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Potential Effects of Toast Bread Supplemented with Lemon Grass Powder on Kidney and Liver Functions in Rats

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Abstract

Lemongrass (LGP) is used in folk medicine, and its decoction and infusion are taken as a diuretic. This study aimed to investigate the effects of toast bread supplemented with lemongrass powder on kidney and liver functions in rats. The toast bread recipes were prepared from wheat flour, 5 and 10 % replacement levels of wheat flour (WF) by LGP. The physicochemical and organoleptic properties of the toasted bread were estimated. Thirty-two Male Sprague Dawley rats were used in the study and randomly divided into four groups. Each group consists of eight rats; the 1st group is control group (group A) and fed on a basal diet; 2nd group is GM (group B) fed on basal diet and injected with gentamicin (GM) in a dose of 100 mg/kg b.wt (positive control); the 3rd group injected with GM and fed on a diet supplemented with 20 % WF toast bread containing 10% LGP (group C); the 4th group injected with GM and fed on a diet supplemented with 20 % WF toast bread containing 5% LGP (group D). The results showed that incorporating toast bread fortified with Lemongrass (5% and 10%) induced significant ($P<0.05$) decreases in serum urea nitrogen, creatinine, ALT, AST, ALP. It also showed hypolipidemic properties and reduced serum cholesterol and triglycerides. These results prove that using toast bread containing Lemongrass might be beneficial for improving vital biomarkers in patient with kidney diseases.

Keywords: physicochemical, sensory properties, gentamycin, kidney diseases, lipid peroxidation

Introduction

Chronic kidney disease (CKD) has been defined as decreased kidney function and kidney damage persistent for at least three months. A glomerular filtration rate (GFR) of kidney dysfunction is indicated by less than 60 mL/min/1.73 m2. In contrast, kidney damage most frequently is displayed as increased urinary albumin excretion [1,2].

In CKD, the kidneys are damaged and/or cannot filter blood normally. CKD increases the risk for many unfavorable health outcomes, including cardiovascular disease, end-stage renal disease (ESRD), and mortality. However, CKD is usually asymptomatic until its most progressive state [3].

National Kidney Foundation [2] recommends plant-based diets, such as the Dietary Approaches to Stop Hypertension (DASH) diet for CKD patients. Dash diet is characterized by its high fiber content, low in saturated fat and processed meats, rich in potassium, phosphorus, magnesium, and calcium, and low in salt. The health benefits of such diets include preventing heart disease and hypertension and delaying the progression of kidney disease. The Mediterranean diet is similar to the DASH diet. It is also associated with a lower risk of mortality from cardiovascular disease [4].

Higher dietary fiber intake was associated with better renal function and lower inflammation. Higher fiber intake was also associated with better survival, especially in individuals with manifest CKD [5].

Lemongrass (Cymbopogon citratus) is a widely used herb in tropical countries, especially in Southeast Asia [6]. The compounds that occur in Lemongrass are mainly alcohols, terpenes aldehyde, ketones, esters, flavonoids and phenolic compounds (i.e. isoorientin, quercetin, luteolin, kaempferol and apigenin). Lemongrass has various pharmacological properties such as antiamoebic, antibacterial, antidiarrheal, antifungal, antioxidants, hypoglycemic and anti-inflammatory [7,8]. Decoction and infusion of Lemongrass are taken as a diuretic to diminish intestine spasm and act against food poisoning, anti-rheumatism, anti-anorexia and digestive disorders [9]. A recent study demonstrated that lemongrass extract and its essential oil have antioxidant and acetylcholinesterase inhibitory properties [9,10].

The popularity of bakery products has contributed to increased demand for ready-to-eat, convenience food products, such as bread, biscuits, snacks and other products [11].

The present study investigated the impact of incorporating lemongrass powder in toast bread, replacing different proportions of wheat flour on its functional properties. Furthermore, the toasted bread fortified by lemongrass powder was tested for its potential biological action and possible use as a functional food for treating nephrotoxicity, artificially induced in rats.

Materials and methods

Materials:

Wheat variety (Triticum sativum) cultivar and wheat grain was milled (Cycrotex sample mill) for 72% extraction using Buhler laboratory pneumatic flour mill. Oil (sunflower oil), salt, sugar and yeast were obtained from the local Zagazig city. , Egypt. Lemongrass powder (Cymbopogon citratus) was purchased from the Egyptian herbal Markets, Dokki,

Giza. The Lemongrass used was defined by specialized professors at the Faculty of Agriculture, Zagazig University. All Kits of Biochemical analyses: AST, ALP, bilirubin, albumin, cholesterol, triglyceride, HDL, LDL, urea, creatinine and urea were purchased from Biomeieux, Laboratory of Reagents and Products (France).

Methods

Preparation of toast bread

Flour blends were baked using the straight–dough method; according to Chauhan et al. [(34)], wheat flour was substituted by two levels of lemongrass powder (5 and 10 % LGP). The baking formula was composed of a 500 g flour blend, 9 g of compressed baker's yeast, 5 g of NaCl, 13 g of cane sugar, 10 g of vegetable oil. The dough was fermented for 90 min at 28±1°C, then punched, scaled to 250 g dough pieces, proofed for 90 min at 30°C, 85% relative humidity and baked at 250°C, for 30 min. Baked toast bread was ground, screened through a 0.25 mm sieve and used for chemical analyses.

Chemical analysis

Total solids, fat, total protein (TN), Carbohydrate, crude fiber, ash, calcium, phosphorus, manganese, potassium and sodium contents were determined according to AOAC [(12)].

Sensory Properties

Toast bread was evaluated for its sensory characteristics by ten trained panelists for appearance 10, Crust color 10, Crumb color 10, flavor 10, texture 10, and overall acceptance 50 on a 10-point hedonic scale [(13)].

Experimental conditions

Male Wistar Albino rats (aged 7-8 weeks) weighing 150-200 g was purchased from the Animal House Colony, National Research Center, Giza, Egypt. All animals were maintained on a standard diet only and housed in a room free from any source of chemical contamination.

Preparation of the basal diet:

The basal diet for rats was prepared using AIN-93 according to Reeves et al. [(14)]. the basal diet consists of the following: Protein (Casein) 20%; Sucrose 10%;Corn Oil 4%; Choline Chloride 0.2%; Vitamin mixture 1%; Salt mixture 3.5%; Fibers (Cellulose) 5% and the remainder is Corn Starch up to 100%.

Experimental design

The animal groups were fed on a standard diet and placed in an atmosphere of filtered, pathogen-free air, water and maintained at a temperature between 20-25ºC for 8weeks, 50% relative humidity and 12 h light and 12 h dark cycle. The rats were adapted for one week as an adaptation period, then were randomly divided into two groups. Thirty-two rats were divided into randomly four groups, each of 8 animals. Group A was injected intraperitoneally with sterile normal saline (0.2 ml) and kept as a normal (negative) control. Group B was injected daily with GM in a dose of 100 mg/kg b.wt during the last

eight days of the experiment to induce nephrotoxicity and kept as a nephrotoxic (positive) control. Groups C and D were injected with GM and fed on basal diet 20 % wheat flour toast read (group C) and 20 % toast bread containing 5 % LGP (group D), respectively. All rats were fasted overnight and then sacrificed at the end of the experimental period. Blood samples were immediately collected from the retro-orbital plexus capillary tubes under mild ether anesthesia into clean dried centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes. Clear serum samples were carefully separated using Pasteur pipettes and frozen at - 20°C until biochemical analysis [(15)].

Biochemical analysis

At the end of the experimental period, animals were lightly anaesthetized with diethyl ether. Blood was collected from the hepatic portal vein. The blood samples were collected and centrifuged at 3000 rpm for 15 min to separate serum, stored at -40° C until biochemical analysis. The method of Caraway [(16)] was used to determine serum uric acid, while serum creatinine level was measured by the method of Bartels [(17)]. Serum urea was determined according to Marsch et al. [(18)]. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes were measured according to the methods described by Bergmeyer and Harder [(19)]. Total cholesterol was determined according to the method of Enzymatic Colorimeter given by R Deeg, and J Ziegenhorn [(20)]. Total lipids and triglycerides was be determined according to the method of Devi and Sharma [(21)]. The LDL was calculated using the Friedewald formula [(22)] as follows:

LDL-cholesterol= Total cholesterol - (HDL-cholesterol) - (Triglycerides/5)

Statistical analysis -:

The obtained results were evaluated statistically using analysis of variance as reported by McClave & Benson [(23)]. In addition, the other reported values were expressed as mean ±SD and ±SE, and a two-tailed Student's t-test was used to compare different groups. The P-value less than 0.05 was considered statistically significant. SPSS (Chicago, IL, USA) software window Version 16 was used.

Results and Discussion

Table (1) summarizes the average moisture, protein, fat, crude fiber and ash contents of the LGP and toast bread treatments. The protein, fat, fiber, ash and carbohydrates levels of LGP were higher than WF. However, the moisture content of WF was higher than LGP. Data presented in Table (1) show the chemical composition of toast bread from WF and (90% WF: 5 % LGP) (90% WF: 10% LGP). LGP toast bread was characterized by higher moisture, fat, ash, and fiber content and lower protein content than other treatments. The increase in fat, fibers and ash of LGP supplemented toast bread can be attributed to the high content of those ingredients in LGP. Such findings were also

obtained by Anne et al. and Abou-Raya, et al. [24,25] who studied the chemical, rheological and sensory properties of wheat-oat flour composite toast bread.

Table (1): Chemical composition of wheat flour (WF), lemongrass powder (LGP) and toast bread

	Moisture %	Protein%	Fat %	Ash%	Fiber%	Carbohydrates%
WF	11.41±0.52 A	12.49±0.95 A	1.4±0.20 B	0.51±0.16 B	0.88±0.12 B	74.19±2.46 AB
LGP	9.00±0.48 B	6.24±0.66 B	3.98±0.65 A	5.20±0.82 A	4.30±0.95 A	75.58±3.16 A
			Toast bread treatments			
C	26.6±2.1A B	12.82±1.14 A	3.06±0.94 AB	1.20±0.87 AB	5.72±0.74 B	53.27±2.24 C
T1	27.6±3.4 A	11.9±1.3AB	3.51±1.05 AB	1.31±0.92 A	6.12±0.68 A	55.56±2.46 B
T2	26.2±2.6 AB	11.26±1.16 B	3.94±1.08 A	1.48±0.84 A	6.34±0.81 A	57.13±2.84 A

Each value represents the mean value ±SD. Means in the column with different letters were significant differences at p≤0.05. WF= Wheat flour (72% ext.) LG = Lemon grass, C: toast bread manufacture with wheat flour (72% ext.), T1: toast bread manufacture with wheat and LGP (95/5 w/w), T2: toast bread manufacture with wheat and LGP (90/10 w/w).

Table (2) shows the average calcium, phosphor and potassium contents of the toast bread treatments. Whereas wheat flour contained the lowest calcium, phosphor, and potassium than LGP. Ranade and Thiagarajan [8] found that lemongrass contains high calcium, phosphor, and potassium values.

Data presented in Table (2) show the minerals content of toast bread from WF and (95% BF: 5% LGP) and (90% WF: 10% LGP). LGP –WF toast bread (90:10) were characterized with higher phosphor, calcium and potassium content than other treatments. The increase in phosphor and potassium content of LGP supplemented toast bread can be attributed to the high content of those ingredients in LGP. Such findings were also obtained by Sangwan et al. [26].

Data presented in Table 3 show the sensory evaluation of toast bread as replacing WF with LGP. Toast bread containing 10% LGP had the lowest scores for all quality attributes (Appearance, flavor, texture, Crust color, Crumb color and overall acceptability); all treatments ranged from "Like slightly" to "Like moderately". Incorporation of LGP recorded the lowest scores for all quality attributes of substitution higher than that control treatment. Moreover, the color appeared to be a fundamental criterion for the consumer's initial acceptability of the baked product. The color of the toasted bread was significantly affected ($P<0.05$) by the addition of LGP. These results are in the same line with El-

Demery [27], .who evaluated of physicochemical properties of toast bread fortified with pumpkin flour.

Table (2) Minerals content of wheat flour, lemon grass powder and toast bread

Samples(mg/100g)	Calcium (Ca)	Phosphor (P)	Potassium (K)
WF	22.13±0.15 B	72.40±0.95 B	67.38±0.88 B
LGP	80.0±1.02 A	150.40±6.14 A	850.24±12.0 A
	Toast bread treatments		
C	16.48±0.2 C	58.34±1.12 C	52.45±0.56 C
T1	37.24±0.34 B	74.25±2.44 B	114.50±4.85 B
T3	50.14±0.75 A	92.14±4.2 A	140.32±5.16 A

Each value represents the mean value ±SD. Means in the column with different letters were significant differences at p≤0.05, WF= Wheat flour (72% ext.) LG = Lemon grass, C: toast bread manufacture with wheat flour (72% ext.), T1: toast bread manufacture with wheat and LGP (95/5 w/w), T2: toast bread manufacture with wheat and LGP (90/10 w/w)

Table (3): Sensory evaluation of toast bread at different levels of wheat flour substituted with lemongrass powder

Treatments	Appearance 10	Flavor 10	Crust color 10	Crumb color 10	Texture 10	overall acceptability 50
C	8.38±0.40 A	8.32±0.62 A	8.38±0.55 A	8.52±0.45 A	8.96±0.43 A	42.56±0.82A
T1	7.42±0.35 B	7.84±0.54 B	8.16±0.48 AB	8.20±0.52 AB	8.14±0.51 B	39.67±0.75B
T2	7.18±0.33 BC	7.12±0.58 C	7.38±0.52 B	7.94±0.48 B	8.46±0.49 C	38.26±0.68C

Each value represents the mean value ±SD. Means in the column with different letters were significant differences at p≤0.05, WF= Wheat flour (72% ext.) LG = Lemon grass, C: toast bread manufacture with wheat flour (72% ext.), T1: toast bread manufacture with wheat and LGP (95/5 w/w), T2: toast bread manufacture with wheat and LGP (90/10 w/w)

Effect of toast bread produced from WFF and LGP on kidney and liver functions of rats

The effect of toast bread produced from WF and LGP on serum urea nitrogen and creatinine in gentamicin-nephrotoxic rats are presented in table (4). The serum levels of urea nitrogen and creatinine of nephrotoxicity rats Group (B) positive control(C+ve) were increased in comparison with Group (A) control negative (C-ve). Administration of toast bread produced from WF and LGP in nephrotoxic rats resulted in decrement of serum levels of urea nitrogen and creatinine compared with Group (B) positive control(C+ve).

Group treated with toast bread fortified with lemongrass powder showed a significant decrease in creatinine and urea compared with the positive control group as a result of the high natural antioxidants content of lemongrass leaves, where those natural antioxidants can reduce uric acid in the blood through direct uricosuric potential or enhancing the glomerular filtration rate moreover, as a potent antioxidant can diminish the oxidative stress and inflammation in the body cells, thereby reducing the synthesis and ultimately blood level of uric acid [28]

Mohamed et al. [9,28] mentioned that the rats treated with lemongrass powder plus gentamicin showed significant changes in serum urea nitrogen and creatinine compared with the gentamicin group alone. The histopathological examination confirmed that the blood biochemical analyses and treatment with CS extract improved the liver and kidney tissues. Celery is rich in B-Complex vitamins, adding stress-reducing and sedative qualities. It is rich in vitamins A and C and is indicated in arthritis and kidney problems.

The same table (4) showed that the nephrotoxicity rats Group (B) positive control(C+ve) were increased in ALT, AST and ALP when compared with Group (A) control negative (C-ve). Oral intake of toast bread produced from WF and LGP in nephrotoxic rats induced a decrease in ALT, AST, and ALP compared with Group (B) positive control(C+ve). This decrease in the values of aminotransferase enzymes and the restoration of some vital functions by the hepatocytes can be attributed to the high content of lemongrass leaves from phenolic and bioactive components, which work to preserve the plasma membrane in hepatocytes and protect it from rupture and the exit of the cytosol loaded with these enzymes [10,29,30]. Such findings were also obtained by Rahim et al. [31], who found that lemongrass attenuated liver damage due to H2O2 administration as indicated by the significant reduction ($p<0.05$), in the elevated levels of ALT, AST, ALP, LDH, TB, and MDA in serum and liver homogenates; increase in TP and GSH levels in serum and liver homogenates; and improvement of liver histopathological changes. These effects of the extract were similar to that of vitamin C, which is used as an antioxidant reference.

The effect of toast bread produced from WF and LGP on total cholesterol and triglyceride in gentamicin-injected rats are presented in Table (5). The results revealed that the total cholesterol was significantly increased in nephrotoxic rats Group (B) positive control(C+ve) compared to Group (A) control negative (C-ve). Oral intake of toast bread produced from WF and LGP in nephrotoxic rats were significantly ($P<0.05$) reduced total serum cholesterol, triglycerides, LDL but increased HDL when compared to Group (B) positive control (C+ve).

Table (4). Effect of toast bread produced from WF and LGP on kidney and liver functions of rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Albumin (mg/dL)	Urea (mg dL)	Creatinine (mg/dL)
Group A	30.20±0.75 C	30.60±1.05 C	115.70±7.12 C	4.66±0.22 AB	42.28±1.4 C	0.66±0.05 C
Group B	39.12±0.98 A	38.70±1.26 A	156.20±7.55 A	3.20±0.18 C	55.12±1.7 A	0.78±0.08 A
Group C	33.40±0.58 B	32.48±1.02 B	126.82±6.34 B	3.96±0.12 B	50.20±1.3 B	0.7±0.06 B
Group D	28.80±0.42 D	30.14±0.96 C	108.40±5.85 D	4.78±0.36 A	41.50±1.0 5D	0.60±0.03 D

Each value represents the mean value ±SD. Means in the column with different letters were significant differences at p≤0.05. Group A: Control negative rats fed on basil diet. Group B: Control positive rats fed on basil diet and treated with gentamicin (100 mg/kg/day.). Group C: Rats fed on basil diet treated with gentamicin and 20% wheat flour toast bread. Group D: Rats fed on basil diet treated with gentamicin and 20% lemon grass toast bread.

Positive control causes oxidative stress, which finally increases the production of reactive oxygen species. Increasing scientific literature provides ample direction. ROS can induce cellular damage via the oxidation of critical cellular components such as membrane lipids, proteins, and DNA. Since the result of the study indicated that lemongrass leaves extract beneficial effect on lipid profile, we have investigated its mechanism of action; this may be due to phenolic compounds as natural antioxidants present in lemongrass leaves [10,29,30]. The results obtained agree with Agbafor and Akubugwo [32], who found that ethanolic extract of fresh leaves of Cymbopogon citratus (lemongrass) had a Hypocholesterolemic effect, this may be due to a high content of lemongrass from dietary fiber and phenolic compounds, which inhibit low-density lipoprotein oxidation and promote scavenging of reactive oxygen species [33].

Table 5. Effect of toast bread produced from WF and LGP on lipid profile in rats

Groups	Cholesterol (mg dL)	Triglycerides (mg dL)	HDL (mg dL)	LDL (mg dL)
Group A	88.30±9.15 D	132.80±11.12 B	32.84±3.45 BC	29.24±0.56 C
Group B	97.52±9.48 A	148.30±11.44 A	26.70±2.66 C	40.60±0.74 A
Group C	94.10±8.52 B	134.42±9.65 B	34.50±3.57 B	32.20±0.65 B
Group D	90.40±8.24 C	128.72±9.23 C	38.12±4.14 A	26.32±0.45 D

Each value represents the mean value ±SD. Means in the column with different letters were significant differences at p≤0.05. Group A: Control negative rats fed on basil diet. Group B: Control positive rats fed on basil diet and treated with gentamicin (100 mg/kg/day.). Group C: Rats fed on basil diet treated with gentamicin and 20% wheat flour toast bread. Group D: Rats fed on basil diet treated with gentamicin and 20% lemon grass toast bread.

Conclusion

Lemongrass powder can be used in toast bread manufacture at a concentration of 10 %, which enhances toast bread's chemical composition. Lemongrass powder may be able to inhibit lipid peroxidation and improve kidney and liver functions.

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التأثيرات المحتملة لخبز القالب المدعم بمسحوق الحلفا بار على وظائف الكلى والكبد في الفئران

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تستخدم النباتات الطبية بهدف الحفاظ على الصحة، على أن تستخدم لظروف معينة، أو كليهما، سواء في الأدوية الحديثة أو التقليدية .ولقد تم تحديد العديد من المواد الكيميائية النباتية ذات النشاط البيولوجي الثابت أو المحتمل في النباتات .يستخدم عشب الحلفابار في الطب الشعبي حيث يتم يتناول مغلي العشب كمدر للبول ولتقليل تشنج الأمعاء وللعمل ضد التسمم الغذائي ومضادات الروماتيزم ومكافحة فقدان الشهية والجهاز الهضمي . لذا بحثت الدراسة الحالية في التأثيرات المحتملة لخبز القالب المدعم بمسحوق عشب الحلفابار على وظائف الكلى والكبد في الفئران. حيث تم صناعة خبز القالب من دقيق القمح فقط كعينة مقارنة والمعاملتين الاخرتين تم استبدال دقيق القمح بمسحوق الحلفا بار بنسبة 5 و10 % على التوالي. واوضحت النتائج ان خبز القالب المصنع باستبدال دقيق القمح ب 5 % مسحوق الحلفا بار اعطي اعلي معدلات للتحكيم الحسي مقارنة بالمعاملة الاخري (10%). تم اختبار الخبز المدعم بمسحوق الحلفا بار كعامل وقائي للكلى في الفئران ,حيث تم تقسيم الفئران المستخدمة في تجربة التغذية الي 4 مجموعات بمعدل 8 فئران لكل مجموعة وكانت المجموعات كالتالي :المجموعة (أ) تم تغذيتها علي الوجبة القياسية : المجموعة (ب) تم تغذيتها علي الوجبة الغذائية مع حقن الفئران بالجينتاميسين بمعدل 100مجم /كجم.: المجموعة (ج) : فئران معاملة بالجينتاميسن مع اضافة 20% خبز القالب الي الوجبة القياسية : المجموعة (د) فئران معاملة بالجينتاميسن مع اضافة 20% خبز القالب المدعم ب 5% مسحوق الحلفا بار الي الوجبة القياسية . اوضحت النتائج ان دمج مسحوق الحلفا بار في خبز القالب المستخدم في تغذية الفئران المصابة ادي الي انخفاض في معدلات اليوريا والكيرياتينين ونشاط انزيمات الكبد والكوليستيرول الكلي والجلسريدات الثلاثية مقارنة بمجموعة التحكم الايجابية. ويرجع الغعل العلاجي للحلفا بار علي قدرته على منع حدوث الاكسدة وتحسين وظائف الكبد والكلي. تشير هذه النتائج الي امكانية استخدام الحلفا بار او الاغذية المدعمة به في الطب الشعبي للوقاية من امراض الكلي.

الكلمات المفتاحية: الطب الشعبي; دهون الدم; الخواص الحسية; الكرياتنين; انزيمات الكبد