Cereals' Products with Red Grape and Walnut Extracts as Functional Foods for Prevention of Kidney Dysfunction

Sahar Y. Al-Okbi, Doha A. Mohamed, Thanaa E. Hamed, and Ahmed Ms Hussein

Abstract—In the present research, two nutraceuticals made from red grape and walnut that showed previously to improve kidney dysfunction were incorporated separately into functional foods' bread made from barley and rice bran. The functional foods were evaluated in rats in which chronic renal failure was induced through feeding diet rich in adenine and phosphate (APD). The evaluation based on assessing kidney function, oxidative stress, inflammatory biomarkers and body weight gain. Results showed induction of chronic kidney failure reflected in significant increase in plasma urea, creatinine, malondialdehyde, tumor necrosis factor-α and low density lipoprotein cholesterol along with significant reduction of plasma albumin, and total antioxidant and creatinine clearance and body weight gain on feeding APD compared to control healthy group. Feeding the functional foods produced amelioration in the different biochemical parameters and body weight gain indicating improvement in kidney function.

Keywords—Functional food, kidney dysfunction, rats.

I. INTRODUCTION

Oxidative stress and inflammation are involved as risk factors of chronic non communicable diseases including renal dysfunction [1], [2]. Increased level of malondialdehyde was reported during renal damage [3]. Both native and oxidized forms of low density lipoprotein-cholesterol (LDL-Ch) may be involved in the glomerular damage mediated by oxidative stress [4]. Inflammatory lesions caused by oxidative stress participate in the glomerular damage [2]. So, antioxidant and anti-inflammatory nutraceuticals could play an important role in the protection from incidence of chronic renal failure. On the other hand some beverages made from barley have been used in Egypt as Folk medicine to alleviate kidney dysfunction. Phytate, β-glucan, tocopherols and tocotrienols were reported to present in barley seeds [5], referring to its potential hypocholesterolemic, anti-inflammatory and antioxidant effect. Also, previously rice bran was shown to contain different bioactive constituents that possess antioxidant and anti-inflammatory activity and LDL-Ch lowering effect [6]. Policosanol, gamma oryzanol, phytosterols, unsaturated fatty acids, phenolic compounds and dietary fibers [6], [7] are examples of such active ingredients that present in rice bran. In a previous work, nutraceuticals prepared from red grape and walnut were proved to improve kidney dysfunction [8]. Red grape with seeds and walnut were reported to contain total phenolic as 6.4 and 97.42g gallic acid equivalent/100g alcohol extract, linolenic as 3.71 and 6.1% of total fatty acids and 15.868 and 11.03 phytosterol % of unsaponifiable portion [8]. Phytosterol, phenolic compounds and omega 3 fatty acids such as linolenic are reported to possess antioxidant, anti-inflammatory and hypocholesterolemic effect [9]-[11]. So, walnut and red grape are important sources of nutraceuticals. Incorporation of barley or rice bran together with such nutraceuticals into functional foods may lead to highly efficient products that might have health effect towards chronic renal dysfunction.

The aim of the present research was preparation and chemical, sensory and biological evaluation of functional foods for protection from chronic renal failure utilizing efficient nutraceuticals from previous research and cereals rich in antioxidant and anti-inflammatory active constituents.

II. MATERIALS AND METHODS

A. Materials

1. Flours: Wheat grains (Giza 168) and naked barley (Hordeum vulgare variety Giza l29) were obtained from Agric. Res. Center, Giza, Egypt. Stabilized rice bran (RB) was supplemented by International Trade and Marketing, Egypt.
2. Nutraceuticals' Sources: Red grape (with seeds) and walnut were purchased from local markets, Cairo, Egypt.
3. Main Chemical: Adenine was obtained from Sigma (Sigma-Aldrich Co., St. Louis, Missouri, USA).
4. Animals: Male Sprague–Dawley rats of 215.8g average body weight were used in the present study. Animals were obtained from animal house of National Research Centre, Cairo, Egypt. They were kept individually in metabolic stainless steel cages; water and food were given ad-libtium all over the experiment.

B. Methods

1. Preparation of Flour: Barley and wheat grain were separately cleaned, tempered (15% moisture) and milled (Quadrmat Junior flour mill) to 100 % extraction flour.
Whole meal wheat flour (WWF) was well blended with rice bran in the ratio of 70:30. The samples were stored in airtight containers and kept at 5-7°C until used.

2. Preparation of Plant Materials: Fresh red grape with seed was washed by tap water and cut into small pieces and dried in an air-circulated oven at 40°C. Both dried grape and walnut were separately reduced into powder.

3. Preparation of Plant Extracts: The dried powder of grape and walnut were separately extracted by continuous extraction apparatus for extraction by petroleum ether (40-60°C) followed by ethanol. The solvent of each extract was removed by evaporation under reduced pressure.

4. Preparation of Extracts Mixtures (Nutraceuticals): Ethanol and petroleum ether extracts of each plant was mixed together in the ratio of their presence in the whole plant.

5. Preparation of Bread: Flat bread was prepared from 100% whole barley flour (WBF) with or without incorporation of grape nutraceutical as functional food I and control, respectively. Balady bread was prepared from 70% WWF and 30% RB with or without walnut nutraceutical as functional food II and control, respectively.

6. Sensory Evaluation of Bread: Each functional food along with its corresponding control was sensory evaluated by 10 trained panelists. Texture, color, odor, taste and appearance were the criteria for evaluation of flat bread. Sensory attributes used for organoleptic assessment of balady bread were appearance, separation of layers, roundness, distribution of crumb, crust color, taste, and odor. Sensory attribute that verify >50% of the score was deemed as accepted.

7. Determination of Proximate Composition of Food Products: Moisture, crude fiber, ash, protein and fat contents of the breads were determined according to AOAC [12]. Total carbohydrate was calculated by difference.

8. Preparation of Experimental Diets: In the present study, renal dysfunction was induced in rats by feeding them high adenine (0.75%) high phosphate (2.4%) diet for 10 days followed by high adenine (0.75%) diet for another consecutive 11 days (APD). The different Types of bread were dried and reduced into powder for preparation of experimental diets as shown in Table I.

9. Design of Animal Experiment: Forty-two rats were divided into two groups. Group one served as normal control (n=6 rats) and fed on balanced diet for three weeks. Group two (n=36 rats) and fed on high phosphate-high adenine diet for 10 days followed by high adenine diet for another 11 days to induce chronic renal failure (APD). During the experiment, body weight and food intake were recorded weekly. At the end of the three weeks (first stage), total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. Also, 24 hour urine was collected from the control normal rats and six rats from APD group. Blood samples were obtained from the same fasted animals. Heparin was used as an anticoagulant and plasma was separated by centrifugation at 3500rpm for 10 min. Plasma malondialdehyde (MDA) was determined according to Satoh [13] as indicator of lipid peroxidation and oxidative stress. Plasma total antioxidant capacity (TAC) as an antioxidant biomarker was assessed according to Stepaniak et al. [14]. Plasma tumor necrosis factor-α (TNF-α), as inflammatory biomarker, was determined according to Satoh et al. [15]. Plasma creatinine [16] and urea [17] were estimated as indicator of kidney function. Plasma albumin [18], plasma total cholesterol (T.Ch) [19] and LDL-Ch [20] were also determined which may serve as extra indicators of kidney function. Creatinine was determined in the collected 24h urine [16] for calculation of creatinine clearance. In the second stage, residual APD rats (n=30) were divided into five groups. Rats of group one were fed on balanced diet, rats of group two and three were fed on balanced diet containing barley bread and rice bran bread, respectively. Rats of groups four and five were fed on balanced diet containing functional food I and II, respectively. This stage of experiment continued for a month. Nutritional parameters, biochemical analysis and creatinine clearance were again assessed as mentioned previously in the end of the first stage.

10. Statistical Analysis: All the results were expressed as mean± SE. Sensory attributes and data of nutritional parameters of the 1st stage of animal experiment were analyzed statistically using t-student test. One-way analysis of variance ANOVA followed by Duncan’s test were used for comparison of all other parameters of the different experimental groups. In all cases p<0.05 was used as the criterion of statistical significance.

### RESULTS

Proximate composition of the prepared functional foods (Table II) showed that the values of both functional food I and II were more or less similar to their respective control bread except for slight change in ash and carbohydrate. In functional food II, fat was slightly more than that of the respective control.

Sensory parameters of functional foods seen in Table III and IV showed no significant changes in all sensory attributes when compared with their respective control. All bread samples showed to be accepted by panelists.

The determined biochemical parameters of the different experimental groups are shown in Table V. APD group showed a significant elevation in plasma creatinine and urea levels as indicator of kidney dysfunction compared with normal control. There were no significant differences in plasma total cholesterol level, while LDL-Ch was elevated significantly in APD group compared to normal group. Plasma MDA and TNF-α showed significant increase in APD group compared to normal group. Plasma total antioxidant capacity and creatinine clearance were significantly reduced in APD group compared to control normal group.
### TABLE I
COMPOSITION OF DIFFERENT DIETS (g/100 g)

<table>
<thead>
<tr>
<th>a) Ingredients</th>
<th>Balanced diet</th>
<th>High phosphate-high adenine diet</th>
<th>High adenine diet</th>
<th>Balanced diet containing barley bread</th>
<th>Balanced diet containing functional food I</th>
<th>Balanced diet containing rice bran bread</th>
<th>Balanced diet containing functional food II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>8.2</td>
<td>8.2</td>
<td>1.44</td>
<td>1.44</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>* Flat barley bread</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>* Functional food I</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>* Rice bran bread</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>* Functional food II</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>23.5</td>
<td>22.8</td>
<td>22.8</td>
<td>13.8</td>
<td>13.8</td>
<td>16.18</td>
<td>16.56</td>
</tr>
<tr>
<td>Starch</td>
<td>47</td>
<td>45.56</td>
<td>46.95</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salt mix.</td>
<td>3.5</td>
<td>-</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>High phosphate salt mix.</td>
<td>-</td>
<td>4.89</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cellulose</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1.7</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenine</td>
<td>-</td>
<td>0.75</td>
<td>0.75</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

12 g casein contains 10 g protein.

\* 70 g of flat barley bread contains 8.47 g protein, 1.75 g fat and 1.302 crude fibers.

\* 70 g of functional food I contains 8.47 g protein, 1.75 fat and 1.302 crude fibers

\* 70 g of rice bran bread contains 8.8 g protein, 2.12 g fat and 3.143 crude fibers.

\* 70 g of functional food II contains 8.8 g protein, 2.5 g fat and 3.143 crude fibers.

### TABLE II
PROXIMATE COMPOSITION OF DIFFERENT BREADS

<table>
<thead>
<tr>
<th>Parameter g/100g dry sample</th>
<th>100%WBF Flat bread</th>
<th>Functional Food I WWF (70%) + (30%) RB</th>
<th>Functional Food II WWF (70%) + (30%) RB + walnut</th>
<th>NS</th>
<th>NS</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>12.1±0.01</td>
<td>12.1±0.02</td>
<td>12.57±0.18</td>
<td>12.57±0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>2.5±0.03</td>
<td>2.5±0.03</td>
<td>3.03±0.12</td>
<td>3.57±0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>2.64±0.07</td>
<td>2.7±0.06</td>
<td>2.95±0.16</td>
<td>3.0±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude fibers</td>
<td>1.8±0.05</td>
<td>1.86±0.06</td>
<td>4.49±0.05</td>
<td>4.49±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>80.9±0.11</td>
<td>80.84±0.12</td>
<td>76.96±1.12</td>
<td>76.37±1.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture (g/100g fresh sample)</td>
<td>14.01±0.12</td>
<td>14.01±0.13</td>
<td>42.0±0.62</td>
<td>42.0±0.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE III
ORGANOLEPTIC PROPERTIES OF FLAT BREAD

<table>
<thead>
<tr>
<th>Samples</th>
<th>Texture (10)</th>
<th>Color (10)</th>
<th>Odor (10)</th>
<th>Taste (10)</th>
<th>Appearance (10)</th>
<th>Overall acceptability (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%WBF</td>
<td>7.4±0.84</td>
<td>7.1±1.73</td>
<td>6.9±1.73</td>
<td>7.0±1.56</td>
<td>6.8±1.62</td>
<td>7.04±1.69</td>
</tr>
<tr>
<td>100%WBF + red grape nutraceutical (Functional food I)</td>
<td>6.9±0.32</td>
<td>6.5±1.01</td>
<td>6.7±0.86</td>
<td>6.6±0.46</td>
<td>6.5±1.13</td>
<td>6.64±1.25</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

WBF: Whole meal barley flour
NS: Non significant

### TABLE IV
ORGANOLEPTIC PROPERTIES OF BALADY BREAD

<table>
<thead>
<tr>
<th>Samples</th>
<th>General appearance (20)</th>
<th>Separation of layers (20)</th>
<th>Roundness (15)</th>
<th>Distribution of crumb (15)</th>
<th>Crust colour (10)</th>
<th>Taste (10)</th>
<th>Odour (10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%WWF + 30% RB</td>
<td>15.79±1.85</td>
<td>20±0.85</td>
<td>12.85±1.23</td>
<td>11.64±2.02</td>
<td>8.07±0.47</td>
<td>7.65±0.46</td>
<td>8.07±0.79</td>
</tr>
<tr>
<td>70%WWF + 30% RB + walnut</td>
<td>15.25±0.72</td>
<td>20±0.46</td>
<td>12.82±1.06</td>
<td>11.42±1.75</td>
<td>7.91±0.22</td>
<td>7.50±0.22</td>
<td>8.15±0.35</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

WWF: Whole meal wheat flour
RB: rice bran
NS: Non significant
Feeding balanced diet after induction of kidney dysfunction produced significant reduction of plasma creatinine, urea, TNF-α and MDA with significant increase in plasma albumin and creatinine clearance which were still not matching the control normal. However no significant change was noticed in plasma TAC, T.Ch and LDL-Ch. Feeding balanced diet mixed with different bread produced significant improvement of the different biochemical parameters reflecting improvement in kidney dysfunction compared to APD group. Also, feeding APD rats balanced diet mixed with different bread produced significant improvement in all biochemical parameters compared to those fed the balanced diet except for creatinine clearance that showed an improvement that was nonsignificant. Also, albumin demonstrated only significant improvement in the group fed functional food I compared to those fed on balanced diet. There was only significant reduction of plasma creatinine in APD rats fed on either barley bread or functional food I compared to those fed on rice bran bread or functional food II, but there was no significant change in all other biochemical parameters among the groups fed on the different bread. However, functional food I seem to be the most efficient in improving kidney dysfunction.

Table VI showed the nutritional parameters of different experimental groups. Nutritional parameters (body weight gain, food efficiency ratio, total food intake) were significantly reduced in APD group compared to normal control. Rats regained weight after stopping APD and feeding either balanced diet or balanced diet containing any of the studied bread. All nutritional parameters among APD fed rats after feeding on balanced diet or balanced diets containing different breads showed non-significant changes when compared with each other.

### IV. DISCUSSION

Kidney is a vital organ for the body, therefore renal impairment results in high mortality and morbidity among population. Different animal models of renal dysfunction have been developed for evaluation of new therapy; high adenine or high adenine high phosphate diet were fed to rats for different periods of time for induction of such models [21], [22]. On feeding adenine it is metabolized to 2, 8-dihydroxyadenine that induced severe chronic renal failure due to degeneration.
of the proximal and distal tubules and interstitial fibrosis [23]. Hyperphosphatemia, is a nearly universal complication of kidney failure [24], therefore high phosphate diet contributed in the induction of renal dysfunction in rats. So in the present research, rats were fed high adenine-high phosphate diet for 10 days followed by feeding high adenine diet for 11 days for induction of chronic renal failure in rats. Results emphasized the induction of chronic renal failure through the changes noticed in the different biochemical parameters.

In the present study, APD produced significant reduction in body weight and food efficiency which might be due to reduction in food intake as shown from the present results. Reduction in body weight may also be ascribed to loss of muscle and visceral protein stores as reported previously [25]. Loss of muscle protein may be related to stimulation of protein degradation and/or reduction of protein synthesis reported in kidney disease [26]. Loss of renal function induced a condition of protein energy wasting as demonstrated previously [25]. Gastrointestinal function and protein metabolism could also be affected by uremia which may share in the resulted poor nutritional status [26].

APD rats showed significant reduction in plasma level of albumin. This reduction in albumin level may be due to loss of albumin in urine due to kidney dysfunction and/or loss of muscle protein. Hypoalbuminemia is a common feature in protein energy wasting and has a strong association with increased mortality [27] and morbidity [28]. Albumin level is a negative acute phase reactant and its serum level is profoundly affected by the presence of an inflammatory response [29] that could be emphasized by the increased TNF- \( \alpha \) in APD group in the present study. Significant increase in the inflammatory cytokines (IL-1, IL-6, TNF-\( \alpha \)) have been reported in dialyzed patients suggesting that a systemic inflammatory response is common in dialysis-treated patients [30], [31]. Inflammation has been accused for serologic and anthropometric evidence of malnutrition [30].

Malondialdehyde as indicator of lipid peroxidation increased while total antioxidant capacity reduced, in APD rats which accounted for increased oxidative stress. Increased levels of malondialdehyde and \( F_2 \)-isoprostanes, two products of lipid peroxidation, were reported to be associated with renal damage [3]. In kidney diseases, oxidative stress results from an imbalance between oxidant generation and anti-oxidant defense mechanisms, leads to cell and tissue injury. Nguyen-Khoa et al. [32] demonstrated a direct correlation between lipid and protein oxidation markers in patient with renal dysfunction.

In the present study, LDL-Ch increased significantly in APD group which together with its oxidized form that may result from elevated oxidative stress might be involved in the glomerular damage of endothelial and mesangial cells. Oxidation of LDL is induced by infiltrating leukocytes. Native LDL can stimulate fibroblast secretion by mesangial cells while oxidized LDL may stimulate the genetic expression of fibroblast [33]. In a previous study, nutraceuticals prepared from red grape and walnut showed to be efficient in prevention of cisplatin induced kidney dysfunction [8]. These nutraceuticals were incorporated in the present study into functional foods (made from barley or rich in rice bran) to test their efficiency in improving chronic renal failure when acting synergistically with the active constituents of barley and rice bran.

Rats fed on balanced diet containing rice bran bread, barley bread, functional food I and functional food II showed significant improvement in the majority of biochemical parameters compared to APD and APD fed balance diet. Also these treatments showed improvement in all the nutritional parameters compared to APD group.

The activity of the prepared functional foods in the reduction of kidney failure may be attributed to the presence of biologically active constituents. In a previous work, walnut and grape nutraceuticals were shown to contain phenolic compounds and unsaturated fatty acids which were of extremely higher level in walnut than in grape while phytosterol content in grape nutraceutical was higher [8].

Red grape was reported to have antioxidant and anti-inflammatory effect [34] due to presence of resveratrol (polyphenolic compound), beta-carotene, tocopherols and dietary fiber [35], [36]. Walnut is a good source of essential fatty acids (linoleic acid), tocopherols, tocotrienols, phytosterols, tannins and other polyphenols [37]. Most phenolic compounds commonly identified in walnut seeds are phenolic acids, namely gallic, ellagic, syringic, 5-O-caffeoylquinic, caffeic, p-coumaric, ferulic and sinapic acids, and tannins, such as glansins A, B and C, casuariin and stenophyllarin [38], [39], therefore it is expected to possess antioxidant and anti-inflammatory effect [40]. So, it was proposed that both nutraceuticals could improve kidney dysfunction through alleviating oxidative stress and inflammation in addition of reducing LDL-Ch.

Rice bran contains an array of bio-active phytochemicals such as \( \gamma \)-oryzanol, phytosterols, tocopherols, tocotrienols, squalene, policosanol, phytic acid and ferulic acid [41], [42]. Also, rice bran is a good source of insoluble dietary fiber, protein and phytic acid [43] that possesses antioxidant, anti-inflammatory and LDL-Ch lowering effect [43]. On the other hand barley contains phytate, beta-glucan, tocopherols and tocotrienols [5] that may reflect its antioxidant and anti-inflammatory activity. This may explain the improvement in kidney dysfunction on feeding the bread made from barley and that rich in rice bran in the present study.

Reported diuretic activity of bioactive constituents that present in functional foods in the current study such as phytosterols and flavonoids [44], [45] may participate in the renal protective effect through increasing creatinine clearance. Reduction of plasma creatinine and increased creatinine clearance on feeding the different bread reflected the improvement of glomerular function and proximal tubules [46]. Acceptance of different bread from sensorial point of view may encourage the possible marketing of such bread for...
preventing renal dysfunction or as dietary intervention during chronic kidney diseases.

V. CONCLUSION

Both barley and rice bran bread with or without the studied nutraceuticals produced improvement in kidney function that might be ascribed to the presence of antioxidant and anti-inflammatory active constituents. It could be noticed that functional food made from barley and red grape nutraceutical was the most promising in improving kidney dysfunction.

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REFERENCES


