

Original Research Article

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

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 than 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

found in CKD [4]. Therefore, there is a strong need for new research discovering new therapeutic strategies and ameliorating agents, especially for 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].

Gum-Arabic Gum (GA) is ~~gotten-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 **guaranteed** that four week supplementation with Gum Arabic (10 g/day) prompted critical increments in Bifidobacteria, Lactobacteria, and Bacteriodes demonstrating a prebiotic impact [8].

Comment [K1]: Odd use of words

The intestinal microflora of humans and animals is a complex ecosystem that contains several species of useful and hurtful **microorganisms**. Recently the concept of 'probiotics' has been the focus of attention for health professionals. 'probiotics' are well-defined as living microorganisms, which confer a benefit on the host, when given in adequate quantities, [9].

Comment [K2]: Be specific. So far there is empty descriptions without scientific explanation of why you are undergoing this study.

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 The Experimental Design

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.

65 Group 1: Continued to receive the same standard diet without treatment until the end of the study (control group)
 66 (C).Group 2: Received standard diet containing adenine at a dose of 0.75%w/w in feed (AD)
 67 Group 3: Was given standard diet and GA in drinking water at a concentration of 15% w/v (GA)
 68 Group 4: Received lactobacillus casei shirota (LcS) supplement orally 1×10^9 colony-forming units (CFU)/day in the
 69 formulation ((LcS)
 70 Group 5: The fifth group was given adenine in the feed as in group 2, plus GA in drinking water at a concentration of 15%
 71 w/v (AD+GA)
 72 Group 6: Received standard diet containing adenine at a dose of 0.75%w/w in feed (AD) + (LcS) as before (AD+LcS).
 73 Group 7: Adenine + GA+ (LcS) as before. (AD+GA+ LcS)
 74 The dose of adenine was chosen from the original method by Yokozawa et al. [12] and the dose of GA according to
 75 [13], the lactobacillus casei shirota (LcS) dose was according to Karimi et al [14].
 76

77 2.3 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 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 At the end of the 8 weeks' experimental period, the rats were fasted overnight and sacrificed under ether anesthesia and
 83 blood samples were collected from the hepatic portal vein. The blood samples were centrifuged at 1500 rpm for 15 min.
 84 The serum samples were stored at -20°C for further assessment. The kidneys were excised, rinsed with saline (0.9% Na
 85 Cl) blotted on filter paper and weighed and stored -20°C for subsequent analysis.

86 2.4 Biological Assay:

87 The rats were weighed weekly and food intake was estimated The Feed Efficiency Ratio (FER) was calculated as
 88 described by the equation of Guo et al. [15].

89 Feed efficiency ratio (FER) = weight gain (g) / feed intake (g).

90 2.5 Biochemical Assays:

91 The concentrations of creatinine in serum and urine were estimated spectrophotometrically using commercial kits Jaffe
 92 [16], Creatinine clearance (CCr) was calculated as reported by Duarte et al. [17]. urea was estimated according to
 93 Fawcett and Scott [18], while uric acid was determined by enzymatic colorimetric kits Gochman and Schmitz [19].
 94 Proteinuria was measured with a kit according to Tietz [20]. serum and urinary potassium concentrations were
 95 determined by [21], while serum and urinary sodium concentrations were measured colorimetrically using kits according to
 96 [22], Calcium was determined in the Serum according to Baginski, [23].

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

102 2.6 Statistical analysis

103 Data are expressed as mean $\pm$ standard error. Statistical comparison between different groups was conducted using one-

way analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparison test to judge the difference between various groups. Significance was accepted at $P \leq 0.05$. Values were expressed as the Mean $\pm$ SEM [28].

Comment [K3]: Did you use bonferonni adjustment?

3. RESULTS AND DISCUSSION

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 novel effect of GA as prebiotic and (LcS) as probiotic on kidney function and antioxidant status in adenine – induced renal failure rat models.

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.

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 by the significant elevation of serum creatinine and urea and the decrease in creatinine clearance as well as in body weight.

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 reduce 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 which reflects a or 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].

LcS has been demonstrated to has beneficial anti-obesity effects but does not have anti-inflammatory or hypolipidemic effects.

Table 1: Biological parameters at the end of the experiment

Comment [K4]: Pre and post testing usually requires a repeated measures ANOVA test.

Parameters Groups	Food intake (g)	Initial Body Weight weight (g)	Final Body Weight (g)	Relative kidneys weight (%)	Fluid Intake (ml)	Urine Output (ml)
Group 1 Normal control(C)	17.30 $\pm$ 0.28 ^a	187.23 $\pm$ 6.68	242.56 $\pm$ 7.11 ^a	0.26 $\pm$ 0.02 a	18.74 $\pm$ 0.61 ^a	10.13 $\pm$ 0.68 ^a
Group 2 (A)	9.49 $\pm$ 0.28	189.15 $\pm$ 5.43 ^a	188.34 $\pm$ 4.7 ^c	0.52 $\pm$ 0.03	25.99 $\pm$ 2.06	21.15 $\pm$ 1.67
Group 3 (GA)	15.96 $\pm$ 0.48	191.89 $\pm$ 4.82 ^a	240.07 $\pm$ 7.35 ^{ab}	0.26 $\pm$ 0.03a	17.34 $\pm$ 0.67 ^b	10.20 $\pm$ 0.61 ^a

Group 4 (LcS)	17.06±0.29 ^a	190.29 ± 5.47 ^a	236.73±1.85 ^b	0.28 ±0.02a	18.35±0.76 ^{ab}	10.64±0.66 ^a
Group 5 (A+GA)	8.27± 0.10 ^b	191.36 ± 6.17 ^a	187.84±6.22 ^c	0.42 ± 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	0.39 ± 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	0.038±0.02b	20.53 ± 1.21	14.18±0.75 ^b

Values are expressed as means ± standard deviation (n = 8). 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, urea and uric acid as shown in table (2). These finding 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].

On the other hand, GA treatment tended to significantly decrease serum urea, creatinine and uric acid concentrations. Within the 8 weeks' treatment period

Table 2: kidney function parameters in the tested groups

Parameters Groups	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Serum Uric acid (mg/dl)	Urinary protein (mg/ dl)	Creatinine clearance (ml/ min.)
Group 1 Normal control(C)	0.72 ± 0.024 ^{ab}	25.23± 1.46 ^a	3.5± 0.246 ^a	82.51 ± 2.09 ^a	0.74 ± 0.22 ^a
Group 2 (A)	2.52 ± 0.074	64.19± 1.55	8.22± 0.211	195.98 ± 5.44	0.21 ± 0.29
Group 3 (GA)	0.68 ± 0.19 ^a	25.30± 1.49 ^a	3.48 ± 0.12 ^a	86.70 ± 6.80 ^a	0.68 ± 0.32 ^b
Group 4 (LcS)	0.73 ± 0.027 ^b	25.32± 1.64 ^a	3.52 ± 0.123 ^a	84.60 ± 6.98 ^a	0.67 ± 0.10 ^b
Group 5 (A+GA)	1.27± 0.048	43.12± 1.70 ^b	4.84 ± 0.41 ^{bc}	145.28 ± 7.64 ^b	0.81 ± 0.24 ^c
Group 6 (A+ LcS)	1.34 ± 0.033	45.7 ± 2.12	5.07 ± 0.37 ^b	165.20 ± 8.70	0.70± 0.028 ^{ab}
Group 7 (A+GA+ LcS)	0.96 ± 0.029	42.30 ± 1.41 ^b	4.62 ± 0.46 ^c	139.84 ± 5.51 ^b	0.78 ± 0.29 ^c

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (P < 0.05).

Comment [K5]: 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.

UNDER PEER REVIEW

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

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

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

176 Proteinuria decreased significantly ($p < 0.05$) by GA. The reduced Proteinuria may be attributed to a decrease of blood
177 pressure.

178 The modulatory effect of GA on blood pressure could have been due to enhanced Ca²⁺ intake which stimulate Ca²⁺
179 sensing receptor leading to Na⁺, K⁺ and Cl⁻ – transport or triggering vasodilation. [37].

180 The mechanism of action of GA as a nephroprotective agent. Gum Arabic (GA), is known to be particularly rich in
181 antioxidants, which have been reported to have antioxidant and lipid peroxidation lowering effects [38]

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

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

186 GA is assumed to act mainly through increasing the fecal nitrogen excretion which in turn reduces serum urea nitrogen
187 levels.

188 The results of the current research suggested that GA may also improves renal function. The finding of the present
189 research are in harmony with those of [40] who demonstrated that, GA has been clinically reported to reduce urea and
190 plasma creatinine concentrations and reduces the need for dialysis from 3 to 2 times per week in chronic renal failure
191 patients [41] GA supplementation significantly increased fecal bacterial mass and fecal nitrogen excretional function.

192 Our results are in accordance with those of Miranda et al., [42] who illustrated that, LcS treatment in patients with stage 3
193 and stage 4 chronic renal failure showed a >10% decrease in serum urea concentrations.

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

197 Chronic kidney failure impacts the intestinal microbiome. Changes in the composition of the intestinal microbiome and
198 disruption of its barrier function may result in the production and absorption of toxic metabolites that can contribute to
199 uremic toxicity, malnutrition, inflammation, and other morbidities in uremic patients [43] (Vaziri et al., 2013). Probiotics
200 modify intestinal microbiome which help to decrease the bacteria producing uremic toxins [44] (Nakabayashi et al., 2011).

201 chronic kidney disease (CKD) patients have altered gut flora. The alteration of gut flora affects the patients through
202 restoring the gastrointestinal flora balance which favorably impacts the CKD patient and improves any GI issues such as
203 constipation or diarrhea as well as promotes healthy digestion and improved immunity. [45]

204 Chronic renal failure is characterized by a progressive retention of several microbial metabolic end products, which are
205 very difficult to be eliminated due to kidney failure and can hardly be removed using dialysis techniques because of their
206 high protein binding [46].

207 Probiotic was proven to minimize inflammation and oxidative stress in CKD patients [47].

208 It has been demonstrated that probiotic supplementation in non-dialyzed CKD patients leads to an improvement in quality
209 of life and a reduction in serum uric acid and creatinine concentrations. Ranganathan et al. [48]

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

Comment [K6]: 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.

Comment [K7]: Doubtful that this is the case. The loss of ammonia attributed to lactulose administration in cirrhotics has little effect on circulating urea.

Comment [K8]: Via what mechanism.

Comment [K9]: Very small effect. Generally the clinical risk associated with high BUN is after tripling the concentration. Why would a 10% reduction matter. What was there explanation of why this would be significant.

Comment [K10]: Please be specific here. What are those organisms.

Comment [K11]: In what way? What was the primary effect that they measured? You are not presenting any data that

Comment [K12]: Again what was the data? Did inflammatory markers decrease post administration? What were those markers?

monitored. The preferred dose of probiotic for GI health is to be a blend of bacteria with at least 10 billion colony forming units taken daily [45].

In contrast to the results of the current study, Hyun et al. [49] illustrated that there was no significant effect of probiotics on the reduction of uraemic toxins in paediatric 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 Ca ²⁺
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±6.19 ^b	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 ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (P< 0.05).

The results of the current study demonstrated that, Adenine significantly (p <0.05) increased the concentrations of serum MDA. In addition, the oxidative stress markers serum and kidney glutathione and superoxide dismutase, serum catalase were, significantly decreased.

Our results agree with those of Anand et al., (2014) who illustrated that, Adenine caused several oxidative and inflammatory reactions in renal tissues which might leading to elevation in several oxidative and inflammatory markers such as GSH, SOD, catalase and TAC [50]. 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 results of the present study showed that, GA and LcS treatment significantly diminished the inflammatory and oxidative stress induced by adenine administration which indicated by the significant reduction of serum and renal MDA paralleled with 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.

GA was considered as an apoptosis scavenger due to its anti-oxidative and anti-inflammatory properties[36].

Our results are in agreement with those of Ghonemy et al., 2014 who confirmed apoptosis scavenger properties of GA, in addition they reported that GA also significantly reduced NGAL, NAG and cystatin C, the biomarkers of acute kidney injury [51]

Comment [K13]: What were the concentrations compared and what was the exact p value.

Comment [K14]: Include this in the beginning not the end.

UNDER PEER REVIEW

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

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, decrease of ascorbate autoxidation, 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 improve the function of biochemical reaction in biological system.

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]

It has been theorized that, probiotics exert their defensive impacts against oxidative stress by reestablishing the gut microbiota [58]. Depending on previous theory, 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) were 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]

268

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

Parameters Groups	Serum SOD	Renal SOD	Blood Reduced glutathione	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 [K15]: 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

270 Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-
271 significant difference (P< 0.05).
272

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

Parameters Groups	Serum Malondialdehyde	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

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277 Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-
278 significant difference (P< 0.05).
279
280

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

Comment [K16]: Odd usage of words.

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

Comment [K17]: What were those markers?

288

289 4. CONCLUSION

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291 In conclusion, the present research reveals many beneficial effects of GA and LcS treatment which include kidney
292 function improvement, most importantly reduction of serum urea, creatinine, Na and K. In addition, GA and LcS HAS a
293 significant antioxidant and anti-inflammatory effects. Thus, GA and LcS treatment significantly ameliorated the a toxic
294 effects associated with use of adenine.

295

296 CONFLICT OF INTEREST AND FUNDING

297 The author declares no conflict of interest

298

299 ETHICAL APPROVAL (WHERE EVER APPLICABLE)

300

301 Author hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed,
302 as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate
303 ethics committee"

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