

Original Research Article

**Effect of Arabic Gum as Prebiotics and
lactobacillus casei shirota (LcS) as probiotic on
Oxidative Stress and Renal function in
Adenine-Induced Chronic Renal Failure in Rats**

ABSTRACT

Objectives: This study aimed at assessing the effect of Gum Arabic as Prebiotics and *lactobacillus casei shirota* (LcS) as probiotic on oxidative stress and renal function in adenine-induced chronic renal failure in rats

Methodology: 70 male albino rats were divided into 7 groups and treated for 8 weeks as follows group1: control basal diet group (BD), group2: adenine in feed (0.75%, w/w), group3: gum Arabic (GA) in drinking water (15%, w/v), group 4: *lactobacillus casei shirota* (LcS) 1×10^9 colony-forming units (CFU) supplement, group 5 adenine + GA, as before, group 6 adenine +(LcS) as before and group 7: adenine + GA+ (LcS) as before. Urine, blood and kidneys were collected from the rats at the end of the treatment for analysis of conventional renal function tests serum creatinine, urea, uric acid , sodium , potassium concentration). In addition, the oxidative stress markers serum and kidney glutathione and superoxide dismutase, serum catalase and malondialdehyde(MDA) were measured.

Results: By the end of the 8 weeks of treatment, Adenine significantly ($p < 0.05$) increased the concentrations of serum creatinine, urea, uric acid , sodium , potassium and serum MDA. In addition, the oxidative stress markers serum and kidney glutathione and superoxide dismutase, serum catalase was, significantly decreased.

Treatment with (GA) and (LcS) significantly ameliorated these actions. The mechanism of the reported salutary effect of GA in adenine-induced CRF is associated with mitigation of the adenine- induced inflammation and generation of free radicals.

Conclusion: The results suggest that that oral administration of gum Arabic and *lactobacillus casei shirota* could conceivably alleviate adverse effects of adenine induced renal toxicity (CRF).

Keywords: Gum Arabic- Prebiotics - *lactobacillus casei shirota* (LcS) - probiotic - Oxidative Stress – Adenine –Chronic Renal Failure

1. INTRODUCTION

Chronic kidney disease (CKD) is a serious worldwide health problem and is now considered a main determining factor of the poor health consequences of major non-infectious diseases [1]. Numerous elements influence the onset and development of this CKD, such as obesity, diabetes mellitus, and hypertension. Further, then these factors, there is evidence of a pathophysiological role for inflammation and oxidative stress in CKD and its complications [2]. These two events are noticeable features of CKD and its complications in human being [3]. Elevated oxygen radical formation was

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Found in CKD [4]. Therefore, there is a strong need for new research discovering new therapeutic strategies and ameliorating agents, especially in the earlier stages of CKD to slow its progression towards ESRD. Among these agents are Gum Acacia (GA) and *Lactobacillus casei shirota* (LcS).

Adenine is used in this study as one of the models used to induce chronic kidney diseases (CKD) in rats. Both adenine and its metabolite, 2,8-dihydroxyadenine (DHA), have low solubility and can precipitate in the renal tubules and form crystals [5].

Arabic Gum (GA) is extracted from exudates of Acacia seyal trees. It comprises of a blend of polysaccharides in addition to oligosaccharides and glycoproteins [6]. Sudan is the world's biggest producer, trailed by numerous other African nations. It promptly disintegrates in water forming low viscosity solutions [7].

Gum Arabic is indigestible to human and animals, but rather fermented in the colon to provide short-chain unsaturated fats, stimulating a numerous medical advantages. One of these advantages is its prebiotic impact. It has been demonstrated that four-week supplementation with Gum Arabic (10 g/day) prompted critical increments in Bifidobacteria, Lactobacteria, and Bacteroides demonstrating a prebiotic impact [8].

The intestinal microflora of humans and animals is a complex ecosystem that contains several species of useful and hurtful microorganisms. Probiotics' are well-defined as living microorganisms, which confer a benefit on the host, when given in adequate quantities, [9].

Probiotics have potential dietary supplement uses (to help maintain healthy organ functions) and drug uses (to prevent or treat disease).

Lactobacilli are gram-positive bacteria characterized by the formation of lactic acid. Numerous Lactobacillus species are also members of the normal microbiota of animal and human gastrointestinal and genitourinary tracts and are reported to have many health benefits. [10].

This study was designed to examine the consequences of oral administration of Gum Arabic as Prebiotics and lactobacillus casei shirota (LcS) as probiotic in rats. Specifically, this involved:(a) assessing kidney function, (b) assessing Oxidative Stress in adenine-induced chronic renal failure rat model.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

2.1 Animals

Healthy Male Wistar rats (weighing 190.20g $\pm$ 5.91) were obtained from the Institute of Ophthalmology (Cairo, Egypt). They were allowed to acclimatize for two weeks. The animals were housed in stainless steel cages at a temperature of (25 $\pm$ 3°C), and light- controlled room (12-12 h), relative humidity of about 60%, w, during acclimatization period animals fed on basal standard diet prepared in accordance with AIN-93 formulation [11] (Reeves *et al.*, 1993) and provided with water *ad libitum*.

2.2 Chemical and drugs

Adenine was obtained from Sigma (St. Louis, MO, USA). Adenine was prepared freshly in 10% Tween 80 obtained from El Gomhouria Co., Zagazig, Egypt. Acacia gum used obtained from Gum Arabic Company in Khartoum, Sudan and verified Aqueous solutions of Acacia gum used were prepared freshly every day. All used chemicals were of analytical reagent grade. Lactobacillus casei is a gram (+) bacteria, and it belongs to the subgenus Streptobacterium. LcS was taken as formulation.

2.3 The Experimental Design

66 After an acclimatization period of 2 weeks to stabilize all metabolic conditions, rats (n= 70) were randomly divided into
 seven equal groups and treated for eight consecutive weeks as shown in the following table:

67 **Table (1)** The Experimental Design.

Group	Treatment
Group 1 (Control group) "C"	Continued to receive the same standard diet without treatment until the end of the study
Group 2 feed (AD)	Received standard diet containing adenine at a dose of 0.75%w/w in feed (AD) Was given standard diet and GA in drinking water at a concentration of 15% w/v (GA)
Group 4(LcS)	Received lactobacillus casei shirota (LcS) supplement orally 1×10^9 colony-forming units (CFU)/day in the formulation (LcS)
Group 5(AD+GA)	The fifth group was given adenine in the feed as in group 2, plus GA in drinking water at a concentration of 15% w/v (AD+GA)
Group 6 (AD+LcS).	Received standard diet containing adenine at a dose of 0.75%w/w in the feed (AD) + (LcS) as before (AD+LcS).
Group 7(AD+GA+ LcS)	Adenine + GA+ (LcS) as before. (AD+GA+ LcS)
The dose of adenine was chosen from the original method by Yokozawa et al. [12] and the dose of GA according to [13], the lactobacillus casei shirota (LcS) dose was according to Karimi et al[14].	

77 **2.4 Handling of Blood, urine and Kidney Samples:**

78 During the treatment, period the rats were weighed weekly, For the collection of urine, they were placed individually in
 79 metabolic cages for 24 h, after the 8 weeks' treatment period. Urine specimens were centrifuged at 900 rpm for 15 min,
 80 filtered to remove sediment (The supernatant remains after centrifugation was transferred by careful decanting or pipetting.
 It is important that the sediment is not disturbed whilst the supernatant is being transferred) and then urine volumes were
 measured. Urine specimens were stored at -20°C until analyzed
 81 for creatinine, total proteins, as well as sodium, potassium and calcium electrolytes.

82
 83 At the end of the 8 weeks' experimental period, the rats were fasted overnight and sacrificed under ether anesthesia and
 84 blood samples were collected from the hepatic portal vein. The blood samples were centrifuged at 1500 rpm for 15 min.
 85 The serum samples were stored at -20°C for further assessment. The kidneys were excised, rinsed with saline (0.9% Na
 86 Cl) blotted on filter paper and weighed and stored -20°C for subsequent analysis.

87 **2.5 Biological Assay:**

88 The rats were weighed weekly and food intake was estimated The Feed Efficiency Ratio (FER) was calculated as
 89 described by the equation of Guo et al. [15].
 90 Feed efficiency ratio (FER) = weight gain (g) / feed intake (g).

91 **2.6 Biochemical Assays:**

92 The concentrations of creatinine in serum and urine were estimated spectrophotometrically using commercial kits Jaffe

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[16], Creatinine clearance (CCr) was calculated as reported by Duarte et al. [17]. urea was estimated according to Fawcett and Scott [18], while uric acid was determined by enzymatic colorimetric kits Gochman and Schmitz [19]. Proteinuria was measured with a kit according to Tietz [20]. serum and urinary potassium concentrations were determined by [21], while serum and urinary sodium concentrations were measured colorimetrically using kits according to [22], Calcium was determined in the Serum according to Baginski, [23].

serum and renal malondialdehyde (MDA) were estimated according to Ohkawa et al. (1979) [24], while Superoxide dismutase (SOD) were determined according to Minami and Yoshikana [25], while reduced glutathione(GSH) were measured chemically according to Beutler et al. [26]. Finally, catalase (CAT) activity was determined by colorimetric kits according to [27].

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2.7 Statistical analysis

Data are expressed as mean $\pm$ standard Error. Statistical comparison between different groups was conducted using one-way analysis of variance (ANOVA). Statistics were performed using the Statistical Package for the Social Science (SPSS soft ware version 17.0). Significance was accepted at $P < 0.05$. Values were expressed as the Mean $\pm$ SEM [28].

3. RESULTS AND DISCUSSION

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In vivo animal, biological experiments are of great importance in examining the physiological and biochemical effects of some possible therapeutic intervention on chronic kidney diseases prior to studies on humans. The present research reveals the novel effect of GA as prebiotic and (LcS) as probiotic on kidney function and antioxidant status in adenine- induced renal failure rat models.

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Chronic kidney disease (CKD) progresses at variable rates, depending on the etiology. It has been reported that, CKD is fatal disorder until the development of renal replacement therapies involving of hemodialysis, peritoneal dialysis, and kidney transplantation allowed decades or longer of survival.

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Kidneys dysfunction is related to disturbed kidney metabolism and to reduced glomerular filtration and tubular secretion/reabsorption problems [29]. Recent studies suggested that, the bacterial load and the adverse products of the intestinal microbiota might influence chronic renal disease pathogenesis[30].

The development of chronic kidney disease upon adenine oral administration is confirmed by subsequent Significant increases in fluid intake and urinary output, in addition to the significant elevation of serum creatinine and urea and the decrease in creatinine clearance as well as in body weight

The data represented in the table (2) indicating that adenine significantly reduced the final body weight of rats, and the concomitant administration of GA or /and LcS with adenine has no significant effect on the reduction in body weight.

The relative weight of kidney significantly increased upon adenine administration, and the supplementation with of GA or /and LcS with adenine significantly diminished that increase

The results of the current study are in harmony with those of Al Za'abi et al., [31] indicating that, GA did not significantly modify the body weight of the treated groups. Despite food intake (FI) was significantly decreased after GA treatment.

GA significantly reduces fluid intake and as a result urinary output. Recent researches highlighted that some bacterial strains, such as Lactobacillus spp. and Bifidobacterium spp., play important role in energy metabolism and weight management in rat models and human being. The beneficial effects of bacteria are strain- dependent [32].

LcS treatment was followed by a significant body weight reduction (Table 2) which reflects energy expenditure prompt [33].;

therefore, It has been also suggested that Probiotics can increase the function of the intestinal barrier, leading to body weight loss [34]. In addition, the oral administration of probiotics increases the activity of the sympathetic nervous system in white and brown adipose tissue. Thus, probiotic consumption facilitates thermogenic and lipolytic responses via stimulating the sympathetic nervous system, which leads to weight reduction [35].

The results in table (1) indicated that there was no significant effect of treatment with GA or/and LcS on feed efficiency of adenine intoxicated rats

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123

124 **Table 2: Biological parameters at the end of the experiment**

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Comment [K1]: Pre and post testing usually requires a repeated measures ANOVA test. All the results at the end of the experiment except for initial body weight.

Parameters	Food intake (g)	Initial Body Weight (g)	Final Body Weight (g)	Mean Feed efficiency	Relative kidneys weight (%)	Fluid Intake (ml/d)	Urine Output (ml/d)
Groups							
Group 1 Normal control(C)	17.30±0.28 ^a	187.23±6.68	242.56±7.11 ^a	0.057±0.004 ^a	0.26±0.02 ^a	18.74 ± 0.61 ^a	10.13±0.68 ^a
Group 2 (A)	9.49 ± 0.28	189.15 ± 5.43 ^a	188.34±4.7 ^c	-0.007±0.008 ^b	0.52 ± 0.03	25.99 ± 2.06	21.15±1.67
Group 3 (GA)	15.96±0.48	191.89 ± 4.82 ^a	240.07±7.35 ^{ab}	0.054±0.011 ^a	0.26 ± 0.03a	17.34 ± 0.67 ^b	10.20±0.61 ^a
Group 4 (LcS)	17.06±0.29 ^a	190.29 ± 5.47 ^a	236.73±1.85 ^b	0.049±0.008 ^a	0.28 ± 0.02a	18.35±0.76ab	10.64±0.66 ^a
Group 5 (A+GA)	8.27± 0.10 ^b	191.36 ± 6.17 ^a	187.84±6.22 ^c	-0.008±0.015 ^b	0.42 ± 0.07	23.45 ± 1.67 ^c	14.65±0.54 ^b
Group 6 (A+LcS)	9.19 ± 0.18	192.31± 5.35 ^a	185.90 ± 5.78 ^c	-0.012±0.06 ^b	0.39 ± 0.08b	22.51 ± 1.87 ^c	16.08 ± 0.65
Group 7 (A+GA+LcS)	8.20± 0.18 ^b	189.18 ± 7.23 ^a	186.93 ± 6.52 ^c	-0.044±0.09 ^b	0.038±0.02b	20.53 ± 1.21	14.18±0.75 ^b

Values are expressed as means ± SEM (n = 10). Means with similar superscript (a, b, c, d) letters in columns indicate non-significant difference (P < 0.05).

By the end of the 8 weeks' experimental period, Adenine tended to significantly (p < 0.05) increased the concentrations of serum creatinine (Fig. 1), urea (Fig. 2) and uric acid (Fig. 3). These findings are in accordance with those of Al Za'abi et al., [31] who reported that, adenine and its metabolite, 2,8-dihydroxyadenine (DHA), have low solubility and can precipitate in the renal tubules and form crystals. Adenine oral consumption might cause the occlusion of renal tubules which hinders the excretion of nitrogenous substances leading to a biochemical and physiological status like chronic kidney disease humans [36].

GA or/ and LcS treatment tended to significantly decrease serum urea, creatinine, uric acid and urinary protein concentrations.

Within the 8 weeks' treatment period as shown in (Fig. 1, 2, 3 and 4 respectively).

On the other hand supplementation with GA or/ and LcS significantly increased Creatinine clearance as compared to adenine intoxicated group.

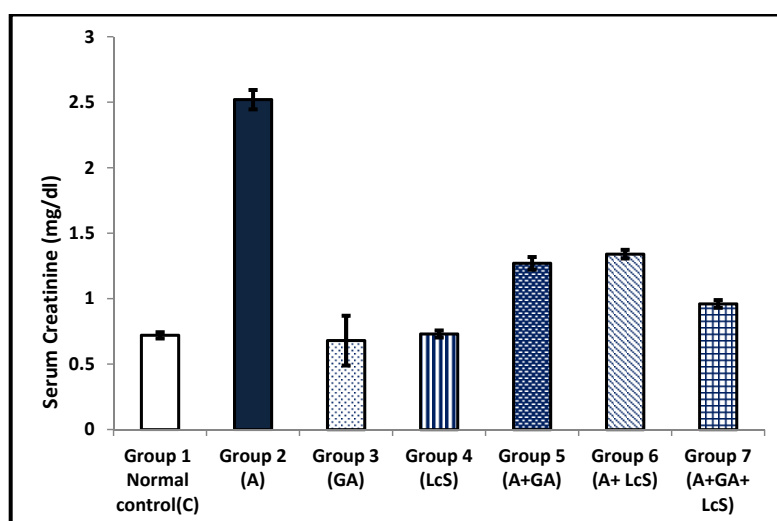


Figure 1. Serum Creatinine concentration in control and treated rats. Each column and vertical bar represents the mean ± SEM (n = 10). P < 0.05 vs. adenine treatment.

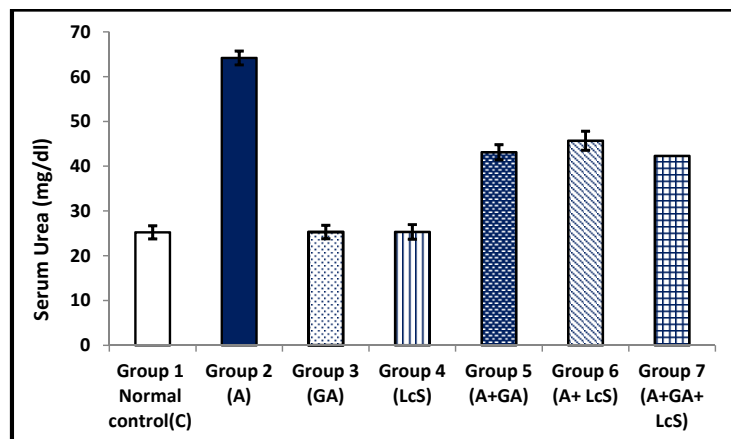


Figure 2:: Serum Urea concentration in control and treated rats. Each column and vertical bar represents the mean $\pm$ SEM (n = 10). P < 0.05 vs. adenine treatment.

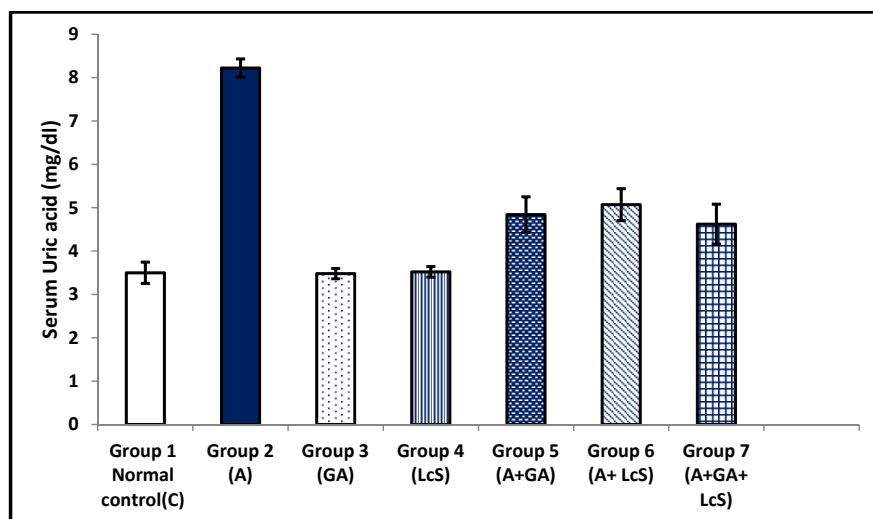
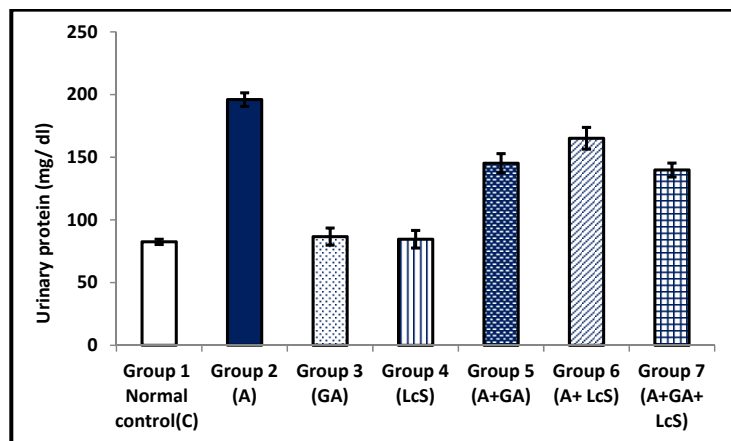
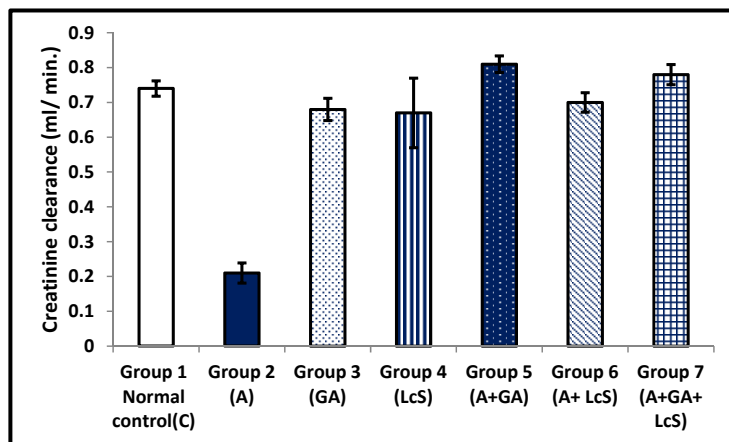


Figure3: Serum Uric Acid concentration in control and treated rats. Each column and vertical bar represents the mean $\pm$ SEM (n = 10). P < 0.05 vs. adenine treatment.



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Figure 4: Urinary Protein concentration in control and treated rats. Each column and vertical bar represents the mean $\pm$ SEM (n = 10). P < 0.05 vs. adenine treatment.



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Figure 5: Creatinine clearance (ml/min.) in control and treated rats. Each column and vertical bar represents the mean $\pm$ SEM (n = 10). P < 0.05 vs. adenine treatment.

144

145 I replaced the table with bar graphs

Comment [K2]: This table would be easier to read as bar graphs with a description of the statistical test used, brackets delineating which groups were compared, and the statistical significance level (p value) listed.
I made it as bar graphs only with some of the results.

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146 The results of the present study indicating that adenine supplementation significantly increased serum and urinary sodium and potassium while it caused a significant decrease in serum Ca²⁺ as illustrated in table 3.

147 Urinary Na⁺ was significantly reduced after GA treatment. On the other hand, the decrease in urinary K⁺ was not statistically significant.

148 Serum Na⁺ and K⁺ were tended to decrease after GA treatment. On the other hand, GA treatment significantly followed by a profound and significant increase in serum Ca²⁺.

150 Gum Arabic (GA), is known to be particularly rich in antioxidants, which have been reported to have antioxidant and lipid peroxidation lowering effects [37]

151 The nephroprotective effect of GA against many nephrotoxic agents was noted in numerous previous researches. [38], [39]. The antioxidation induced by GA might be one of the most likely mechanisms contributing to its beneficial effect against renal injury. This antioxidant effect of GA was confirmed previously by in vitro studies, which showed that AG had a dose-dependent scavenging of superoxide radicals generated enzymatically and nonenzymatically. It could be suggested that AG scavenges Adenine free-radical generation and, in turn, inhibits lipid peroxidation-induced injury in renal tissues, which has been suggested to protect renal structure and function. Therefore, the protective effect is provided by GA on renal tissue through antioxidants as well as by scavenging free radicals in vivo.

152 GA has many health benefits, including beneficial effects on the kidney. GA is thought to act primarily via increased nitrogen excretion which in turn lowers serum urea nitrogen concentration.

154 GA is a dietary fiber rich in Ca²⁺, Mg²⁺, and K⁺. It has been demonstrated that GA increased creatinine clearance in healthy mice. Also, it increases ADH excretion, as well as intestinal and renal excretion of Ca²⁺ [39]

156 One of the mechanisms of GA beneficial effects is assumed to be through increasing the fecal nitrogen excretion which in turn reduces serum urea nitrogen levels.

157 It has been demonstrated that chronic renal failure (CRF) patients consuming low protein diet (LPD) supplemented with 50 g Arabic gum/d had greater fecal bacterial masses, greater fecal nitrogen excretion, and lower serum urea nitrogen than those consumed the LPD alone or supplemented with 1 g pectin/d. Because elevated concentrations of serum urea nitrogen have been associated with adverse clinical symptoms of CRF, the results suggest that Arabic gum may be a useful adjunct to an LPD for increasing excretion of nitrogenous wastes in feces [40]. Another study by Ali et al. [41] on rat models of acute renal failure showed that Arabic gum may also improve renal function independently of its action on fecal bacterial ammonia metabolism, but its effect is attributed to a decrease in the generation of free oxygen radicals.

158 The results of the current research suggested that GA may also improve renal function. The finding of the present research is in harmony with those of [42] who demonstrated that GA has been clinically reported to reduce urea and plasma creatinine concentrations and reduces the need for dialysis from 3 to 2 times per week in chronic renal failure patients [43] GA supplementation significantly increased fecal bacterial mass and fecal nitrogen excretion function.

162 Our results are in accordance with those of Miranda et al., [44] who illustrated that, LCS treatment in patients with stage 3 and stage 4 chronic renal failure showed a >10% decrease in serum urea concentrations.

164 The gastrointestinal microflora represents a complex ecosystem containing both beneficial and harmful microorganisms. The composition and function of the intestinal flora is influenced by several factors including age, diet, and antibiotic Treatment.

167 Chronic kidney failure impacts the intestinal microbiome. Changes in the composition of the intestinal microbiome and disruption of its barrier function may result in the production and absorption of toxic metabolites that can contribute to uremic toxicity, malnutrition, inflammation, and other morbidities in uremic patients [45] (Vaziri et al., 2013). Probiotics modify intestinal microbiome which helps to decrease the bacteria producing uremic toxins [46] (Nakabayashi et al., 2011).

171 chronic kidney disease (CKD) patients have altered gut flora. The alteration of gut flora affects the patients through restoring the gastrointestinal flora balance which favorably impacts the CKD patient and improves any GI issues such as constipation or diarrhea as well as promotes healthy digestion and improved immunity.[47]

174 Chronic renal failure is characterized by a progressive retention of several microbial metabolic end products, which are very difficult to be eliminated due to kidney failure and can hardly be removed using dialysis techniques because of their high protein binding [48].

177 Probiotic was proven to minimize inflammation and oxidative stress in CKD patients. It has been demonstrated that probiotic

Comment [K3]: Without a mechanism this claim is more hand waving. Better to discuss perhaps a particular component to Arabic Gum that would support your claim of prevention of toxicity when exposed to oxidative stressor.
I discussed the possible mechanism

Comment [K4]: Doubtful that this is the case. The loss of ammonia attributed to lactulose administration in cirrhotics has little effect on circulating urea.
This might be one of the effects of GA

Comment [K5]: Via what mechanism.

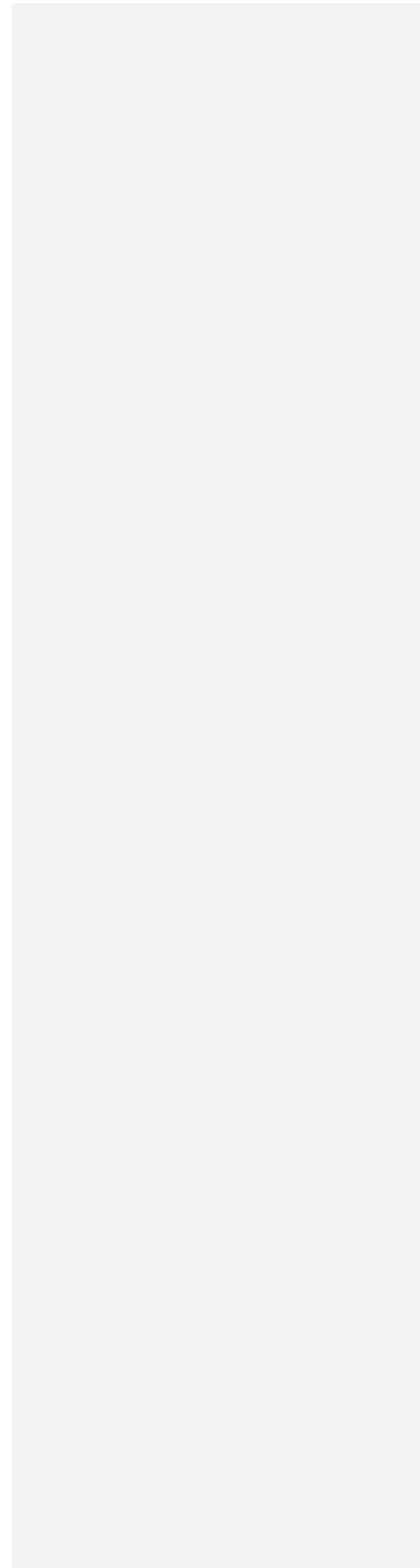
Comment [K6]: Please be specific here. What are those organisms.
This statement just to start discussion then I. Specified the organism used in this study.

Comment [K7]: In what way? What was the primary effect that they measured? You are not presenting any data that
I used this just as entrance to discuss later the specific beneficial effect of. LcS

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supplementation in non- dialyzed CKD patients leads to an improvement in the quality of life and a reduction in serum uric acid and creatinine concentrations. Ranganathan et al. [49]

178 Probiotic is preferred over food sources due to the high potassium, phosphorus, sodium, and sugar content of many foods
179 containing probiotics. In addition, when using quality supplements, the probiotic dose can be accurately estimated and



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monitored. The preferred dose of probiotic for GI health is to be a blend of bacteria with at least 10 billion colonies forming units took daily [47].

In contrast to the results of the current study, Hyun et al. [50] illustrated that there was no significant effect of probiotics on The reduction of uraemic toxins in pediatric dialysis patients.

There is a decrease in urinary N excretion observed after the administration of GA, LcS or the synbiotic combination of GA and L. casei Shirota.

Table 3: Serum and urine electrolytes in the tested groups

Parameters Groups	Serum Na ⁺ (mmol/ L)	Urinary Na ⁺ (mmol/L)	Serum K ⁺ (mmol/ L)	Urinary K ⁺ (mmol/L)	Serum Ca2+ (mmol/L)
Group 1 Normal control(C)	141.78±3.74 ^c	130.68±5.53 ^a	7.67 ± 0.51 ^a	81.18±3.06 ^a	10.19 ± 0.55 ^a
Group 2 (A)	165.23 ± 4.15 ^a	179.80±6.27	15.70 ± 0.77	128.24±6.15 ^c	8.56 ± 0.12
Group 3 (GA)	159.51± 4.73 ^{bd}	132.07±6.56 ^a	7.18 ± 0.51	78.05±6.58 ^a	10.46 ± 0.21
Group 4 (LcS)	163.61± 6.58 ^{ab}	133.57±6.09 ^a	7.68 ± 0.53 ^a	79.17±3.81 ^a	10.21 ± 0.096 ^a
Group 5 (A+GA)	152.88 ± 6.98 ^e	152.60±6.19b ^c	10.66 ± 0.60 ^b	90.49±4.96 ^b	9.21 ± 0.15
Group 6 (A+ LcS)	155.15 ±5.49 ^{de}	155.97±5.23 ^b	11.18 ± 0.31	88.71±6.41 ^b	10.12 ± 0.178 ^{ab}
Group 7 (A+GA+ LcS)	146.45 ± 5.69 ^c	148.80±7.46 ^c	10.44 ± 0.36 ^b	83.71±6.38 ^c	9.93 ± 0.24 ^b

Values are expressed as means ± SEM (n = 10). Means with similar superscript (a, b, c, d) letters in columns indicate non-significant difference (P< 0.05).

The results represented in the table (4) demonstrated that, Adenine significantly (p <0.05) increased the concentrations of serum MDA as compared to normal control group. In addition, the oxidative stress markers serum and kidney glutathione and superoxide dismutase, serum catalase were, significantly decreased.

Our results agreed with those of Anand et al., (2014) who illustrated that, Adenine caused several oxidative reactions in renal tissues which might lead to an elevation in several oxidative markers such as GSH, SOD, catalase and TAC [51]. Furthermore, Adenine and its metabolite, DHA, have low solubility and precipitate in the renal tubules leading to their occlusion and the development of uremia[39].

The obvious antioxidant properties of GA have been described before by Ali et al., who confirmed the salutary action of GA in adenine-induced chronic failure in both mice and rats [5].

Ali et al., reported an explanation of the beneficial actions of GA as a dietary supplementation in patients suffering from chronic kidney disease, that GA could ameliorate a loss of antioxidant defense and decrease adenine-induced superoxide production [5].

The results of the present study showed that, GA and LcS treatment significantly diminished the oxidative stress induced by adenine administration which indicated by the significant reduction of serum and renal MDA paralleled with a statistically significant increase in serum and renal reduced GSH, SOD and CAT. The best improvement in antioxidant status was observed in group 7 supplemented with both GA and LcS.

The major mechanism of the valuable action of GA in adenine-induced CKD might be due to its antioxidative properties.

Comment [K8]: What were the concentrations compared and what was the exact p value.

As compared to normal control group (p<.005)

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On the other hand, Ali., 2004 demonstrated that gum Arabic did not significantly affect any of the antioxidant enzymes [52].

Probiotics used popularly in humans due to their antimicrobial and antioxidative properties. In addition, Probiotics was considered as cancer prevention agent which might be due to metal particle chelation, compound hindrance, a decrease of ascorbate autooxidation, and ROS searching [53]. The causes of this decline have been suggested to be increased oxidative stress and disorders in energy metabolism, which might participate in important functions [54]. Oxidative stress arises when there is a marked imbalance between the production and the elimination of ROS. results in the urgent formation of free radicals that last for a matter of milliseconds

Probiotic have antioxidant activity which improves the function of biochemical reaction in a biologicalsystem.

Previous research has proposed that specific probiotics assume different biological roles through various mechanisms, [55].

“ROS generation overwhelms antioxidant defenses, and ROS can interact with endogenous macromolecules and change cellular functions” [56]. Elevated level of ROS can also result in protein oxidation (PO) and lipid peroxidation (LPO) which can be used as biomarkers of ROS-induced tissue damage in various diseases [57]

Probiotics may exert their defensive impacts against oxidative stress by reestablishing the gut microbiota].

Probiotic strains can be chosen and explored as promising agents for the prevention and control of several free radical-related disorders [59]

Our study in agreements with AOAC [60] which illustrated that lactobacilli have excessive antioxidant activity while this was greatly strain dependent among facultative and obligatory hetero-fermentative lactobacilli.

Also, Gao J demonstrated that antioxidant activity of four Lactobacillus rhamnosus (B7, B8, B10, B44) was detected by DPPH method. The DPPH scavenging ability of cell-free extracts. The Lactobacillus rhamnosus B10 strain showed a relatively high antioxidant activity.[61]

Yoghurt bacteria such as S. salvarius ssp. thermophilus ATCC 19258 and L. delbrueckii ssp. bulgaricus ATCC 11842 have been described to have certain effect on oxidative stress [62]. The peptide hydrolyzed from fermentation could be responsible for minimizing oxidative stress when analyzed for their antioxidant activity by the β -Carotene bleaching assay [63]

several studies confirmed antioxidant properties of Lactobacilli . [64]

Table 4. Reduced Glutathione, catalase and superoxide dismutase enzymes activity in the tested groups

Parameters Groups	Erythrocyte SOD activity (U/mL)	Renal SOD (U/100mg)	Blood Reduced glutathione (mg/dL)	Renal Reduced Glutathione (mg/ g tissue)	Serum Catalase (U/ L)	Renal Catalase (U/ g tissue)
Group 1 Normal control(C)	279.46±9.99a	8.59 ± 0.20 a	15.75 ± 0.61	96.43 ± 2.95 a	1593.98 ± 17.06 a	8.29± 0.21 a
Group 2 (A)	185.62±8.79	4.60 ± 0.26	8.18 ± 0.10	60.18 ± 3.29	1019.67 ± 20.19	3.92 ± 0.31
Group 3 (GA)	278.66±9.22a	8.28 ± 0.26 b	15.23 ± 0.23 a	93.93 ±4.73 a	1599.59± 15.59 a	8.05 ± 0.16

Comment [K9]: Same comments from prior apply to all your tables.

Group 4 (LcS)	278.90±11.35a	8.47 ± 0.28 ab	15.21 ± 0.16 a	94.68 ± 3.78 a	1596.39 ± 19.65 a	8.33 ± 0.12 a
Group 5 (A+GA)	246.98±10.7b	6.97 ± 0.33 c	11.25 ± 0.41 b	75.37 ± 04.9 bc	1192.97± 12.11	5.42 ± 0.24 b
Group 6 (A+ LcS)	249.04±12.06b	6.37 ± 0.20	10.90 ± 0.37 c	72.78 ± 4.43 b	111.39± 14.18	5.37 ± 0.18 b
Group 7 (A+GA+ LcS)	257.10±13.23b	6.78 ± 0.12 c	11.03 ± 0.26 bc	78.68 ± 5.09 c	1218.11 ± 17.63	5.52 ± 0.25 b

269 Values are expressed as means ± SEM (n = 10). Means with similar superscript (a, b c, d) letters in columns indicate non-
270 significant difference (P< 0.05).
271

272 Table 5. Serum and Renal Malondialdehyde in the tested groups

Parameters Groups	Serum Malondialdehyde (nmol/ml)	Renal Malondialdehyde (mmol/ g tissue)
Group 1 Normal control(C)	1.82 ± 0.19 ^{ab}	3.46 ± 0.121 ^a
Group 2 (A)	4.25 ± 0.086	7.84 ± 0.068
Group 3 (GA)	1.85 ± 0.022 ^a	3.41 ± 0.096 ^{ab}
Group 4 (LcS)	1.79 ± 0.038 ^b	3.35 ± 0.045 ^b
Group 5 (A+GA)	3.09 ± 0.086	5.63 ± 0.153
Group 6 (A+ LcS)	3.20 ± 0.051	5.82± 0.157
Group 7 (A+GA+ LcS)	2.58 ± 0.057	5.17 ± 0.127

273 Values are expressed as means ± SEM (n = 10). Means with similar superscript (a, b c, d) letters in columns indicate non-
274 significant difference (P< 0.05).
275

The results of the current study agreed with those of Leila et al., 2015 who showed that Lactobacilli induced a high statistically significant reduction in lipid peroxidation in kidney and liver as well as a reducing in MDA concentration, supporting that Lactobacilli presented effective antioxidative properties and could scavenge the excess of free radicals. [65]

Comment [K10]: Odd usage of words.
I deleted the word

277 A recent study by Kaddam et al., 2017 provided more evidence that GA has potent antioxidative effects in humans as
278 demonstrated by its ability to increase TAC and to decrease oxidative stress markers in humans. Thus, the increased
279 intake of dietary antioxidants from GA may help to maintain an adequate antioxidant defense status and consequently
contribute to the management of several oxidative stress-related disorders[66].

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4. CONCLUSION

In conclusion, the present research reveals many beneficial effects of GA and LcS treatment which include kidney function improvement, most importantly reduction of serum urea, creatinine, Na and K. In addition, GA and LcS have a significant antioxidant effects in adenine-induced chronic renal failure in rat models. Thus, The results suggest that that oral administration of gum Arabic and lactobacillus casei shirota could conceivably alleviate adverse effects of adenine-induced renal toxicity and can be considered as promising treatment patients with chronic kidney diseases.

CONFLICT OF INTEREST AND FUNDING

The author declares no conflict of interest

ETHICAL APPROVAL

291 Procedures involving animals and their care were carried out in accordance with international laws and policies (EEC Council directives 86/609, OJL 358, 1 December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publications No. 85–23, 1985). The study was approved by the University Animal Ethical Committee

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