

Received: ***Accepted:*** ***Published:***

INTRODUCTION

Diabetes mellitus (DM), or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger ***(Chinmay and Anurekha, 2015).***

Onions are found in a large number of recipes and preparations spanning almost the totality of the world's cultures. The whole plant is edible and is used as food in some form or another. Onion has been found to contain quercetin, fructose, quercetin-3-glucoside,isorhamnetin-4-glucoside,xylose,galactose,glucose, mannose, organosulfur compounds, allylsulfides,flavonoid, flavenols, S-alk(en)yl cysteine sulfoxides, cycloalliin, selenium, thiosulfinates, and sulfur and seleno compounds ***(Lenka et al., 2016).***

Red onion peels, (Allium cepa L.) contain anthocyanins and colourless phenols in their dried outer skins and the epidermal layer of their fleshy scales. That the major pigment in red onions was cyaniding 3-glucoside,with lesser amounts of cyaniding 3-laminaribioside and other minor unidentified cyaniding, peonidin and pelargonidin glucosides ***(Om Hashem et al., 2016).***

Vegetables like red onions, which are deeper in color, contain a variety of phytochemicals, including flavonoids (quercetin) and phenolic compounds that can be employed in medicines and nutraceuticals ***(Packia et al., 2015)***. People who consume red onion peel are thought to have a lower risk of developing stomach, brain, and liver cancer as well as heart attacks and strokes through decreasing platelet-mediated thrombosis ***(Asmah et al., 2018).***

Many studies also showed that red onion peel contained higher antioxidant activity as compared to white and yellow onions. By considering the presence of various phytochemicals in onion ***(Asmah et al., 2018).***

Wahyu et al., (2022) reported that quercetin could protect human renal proximal tubular cells against the toxicity of radio contrast medium in a recent study ***(Andreucci et al., 2018).*** The previous studies showed that quercetin could improve renal function and reduce oxidative stress factors, serum level of Fibroblast Growth Factor 23 (FGF23), renal inflammation, and renal tubular damage in a rat adenine-induction CKD model ***(Yang et al., 2018).*** The purpose of this study was to investigate the impact of diets containing some levels of red onion peels and bread fortified with these peels on kidney function, liver enzymes, and antioxidant enzymes in diabetic rats.

MATERIALS AND METHODS

Materials:

- Casein, alloxan, vitamins, minerals, cellulose, and choline chloride were purchased from EL- Gomhoria Company, Cairo, Egypt.

- Soy oil and red onion were obtained from an agricultural research centre in Giza, Egypt.
- Starch, wheat flour (80% extraction), and yeast were obtained from the local market, in Cairo, Egypt.

- Rats: Male albino rats Sprague Dawley strain weighting 150 ± 10g were purchased from the Helwan farm of experimental animals, Ministry of Health and Population, Helwan, Cairo, Egypt.

- Kits for biochemical analysis were obtained from Alkan for pharmaceutical and chemical Dokki, Egypt.

Methods:

Preparation of Bread: Normal bread consists of wheat flour (91 g), yeast (5 g), salt (2 g), and sugar (2 g).

Fortified of Bread with Dried Red Onion Peels:

-In this study, white flour was fortified with red onion peels by partially replacing the wheat flour with red onion peels.

Samples \ Ingredients	Red onion peels	Sugar	Salt	Yeast	Wheat Flour
Control	**--**	**2.0**	**2.0**	**5**	**91**
Fortified bread with 10% red onion peels	**10**	**2.0**	**2.0**	**5**	**81**
Fortified bread with 15% red onion peels	**15**	**2.0**	**2.0**	**5**	**76**
Fortified bread with 20% red onion peels	**20**	**2.0**	**2.0**	**5**	**71**

Sensory evaluation of all samples was used to identify the best two samples from bread, which were fortified with red onion peels.

Sensory evaluation:

The sensory properties of the produced bread were measured by twenty personally trained judges. The judges were asked to give a score from zero to 100 for colour, odour, taste, texture, and general acceptance as reported by, ***Abd El-latif (1990).***

Chemical analysis:

Chemical analysis*:* moisture content, total protein, fat, fiber, and ash were determined in unfortified bread, and the best two samples from fortified bread with red onion peels powdered "fortified bread with 10% and 15% red onion peels", according to the methods outlined in ***A.O.A.C (1990),*** while the carbohydrates content will be calculated by difference.

Moisture, protein, fat, fiber, ash, carbohydrate, and active components (anthocyanins, Total flavonoids, tannins and total phenolic content) were determined in red onion peels powdered in Agricultural Research Center, Giza, Egypt, according to ***(A.O.A.C. 1990).***

Biological Part:

Forty-eight male albino rats, Sprague Dawley Strain, weighing 150 ± 10 g, were housed in well-aerated wire cages. All animals were kept under normal healthy conditions and fed on a basal diet for one week for adaptation ***(Reeves et al, 1993).*** After the adaptation period, rats were divided into two main groups, as follows: The first main group (12 rats) was divided into two subgroups. Subgroup (1): fed on a basal diet as (a control negative group). Subgroup (2): fed on a diet containing 250 g of unfortified bread/ kg of diet (a control negative group also). The second main group: 36 rats. Injected with alloxan (150 mg/kg b.w.) to induce hyperglycemia and fed on a basal diet for four days ***(Buko et al., 1996*****).** After this period, serum glucose was determined in each rat in the first and second main groups, to ensure the induction. The rats in the second main group were divided into six subgroups as follows: *The first subgroup (6 rats)* fed on a diet containing 250 g of unfortified bread / kg of diet (as a control positive group). *The second and third subgroups (12 rats)* were fed diets containing the best two samples from bread, which were estimated by the sensory evaluation (250 g fortified bread with 10% and 15% red onion peels / 1000g diets), respectively. *The fourth subgroup (6 rats)* fed on a basal diet (as a control positive group also). The fifth and sixth subgroups (12 rats) were fed diets containing the levels of red onion peels that are present in 250 g fortified bread with 10% and 15% red onion peels/kg diet, respectively.

During the experimental period (4 weeks), the diets consumed were recorded every week. At the end of the experiment, the rats were fasted overnight, and then the rats were anaesthetized and sacrificed. Blood samples were collected from the aorta of all rats. The blood samples were centrifuged, and serum was separated to estimate some biochemical parameters, i.e., serum glucose (***Trinder, 1959)***, uric acid ***(Fossati et al, 1980)***, urea nitrogen ***(Patton and Crouch, 1977)***, creatinine ***(Bohmer, (1971)***, [aspartate aminotransferase and alanine aminotransferase AST and ALT ***(Henry 1974)***, alkaline phosphates (ALP) (Belfield and Goldberg 1971), and [glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) ***(Aebi, 1984; Beauchamp and Fridovich 1971 and Paglia & Valentine 1967)*****, respectively.**

The kidney was separated from each rat and weighted to calculate the kidney weight to body weight %. The liver in each group was examined histopathologically in the Faculty of Veterinary Medicine. The kidneys of sacrificed rats were taken and immersed in a 10% formalin solution. The fixed specimens were then trimmed, washed, and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns' thickness, and stained with hematoxylin and eosin according to ***(Sheehan and Hrapchak, 1980).***

The results of the biological evaluation of each group were statistically analyzed (mean ± standard deviation and one-way ANOVA test) using the SAS package and compared with each other using the suitable test (least significant differences at $P< 0.05$ ***(SAS, 1996).***

RESULTS AND DISCUSSION

Chemical Composition of Red Onion Peels Powdered (100 g dry weight basis)

Table 1 presents the proximate chemical composition of red onion peels powdered (on a 100-g). The data in this table showed that the mean values of moisture, total protein, fat, ash, crude fiber, and carbohydrate were 8.16, 0.90, 0.13, 0.7, 0.30, and 89.81g / 100 g dry weight, respectively. From this data, it was revealed that red onion peel powder is a rich source of carbohydrates. These findings showed that powdered red onion peel is a rich source of carbohydrates.

The data in this table also revealed that the mean concentrations of anthocyanins, total flavonoids, total phenolic content, and tannins in powdered red onion peels were 0.105 mg/100g, 112.53 µg QUE / ml, 550.73 µg ml/GAE and 1.30 mg/100g, respectively. In this regard, ***Beatrice, (2017)*** discovered that onion peel has a very low protein content (0.88%) and a very high carbohydrate content (88.56%). Onion peel extract yielded 98.52 µg QUE / ml total flavonoid, 664.30 µg / ml GAE total phenol, total antioxidant property (1338.15 µg / ml) and scavenged DPPH radical of 27.76 µg / ml.

According to ***Manoj et al. (2022)***, onion peel has a variety of therapeutic benefits for treating conditions like cancer, obesity, diabetes, neurological disorders, cardiovascular disorders, microbiological damage, and erectile dysfunction due to the presence of bioactive substances. As a result, onion peel can be used as a medicinal and therapeutic agent in a variety of sectors in the form of powders or extracts. On the other hand, according to the same authors, numerous thorough studies have confirmed that onion peel is a concentrated source of bioactives and provides a variety of medicinal advantages.The biochemical components, particularly phytochemicals like quercetin and its derivatives, total flavonoids, total polyphenols, and other phytochemicals found in onion peel, make its use in the biomedical and pharmaceutical industries possible.

Sensory Evaluation of Bread Fortified with Three Levels of Onion Peel Powder

One of the bakery goods that is regularly consumed is bread. Considering the crucial role that nutrition plays in many diseases, including diabetes, a whole-wheat bread diet improves postprandial blood glucose levels as compared to a diet high in refined grains, such as white bread. On the other hand, adding red onion peel powder when preparing bread is a good way to increase the bread's nutritional value. The sensory properties of prepared bread fortified with red onion peel powder are displayed in Table 2.

The results revealed that bread fortified with 10% and 15% red onion peel powder showed a non-significant difference in colour and texture, while fortified bread with 20% red onion peel powder decreased the score of color and texture significantly ($p \leq 0.05$), as compared to un-fortified bread. On the other hand, the data presented in this table revealed that, the mean score of taste, odour, and general acceptance in fortified bread decreased gradually with increasing levels of red onion peels. The highest score of the sensory evaluation was recorded for the control sample (un-fortified bread), followed by the bread fortified with 10%, 15%, and 20% red onion peels, respectively. Therefore, the biological part of this study was done to determine the effect of fortified bread with 10% and 15% red onion peel powder on diabetic rats.

In this respect, ***Jung et al., (2011)*** reported that bread with onion peel and onion powder added is higher in nutritional value than normal bread because it contains active ingredients like quercetin and allyl sulphur compounds. On the other hand, ***Sara et al., (2020)*** revealed that the organoleptic characteristics of onion peel extract-supplemented bread were superior to those of plain white flour bread in terms of aroma, appearance, texture, and taste. Therefore, there is a potential to testify to the use of onion peel in other types and forms of bread to drive the idea of food supplementation parallel to food innovation.

Chemical Composition of Bread Fortified with 10% and 15% Red Onion Peel Powder (% Fresh Weight and Dry Weight Basis).

The data in Table 3 showed the approximate chemical composition of unfortified and fortified bread with 10 and 15% red onion peels, respectively. The information in this table showed that the average amounts of moisture, total protein, fat, ash, crude fiber, and carbohydrates in 100 g of unfortified bread were 35, 9.73, 2.44, 1.62, 3.61, and 47.60 g, respectively, and 6.05, 14.96, 3.75, 2.49, 5.56, and 67.19 g dry weight, respectively.

On the other hand, fortified bread with 10% red onion peel powder showed changes in moisture, total protein, fat, ash, crude fiber, and carbohydrate, which were recorded (34.73, 9.66, 2.21, 1.50, 3.48, and 48.42 g/100 g fresh weight, respectively) and (5.92, 14.80, 3.38, 2.29, 5.33, and 68.28 g/100g dry weight, respectively). While these nutrients in fortified bread with 15% red onion peel powder were recorded (34.50, 9.42, 2.12, 1.40, 3.36, and 49.2g/100 fresh weight basis, respectively) and (5.75, 14.38, 3.23, 2.14, 5.13, and 69.37g/100g dry weight basis, respectively).

The data in this table showed that the highest amounts of nutrients were presented in un-fortified and fortified bread, with 10% and 15% red onion peels recorded for carbohydrates, followed by protein, crude fiber, fat, and ash, respectively.

Effect of Fortified Bread with Two Levels from Red Onion Peels and Also the Peels Used in Preparing This Bread on Feed Intake, Kidney Weight/Body Weight% and Serum Glucose of Diabetic Rats.

Data in Table 4 demonstrated how fortified bread with two levels of red onion peels, as well as the peels used to make this bread, affected diabetic rats' feed intake, kidney weight/body weight%, and serum glucose.

The mean value of feed intake (FI) of diabetic rats fed on a diet containing 250g / kg diet ("positive control group") decreased significantly $p \leq 0.05$ as compared to the negative control group fed on the same diet (15.466 ± 0.503 vs. 17.600 ± 0.655 g / each rat), respectively. The same trend was observed when feeding diabetic rats on a basal diet as compared to healthy rats who were fed on a basal diet (15.200 ± 0.458 vs. 16.766 ± 0.665 g / rat), respectively. According to the results in this table, there were no statistically significant differences in the mean value of FI between any of the treated diabetic rat groups fed either a diet consisting of 250 g of bread fortified with 10% or 15% red onion peels or a basal diet consisting of the amounts of ROP used for preparing the fortified bread. On the other hand, the mean values of feed intake in all treated groups showed non-significant changes, as compared to the positive control groups.

Kidney weight/body weight % of diabetic rats fed on a diet containing 250g of unfortified bread / kg diet increased the mean values of this organ, as compared to normal rats fed on the same diet. Treating diabetic rats with diets containing 250 g of fortified bread with 10 and 15% ROP led to a significant decrease in kidney weight/body weight %, as compared to the positive control group fed on a diet containing unfortified bread. On the other hand, the group of rats fed on a diet containing 250 g of fortified bread with 15% ROP recorded the highest decrease in kidney weight/ body weight %, as compared to the group treated with 250g of fortified bread with 10%. The same trend was observed when using the basal diet, which contained ROP that was used in preparing fortified bread with 10% and 15%.

The effect of fortified bread with two levels of red onion peels ("ROP") (10 and 15%) and also the peels used in preparing this bread on the serum glucose of diabetic rats is presented in Table (4). Injecting rats with alloxan and feeding them a diet containing 250 g of unfortified bread per kg diet led to a significant increase ($P \leq 0.05$) in serum glucose as compared to the negative control group fed on the same diet. The mean value of serum glucose increased by about 103.435% in the positive control group compared to that of the negative control group. Feeding diabetic rats with a diet containing fortified bread with 15% ROP led to a significant decrease in serum glucose, decreasing by 36.723% compared to the control positive group which fed on a diet containing unfortified bread.

On the other side, the data in the same Table revealed that injected rats with alloxan and who fed on a basal diet increased the mean value of serum glucose significantly ($P \leq 0.05$), as compared to non-injected rats. Diabetic rats which were fed on a basal diet containing the amounts of ROP that were used in preparing fortified bread with 10% & 15% showed a significant decrease ($P \leq 0.05$), as compared to the positive control group fed on a basal diet.

Quercetin has been found in previous research to protect the kidneys by reducing glucose levels ***(Vera et al., 2018).*** By enhancing the antioxidant capacity of cells, quercetin can help minimize oxidative stress ***(Wu et al., 2017).*** Moreover, by suppressing inflammatory factors, quercetin can lessen inflammatory stimulation ***(Tan et al., 2020).*** Quercetin inhibits the transformation of renal tubular epithelial cells, which can postpone fibrosis and modulate macrophage polarization ***(Lu et al., 2018 and Yang et al., 2018).*** Moreover, it has been proven that quercetin could protect human renal proximal tubular cells against toxicity ***(Andreucci et al., 2018).***

Red onion peels contain a high amount of quercetin; Researchers indicate that quercetin has good anti-diabetic effects. Quercetin may exert antidiabetic effects through various mechanisms, including promoting insulin secretion, improving insulin resistance, maintaining glucose homeostasis, and inhibiting inflammation, oxidative stress, and apoptosis ***(Huan et al., 2021).*** On the other hand, ***Carrasco-Pozo et al., (2016)*** reported that quercetin could resist cholesterol-induced pancreatic β cell dysfunction, thereby maintaining glucose-stimulated insulin secretion and glycemic control. ***Jung et al., (2011)*** reported that phytochemicals such as quercetin and allyl-propyl disulfides found in onion peel and bulb might be responsible for the beneficial effect on blood glucose levels by up-regulating the expression of insulin receptors and glucose transporters, improving insulin sensitivity and promoting glucose metabolism in peripheral tissues in diabetic rats.

Effect of Fortified Bread with Two Levels of Red Onion Peels and the Peels Used in Preparing This Bread on Kidney Functions of Diabetic Rats

The effect of fortified bread with two levels of red onion peels ("ROP") (10 and 15%) and also the amounts of the peels that were used in preparing this bread on the serum uric acid, urea nitrogen, and creatinine (mg/dl) of diabetic rats is presented in Table 5. Feeding diabetic rats on a diet containing 250 g of fortified bread with 10% and 15% ROP led to a significant decrease $p \leq 0.05$ in serum uric acid, urea nitrogen, and creatinine, as compared to the positive control group. On the other hand, treating diabetic rats with a diet containing fortified bread with 15% ROP recorded a significant decrease in the mean value of serum uric acid and urea nitrogen, while serum creatinine showed a non-significant change, as compared to the rats treated with a diet containing fortified bread with 10% ROP. The best results in decreasing the mean values of serum uric acid, urea nitrogen, and creatinine were recorded in the diabetic group fed on a diet containing fortified bread with 15% ROP. This treatment decreased the mean values of serum uric acid, urea nitrogen, and creatinine by about 24.98%, 29.56%, and 32.697%, respectively, compared to the control positive group which was fed on unfortified bread.

On the other side, the data in the same table revealed that injected rats with alloxan who fed on a basal diet significantly increased the mean values of serum uric acid, urea nitrogen, and creatinine ($P \leq 0.05$), as compared to non-injected rats that fed on a basal diet. Diabetic groups that were fed on a basal diet containing the amounts of ROP that were used in preparing fortified bread at 10% and 15% showed a significant decrease in serum uric acid, urea nitrogen, and creatinine ($P \leq 0.05$) as compared to the positive control group fed on a basal diet.

From the above-mentioned data, it could be concluded that, the mean values of serum uric acid, urea nitrogen, and creatinine increased significantly in the control positive groups as compared to the healthy groups (control negative groups). In this respect, ***Ayoade et al., (2009)*** reported that increasing the mean values of serum urea and creatinine in rats that were treated with alloxan might be due to the damage that occurred to pancreatic cells and not as a result of kidney damage. All treated diabetic groups with fortified bread with 10% and 15% ROP and the peels used in preparing fortified bread with 10% and 15% ROP showed a significant decrease in all kidney function parameters as compared to the positive control groups. ***Atallah et al., (2017)*** suggest that red onion peels can serve as a convenient and cost-effective source of high-value antioxidant nutraceuticals for protection against oxidative stress-related disorders.

Quercetin has been found in previous research to protect the kidneys by reducing glucose levels ***(Vera et al., 2018).*** By enhancing the antioxidant capacity of cells, quercetin can help minimize oxidative stress ***(Wu et al., 2017).*** Moreover, by suppressing inflammatory factors, quercetin can lessen inflammatory stimulation ***(Tan et al., 2020).*** Quercetin inhibits the transformation of renal tubular epithelial cells, which can postpone fibrosis and modulate macrophage polarization ***(Lu et al., 2018 and Yang et al., 2018).*** Moreover, it has been proven that quercetin could protect human renal proximal tubular cells against the toxicity ***(Andreucci et al., 2018).***

The Effect of Red Onion Peels and Fortified Bread with These Peels on Liver Enzymes in Diabetic Rats

The effect of fortified bread with two levels of red onion peels ("ROP") (10 and 15%) and also the amounts of the peels that were used in preparing these breads on liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphates (ALP) (U/l) of diabetic rats is presented in Table (6).

Feeding diabetic rats on a diet containing 250 g of fortified bread with 10% and 15% ROP caused a significant decrease $p \leq 0.05$ in serum AST, ALT, and ALP as compared to the positive control group. On the other hand, treating diabetic rats with a diet that contained fortified bread with 15% ROP recorded a significant decrease (p 0.05) in the mean value of these parameters as compared to diabetic rats treated with a diet containing fortified bread with 10% ROP.

Diabetic rats fed on a basal diet containing the amounts of ROP that were used in preparing fortified bread with 10% and 15% showed a significant decrease in serum AST, ALT, and ALP ($P \leq 0.05$), as compared to the positive control group fed on a basal diet. On the other hand, treating diabetic rats with the amount of ROP that was used in preparing fortified bread with 15% recorded a significant decrease $P \leq 0.05$ in the mean values of AST, ALT, and ALP as compared to diabetic rats treated with the amount of ROP that was used in preparing fortified bread with 10%.

From these results, it could be concluded that treating diabetic rats with a diet containing 250 g of fortified bread with 10% and 15% ROP or with basal diets containing the amounts of ROP that were used in preparing fortified bread with 10% and 15% improved the mean values of AST, ALT, and ALP as compared to the positive control groups. On the other hand, feeding diabetic rats with basal diets containing the amounts of ROP that were used in preparing fortified bread with 10% and 15% was more effective in reducing these parameters. After 2 weeks of quercetin combined with basic treatment, the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) were significantly reduced. These results suggest that quercetin has potential therapeutic value for NAFLD ***(Huan et al., 2021).***

Marwa, (2015*)** reported significant decreases in the elevated serum levels of liver enzymes (AST, ALT, and ALP), total cholesterol, and triglycerides in obese diabetic rats by treating them with onion peel extract OPE. The hepatoprotective activity of OPE could possibly be attributed to their antioxidant effect, which was previously reported by ***Park et al., (2007). Concerning OPE, its hypolipidemic activity was explained by its high content of quercetin, which has been reported to have lipid-lowering properties ***(Bae et al., 2014).*** On the other hand, ***Abd Elsabor et al., (2019)*** reported that ibuprofen induced a significant increased ($p \leq 0.05$) in TG, TC and LDL, also ALT and AST levels compared to normal group. Feeding rats on a diet containing 10% of onion peel bead OPB and garlic peel bread GPB exhibited a significant improvement ($p \leq 0.05$) in these parameters.

The Effect of Red Onion Peels and Fortified Bread with These Peels on the Antioxidant Enzymes of Diabetic Rats

The effect of fortified bread with two levels of red onion peels ("ROP") (10 and 15%) and also the amounts of the peels that were used in preparing these breads on antioxidant enzymes in the liver, including glutathione peroxidase (GSH-Px) ng/g liver, superoxide dismutase (SOD) U/g liver, and catalase (CAT) mmol/g liver, in diabetic rats is presented in Table 7**.** Antioxidant enzymes, including glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT), decreased significantly in injected rats with alloxan (the positive control groups) as compared to healthy rats (the negative control groups). in this respect, ***Stanely et al., (2001)*** reported a decrease in the activities of these antioxidant enzymes (SOD, catalase, GPx and GST) in the liver and kidneys of diabetic rats. All treated groups with fortified bread with 10% and 15% red onion peels or the peels that were used in preparing this bread increased the mean values of GSH-Px, SOD, and CAT as compared to the positive control groups. The best results in increasing the mean values of GSH-Px, SOD, and CAT were recorded in the diabetic rats fed on a basal diet containing ROP, which is present in 250 g fortified bread with 15%, followed by the group fed on a diet containing 250 g fortified bread with 15% ROP.

All treated groups with fortified bread with 10% and 15% red onion peels or the peels that were used in preparing this bread increased the mean values of GSH-Px, SOD, and CAT as compared to the positive control groups. In this context, ***Sara et al., (2020)*** reported that researchers in some studies reported red onion skin to be a good source of natural antioxidants (flavonoids) with very high antioxidant activities. *Sara* also reported that some researchers extracted and evaluated different onion skin phenolic compounds for antioxidant activities and observed the highest amount of anthocyanin and antioxidant activities in red onion skin. ***Masood et al., (2021)*** reported that when diabetic rats were fed bread supplemented with onion peel extract or onion powder, the altered activities of the antioxidant enzymes such as SOD, CAT, GPx, and GR were significantly restored ($p < 0.01$). The antioxidant potential of various nutraceuticals such as quercetin, isorhamnetin, kaempferol, and allyl-propyl disulfides might have contributed to this effect. All these constituents previously showed a potent effect on reducing blood glucose levels and oxidative stress by modulating various antioxidant enzymes in diabetic rats ***(Senyigit et al., 2019 and Campos et al., 2003).***

Histopathological examination of the Kidney:

Microscopically, kidneys of rats from the control negative group fed on a diet containing 250 g unfortified bread / kg diet revealed the normal histological structure of renal parenchyma (normal renal cortex and renal medulla) (Photo 1). On the other hand, kidney sections of rats from diabetic group which was fed on the same diet in the negative control group showed vacuolar degeneration of epithelial lining renal tubules and necrobiosis of renal tubular epithelium (Photo 2). Meanwhile, kidneys of rats from diabetic group fed on a diet containing 250g fortified bread with 10% ROP / kg diet described no histopathological changes (Photo 3). Otherwise, kidneys of rats from diabetic group fed on a diet containing 250g fortified bread with 15% ROP / kg diet exhibited necrobiosis of renal tubular epithelium with pyknosis of their nuclei (Photo 4 and 5). On the other hand, kidneys of rats from the control negative group fed on a basal diet showed the normal histological structure of renal parenchyma (normal renal cortex and renal medulla) (Photo 6). While kidneys of rats from the positive control group revealed vacuolar degeneration of epithelial lining some renal tubules (photo 7 & 8). Examination of sections from the groups fed on a basal diet containing ROP which is present in 250 g fortified bread with 10% & 15% manifested no histopathological changes (Photo 9, 10 and 11).

Figures and Tables

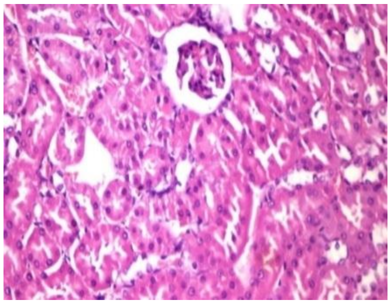

Figure1. Photomicrograph of kidney of the control negative group of rats fed on a diet containing 250 g unfortified bread / kg diet showing the normal histological structure of renal parenchyma (H & E X 400).

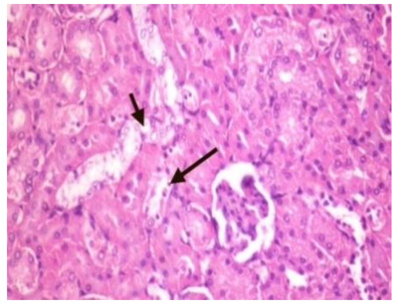

Figure2. Photomicrograph of kidney of rat from the positive control group fed on a diet containing 250 g unfortified bread / kg diet showing vacuolar degeneration of epithelial lining renal tubules and necrobiosis of renal tubular epithelium (H & E X 400).

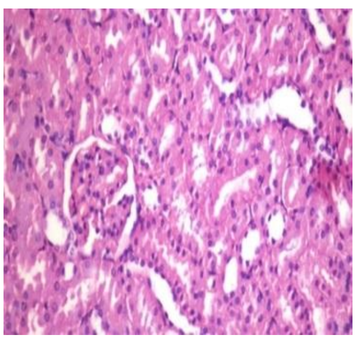

Figure3. Photomicrograph of kidney of diabetic rats fed on a diet containing 250g fortified bread with 10% ROP / kg diet showing no histopathological changes (H & E X 400).

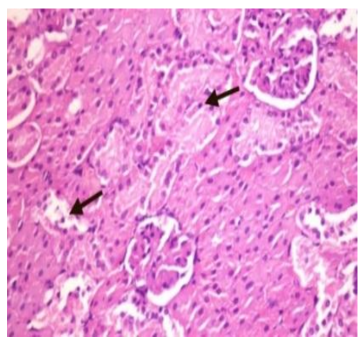

Figure4. Photomicrograph of kidney of diabetic rats fed on a diet containing 250g fortified bread with 15% ROP / kg diet showing necrobiosis of renal tubular epithelium with pyknosis of their nuclei (H & E X 400).

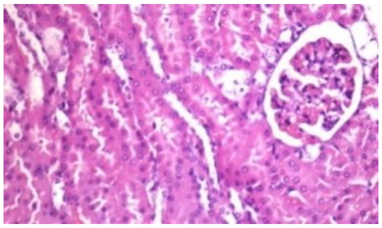

Figure5. Photomicrograph of kidney of diabetic rats fed on a diet containing 250g fortified bread with 15% ROP / kg diet showing necrobiosis of renal tubular epithelium with pyknosis of their nuclei (H & E X 400).

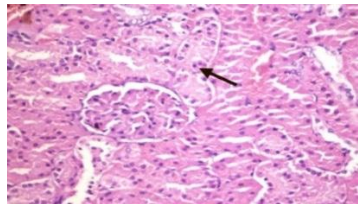

Figure6. Photomicrograph of kidney of rat from the control negative group fed on a basal diet showing the normal histological structure of renal parenchyma (H & E X 400).

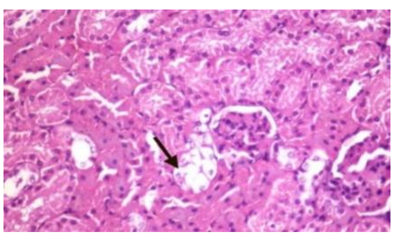

Figure7. Photomicrograph of kidney of rats from the positive control group fed on basal diet showing vacuolar degeneration of epithelial lining some renal tubules (H & E X 400).

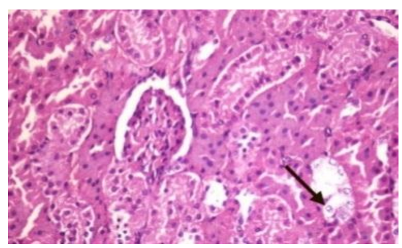

Figure8. Photomicrograph of kidney of rat from the positive control group fed on basal diet showing vacuolar degeneration of epithelial lining some renal tubules (H & E X 400).

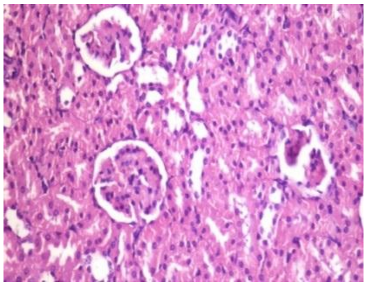

Figure9. Photomicrograph of kidney of diabetic rat basal diet containing ROP which is present in 250 g fortified bread with 10% showing no histopathological changes (H & E X 400).

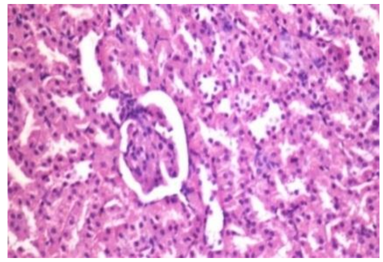

Figure10. Photomicrograph of kidney of diabetic rat fed on basal diet containing ROP which is present in 250 g fortified bread with 15% manifested no histopathological changes (H & E X 400).

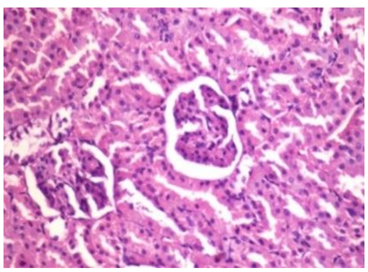

Figure11. Photomicrograph of kidney of diabetic rat fed on basal diet containing ROP which is present in 250 g fortified bread with 15% manifested no histopathological changes (H & E X 400).

Table 1. Chemical Composition of Red Onion Peels Powdered (100g dry weight basis).

Components (g)	**Ingredients**
	Dry weight
Moisture (g)	8.16
Total Protein (g)	0.90
Fat (g)	0.13
Ash (g)	0.7
Crude Fiber (g)	0.30
Carbohydrate*(g)	89.81
Anthocyanins (mg/100g)	0.105
Total flavonoids (µg QUE / ml)	112.53
Total phenolic content (µg ml/GAE)	550.73
Tannins (mg/100g)	1.30

Each value represents the mean of two determinations.
* Total carbohydrates were calculated by differences.

Table 2: Sensory Evaluation of Bread Fortified with Three Levels of Onion Peel Powder

Sensory Characteristics	**Control**	**Bread with Onion Peels Powdered %**		
		10%	**15%**	**20%**
Color (20)	19.44 [a] ± 0.341	18.53 [a] ± 0.410	18.43 [a] ± 0.442	11.82 [b] ±.581
Taste (20)	19.35 [a] ± 0.229	18.32 [a] ± 0.270	17.52 [b] ± 0.733	13.61 [c] ± 0.389
Odor (20)	19.09 [a] ± 0.220	18.44 [a] ± 0.461	17.03 [b] ± 0.430	13.41 [c] ± 0.511
Texture (20)	19.41 [a] ± 0.352	18.72 [a] ± 0.353	18.74 [a] ± 0.334	15.24 [b] ± 0.45
General Acceptance (20)	19.49 [a] ± 0.280	18.13 [a] ± 0.324	17.51 [b] ± 0.550	13.26 [c] ± 0.77
Total Score (100)	96.78 [a] ± 0.361	92.14[b] ± 0.448	89.23[c] ±1.371	67.45[d] ±1.238

Mean values in the same row which is not followed by the same letter are significantly different ($p \leq 0.05$).

Table 3 Chemical Composition of Bread Fortified with 10% and 15% Red Onion Peel Powder (% Fresh Weight and Dry Weight Basis)

Components (g)	Unfortified Bread		Fortified Bread with Red Onion Peels Powdered			
			10%		**15%**	
	FW	DW	FW	DW	FW	DW
Moisture	35	6.05	34.73	5.92	34.50	5.75
Total Protein	9.73	14.96	9.66	14.80	9.42	14.38
Fat	2.44	3.75	2.21	3.38	2.12	3.23
Ash	1.62	2.49	1.50	2.29	1.40	2.14
Crude fiber	3.61	5.56	3.48	5.33	3.36	5.13
Carbohydrate*	47.60	67.19	48.42	68.28	49.2	69.37

Each value represents the mean of two determinations.
*Total carbohydrates were calculated by differences
FW: Fresh weight DW: dry weight

Table 4 Effect of Fortified Bread with Two Levels from Red Onion Peels and Also the Peels Used in Preparing This Bread on Feed Intake, Kidney Weight/Body Weight% and Serum Glucose of Diabetic Rats

Groups		Parameters	Feed intake g/day/each rat	Kidney weight / body weight%	Serum glucose mg/dl
1	Control (-ve) fed on diet containing 250 g unfortified bread / kg diet		17.600 [a] ± 0.655	0.601 [e] ± 0.018	91.667 [f] ± 6.951
2	Control (+ve) fed on diet containing 250 g unfortified bread / kg diet		15.466 [b] ± 0.503	1.263 [a] ± 0.076	186.483 [a] ± 5.354
3	Diabetic rats fed on diet containin	250g fortified bread with 10% ROP / kg diet	15.200 [b] ± 0.458	1.064 [b] ± 0.077	131.000 [c] ± 6.557
4		250g fortified bread with 15% ROP / kg diet	15.066 [b] ± 0.404	0.860 [c] ± 0.069	118.000 [d] ± 9.165
5	Control (-ve) fed on basal diet (BD)		16.766 [a] ± 0.665	0.580 [e] ± 0.019	79.773 [g] ± 1.988
6	Control (+ve) fed on (BD)		15.200 [b] ± 0.458	1.094 [b] ± 0.075	169.673 [b] ± 5.816
7	Diabetic rats fed on BD containing	ROP which present in 250 g fortified bread with 10%	14.966 [b] ± 0.472	0.842 [c d] ± 0.052	119.613 [d] ± 4.960
8		ROP which present in 250 g fortified bread with 15%	14.633 [b] ± 0.513	0.747 [d] ± 0.047	106.253 [e] ± 7.101

ROP: Red onion peel. BD: Basal diet Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Table 5. The Effect of Red Onion Peels and Fortified Bread with These Peels on Kidney Functions of Diabetic Rats

Groups		Parameters	Uric acid (mg/dl)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)
1	Control (-ve) fed on diet containing 250 g unfortified bread / kg diet		2.003 [c] ± 0.127	39.666 [d] ± 2.621	0.693 [d e] ± 0.040
2	Control (+ve) fed on diet containing 250 g unfortified bread / kg diet		2.710 [a] ± 0.110	69.440 [a] ± 3.197	1.153 [a] ± 0.181
3	Diabetic rats fed on diet	containing 250g fortified bread with 10% ROP / kg diet	2.460 [b] ± 0.043	58.606 [b] ± 3.134	0.930 [b c] ± 0.121
4	Diabetic rats fed on diet	containing 250g fortified bread with 15% ROP / kg diet	2.033 [c] ± 0.152	48.913 [c] ± 3.420	0.776 [c d] ± 0.025
5	Control (-ve) fed on basal diet (BD)		1.686 [e] ± 0.102	33.126 [e] ± 2.348	0.556 [e] ± 0.060
6	Control (+ve) fed on (BD)		2.313 [b] ± 0.090	58.540 [b] ± 4.234	1.000 [a b] ± 0.160
7	Diabetic rats fed on BD	containing ROP which present in 250 g fortified bread with 10%	1.923 [cd] ±0.092	46.333 [c] ± 4.509	0.800 [c d] ± 0.080
8	Diabetic rats fed on BD	containing ROP which present in 250 g fortified bread with 15%	1.783 [de] ± 0.076	39.116 [d] ± 2.781	0.676 [d e] ± 0.068

ROP: Red onion peel. BD: Basal diet
Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Table 6. The Effect of Red Onion Peels and Fortified Bread with These Peels on Liver Enzymes of Diabetic Rats.

Groups		Parameters	AST (U/L)	ALT (U/L)	ALP (U/L)
1	Control (-ve) fed on diet containing 250 g unfortified bread / kg diet		87.667 [f] ± 4.725	32.743 [e] ± 2.637	113.936 [f] ± 3.290
2	Control (+ve) fed on diet containing 250 g unfortified bread / kg diet		170.900 [a] ± 1.873	52.243 [a] ± 1.825	269.773 [a] ± 4.227
3	Diabetic rats fed on diet	containing 250g fortified bread with 10% ROP / kg diet	147.333 [b] ± 7.505	45.610 [b c] ± 2.235	236.210 [c] ± 5.224
4	Diabetic rats fed on diet	containing 250g fortified bread with 15% ROP / kg diet	117.667 [d] ± 4.725	39.360 [d] ± 2.653	214.503 [d] ± 5.416
5	Control (-ve) fed on basal diet (BD)		80.333 [f] ± 2.309	30.366 [e] ± 3.208	100.323 [g] ± 5.110
6	Control (+ve) fed on (BD)		152.333 [b] ± 5.859	49.500 [a b] ± 2.233	256.620 [b] ± 5.746
7	Diabetic rats fed on BD	containing ROP which present in 250 g fortified bread with 10%	132.333 [c] ± 5.859	43.566 [c d] ± 3.187	221.803 [d] ± 5.203
8	Diabetic rats fed on BD	containing ROP which present in 250 g fortified bread with 15%	107.000 [e] ± 7.211	32.393 [e] ± 2.569	189.800 [e] ± 8.292

ROP: Red onion peel. BD: Basal diet
Means in the same column with different letters are significantly different at ($p \leq 0.05$).

Table 7. The Effect of Red Onion Peels and Fortified Bread with These Peels on the Antioxidant Enzymes of Diabetic Rats.

Groups		Glutathione peroxidase (GSH-Px) ng/g Liver	Superoxide dismutase (SOD) U/g liver	Catalase (CAT) mmol/g liver
1	Control (-ve) fed on diet containing 250 g unfortified bread / kg diet	0.490^{a} ± 0.009	0.402^{b} ± 0.006	0.396^{a} ± 0.004
2	Control (+ve) fed on diet containing 250 g unfortified bread / kg diet	0.207^{e} ± 0.021	0.236^{g} ± 0.009	0.177^{e} ± 0.009
3	Diabetic rats fed on diet containing 250g fortified bread with 10% ROP / kg diet	0.306^{d} ± 0.017	0.316^{e} ± 0.011	0.230^{d} ± 0.016
4	Diabetic rats fed on diet containing 250g fortified bread with 15% ROP / kg diet	0.373^{c} ± 0.017	0.354^{d} ± 0.008	0.262^{c} ± 0.015
5	Control (-ve) fed on basal diet (BD)	0.517^{a} ± 0.015	0.425^{a} ± 0.007	0.412^{a} ± 0.011
6	Control (+ve) fed on (BD)	0.232^{e} ± 0.027	0.266^{f} ± 0.016	0.195^{e} ± 0.006
7	Diabetic rats fed on BD containing ROP which present in 250 g fortified bread with 10%	0.330^{d} ± 0.011	0.360^{d} ± 0.011	0.263^{c} ± 0.008
8	Diabetic rats fed on BD containing ROP which present in 250 g fortified bread with 15%	0.410^{b} ± 0.022	0.382^{c} ± 0.004	0.308^{b} ± 0.009

ROP: Red onion peel.BD: Basal diet Means in the same column with different letters are significantly different at ($p \leq 0.05$).

CONCLUSION

Red onion peels and bread fortified with 10% and 15% of these peels improved kidney functions including (uric acid, urea nitrogen, and creatinine); liver enzymes (AST, ALT, and ALP), and antioxidant enzymes (GSH-Px, SOD, and CAT) in diabetic rats. Therefore, these peels and the bread that was fortified with red onion peels can be used to reduce the side effects of diabetic Patients.

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تأثير قشر البصل الأحمر والخبز المدعم بهذه القشور على وظائف الكلي، انزيمات الكبد والانزيمات المضادة للأكسدة في الفئران المصابة بمرض السكر

أ.د/ سونيا صالح المراسي
أ.د/ أشرف عبدالعزيز عبدالمجيد

دعاء عبدالبديع محمد موسى مندور

قسم التغذية وعلوم الأطعمه كلية الإقتصاد المنزلي جامعة حلوان

الملخص

أجريت هذه الدراسة لمعرفة تأثير الأنظمة الغذائية المحتوية على بعض مستويات قشور البصل الأحمر والخبز المدعم بهذه القشور على التقييم البيولوجي والتحليل الكيميائي الحيوي والفحص التشريحي للكلى لدى الفئران المصابة بداء السكري. . استخدمت في هذه الدراسة عدد 48 فأرا من ذكور الفئران البيضاء، تم تقسيم الفئران إلى مجموعتين رئيسيتين على النحو التالي: المجموعة الرئيسية الأولى (12 فأر): تم تقسيمهم إلى مجموعتين فرعيتين:المجموعة الفرعية الأولى: تم تغذيتها على الوجبة الأساسية (استخدمت كمجموعة ضابطة سالبة)،المجموعة الفرعية الثانية: تم تغذيتها على غذاء يحتوي على 250جم خبز غير مدعم/ كيلو جرام غذاء (استخدمت كمجموعة ضابطة سالبة، ايضا)، المجموعة الرئيسية الثانية (36 فأر): تم حقنهم بمادة الالوكسان لإحداث ارتفاع مستوى الجلوكوز في الدم. تم تقسيم فنران المجموعة الرئيسية الثانية إلى (ست مجموعات فرعية متساوية) كالتالي: المجموعة الفرعية الأولى: تم تغذيتها على غذاء يحتوي على 250 جم من الخبز غير المدعم/ كجم غذاء (استخدمت كمجموعة ضابطة ايجابية). المجموعات الفرعية الثانية والثالثة: تم تغذيتهم على غذاء يحتوي على أفضل عينتين من الخبز والتي تم تحديدهم بواسطة التقييم الحسي (250 جرام من الخبز مدعم بنسبة 10% و 15% قشور البصل الاحمر / كيلو جرام)، على التوالي. المجموعات الفرعية الرابعة: تم تغذيتها على غذاء أساسي (استخدمت كمجموعة ضابطة ايجابية). المجموعات الفرعية الخامسة والسادسة: تم تغذيتهم على وجبات تحتوي علي مستويات قشور البصل الاحمر والموجودة في 250 جم من الخبز المدعم بنسبة (10% و15 %) من قشور البصل الأحمر / كيلوجرام غذاء. أشارت النتائج الي أن حقن الفئران بمادة الألوكسان أحدثت زيادة في (وزن الكلى وايضا مستوي الجلوكوز في سيرم الدم، ووظائف الكلي، وانزيمات الكبد)، بينما أحدثت هذه المعاملة الي تناقص كمية الطعام المتناول والانزيمات المضادة للأكسدة في الكبد. تغذية الفئران المصابة بالسكر بالوجبات المحتوية علي الخبز المدعم بمستويين (10% و15 %) من قشور البصل الأحمر وكذلك القشور المستخدمة في تحضير هذا الخبز يحسن من جميع القياسات والتغييرات الفسيولوجية للكلى مقارنة بالفئران المصابة بالسكر الغير معاملة. الخلاصة: استخدام قشور البصل الأحمر والخبز المدعم بهذه القشور لتقليل الآثار الجانبية لمرض السكري

الكلمات المفتاحية: داء السكر – قشور البصل الاحمر – خبز معزز بقشور البصل – فئران تجارب