



## **Protective Effects of Alcoholic and Aqueous Extracts of Lyophilized Kiwifruit on Acute Kidney Injury (AKI) Induced by Glycerol in Male Albino Rat**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author RMH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FKK and AAA managed the analyses of the study. Author AAA managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Objectives:** The study aimed to evaluate and compare the protective effects of alcoholic and aqueous extracts of lyophilized kiwifruit on acute kidney injury (AKI) induced by glycerol in male albino rats.

**Methods:** Rats were divided into four groups: negative control healthy group, acute kidney injury group (administered glycerol), AKI rats treated with ethanolic kiwifruit extract group, and AKI rats treated with aqueous kiwifruit extract group. Kidney biomarkers, oxidative stress, and inflammatory markers were measured and histological examination was performed.

**Results:** We observed that glycerol induced acute renal injury and as a result there was an increase in the levels of blood urea nitrogen, creatinine,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , uric acid, cystatin-C, neutrophil gelatinase-associated lipocalin, C-reactive protein, tumour necrosis factor- $\alpha$ , interleukin-6, and malondialdehyde as well as a decrease in the levels of reduced glutathione and superoxide dismutase compared to that in the negative control group. Ethanolic and aqueous kiwifruit extracts

improved all the kidney biomarkers in AKI rats. Histological examination showed acute tubular necrosis in the AKI group. However, there was an improvement in the renal tissue, represented by epithelial cell regeneration, in rats that were administered both the extracts.

**Conclusion:** Kiwifruit extracts have a positive effect on serum biochemical parameters and renal tissue, which can be beneficial in the treatment of AKI. The treatment with the ethanolic extract is more potent than with the aqueous extract.

*Keywords:* Kiwifruit; ethanolic extract; acute kidney injury; oxidative stress.

## 1. INTRODUCTION

Acute kidney injury (AKI) is a serious clinical issue that nephrologists, intensivists, general physicians, and surgeons are faced with [1]. AKI is a condition in which there is a sudden loss of kidney function, and it is associated with increased risk of death, extended hospital stays, and incidences of worsening chronic kidney disease [2]. A severe reduction and persistent decline in the kidney function is characteristic of AKI [3,4]. It results in depletion of nephrons and waste accumulation and electrolyte disruption [4,5]. This type of renal failure is reversible in most cases but can progress to chronic kidney disease (CKD) if the patient is not responsive to the treatment. The causes for AKI can mostly be of three major types—pre-renal or haemodynamic (e.g., renal hypo-perfusion), intrinsic (e.g., damage of the renal structure), and post-renal (e.g., urinary outflow blockage). Determining the cause of the disease is critical in determining effective methods for reducing the seriousness of injury. In rats, AKI induced by glycerol is a result of renal ischaemia and myoglobin nephrotoxicity. The presence of myoglobin haeme in the redox cycle induces proximal tubular cell oxidative stress and lipid peroxidation, which stimulates the secretion of a variety of mediators, including cytokines and chemokines, resulting in cortical tubular necrosis by leucocyte activation [6].

Kiwifruit is well known for its quality in terms of flavour and vitamin C content [7]. Kiwifruit is renowned for its antioxidant qualities, owing to the presence of biologically active compounds. A strong correlation between the total polyphenol content and the antioxidant activity has been identified [8]. Various studies have revealed the presence of higher polyphenol levels in kiwifruit relative to those in other fruits [9]. The fruit also contains peptides, which have both anti-inflammatory and antioxidant effects. Kiwi's popular therapeutic profile includes anti-hypertensive, antidiabetic, anti-carcinogenic, antifungal, anti-asthma, hepatoprotective, anti-

platelet, antinociceptive, anti-HIV, among other properties [10,11]. Therefore, Kiwifruit is cultivated for its nutritious advantages and beneficial medicinal properties [12,13]. Medicinal herbs have recently been used in the treatment of diseases, owing to their integrity and efficacy, as well as because of the presence of effective compounds used in the manufacture of various pharmaceutical products [14].

The effects of kiwifruit in protecting against kidney injury are not known. In the present study, we evaluated and compared the therapeutic and protective effects of alcoholic and aqueous extracts of lyophilized kiwifruit on AKI induced by glycerol in albino rats.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Freeze-dried kiwi powder was obtained from Honeyberry Company (England), and was imported from the UK. The nutritional values (per 100 g) of different components present in the powder were as follows: sugars, 52 g; proteins, 5.7 g; fat saturated, 0.0 g; fats, 3.6 g; total dietary fibre, 12.1 g; minerals, 4.1 g; sodium, 0.02 g; salt, 0.04 g; organic acids, 8.5 g; energy, 1318 kJ/308 kcal; and moisture < 5%.

### 2.2 Chemicals

Glycerol, formaldehyde solution (10% formalin), and absolute ethanol were purchased from Sigma-Aldrich (St. Louis, MO). Kits for the estimation of creatinine (Cr), uric acid (UA), blood urea nitrogen (BUN), sodium ( $\text{Na}^+$ ), chloride (Cl), and potassium ( $\text{K}^+$ ) were purchased from Centronic GmbH (Germany). Kits of cystatin-C (CYS-C), malondialdehyde (MDA; used as a lipid peroxidation marker), neutrophil gelatinase-associated lipocalin (NGAL), C-reactive protein (CRP), interleukin-6 (IL-6), superoxide dismutase (SOD), tumour necrosis factor (TNF- $\alpha$ ), and reduced glutathione (GSH)

were purchased from Bioassay Technology Laboratory (China).

## 2.3 Preparation of Kiwifruit Extracts

### 2.3.1 Aqueous extract of kiwi powder

The aqueous extract was prepared by boiling 10 g of kiwifruit powder in 100 mL of distilled water. It was left for 15 min to infuse, and then cooled and filtered to eliminate residues before use [15].

### 2.3.2 Ethanolic extract of kiwi powder

The ethanolic extract was prepared by dissolving 10 g kiwifruit powder in 100 mL of 95% methanol at room temperature (25°C) for 72 h until the soluble matter was dissolved. The mixture was then filtered. The filtrate was evaporated to dryness using a rotavapor and water bath and kept at 4°C. After complete evaporation, the deposit was dissolved in 100 mL of distilled water [15].

## 2.4 Experimental Animals

In this study, 40 adult male albino rats of 150–190 g were used. The rats were collected from the King Fahd Medical Research Center (KFMR) Animal House Colony, Jeddah, Saudi Arabia. For 1 week prior to the start of the tests, the animals were acclimated to laboratory conditions. The animals were grouped and housed in environmentally controlled (24±1°C, 45±5% relative humidity, and 12 h light/dark cycle) cages. Throughout the experiment period, the rats had access to a commercially available diet and tap water. This study complied with the recommendations of the National Research Council (NRC) on animal experiments [16].

### 2.4.1 Experimental design

In this study four groups, each composed of ten rats, were appointed. Group 1 (C) is the healthy rats (negative control) that received 1 mL of oral saline daily. Group 2 (RI) is the AKI rats (positive control), that received 25% glycerol as a first dose of 10 ml/kg.bw (body weight) after 24 h of water deprivation, a second dose was administered 3 weeks after the first. Group 3 (RI-EE) is the renal-injury rats that were administered of the ethanolic kiwifruit extract (EKE) at dose of 5 mL/kg.bw. Group 4 (RI-AE) is the renal-injury rats that were administered of the aqueous kiwifruit extract (AKE) at dose of 5 mL/kg.bw.

## 2.5 Sample Collection

At the end of the experimental duration (4 weeks), the rats were fasted overnight before collection of blood. The rats were anaesthetised with diethyl ether and blood was collected by cardiac puncture or retro-orbital bleeding. Thereafter, the rats were sacrificed by cervical dislocation. Serum was isolated by placing the blood samples at room temperature for 30 min and then centrifuging them for 20 min at 3000 rpm. The serum samples were split into multiple aliquots and stored at -20°C before the tests were performed. The kidneys of rats were removed immediately by careful dissection and washed with saline, blotted dry, and weighed separately to calculate the relative weight. The left kidneys were cut length-wise, and right kidneys were cut cross-wise for histological studies as well as for the analysis of damage score. For histopathological examinations, the kidney tissue was retained in 10% formal saline.

## 2.6 Statistical Analysis

The statistical analysis was carried out using the Statistical Package for Social Science (SPSS windows software, version 25) (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± standard deviation. One-Way ANOVA (LSD) was used to analyse the differences between the various study groups. A *P*-value ≤ 0.05 was considered to be statistically significant.

## 3. RESULTS

### 3.1 Effect of Different Treatments on Body Weight (bw) and Kidney Weight in the Treated Rat Groups

Administration of glycerol reduced the BW while increased the kidney weight significantly (*P* ≤ 0.05) in G2 (RI) as compared to G1(C). Treatment of rats with RI-EE caused a reduce significantly in body and kidney weights when compared to RI group. On the contrary, treatment of rats with RI-AE caused no significant difference in BW and kidney weights when compared to RI group. There was a significant difference in the BW and kidney weights between RI-EE and RI-AE groups. The ethanolic extract reduced the BW and kidney weights more significantly than the aqueous extract (Table 1).

### **3.2 Effect of Different Treatments on Serum Electrolytes Levels in the Treated Rat Groups**

The administration of glycerol resulted in an increase significantly ( $P \leq 0.05$ ) in serum levels of  $\text{Na}^+$ ,  $\text{Cl}$ , and  $\text{K}^+$ . Treatment of rats with either RI-EE or RI-AE caused a significant reduction in sodium, chloride, and potassium levels when compared to the RI group. The ethanolic extract reduced the serum level of all the three electrolytes in RI-EE rats more significantly than the aqueous extract in RI-AE rats (Table 2).

### **3.3 Effect of Different Treatments on Serum Kidney Function in Treated Rat Groups**

The administration of glycerol increased the serum (BUN), Cr, UA, CYS-C, and (NGAL) levels significantly ( $P \leq 0.05$ ) in RI rats compared to the corresponding levels in the control. On the other contrary, treatment with either ethanolic (RI-EE) or aqueous (RI-AE) kiwifruit extract caused a significant reduction in BUN, Cr, UA, CYS-C, and NGAL levels when compared to the levels in the RI group. The ethanolic extract reduced the serum levels of BUN, Cr, UA, CYS-C, and NGAL in the RI-EE rats more significantly than the aqueous extract in the RI-AE rats (Table 3).

### **3.4 Effect of Different Treatments on Complete Blood Count (CBC) Marker Levels in Treated Rat Groups**

The administration of glycerol in the G2 (RI) group caused a significant increase in serum white blood cells (WBCs) and platelets (PLT) levels and a significant decrease in serum red blood cells (RBCs) and haemoglobin (HB) levels ( $P \leq 0.05$ ) compared with the relevant levels in G1(C). In addition, treatment with either ethanolic (RI-EE) or aqueous (RI-AE) kiwifruit extract caused an increase significantly in the RBC count and HB levels when compared to that in the RI group. The ethanolic extract increased the serum level of RBCs significantly when compared to that in the RI-AE group (water extract). On the contrary, treatment with ethanolic or aqueous kiwifruit extract caused a significant reduction in the WBC count. The ethanolic extract decreased the serum level of PLT significantly when compared to that in the RI-AE group (water extract) (Table 4).

### **3.5 Effect of Different Treatments on Serum Levels of Oxidative Stress Biomarkers in Treated Rat Groups**

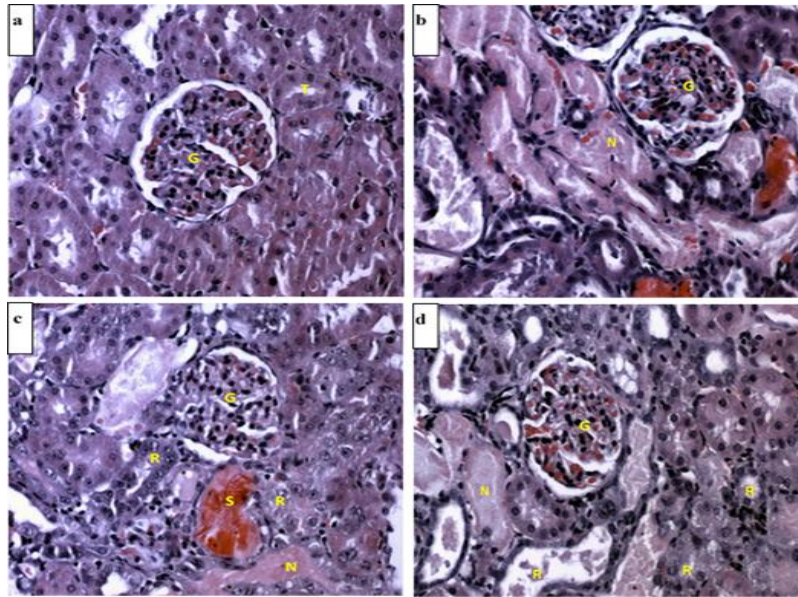
Treatment with ethanolic (RI-EE) or aqueous (RI-AE) kiwifruit extracts caused an increase significantly in serum levels of SOD and GSH as compared to the respective levels in the RI group. The ethanolic extract increased the serum level of SOD more significantly toward the normal level than the water extract. In contrast, treatment with either of the kiwifruit extracts caused a decrease significantly in serum levels of MDA as compared to the levels in the RI group (Table 5).

### **3.6 Effect of Different Treatments on Serum Levels of Inflammatory Biomarkers in Treated Rat Groups**

The results presented in Table 6 show the influence of various therapies on serum levels of IL-6, TNF- $\alpha$ , and CRP. The administration of glycerol increased the serum levels of TNF- $\alpha$ , IL-6, and CRP significantly ( $P \leq 0.05$ ) in the RI compared to that in the C group. In contrast, treatment with ethanolic RI-EE or aqueous RI-AE kiwifruit extracts caused a significant reduction in levels of TNF- $\alpha$ , IL-6, and CRP when compared to the respective levels in the RI group. There were differences statistically significant in the levels of IL-6, TNF- $\alpha$ , and CRP between the RI-EE and RI-AE groups. The serum TNF- $\alpha$  and IL-6 and CRP levels in compared to that of the RI-AE group, the RI-EE group was reduced significantly.

### **3.7 Histopathological Examination of Kidney Tissue in the Studied Groups**

Renal histopathology was essentially normal in the negative control group (C) (Fig. 1a). In contrast, rats in the positive control group (RI) showed the typical histology of acute tubular necrosis (ATN), which is characterized by extensive necrosis on along the proximal tubule in tubular cells. There were no inflammatory cell infiltrates and the glomeruli appeared normal in the RI group (Fig. 1b). The histological features were similar in the RI-EE and RI-AE groups with tubular necrosis, dystrophic calcification, intratubular cast formation, and epithelial regeneration (dilated tubular lumina, flattened epithelium, and mitotic activity, large nuclei with prominent nucleoli) (Fig. 1c and 1d).



**Fig. 1.** Histopathological examination of kidney in all the studied groups. Sections were stained with haematoxylin and eosin ( $\times 400$ ). Kidney tissue sections obtained from (a) negative control rats showed normal renal tissue architecture, characterised by an intact renal tubular epithelium and no evident pathological alteration in the glomerular or renal interstitium. In contrast, kidney sections from (b) glycerol-injected rats (positive control) showed widespread damage, evidenced by proximal tubular necrosis and epithelial vacuolisation. (c) Acute kidney injured rats treated with kiwifruit ethanolic extract and (d) acute kidney injured rats treated with kiwifruit aqueous extract showed and similar histological features as shown in (b), in addition to regenerating tubular cells and intratubular cast formation. G: glomerulus; T: tubule; N: necrotic tubule; R: regenerating tubule; S: cast formation

**Table 1.** Effect of different treatments on body weight and Kidney weight in treated rat groups

Groups	Weights (g)	Gain in body weight (g)	Kidney weight (g)
G1 (C)	180.7 $\pm$ 9.01		1.20 $\pm$ 0.04
G2 (RI)	173.8 $\pm$ 6.3 <sup>a</sup>		1.75 $\pm$ 0.18 <sup>a</sup>
G3 (RI-EE)	150.6 $\pm$ 10.4 <sup>ab</sup>		1.61 $\pm$ 0.23 <sup>ab</sup>
G4 (RI-AE)	169.3 $\pm$ 9.2 <sup>ac</sup>		1.75 $\pm$ 0.22 <sup>ac</sup>

Data are expressed as means  $\pm$  SD; a: significant versus G1; b: significant versus G2; c: significant versus G3. A P-value  $\leq$  0.05 was recognized as statistically significant. (C): control; (RI): Renal injury; (RI-EE): Renal-injury rats treated with ethanolic extract of kiwi; (RI-AE): Renal-injury rats treated with aqueous extract of kiwi

**Table 2.** Effect of different treatments on serum electrolytes levels in treated rat groups

Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
G1 (C)	142.4 $\pm$ 0.9	4.33 $\pm$ 0.24	88.86 $\pm$ 2.7
G2 (RI)	181.0 $\pm$ 2.5 <sup>a</sup>	7.76 $\pm$ 0.07 <sup>a</sup>	134.71 $\pm$ 3.7 <sup>a</sup>
G3 (RI-EE)	149.0 $\pm$ 2.0 <sup>ab</sup>	5.43 $\pm$ 0.21 <sup>ab</sup>	84.14 $\pm$ 2.8 <sup>ab</sup>
G4 (RI-AE)	166.4 $\pm$ 1.5 <sup>ab</sup>	6.26 $\pm$ 0.09 <sup>abc</sup>	115.86 $\pm$ 4.1 <sup>abc</sup>

Data are expressed as means  $\pm$  SD; a: significant versus G1; b: significant versus G2; c: significant versus G3. A P-value  $\leq$  0.05 was recognized as statistically significant. (C): control; (RI): Renal injury; (RI-EE): Renal-injury rats treated with ethanolic extract of kiwi; (RI-AE): Renal-injury rats treated with aqueous extract of kiwi

**Table 3. Effect of different treatments on serum kidney functions in treated rat groups**

Parameters Groups	BUN (mmol/L)	Creatinine (mmol/L)	Uric acid (mmol/L)	Cystatin C (ng/mL)	NGAL (ng/mL)
G1 (C)	7.40 ± 0.7	51.1 ± 4.2	73.57 ± 5.09	33.5 ± 2.4	10.97 ± 0.9
G2 (RI)	27.0 ± 1.5 <sup>a</sup>	159.9 ± 4.7 <sup>a</sup>	167.86 ± 6.03 <sup>a</sup>	144.9 ± 1.6 <sup>a</sup>	45.61 ± 1.4 <sup>a</sup>
G3 (RI-EE)	13.3 ± 1.0 <sup>ab</sup>	84.1 ± 2.2 <sup>ab</sup>	124.71 ± 3.1 <sup>ab</sup>	109.5 ± 3.9 <sup>ab</sup>	33.51 ± 1.6 <sup>ab</sup>
G4 (RI-AE)	18.9 ± 1.1 <sup>abc</sup>	121.3 ± 6.6 <sup>abc</sup>	143.86 ± 2.8 <sup>abc</sup>	124.7 ± 2.8 <sup>abc</sup>	39.29 ± 1.2 <sup>abc</sup>

Data are expressed as means ± SD; a: significant versus G1; b: significant versus G2; c: significant versus G3. A P-value ≤ 0.05 was recognized as statistically significant. (C): control; (RI): Renal injury; (RI-EE): Renal-injury rats treated with ethanolic extract of kiwi; (RI-AE): Renal-injury rats treated with aqueous extract of kiwi. LSD; Least significant difference

**Table 4. Effect of different treatments on complete blood count (CBC) marker levels in treated rat groups**

Parameters Groups	RBCs (million/ $\mu$ L)	WBCs (X 10 <sup>3</sup> / $\mu$ L)	HB (g/dL)	PLT (X 10 <sup>3</sup> / $\mu$ L)
G1 (C)	5.1 ± 0.25	5.95 ± 0.98	13.5 ± 0.6	248.0 ± 31.2
G2 (RI)	3.0 ± 0.35 <sup>a</sup>	16.7 ± 0.95 <sup>a</sup>	8.3 ± 1.0 <sup>a</sup>	362.4 ± 26.2 <sup>a</sup>
G3 (RI-EE)	5.6 ± 0.8 <sup>b</sup>	11.14 ± 1.9 <sup>ab</sup>	12.5 ± 1.5 <sup>b</sup>	318.9 ± 52.5 <sup>ab</sup>
G4 (RI-AE)	4.4 ± 1.3 <sup>bc</sup>	12.91 ± 2.9 <sup>ab</sup>	11.4 ± 2.5 <sup>ab</sup>	363.1 ± 16.4 <sup>abc</sup>

Data are expressed as means ± SD; a: significant versus G1; b: significant versus G2; c: significant versus G3. A P-value ≤ 0.05 was recognized as statistically significant. (C): control; (RI): Renal injury; (RI-EE): Renal-injury rats treated with ethanolic extract of kiwi; (RI-AE): Renal-injury rats treated with aqueous extract of kiwi.

**Table 5. Effect of different treatments on serum levels of oxidative stress biomarkers in treated rat groups**

Parameters Groups	SOD (ng/mL)	MDA (nmol/mL)	GSH (mg/L)
G1 (C)	28.77 ± 3.5	1.19 ± 0.15	434.6 ± 23.1
G2 (RI)	13.13 ± 0.2 <sup>a</sup>	3.84 ± 0.33 <sup>a</sup>	126.0 ± 20.6 <sup>a</sup>
G3 (RI-EE)	22.46 ± 3.6 <sup>ab</sup>	2.16 ± 0.71 <sup>ab</sup>	324.6 ± 19.5 <sup>ab</sup>
G4 (RI-AE)	18.16 ± 1.1 <sup>abc</sup>	3.20 ± 0.41 <sup>abc</sup>	327.4 ± 20.6 <sup>ab</sup>

Data are expressed as means ± SD; a: significant versus G1; b: significant versus G2; c: significant versus G3. A P-value ≤ 0.05 was recognized as statistically significant. (C): control; (RI): Renal injury; (RI-EE): Renal-injury rats treated with ethanolic extract of kiwi; (RI-AE): Renal-injury rats treated with aqueous extract of kiwi.

**Table 6. Effect of different treatments on serum levels of inflammatory biomarkers in treated rat groups**

Parameters Groups	IL-6 (ng/L)	TNF- $\alpha$ (ng/L)	CRP ( $\mu$ g/L)
G1 (C)	8.68 ± 1.0	54.45 ± 3.1	419.53 ± 9.4
G2 (RI)	28.33 ± 1.2 <sup>a</sup>	113.98 ± 2.2 <sup>a</sup>	516.42 ± 16.7 <sup>a</sup>
G3 (RI-EE)	17.61 ± 1.0 <sup>ab</sup>	74.73 ± 2.3 <sup>ab</sup>	462.67 ± 12.5 <sup>ab</sup>
G4 (RI-AE)	20.73 ± 0.5 <sup>abc</sup>	84.30 ± 2.9 <sup>abc</sup>	482.37 ± 10.2 <sup>abc</sup>

Data are expressed as means ± SD; a: significant versus G1; b: significant versus G2; c: significant versus G3. A P-value ≤ 0.05 was recognized as statistically significant. (C): control; (RI): Renal injury; (RI-EE): Renal-injury rats treated with ethanolic extract of kiwi; (RI-AE): Renal-injury rats treated with aqueous extract of kiwi.

#### 4. DISCUSSION

The results of the present study show the effectiveness of kiwifruit extract in protecting against glycerol-induced acute kidney injury in rats. Marked loss in body weight was observed in

response to the glycerol injection. This is consistent with the results of Albeladi [17], who showed the loss in body weight is linked to the inhibition of protein synthesis and exacerbation of catabolism by glycerol, resulting in acidosis and anorexia and consequently in reduced food

consumption. The rat groups administered the ethanolic (RI-EE) kiwifruit extracts exhibited a significant decrease in body weight when compared to the positive control (RI). This result agrees with the study of Alim et al. [18], who showed that the consumption of kiwifruit, which is rich in dietary fibre and phenolic contents, improved the lipid homeostasis. Alim et al. [18] study showed that the consumption of kiwifruit exhibits significant cholesterol-lowering effects and a reduce significantly in levels of total triglyceride (TG), and increase significantly in high density lipoprotein (HDL) concentration. However, the rat groups administered the aqueous (RI-AE) kiwifruit extracts showed no significant difference as compared to the positive control (RI) which indicates that the ethanolic method is more potent than the aqueous method in extracting the botanical components.

The rat groups administered the ethanolic (RI-EE) and aqueous (RI-AE) kiwifruit extracts exhibited a significant decrease in serum  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  levels. Similar results were found in a previous study by Svendsen et al. [19] showed that in the group consuming kiwifruit, reduced potassium consumption was associated with decrease in blood pressure (BP). Diuretic mechanism is the most important in the treatment of high BP. Diuresis removes excess sodium ( $\text{Cl}^-$  is associated with  $\text{Na}^+$ , which helps maintain fluid equilibrium when  $\text{Na}^+$  is found outside of the cells and  $\text{K}^+$  stays within the cells) and water in the form of urine through renal excretion, thereby lowering its volume of in blood circulation, increasing the dilation of blood vessels and glomerular filtration rate, and reducing the pressure on the walls of blood vessels; it also decreases the levels of renin, reactive oxygen species production, and platelet aggregation [20].

Administration of ethanolic and aqueous kiwifruit extracts to AKI-rats resulted in a significant decrease in the serum levels of BUN, Cr, UA, Cys C, and NGAL. Similar results were found in a previous study wherein it was observed that the aqueous and methanolic kiwifruit extracts resulted in a significant reduction in serum creatinine, urea, and uric acid levels [15]. Kiwi fruits are nutrient-dense and full of vitamin C. Thus, our results demonstrate the protective effect of vitamin C against nephrotoxicity. Vitamin C shows protection against nephrotoxicity by a compensatory mechanism involving the induction of antioxidant enzyme activities as a defence

against reducing reactive oxygen species and by increasing the levels nitric oxide that prevents free radical-induced cellular transformation. Vitamin C has been proposed as a potent antioxidant agent that is capable of uptake of reactive oxygen species in plasma, thereby, playing a crucial role in the preventing their entry into the cells and subsequent damage of cellular components[21].

The concurrent administration of kiwifruit and glycerol in this study resulted in a substantial decrease in nephrotoxicity by reducing the NGAL levels. NGAL is a protein expressed in convoluted renal proximal tubules, and is also regulated after renal ischaemia and injury. It detects *in vitro* and *in vivo* tubular damage and increases within 2 h of renal ischaemia; for this reason, it is considered a valid biomarker for early diagnosis of AKI and its levels are associated with the magnitude of renal damage [22].

Cystatin-c is a substitute biomarker of glomerular filtration rate (GFR) that is not effected by the quality of diet, muscle mass, gender, and age, and is better compared to serum creatinine. In renal injury, cystatin-C levels can be detected two days prior to the increase in the levels of blood urea and serum creatinine. Usually, cystatin-C is filtered by glomeruli and is then reabsorbed by proximal convoluted tubules; the increase in serum cystatin-C levels suggests harm to the glomeruli [22]. In this study, glycerol resulted in nephrotoxicity and AKI, which was attributable to decreased GFR levels and increased glomerular and tubular biomarker disruption in rats. The co-administration of kiwifruit with glycerol contributed to a significant decrease in nephrotoxicity through the amelioration of estimated GFR and reduction of blood urea.

Administration of the aqueous and ethanolic kiwifruit extracts to AKI rats resulted in a significant decrease in WBC as well as a significant increase in RBC and HB levels. The ethanolic extract decreased the serum level of PLT significantly when compared to that in the aqueous extract. These results are in agreement with those of a previous study in which it was shown that oral administration of kiwifruit induced a significant increase in RBC count and HB levels, and a significant decrease in the WBC count. This could be due to the high amounts of vitamin C in kiwifruit that may increase the

bioavailability of iron and improve the immune function [23].

We also observed a significant decrease in serum MDA levels as well as a significant increase in serum GSH and SOD levels in rats that were administered aqueous and ethanolic kiwifruit extracts. Ali and Al-Firdous [15] showed that administration of aqueous and methanolic kiwifruit extracts increased the serum SOD levels but decreased the MDA levels compared to the respective levels in the positive control as was observed in the present study. Also, this result is consistent with the finding of Shehata and Soltan [24], who showed that rats eating kiwi fruit had a substantial increase in their glutathione (GSH) content relative to that in control rats.

The effect of kiwifruits is attributable to the high concentration of antioxidants, such as beta carotene, lutein, xanthin, vitamin C, and polyphenols. A verified not only the *in vitro* but also the antioxidant role of kiwifruit *in vivo*. These findings indicate that intake of kiwifruit improves the cell-mediated immune function. Thus, consumption of kiwifruit can lower the levels of oxidative stress indicators in healthy subjects. The results suggest that daily intake of kiwifruit can potentially be effective in reducing oxidative stress and the onset of diseases. The antioxidant effects evaluated *in vitro* show that green kiwifruits have lower antioxidant effects than the gold kiwifruit but have more powerful antioxidant effects than the other fruits. The intake of kiwifruit decreased the levels of oxides, especially oxidized lipids, *in vivo*. Consequently, kiwifruit intake should inhibit the oxidation in body and can prevent the onset and deterioration of arteriosclerosis, mostly by inhibiting lipid oxidation and rapid phagocytosis by activated macrophages [9,25].

A significant decrease in serum CRP, TNF- $\alpha$ , and IL-6 levels was observed upon administration of aqueous and ethanolic kiwifruit extracts. Similar to our study, in a previous study, the inhibitory effects of kiwifruit phenolic extracts on the generation of pro-inflammatory cytokines IL-6 and TNF- $\alpha$  were shown in male mice. Increased production of mediators, such as TNF- $\alpha$  and IL-6, is linked with certain inflammatory diseases. Inflammation is controlled by multiple immune cells and mediator substances, including pro-inflammatory cytokines. Pharmacological inhibition of these mediators is a successful clinical approach for reducing inflammatory processes and the dangers of inflammation.

Macrophages perform a vital function in the immune system and produce a variety of cytokines involved in inflammatory and immune responses to harmful stimuli, such as pathogenic lipopolysaccharides [26].

Additionally, in a previous study, it was shown that in a medium inflammatory group, kiwifruit substantially decreased the plasma hs-CRP and IL-6 levels relative to that in the control groups, suggesting a reduction in total inflammation. Previous studies have shown that quercetin (found in kiwifruit), resveratrol, and epicatechin prevent nuclear factor-kappa B (NF- $\kappa$ B) stimulation. The replication factor, NF- $\kappa$ B, is a major regulator of pro-inflammatory signalling molecules, like IL-6 and CRP. Likewise, vitamins C and E present in high concentrations in kiwifruit have been shown to suppress the NF- $\kappa$ B activity [27].

Macroscopic examination of rats injected with glycerol revealed pale, bloated, and oedematous kidneys with increased weight. Oedematous kidneys could be attributed to the inhibition of cell membrane transporters by glycerol, which affects tubular reabsorption and causes cell swelling [28]. Moreover, we observed the typical histology of acute tubular necrosis (extensive necrosis of tubular cells along the proximal tubule) and minimal tubular epithelial vacuolation. However, there no regenerating tubular cells and inflammatory cell infiltrates were observed, and glomeruli appeared normal. Similar findings were also reported in a previous study by Al Asmari et al. [6], who showed that glycerol injection in rats has contributed to the kidneys enlargement, which was evidence from increase in kidneys weight / body weight ratio indicating a major toxic insult to the renal tissue. The histopathological study demonstrated major structural alterations in the kidneys of rats injected with glycerol, which include tubular dilatation, vacuolation, and necrosis.

The mechanism of renal injury caused by glycerol is not totally clear. A range of cell-mediated immune responses and inflammatory mediators are thought to be included in the pathophysiology of AKI. A substantial increase in the neutrophil-derived enzyme, myeloperoxidase, in the kidneys of glycerol-treated rats has been noticed, demonstrating vigorous neutrophil activity in the tissues. It has been shown that neutrophil build up in kidneys after an ischaemic insult is attributable to their release into the interstitium. Modification in epithelial and



endothelial cells through neutrophils contributes to kidney injuries. It was proposed that neutrophil induces kidney damage due to the superfluous secretion of proteases and oxygen radicals [6]. In addition, kidney injury induced by glycerol can be associated with elevated oxidative stress. Injection of glycerol causes the release of iron from haeme pigment myoglobin and the production of free radicals, lipid peroxidation, and changes in the structure and function of kidney [29].

In the present study, the administration of the ethanolic kiwifruit extract after the glycerol treatment, resulted in a significant reduction in kidney weight, which resulted in a significant decrease in body weight, whereas the administration of aqueous kiwifruit extract after glycerol treatment, did not result in any change in the weight of kidneys and in the body weight. Moreover, histological examination of the kidneys showed tubular necrosis, dystrophic calcification, intratubular cast formation, and epithelial regeneration (flattened epithelium, dilated tubular lumina, big nuclei with conspicuous nucleoli, and mitotic activity). However, there was no inflammatory cell infiltration and glomerular atrophy. Similar results were found in study by Mahmoud and Farag [30], who showed that administration of kiwifruit following gentamicin therapy restored the normal histology and histochemistry of kidneys, and decreased epithelial vacuolation, glomerular atrophy, necrosis of the tubules and expanded the urinary space. The ameliorative effect of kiwifruit can be related to its antioxidant activity.

It has been previously shown that the pathophysiological amelioration effects of kiwifruit are induced by the inhibition of NF- $\kappa$ B, which regulates the development of cytokines and cell survival. Studies have found that kiwifruit can modify the generation of inflammatory cytokines and mediators in inflammatory diseases. The anti-inflammatory effect of kiwifruit might be attributed to its phenolic compounds that inhibit the secretion of NF- $\kappa$ B, which controls the expression of pro-inflammatory proteins and enzymes. Also, the concomitant use of kiwi with gentamicin resulted in a decreased expression of Nrf2, the main regulator of cytoprotective responses to oxidative stress. However, electrophiles or oxidants stimulate the nuclear translocation of Nrf2 and the transcription of different antioxidants and detoxifying enzymes. This might be due to the antioxidant influence of kiwifruit and its potential to improve the synthesis

of antioxidant enzymes, that are likely to rely on the direct induction of Nrf2 as the first level of protection [28].

#### 4.1 Practical Application

Kiwifruit can potentially be used in the treatment of patients with AKI. The ethanolic extract of kiwifruit is better than its water extract.

### 5. CONCLUSION

In conclusion, the results of our study show the ameliorative effect of kiwifruit extract on glycerol-induced nephrotoxicity primarily by reducing oxidative stress, which counteracts the accumulation of free radicals and suppresses the production of inflammatory cytokines. These effects make kiwifruit a potential food that can be used to reduce complications associated with AKI. Kiwifruit extracts have a positive effect on the serum biochemical parameters, which indicates that it has a healing property for renal tissue and can be beneficial in the treatment of AKI. Treatment with ethanolic kiwifruit extract was more potent than the treatment with aqueous kiwifruit extract, which indicates that the ethanolic extract has higher concentrations of phytochemicals compared to the water extract.

### CONSENT

It is not applicable.

### ETHICAL APPROVAL

The study was accepted by the Ethical Committee of the King Abdul-Aziz University Faculty of Medicine (Ethical approval reference No. 166-19).

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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