The Protective Effect of the Fortified Bread with Green Tea Against Chronic Renal Failure Induced by Excessive Dietary Arginine in Male Albino Rats

Ashraf A. Abd El-Megeid, Inas Z.A. AbdAllah, M.F. Elsadek and Yasmeen F. Abd El-Moneim

Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt
Department of Home Economics, Faculty of Specific Education, Fayoum University, Egypt

Abstract: Recently, considerable attention has been focused on dietary and medicinal phytochemicals that inhibit or retard diseases caused by oxidative processes. Green tea (GT) polyphenols have antioxidant properties. The present work was carried out to study the potential use of ground dried green tea leaves for fortification of pan bread and evaluate the sensory characteristics and the nutritive value of the fortified bread. The results showed that the best level of fortification which had the highest scores was recorded for 2% GT-fortified bread followed by 4% GT-fortified bread, while 6% GT-fortified bread had the lowest scores so, it was excluded from the biological study. The present work was also designed to investigate the protective effect of the fortified bread against renal failure induced by excessive dietary arginine (20g/kg diet). The possible beneficial role of 2% and 4% GT-fortified bread was evaluated by dietary supplementation in arginine diet to chronic renal failure (CRF) rats. The results revealed that, feeding CRF rats on arginine diet containing un-fortified bread resulted in significant increase in serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), uric acid, urea nitrogen and creatinine and significant reduction in serum high-density lipoprotein cholesterol (HDL-C). Dietary supplementation of arginine diet with GT-fortified bread resulted in sufficient amelioration against the effects of excessive dietary arginine. GT-fortified bread showed improvement in all tested biochemical parameters which was more pronounced in 4% GT-fortified bread feeding group than 2% GT-fortified bread feeding group. Based on the evidence available it appeared that GT-fortified bread was beneficial to renal function and eliminated oxidative stress by virtue of its antioxidant properties. Utilization of GT could be recommended for pan bread fortification.

Key words: Green tea • Rats • Arginine • Chronic renal failure • Lipid profiles • Kidney function and liver function

INTRODUCTION

Chronic renal failure (CRF) is a progressive and irreversible loss of large numbers of functioning nephrons caused by wide variety of disorders of the blood vessels, glomeruli, tubules and renal interstitium. CRF is characterized by structural and functional responses of remnant nephrons, which ultimately lead to glomerulosclerosis [1]. The diseased kidney showed reduction in kidney size and presence of broad casts in the urine sediment, reflecting the dilated hypertrophied remaining nephrons [2]. When the kidneys no longer have enough functioning nephrons to effectively rid the body of toxins, uremic poisoning results [3].

Arginine, a semi essential amino acid is used in the synthesis of body proteins and it is a material for transport, storage and excretion of nitrous products. In particular, arginine is essential for ammonia detoxification via urea synthesis. Physiological requirement of tissues and organs for arginine should be supplied by endogenous synthesis and the diet [4]. Kidney is the main site of endogenous L-arginine synthesis. However, administration of excess arginine causes imbalance of amino acids and changes in protein metabolism. In addition arginine is the key substance of nitric oxide and guanidine compounds such as creatinine and methylguanidine which are considered to be uremic toxins responsible for renal failure [5-7].
There has been a great deal of interest recently in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases [8]. Of the various herbal and the botanical agents, available interest has focused on the anti-inflammatory and antioxidant properties of polyphenols found in green tea (GT) [9, 10]. GT is produced from the dried leaves of the plant *Camellia sinensis* and contains several polyphenolic components. Most of these polyphenols are flavonols, which is usually called catechins such as (-) epigallocatechin gallate (EGCG), (-) epigallocatechin, (-) epicatechin gallate, (-) epicatechin and (-) gallocatechin gallate (GCG) [11]. EGCG is the most abundant catechin, accounting for about 65% of GT’s catechin content and is also the component with the highest antioxidant properties [12]. It was found that tea catechins were relatively stable in breadmaking, having 84% of the total tea catechins remained after baking as well as during its shelf life [13].

Many studies have found beneficial effects associated with the consumption of GT. Scientists have cited these beneficial effects in fighting obesity, liver, stomach, breast, prostate, lung and skin cancers [14], decreasing the risk of atherosclerosis and heart disease [15] and protecting against the development of neurodegenerative diseases as Parkinson’s disease and Alzheimer’s disease [16]. It has been reported that GT consumed within a balanced controlled diet improve the overall antioxidative status and protect against damage in humans [17].

Very little attention was focused on the addition of tea to bread which is consumed daily. Therefore, the present study was designed to investigate the potential use of ground dried GT leaves for fortification of pan bread and evaluate the sensory characteristics and the nutritive value of the fortified bread. The protective effect of the fortified bread with green tea against chronic renal failure induced by excessive dietary arginine in male albino rats was also assessed as a main target of the present study.

**MATERIALS AND METHODS**

**Material**

**Plant Material and Ingredients for Bread Preparation:** Samples of *Camellia sinensis* L. were obtained from the local market and ground to fine powder. Bread ingredients (wheat flour, sugar, salt and yeast) were purchased from the local market.

**Chemicals:** Arginine, casein, vitamins, minerals, cellulose and choline chloride were purchased from El-Nasr Pharm. and Chem. Ind. Comp. Cairo, Egypt. Corn oil and corn starch were obtained from local market. Kits used to determine serum biochemical parameters were purchased from Alkan Pharm. Ind. Comp. Cairo, Egypt.

**Experimental animals:** Forty male albino rats of Sprague Dawley Strain weighing 220 ± 10g. were obtained from Helwan farm.

**Methods**

**Preparation of Green Tea Fortified Bread:** Pan Bread was prepared using the method adapted by Penfield and Campbell [18].

**Normal Bread Was Prepared as Follows (g %):** All dried materials (flour 80 g., sugar 5g. and salt 5g.) were added together in a bowl and mixed well. The fermented yeast (10g.) was added to flour and the remaining water was added while mixing all the ingredients together to make a ball of dough. The dough kept in a warm place to rise, then kneaded again and kept for the second fermentation time in a warm place. Gently the dough was punched down; turning the edges toward the center, then the dough was shaped into a rectangle and put in a Tefal pan about 19/9/6 cm. The dough was allowed to rise in the pan and was baked at 250°C for 25 min. The bread was removed from the pan and cooled at room temperature before carefully getting sliced. Sensory characteristics of the products were evaluated.

**Supplementation of Bread:** In this study, bread ingredients were altered by partially replacing the wheat flour by 2, 4 and 6% of powdered dried GT leaves.

**Sensory Evaluation:** Sensory evaluation of all samples (fortified and un-fortified bread with green tea) were carried out according to Molander [19].

**Chemical Analysis of Bread:** Moisture, crude protein, crude fat, ash and crude fiber contents were determined in pan bread, according to A.O.A.C [20], while total carbohydrates content were calculated by difference.

**Induction of Chronic Renal Failure:** CRF was induced by feeding rats on basal diet containing 2% (w/w) arginine according to Yokozawa [21].
Experimental Design: Forty male albino rats of Sprague Dawley Strain weighing 220 ± 10g were kept in stainless steel cages under hygienic conditions. They were acclimatized for one week on a basal diet with free access to food and water through the study in the animal house of Faculty of Home Economics, Helwan University. The basal diet in the preliminary experiment consists of 20% casein (protein > 80% ), corn oil 4%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.2% and the remainder is corn starch [22].

After a period of adaptation on basal diet, the rats were divided into two main groups as follows:

The First Main Group: Ten rats fed on basal diet (negative control).

The Second Main Group: Thirty rats fed on basal diet containing 2% (w/w) arginine (2% arginine diet) (20g/kg diet) for two weeks to induce CRF according to the method described by Yokozawa [21]. After this period, rats in both groups were fasted overnight. Blood samples were collected from the eye in each rat to make sure the induction of CRF in arginine group, the mean values of serum uric acid, urea nitrogen and creatinine were (1.623 ± 0.032, 20.311 ± 1.416 and 0.516 ± 0.023 mg/dl) and (2.664 ± 0.032, 35.779 ± 2.115 and 1.205 ± 0.016 mg/dl) in the negative group and arginine group, respectively. Then, the second main group divided into three subgroups as follows:

The First Subgroup: ten rats fed on arginine diet containing half amount of protein from un-fortified bread (as a positive control group).

The Second Subgroup: ten rats fed on arginine diet containing half amount of protein from ground dried bread fortified with 2% GT.

The Third Subgroup: ten rats fed on arginine diet containing half amount of protein from ground dried bread fortified with 4% GT.

During the experimental period (28 days), the diets consumed and body weights were recorded twice weekly. Biological evaluation for different groups was carried out by determination of food intake, body weight gain% and kidney weight/body weight%. At the end of the experimental period the rats were fasted overnight and sacrificed from the abdominal aorta under ether anesthesia. Blood samples were collected in dry clean centrifuge tubes, then centrifuged to separate the serum which kept frozen till analysis. Kidneys were removed, cleaned in saline solution and weighed to calculate the relative organ weight.

Serum samples were used for determination of total cholesterol (TC) [23], triglycerides (TG) [24], high-density lipoprotein cholesterol (HDL-C) [25]. While serum low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated according to the equation of Friedwald [26]. Serum samples were also used for determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities [27], Uric acid [28], urea [29] and creatinine [30].

Statistical Analysis: Statistical analysis was carried out using SPSS statistical software version 11. The results were expressed as mean ± SD. Data were analyzed by one way analysis of variance (ANOVA). The differences between means were tested for significance using least significant difference (LSD) test at p< 0.05.

RESULTS AND DISCUSSION

Sensory Evaluation of Bread: Sensory evaluation of un-fortified and fortified bread with different percentages of GT was performed for appearance, taste, odor, color, volume, texture and general acceptability (GA). Samples of bread which have the best sensory characteristics were used in the biological experiment. Table 1 expressed the results of all sensory characteristics, the control sample (bread without fortification) recorded the highest scores in comparison with the other samples (2%, 4% and 6% GT-fortified bread). Appearance and color scores of 2% GT-fortified bread decreased significantly (P<0.05), as compared to the control. Meanwhile, there were no significant differences in taste, odor, volume, texture and GA between control and 2% GT-fortified bread. Wang et al. [31] found that with an increase in the level of GT extract, the brightness of the bread decreased. The difference in brightness could be detected with GT extract at 1.5g/kg flour. Based on their previous study [13], the value of the GT extract bread fell into the range of market products, such as whole meal bread and fiber enriched bread. Therefore, no consumer acceptability problem due to color was observed for bread containing GT extract up to the level of 5g/kg flour. On the other hand, there was
Table 1: Sensory Evaluation Of Un-Fortified And Fortified Bread With Different Percentages Of Powdered Green Tea (GT)

<table>
<thead>
<tr>
<th>Characters</th>
<th>Appearance (20)</th>
<th>Taste (15)</th>
<th>Odor (10)</th>
<th>Color (15)</th>
<th>Volume (15)</th>
<th>Texture (10)</th>
<th>GA (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-fortified bread (control).</td>
<td>17.72 ±1.127</td>
<td>12.78 ±1.215</td>
<td>8.78 ±1.06</td>
<td>13.78 ±1.060</td>
<td>13.33 ±1.084</td>
<td>8.22 ±1.215</td>
<td>13.00 ±1.715</td>
</tr>
<tr>
<td>2% GT-fortified bread.</td>
<td>16.00 ±1.495</td>
<td>11.94 ±1.514</td>
<td>8.00 ±0.840</td>
<td>12.22 ±1.437</td>
<td>12.89 ±1.491</td>
<td>7.56 ±1.293</td>
<td>12.17 ±2.202</td>
</tr>
<tr>
<td>4% GT-fortified bread.</td>
<td>14.44 ±2.202</td>
<td>10.72 ±1.708</td>
<td>7.94 ±1.304</td>
<td>11.33 ±1.645</td>
<td>12.72 ±1.487</td>
<td>7.11 ±1.022</td>
<td>12.11 ±1.936</td>
</tr>
</tbody>
</table>

GA: General acceptability Values are expressed as mean±SD. Number of panelists = 20.

Values in each column which have different letters are significantly different at (p< 0.05).

Table 2: Chemical Composition Of Bread

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture g/100g.</th>
<th>Crude Protein g/100g.</th>
<th>Crude Fat g/100g.</th>
<th>Ash g/100g.</th>
<th>Crude Fiber g/100g.</th>
<th>Carbohydrates g/100g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>29.615</td>
<td>8.624</td>
<td>1.613</td>
<td>0.712</td>
<td>0.522</td>
<td>58.914</td>
</tr>
</tbody>
</table>

Each value represents the average of three determinations.

Table 3: Effect of fortified bread with green tea on food intake, body weight gain% and kidney weight/body weight % in rats suffering from chronic renal failure (CRF)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Food intake (g/day/rat)</th>
<th>Body weight gain %</th>
<th>Kidney weight/body weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(-) Control</td>
<td>14.729</td>
<td>26.03±5±7.170</td>
<td>0.67±0.04</td>
</tr>
<tr>
<td></td>
<td>(+) Control</td>
<td>15.213</td>
<td>29.30±7.697</td>
<td>0.78±0.027</td>
</tr>
<tr>
<td></td>
<td>2% GT fortified bread.</td>
<td>15.621</td>
<td>21.22±4.869</td>
<td>0.67±0.047</td>
</tr>
<tr>
<td></td>
<td>4%GT fortified bread.</td>
<td>14.423</td>
<td>19.12±2.747</td>
<td>0.66±0.077</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at (p< 0.05).

a significant decrease in the mean scores of all sensory characteristics for the 4% GT-fortified bread, except the volume and GA, as compared with the control group. While all sensory characteristics for the 6% GT-fortified bread recorded significant decrease (P<0.05), as compared to the control sample. Wang et al. [31] stated that GT extract at only the level of 5g/kg flour significantly reduced the sweetness and increased the astringency, hardness and stickiness of the bread with GT extract compared with the control.

The best level of fortification which had the highest scores was the 2% GT-fortified bread followed by the 4% GT-fortified bread. The 6% GT-fortified bread recorded the lowest scores, so it was excluded from the biological study.

Chemical Composition of Bread: In the present study bread was analyzed for its content and illustrated in Table 2. The content of moisture, Crude protein, Crude fat, ash, Crude fiber and carbohydrates were 29.615, 8.624, 1.613, 0.712, 0.522 and 58.914 g., respectively.

Biological Evaluation of Fortified Bread: Effect of fortified bread with green tea on food intake, body weight gain% and kidney weight/body weight % in rats suffering from chronic renal failure (CRF).

Table 3 represented effect of GT fortified bread feeding on food intake, body weight gain% and kidney weight/body weight % in CRF rats. The mean value of food intake (g/day/rat) for all groups was slightly increased, except for the 4% GT-fortified bread feeding group, as compared to the negative control group. On the other hand, the body weight gain% showed non-significant changes between all groups except group of rats fed on arginine diet containing 4% GT-fortified bread which showed a significant decrease.

This finding was in agreement with the result of Dhanakoti et al. [32] who reported that adult rats fed a
diet devoid of arginine did not gain body weight and the food intake of these animals was less than that of rats fed 2% arginine diet. Feeding CRF rats on arginine diet containing 2% GT- fortified bread tended to decrease body weight gain% but not significantly compared to both the negative and positive control groups. Meanwhile, 4% GT- fortified bread feeding group revealed significant decrease in body weight gain%, as compared to both the negative and positive control groups.

In the present study, GT fortified bread feeding induced progressive decline in weight gain values. This reduction was more pronounced in 4% GT- fortified bread feeding group than 2% GT- fortified bread feeding group, where weight reduction recorded significant difference between them. Thus agreeing with Lin and Lin- Shiau [33] who reported that the body weights of rats have been significantly reduced by feeding GT leaves to the animals. Moreover, Wolfram et al. [34] found that feeding mice on diets supplemented with GT at levels from 1% to 4% had significantly decreased food intake, body weight gain and fat mass. Serum leptin levels were also lower and that decreases appetite. Sayama et al. [35] reported that addition of 2% GT powder to the diet suppressed fat accumulation and body weight by reduction of food intake. The reduced food intake in 4% GT- fortified bread group may influence the reduced body weight gain.

Long term consumption of GT may decrease the incidence of obesity and perhaps, GT components such as EGCG may be useful for treating obesity. GT catechins influence metabolism in several ways. Klaus et al. [36] reported that dietary supplementation with EGCG purified from GT for 1 month attenuated diet- induced obesity and decreased body weight of mice by decreasing energy absorption and increasing fat oxidation. Supplementation with GT catechins resulted in a significant reduction of high- fat diet induced body weight gain, visceral and liver fat accumulation and the development of hyperlipidemia in mice [37]. GT catechins are known to activate many cellular mechanisms for reducing body weight. Studies in adipocyte cell lines and animal models have demonstrated that EGCG inhibits extracellular signal related kinases, activates AMP-activated protein kinases, modulates adipocyte marker proteins and down- regulates lipogenic enzymes [38].

Concerning kidney weight/body weight%, it could be observed that CRF (positive control) produced significant increase P<0.05 in kidney weight/body weight% as a result of feeding on arginine supplemented standard casein diet, as compared to the negative control group. There were significant decrease P<0.05 in kidney weight/body weight % between both 2% and 4% GT- fortified bread feeding groups, as compared to the positive control group. On the other hand non significant differences were observed between both 2% and 4% GT- fortified bread feeding groups as compared to the negative control group. Kao et al. [39] found that in male Sprague Dawley and lean Zucker rats treated with EGCG for 7-8 days, the weight of the liver were reduced by about 10-20%. He concluded also that green tea administration led to decrease in body weight; he mentioned that this effect may be due to the thermogenesis, inhibition of lipogenesis in adipose tissue, decrease food intake, decrease blood glucose, triglycerides and insulin dependant growth factor.

**Effect of Fortified Bread with Green Tea on Lipid Profile in Rats Suffering from Chronic Renal Failure (CRF):**

The effect of GT fortified bread feeding on serum lipids profile in CRF rats is presented in Tables 4&5. The results indicated significant elevation P<0.05 in both total cholesterol (TC) and triglyceride (TG) in CRF group (positive control), as compared to negative control group. Feeding CRF rats on arginine diet containing GT fortified bread at levels of 2% and 4% induced significant reduction (P<0.05) in both TC and TG with increasing the level of fortification in the consumed diet. It was clearly observed that 4% GT fortified bread feeding group had the lowest values of TC and TG, compared with 2% GT- fortified bread feeding group and positive control group.

Results in Table 5 revealed significant decrease P<0.05 in high density lipoprotein-cholesterol (HDL-C) and significant increase P<0.05 in low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) in CRF group (positive control), as compared to negative control group. Feeding CRF rat groups on arginine diet containing GT fortified bread at levels of 2% and 4% induced significant improvement in all lipoprotein parameters. There were significant increase P<0.05 in HDL-C and significant decrease P<0.05 in LDL-C and VLDL-C (P<0.05) in both GT fortified bread feeding groups, as compared to the positive control group. The values of HDL-C, LDL-C and VLDL-C tended to match the control values in rat group fed on basal diet containing GT fortified bread at level of 4%. This means that when the level of bread fortification increases, the rate of improvement increases.

In the present study, GT fortified bread feeding imposed significant beneficial changes in the lipid profile of rat groups. The same observation were reported by Lin et al. [40], Yokozawa et al. [41], Murakami and
Table 4: Effect of fortified bread with green tea on total cholesterol and triglycerides in rats suffering from chronic renal failure (CRF)

<table>
<thead>
<tr>
<th>Parameters mg/dl</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total cholesterol</td>
</tr>
<tr>
<td></td>
<td>(-) Control</td>
</tr>
<tr>
<td></td>
<td>(+) Control</td>
</tr>
<tr>
<td></td>
<td>2% GT fortified bread</td>
</tr>
<tr>
<td></td>
<td>4% GT fortified bread</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at (p< 0.05).

Table 5: Effect of fortified bread with green tea on lipoprotein fraction in rats suffering from chronic renal failure (CRF)

<table>
<thead>
<tr>
<th>Parameters Mg/dl</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HDL-C</td>
</tr>
<tr>
<td></td>
<td>(-) Control</td>
</tr>
<tr>
<td></td>
<td>(+) Control</td>
</tr>
<tr>
<td></td>
<td>2% GT fortified bread</td>
</tr>
<tr>
<td></td>
<td>4% GT fortified bread</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at (p< 0.05).

Table 6: Effect of green tea fortified bread feeding on liver enzymes activities in rats suffering from chronic renal failure(CRF)

<table>
<thead>
<tr>
<th>Parameters U/l</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST</td>
</tr>
<tr>
<td></td>
<td>(-) Control</td>
</tr>
<tr>
<td></td>
<td>(+) Control</td>
</tr>
<tr>
<td></td>
<td>2%green tea fortified bread</td>
</tr>
<tr>
<td></td>
<td>4% green tea fortified bread</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

AST: Aspartate Amino Transferase ALT: Alanine Amino Transferase

Values in each column which have different letters are significantly different at (p< 0.05).

Ohsato [42] and Lin and Lin-Shiau [36] who stated that GT leaves in diet was associated with lower serum levels of TC, LDL-C and TG but higher serum levels of HDL-C. This effect is attributed to a reduction in cholesterol absorption and to an increased excretion of biliary acids and cholesterol; another proposed action is the inhibition of cholesterol synthesis in the liver [43, 44]. GT is particularly rich in EGCG, a powerful antioxidant [45]. Some in vitro studies showed indicate the EGCG exerts a protective effect against lipoprotein oxidation, namely, against LDL oxidation [46]. GT catechins affects lipid metabolism by different pathways and prevent the appearance of atherosclerotic plaque. Its intake decreases the absorption of TG and TC and these findings are in accordance with the fact that it increases the excretion of fat [47]. GCG-rich tea catechins have strong effects on lowering TC and TG concentrations in hyperlipidemic rats [48].

Effect of Fortified Bread with Green Tea on Liver Enzymes in Rats Suffering from Chronic Renal Failure (CRF): Data represented in Table 6 showed effect of GT fortified bread feeding on liver enzymes activities in CRF rats. There was significant increase in tested liver enzyme (AST & ALT activities) in CRF rats (positive control group), compared to negative control group. Feeding CRF rats on arginine diet containing GT fortified bread at levels of 2% and 4% resulted in sufficient amelioration in both AST and ALT activities, recording significant reduction when compared with positive control.
group. The activities of ALT and AST tended to match the negative control values in rat group fed on arginine diet containing GT fortified bread at level of 4%.

An excessive dietary arginine induced hepatotoxicity as represented by markedly elevated levels of AST and ALT. Varga et al. [49] and Czako et al. [50] found that excessive administration of arginine induced pancreatitis in rats and enhanced hypoxia/reoxygenation injury. Feeding CRF rats on arginine diet containing GT fortified bread at level of 2% and 4% resulted in sufficient amelioration in both AST and ALT. GT is an important source of flavonoids, namely catechins, which are strong antioxidants. The same results were obtained by Sugiyama et al. [51] who reported that feeding rats on diet supplemented with the powder of GT extract showed a significant decrease in serum AST and ALT compared with control. Studies by Arteel et al. [52] also noted that there was a significant lowering in the activities of AST and ALT in rats treated with GT extract in the diet.

Reactive oxygen species and other free radicals cause hepatocellular damage, the treatment with phenolic compounds was found to be hepatoprotective agents, because it acts as free radicals scavenging [53]. El-Beshbishy et al. [54] observed that the antioxidant property of flavonoidal compounds of GT extract contributes to decrease the oxidative stress in liver and increase the levels of antioxidant enzymes, superoxide dismutase, catalase and glutathione. Abe et al. [55] suggested that the drinking of green tea with a high catechin content may help to prevent and/or attenuate the development of a certain type of hepatitis. Almurshed et al. [56] suggested also that both black and green tea possess preventive effects against carbon tetrachloride CCl4 induced liver damage in rats.

### Effect of Fortified Bread with Green Tea on Kidney Functions in Rats Suffering from Chronic Renal Failure (CRF):

Table 7 illustrated the effect of GT fortified bread feeding at levels of 2% and 4% on kidney function of CRF rats. Serum uric acid, urea nitrogen and creatinine exhibited significant elevation in CRF rats (positive control group), in comparison with the negative control group. In this respect Penny and Debbie [57] reported that, in Patients with CRF, blood urea nitrogen (BUN) and creatinine levels are the most significant blood test results. BUN is a protein metabolite and although lab values differ, the approximate range is between 14-36 mg/dl (5-12.9 nmol/L) for healthy kidneys. BUN is related more to diet and if dehydration is present, will show increased values. Creatinine is thought to be a more reliable indicator of kidney function as it shows how well the kidneys are filtering out the toxins and is less dependent on dietary factors and hydration status. Ma et al. [58] reported that, the levels of serum BUN, creatinine were markedly elevated, while the levels of albumin markedly failed in Sprague-Dawley rats received diet containing 0.5% adenine to induce CRF. Yokosawa et al. [21] also demonstrated that the serum levels of creatinine and urea nitrogen were increased by the 2% arginine diet. These results provide evidence that arginine administered to rats is metabolized to guanidino compounds, which accumulate in the serum leading to renal impairment. In addition, Shin et al. [59] showed that the administration of excess L-arginine inhibited the proliferation of human mesangial cells and increased the concentration of nitric oxide. Noris et al. [60] reported that arginine levels and nitric oxide synthesis were higher in uremic patients than in healthy volunteers, suggesting an explanation for the increased nitric oxide synthesis in uremia. It raises the possibility that the increase in nitric oxide production may be attributable to dietary arginine and that may cause renal injury.

Feeding chronic renal failure rats on arginine diet containing GT fortified bread at the two levels 2% and 4% recorded significant noticeable improvement in these parameters when compared them with the positive control group.

### Table 7: Effect of fortified bread with green tea on kidney functions in rats suffering FROM CHRONIC renal failure (CRF)

<table>
<thead>
<tr>
<th>Parameters mg/dl</th>
<th>Uric acid</th>
<th>Urea nitrogen</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-) Control</td>
<td>1.782±0.081</td>
<td>20.443±2.005</td>
<td>0.532±0.061</td>
</tr>
<tr>
<td>(+) Control</td>
<td>3.229±0.175</td>
<td>42.414±4.115</td>
<td>1.403±0.164</td>
</tr>
<tr>
<td>2% green tea fortified bread</td>
<td>2.770±0.192</td>
<td>38.190±3.162</td>
<td>1.182±0.164</td>
</tr>
<tr>
<td>4% green tea fortified bread</td>
<td>2.353±0.116</td>
<td>33.780±2.797</td>
<td>0.923±0.099</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD.

Values in each column which have different letters are significantly different at (p< 0.05)
There was significant decrease p< 0.05 in serum uric acid, urea nitrogen and creatinine in groups received diets containing GT fortified bread at the two levels when compared with (+) control group. The same results were obtained by Yokozawa et al. [21] who reported that GT polyphenol is effective against renal failure in rats. GT polyphenol administered to rats at a daily dose of 50 or 100 mg/kg body weight for 30 days with a 2% arginine diet decreased serum levels of creatinine and urea nitrogen. The protective effect can be attributed to a decrease in uremic toxins and nitric oxide production and an increase in the renal activities of the oxygen species-scavenging enzymes, superoxide dismutase and catalase. The antioxidant properties of GT likely play a significant role in protecting the kidneys. Since GT has been shown to lower the concentrations of free radicals and lipid peroxides in organs such as the liver [61] and the pancreas [62, 63], this is likely to be true in the kidneys as well. EGCG was shown to have antioxidant effect on creatinine oxidation in rats with CRF and thus inhibited methylguanidine production in an adenine induced renal failure model [64].

Takako et al. [65] suggested that green tea polyphenol could ameliorate renal failure induced by excessive dietary arginine by decreasing uremic toxins and nitric oxide production and increasing radical-scavenging enzyme activity. Leena and Balaraman[66] found that orally administration of green tea extract to rats at the dose levels of 25, 50,100 mg/kg to investigate its effect on cisplatin (3mg/kg) induced nephrotoxicity, restored the level of creatinine, urea, BUN and uric acid in serum of animals treated with cisplatin as compared to the animals treated with cisplatin alone. Shin et al. [59] reported that green tea extract might play a crucial role of nitric oxide inhibition as free radical scavenging effect and ameliorate anti-proliferative effect of L-arginine in mesangial cells suggesting that GT extract and probably its polyphenol, can protect renal cells against oxidative injury.

In conclusion, Kidney function appears to be improved with GT fortified bread consumption which also improve liver function and lipid parameters. If these data are confirmed in humans, GT may ultimately prove to be a useful dietary supplement in patients with CRF to attenuate the symptoms of chronic renal diseases and protect against its related complications. Further studies are needed to demonstrate the antioxidant effect of GT on renal diseases.

REFERENCES


