Original Research

Fisetin Treats Kidney Oxidative Stress, Inflammation, and Apoptosis in Rat Diarrhea

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Abstract

Background: Diarrhea is the increase in the excretion of human water; meanwhile, fisetin, a bioactive flavonol molecule, is widely used in the treatment/prevention of gastrointestinal disorders. The purpose of this study is to investigate the anti-diarrheal activity of fisetin by restoring kidney function, antioxidant activity, inflammatory markers, Na^+/K^+-ATPase level, apoptosis, and protein content in diarrheal rats. **Methods**: A total of 36 male rats were distributed into two groups (18 rats/group): normal and diarrheal. The normal group was further divided into three subgroups (6 rats/subgroup): Control, fisetin, and desmopressin drug subgroups, consisting of normal rats orally treated once a day for 4 weeks with 1 mL distilled water, 50 mg/kg fisetin, and 1 mg/kg desmopressin drug, respectively. A lactose diet containing 35% lactose was fed to the normal rats for a month. The diarrheal rats were also divided into three subgroups (6 rats/subgroup): diarrheal rats, diarrheal rats + fisetin, and diarrheal rats + desmopressin drug groups, whereby the diarrheal rats were orally treated once a day for 4 weeks with 1 mL distilled water, 50 mg/kg fisetin, and 1 mg/kg desmopressin drug, respectively. **Results**: Fisetin significantly decreased serum urea (41.20 ± 2.6–29.74 ± 2.65), creatinine (1.43 ± 0.05–0.79 ± 0.06), and urinary volume (1.30 ± 0.41–0.98 ± 0.20), while significantly increasing kidney weight (0.48 ± 0.03–0.67 ± 0.07), sodium, potassium, and chloride balance in both serum and urine. Fisetin significantly increased the antioxidant activity (superoxide dismutase (1170 ± 40–3230 ± 50), glutathione peroxidase (365 ± 18–775 ± 16), catalase (0.09 ± 0.03–0.14 ± 0.06), and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase activity (8.6 ± 1.31–10.5 ± 1.25), while significantly decreasing malondialdehyde (19.38 ± 0.54–9.54 ± 0.83), conjugated dienes (1.86 ± 0.24–1.64 ± 0.19), and oxidative index (0.62 ± 0.04–0.54 ± 0.05)), alongside the inflammatory markers (tumor necrosis factor-α (65.2 ± 2.59–45.3 ± 2.64), interleukin-1β, interleukin-6 (107 ± 4.5–56.1 ± 7.2), and interleukin-10) in the diarrheal rats to values approaching the control values. Fisetin also restored the Na^+/K^+-ATPase level, apoptosis, protein content, and kidney architecture in diarrheal rats to be near the control group. **Conclusions**: Fisetin treated diarrhea in rats by restoring kidney function, antioxidant activity, inflammatory markers, apoptosis, proteome content, and histology.

Keywords: fisetin; diarrhea; sodium; potassium; oxidative stress; apoptosis

1. Introduction

Diarrhea is a gastrointestinal disease that accounts for around 5 million deaths annually worldwide [1]. An increase in human water excretion and an imbalance in the physiological functions of the small and large intestines are the main causes of diarrhea. While chronic diarrhea lasts longer than 14 days, acute diarrhea normally lasts for 14 days or less. Acute diarrhea is a symptom of acute gastroenteritis [2]. Since electrolytes are essential for preserving the body's homeostasis, disturbances in the balance of electrolytes can result in a variety of human disorders, such as diarrhea. Diarrhea leads to further electrolyte imbalance and water–electrolyte imbalance occurs. The most severe electrolyte imbalance in diarrhea includes abnormal sodium, potassium, and chloride levels [3,4]. Human malnutrition, digestive problems, and a decline in growth and productivity can all be caused by diarrhea [5,6]. It has been demonstrated in earlier studies that rats with diarrhea experience oxidative stress [1,7]. Conversely, preventing ox-

idative stress helped to alleviate rat diarrhea [8]. Oxidative stress and inflammatory cytokines are linked to diarrhea [9,10]. Therefore, newborn goats with diarrhea had increased intestinal oxidative stress, compared to the controls [11]. Moreover, inflammatory markers are elevated in diarrhea [12]. Acute renal damage is brought on by diarrhea [13]. Lactose affects food alimentary passage, which occurs through the digestive tract. The primary source of lactose is milk and dairy products, where it hydrolyzes to produce glucose and galactose [14]. Many studies have noted that consuming different levels of lactose can result in diarrhea, although no effort has been made to determine the precise lowest amount of lactose consumption that can produce diarrhea. Few research studies have been performed on the levels of lactose that cause diarrhea in humans. Robinson [15] discovered in a clinical study that diarrhea occurred when humans consumed 200–250 g of lactose. Additionally, diarrhea was reported by Koehler *et al.* [16] following the consumption of 80–120 g of lactose. Furthermore,

300 g of lactose per day tends to cause diarrhea in humans, according to a study by Rettger and Cheplin [17]. Furthermore, Kopeloff and Cohen [18] found that consuming 100 g of lactose resulted in diarrhea. After consuming lactose, people suffer from diarrhea because lactose stimulates digestive movement in people [19]. Lastly, Traube [20] found that consuming just 9–15 g of lactose in the morning on an empty stomach resulted in a mild laxative effect or mild diarrhea.

Natural herbs, such as fisetin, which is utilized in this study, are inexpensive, readily available, and harmless substances with no known side effects. Fruits and vegetables, such as strawberries, apples, persimmons, grapes, onions, and cucumbers all contain the bioactive flavonol molecule known as fisetin [21]. The average daily intake of fisetin was calculated to be 0.4 mg, while fisetin is most abundant in strawberries (160 g/g), apples (26.9 g/g), and persimmons (10.5 g/g) [22]. Fisetin has antiproliferative [23], lipid-lowering [24], apoptotic [25], and antioxidant [26] effects, and it is found in a variety of foods that are consumed by humans. Fisetin is used to treat psoriasis and other inflammatory skin diseases because it inhibits the interleukin pathway and increases keratinocyte differentiation, in addition to autophagy in mice [27]. Fisetin possesses anticancer, anti-inflammatory, and antioxidant properties. It prevents the division of caspase-9 and caspase-8 during cell division; it also induces apoptosis and prevents the rapid growth, invasiveness, and metastasis of tumors [28]. Fisetin protects the liver by increasing glutathione levels and decreasing inflammatory markers [29]. Moreover, it inhibits the vascular endothelial growth factor, thereby reducing angiogenesis in diabetic retinopathy [30]. Additionally, fisetin improves neuronal protection and treats impairments in cognition [31]. Over the last forty years, there have been significant attempts to decrease the high rates of morbidity and death linked to diarrheal diseases. One such attempt has been to discover natural herbs that can alleviate diarrhea, particularly herbs that contain medicinal ingredients to prevent oxidative stress, which is linked to intestinal disturbance. This study postulated that fisetin can alleviate oxidative stress and improve the inflammatory state of rats with diarrhea. Therefore, the purpose of this study was to investigate the anti-diarrheal activity of fisetin by treating kidney function and weight, urinary volume, electrolyte balance in serum and urine, antioxidants, inflammatory markers, Na^+/K^+-ATPase level, apoptosis, and the kidney tissue in male rats with diarrhea.

2. Materials and Methods

2.1 Animals

Thirty-six male albino Sprague-Dawley (145 ± 10 g, 12 weeks old) strain rats were purchased from the National Research Centre's animal house in Dokki, Giza, Egypt. The animals were housed in experimental plastic cages and provided drinking tap water and a commercially available rat diet. The investigation was carried out after receiving approval from the National Research Centre's ethical committee in Egypt (approved number 12032627), and in compliance with the guidelines for the appropriate treatment and use of laboratory animals as outlined in NIH publication no. 85:23, updated 1985.

2.2 Materials

Fisetin (CAS No.: 528-48-3; purity: 98%; yellow-brown powder; IUPAC name: 2-(3,4-Dihydroxyphenyl)-3,7-dihydroxy-4H-1-benzopyran-4-one) was purchased from NutriHerb Company (BioTech and Pharmaceutical Valley, Nanjing, 210000, China). Nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) (IUPAC name: [(2R,3R,4R,5R)-5-(6-aminopurin-9-yl)-3-hydroxy-4-phosphonooxyoxolan-2-yl]methoxy-hydroxyphosphoryl), EGTA (IUPAC name: ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid), lubrol (IUPAC name: 2-dodecoxyethanol), potassium phosphate monobasic (KH_2PO_4; IUPAC name: Potassium dihydrogen phosphate), and lucigenin (9, 9′-bis[N-methyl acridinium nitrate]; IUPAC name: 10,10′-dimethyl[9,9′-biacridine]-10,10′-diium dinitrate) were all purchased from Sigma-Aldrich Company in the United States. Desmopressin (1-deamino-8-D-arginine vasopressin, ddAVP; IUPAC name: (2S)-N-[(2R)-1-[(2-amino-2-oxoethyl)amino]-5-(diaminomethylideneamino)-1-oxopentan-2-yl]-1-[(4R,7S,10S, 13S,16S)-7-(2-amino-2-oxoethyl)-10-(3-amino-3-oxopropyl)-16-[(4-hydroxyphenyl) methyl]-6,9,12,15,18-pentaoxo-13-(phenylmethyl)1,2-dithia-5,8,11,14,17-pentazacycl oicosane-4-carbonyl] pyrrolidine-2-carboxamide) was used as a common antidiuretic drug and was bought from El-Kahera Pharmaceutical Industrial Company in Egypt. All of the kit reagents used in the biochemical analysis of the study was procured from Bio-diagnostics Company in the United Kingdom, via a regional Egyptian source.

2.3 Experimental Design

Depending on the previous study [32], five doses (10, 20, 30, 40, and 50 mg/kg) were tested in this study. All these doses were orally administrated separately to diarrheal rats (6 rats/group) for 2 weeks before blood serums were obtained from the rats to evaluate kidney functions. The oral dose of fisetin utilized in the prior study was 50 mg/kg. In order to support and validate this dosage, the authors of this study started with a fisetin dose of 10 mg/kg and continued to increase the dose until they reached 50 mg/kg. Then, they gathered data from these doses to determine the suitable dosage for the investigation. A fisetin dose of 50 mg/kg was considered the best concentration as determined by the resulting kidney function.

Based on the previous study, 36 male rats were divided into 6 groups with 6 rats in each group. Control group: Normal animals were administered 1 mL of distilled

water orally. Fisetin-treated group: Normal animals were administered 50 mg/kg of fisetin dissolved in 1 mL distilled water orally. Desmopressin-treated group: Normal animals were administered 1 mg/kg of the desmopressin [33] drug dissolved in 1 mL distilled water orally. Diarrheal group: Diarrheal rats were administered 1 mL of distilled water orally. Diarrheal group + fisetin-treated group: Diarrheal rats were administered fisetin (50 mg/kg) dissolved in 1 mL distilled water orally. Diarrheal group + desmopressin drug-treated group: Diarrheal rats were administered 1 mg/kg of desmopressin drug dissolved in 1 mL distilled water orally.

These treatments were received once a day by oral gavage for 4 weeks. The animals were observed carefully during the whole study period for any abnormal physical and clinical signs, such as hair loss, skin patch formation, convulsions, and any mortality during the experimental study.

2.4 Induction and Assessment of Diuretic Activity

The experimental animals in the study were divided into two equal (normal and diarrheal) groups. The rats with diarrhea were fed a rat diet containing lactose for a month in order to induce diarrhea, whereas the normal animals were fed a standard rat diet. The diuretic activity of the animals was detected using a previous method by Kau *et al*. [34]. All rats were kept in the metabolic cage for 7 days before the research protocol started to allow the animals to adapt to their surroundings. All of the rats received saline (0.9% NaCl) solution orally prior to the experiment. The rats were administered a lactose diet (3000 calories; Purina Mills; Richmond, IN, USA) that included 35% lactose instead of starch. Changes in the consistency of the urine volume by the rats were observed. If rats produced 50% more urine than the control group, they were considered to be diarrheic rats [35,36]. Urinary volume was collected and assessed after the research study period. Additionally, the levels of urinary Na^+, K^+, and Cl^- ions were measured as mmol/L.

2.5 Experimental Procedure
2.5.1 Serum Collection

Through the retro-orbital plexuses, capillary tubes containing ethylenediamine tetra-acetic acid were used to collect the blood samples. Centrifugation was used to produce the serum (6000 g at 4 °C), which was then frozen at –80 °C in a deep freezer. The levels of Na^+, K^+, and Cl^- ions in the serum and urine were measured using the techniques described by Jooste and Strydom [37], Wang *et al*. [38], and Hassan *et al*. [39], respectively. According to the protocols outlined by Orsonneau *et al*. [40] and Myre *et al*. [41], kidney function was determined alongside serum urea and creatinine levels, respectively. Following the manufacturer's instructions, commercial spectrophotometric kits were used to detect all the above biochemical parameters.

2.5.2 Kidney Tissue Preparation

All of the rats were administered a diethyl ether solution anesthesia in the final step of the experimental study. Kidneys were obtained and the kidney tissue was cleaned using saline. Filter papers were utilized to dry the kidney tissue. The kidney tissue was separated into two parts; the first part of the kidney tissue was first dissolved in 2.5 mL of Tris buffer solution. After that, these tissues were homogenized using an automated homogenizer at room temperature for ten minutes. The kidney tissue was centrifuged at –4 °C and 7000 rpm for 15 minutes to obtain the supernatant, which was used to evaluate the biochemical parameters. The second part of the kidney tissue was utilized for histological examination.

2.6 Biochemical Analysis
2.6.1 Kidney Antioxidants Detection

The Suttle method [42] was used to estimate the superoxide dismutase (SOD) enzymatic activity. This technique relies on the enzyme's capacity to prevent phenazine methosulphate (PMS) from reducing nitroblue tetrazolium dye (NBT). The Paglia and Valentine [43] approach was used to identify glutathione peroxidase (GPx) activity. Tissue homogenate was added to a solution containing glutathione reductase, glutathione, and NADPH, to assess GPx. Oxidized glutathione is created when GPx reduces organic peroxide and is recycled to its reduced state by glutathione reductase. Substrates and hydrogen peroxide are added to start the enzyme reaction, which is then detected at 340 nm. The Aebi [44] technique was used to measure the catalase (CAT) activity. The basic core of this method depends on combining CAT and H_2O_2 together to form 4-aminophenazone and 3,5-dichloro-2-hydroxybenzene sulfonic acid, while a chromophore is produced, and the color intensity of the chromophore is inversely proportional to the level of CAT present in the sample. Using the Ohkawa *et al*. [45] technique, malondialdehyde (MDA) levels were also measured as a marker of lipid peroxidation. The basic core of this method is thiobarbaturic acid, which reacts with MDA in the presence of an acidic medium at 59 °C, to form thiobarbaturic acid reactive (pink solution). Spectrophotometric measurements of these antioxidants were completed following the commercial indicators and the manufacturer's steps.

According to the method by Kogure *et al*. [46], conjugated dienes (CD) were made by adding kidney homogenate (0.01 mg of protein) to 1 mL of 10 mmol/L phosphate buffer (pH 7.4) with 1% lubrol. The absorbance ratio A_{233}/A_{215} (oxidative index) was measured by a spectrophotometer to calculate the production of conjugated dienes [47,48].

A lucigenin-enhanced chemiluminescence test was used to measure NADPH oxidase activity to assess NADPH oxidase-mediated superoxide radical (O_2^-) generation [49]. Lucigenin (5 mol/L) was added following the addition

of NADPH (0.1 mmol/L) to the kidney homogenate (250 μL), which already included phosphate buffer (50 mmol/L KH$_2$PO$_4$, 1 mmol/L EGTA, 150 mmol/L sucrose, pH 7.4). A Tecan Infinite M200 multimode microplate fluorometer was used to measure the luminescence at 30 °C every 5 seconds for 10 minutes. NADPH oxidase activity is reported as mg/mg protein [50].

2.6.2 Kidney Inflammatory Markers Determination

The technique by Matalka *et al*. [51] was used to assess the tumor necrosis factor-α (TNF-α). The scheme reported by DeCicco *et al*. [52] was used to determine interleukin-1β (IL-1β) levels. Interleukin-6 (IL-6) and interleukin-10 (IL-10) were detected by following the Stelmasiak *et al*. [53] technique. Following the instructions provided by the manufacturer, ELISA kits were utilized to measure every inflammatory marker. This method depends on using the tested parameter's antibody to coat the microtiter, thereby allowing the parameter to be quantitatively evaluated. The tested sample was combined with the labeled conjugate solution to produce the antibody–antigen–enzyme–antibody mixture. Subsequently, a substrate solution was added to the above mixture to form a product that provides the mixture with a blue color. Finally, a stop solution was added to terminate the reaction. At 450 nm, the color change was noticeable, and the standard curve was used to calculate the tested sample's absorbance.

2.6.3 Kidney Sodium/Potassium-ATPase Determination

A solution containing 80 mM NaCl, 20 mM KCl, 5 mM MgCl$_2$, 50 mM Tris HCl (pH 7.4), and 3 mM ATP disodium salt was produced to identify the sodium/potassium-ATPase activity. The reaction was ceased by adding 50 mL of trichloroacetic acid after incubating at 37 °C with 50 mL of homogenate for 10 minutes. The mixture was centrifuged for 15 minutes at 3000 rpm before 250 mL of ammonium molybdate, 500 mL of trichloroacetic acid, and 250 mL of ascorbic acid were prepared in 1 mL of the kidney supernatant. The resulting color was measured at 680 nm by a spectrophotometer [54].

2.7 Molecular Analysis

2.7.1 Kidney DNA Damage Investigation

DNA fragmentation analysis was performed by agarose gel electrophoresis. Following earlier studies [55, 56], DNA was extracted. A Tris–EDTA buffer (pH 8.0) was used to dissolve the DNA. Tris-boric acid–EDTA buffer (pH 8.3) was used to electrophorese the DNA samples on a 0.7% agarose gel at 40 volts for 4 hours. DNA damage was observed following ethidium bromide staining. UV-transillumination was used to observe the bands (version number:10016027 Rev C US/EG, lot number: 10-0082 0100 Sig 1109, Bio-Rad Laboratory, Hercules, CA, USA) and photograph them.

2.7.2 Analysis Using Gas Chromatography–Mass Spectrometry (GC–MS)

2.7.2.1 Preparing Kidney Tissue for GC–MS Analysis. Firstly, 2 mL of freshly made solution (including 2 mL each of water, methanol, and chloroform) and 1 mL of kidney tissue were added. To make an internal standard, 10 milliliters of alanine-d$_4$ and 10 milliliters of ribitol were subsequently added. Afterward, the solution was centrifuged at 12,300 g for 10 minutes. Next, 10 milliliters of the supernatant were immediately exposed to 37 °C nitrogen gas until it dried. Then, 5 milliliters of a methoxyamine hydrochloride solution was added, and the mixture was kept at 37 °C for 2 hours. Then, 50 milliliters of N,O-bistrimethyl silyltrifluoro-acetamide (BSTFA) was added and maintained at 37 °C for 4 hours. Next, the resulting solution was dried at 37 °C for 24 hours.

2.7.2.2 GC–MS for the Identification of Kidney Proteins. Hewlett-Packard GC equipment 6890 was used to detect the different kidney proteins. The Enhanced ChemStation Version was the instrument utilized. The capillary column utilized was an Agilent HP-5MS UI (30 m 0.25 mm, coated with 5% diphenyl and 95% dimethylpolysiloxane). Helium gas was used as a carrier gas at a rate of 1 mL/min. The injection temperature was set to 250 °C. The column temperature was initially set to 80 °C for 3 minutes before it was raised to 280 °C at a rate of 10 °C/min for 5 minutes. The ion source, MS quadruple, and MS transfer line were arranged at 230 °C, 150 °C, and 280 °C, respectively.

2.8 Kidney Tissue Examination Using Histopathology

Kidney specimens were prepared for routine embedding in paraffin after being preserved in a 10% formalin solution. For histological analysis, hematoxylin and eosin solution was used to stain the blocks after they were sectioned at a thickness of 5 μm.

2.9 Statistical Evaluation

The findings were displayed in tables as mean ± standard error mean (SEM). To ascertain the differences between the various groups, a one-way analysis of variance (ANOVA) test was conducted using the SPSS 13 software (SPSS Incorporation's headquarters located in Chicago in the United States). The Fisher least significant difference (FLSD) test was applied as the *post hoc* analysis to compare the differently used groups. Statistical significance was attained at $p \leq 0.05$.

3. Results

3.1 Body Weight and Organs Results

In comparison to the control group, the body weight, food intake, weight of the spleen, liver, pancreas, and heart, as well as fecal pellet count in the diarrheal rats were significantly decreased, although an increase in water intake was observed (Table 1). Treatment with either fisetin or desmo-

Table 1. Effect of fisetin on body weight and organs of normal and diarrheal rats.

Parameters	Control	Fisetin	Desmopressin drug	Diarrheal rats	Diarrheal rats + fisetin	Diarrheal rats + desmopressin
Total body weight (g)	165 ± 6.24	167 ± 6.13	164 ± 6.52	128 ± 5.09^a	163 ± 6.24^b	162 ± 6.24^b
Food consumption (g/day)	11.6 ± 1.2	11.4 ± 1.4	11.5 ± 1.6	7.2 ± 1.3^a	11.3 ± 1.6^b	11.2 ± 1.5^b
Water intake (mL/day)	12.4 ± 1.7	12.6 ± 1.5	12.3 ± 1.2	17.8 ± 1.3^a	12.2 ± 1.7^b	12.1 ± 1.7^b
Liver weight (g/100 g bw)	2.7 ± 0.08	2.6 ± 0.06	2.5 ± 0.09	1.8 ± 0.05^a	2.4 ± 0.08^b	2.3 ± 0.08^b
Pancreas weight (g/100 g bw)	0.25 ± 0.03	0.27 ± 0.02	0.24 ± 0.04	0.16 ± 0.02^a	0.23 ± 0.03^b	0.22 ± 0.03^b
Spleen weight (g/100 g bw)	0.34 ± 0.06	0.33 ± 0.04	0.36 ± 0.05	0.21 ± 0.03^a	0.32 ± 0.05^b	0.31 ± 0.07^b
Heart weight (g/100 g bw)	0.36 ± 0.04	0.35 ± 0.06	0.38 ± 0.05	0.23 ± 0.03^a	0.34 ± 0.03^b	0.33 ± 0.03^b
Fecal pellet count	38 ± 4.07	37 ± 3.86	36 ± 4.20	25 ± 3.14^a	35 ± 4.35^b	34 ± 4.26^b

There were six rats in each group. The data are shown as mean $\pm$ SEM. ^{a}Highly significant change ($p \leq 0.01$) compared to the control. ^{b}Highly significant change ($p \leq 0.01$) compared to diarrheal rats.

Table 2. Effect of fisetin on kidney function, electrolytes, and weight in normal and diarrheal rats.

Groups	Serum electrolytes levels		Serum urea and creatinine levels		Kidney weight (g/100 g bwt)	Serum chloride (mmol/L)
	Sodium (mmol/L)	Potassium (mmol/L)	Urea (mg/dL)	Creatinine (mg/dL)		
Control	152.32 ± 4.64	5.58 ± 0.26	24.81 ± 2.71	0.82 ± 0.07	0.69 ± 0.05	104.29 ± 2.35
Fisetin	150.46 ± 3.85	5.56 ± 0.31	24.75 ± 2.39	0.81 ± 0.05	0.70 ± 0.07	102.87 ± 2.46
Desmopressin drug	151.71 ± 4.53	5.59 ± 0.27	24.84 ± 2.80	0.80 ± 0.06	0.67 ± 0.09	105.12 ± 2.18
Diarrheal rats	98.76 ± 4.03^b	2.52 ± 0.34^b	41.20 ± 2.67^b	1.43 ± 0.05^b	0.48 ± 0.03^b	79.61 ± 1.65^b
Diarrheal rats + fisetin	151.8 ± 3.75^c	5.19 ± 0.24^c	29.74 ± 2.65^c	0.79 ± 0.06^d	0.67 ± 0.07^d	$102..8 \pm 2.41^c$
Diarrheal rats + desmopressin drug	153.1 ± 3.50^d	5.49 ± 0.32^d	25.26 ± 3.59^d	0.81 ± 0.04^d	0.71 ± 0.06^d	103.5 ± 1.86^d

There were six rats in each group. The data are shown as mean $\pm$ SEM. Statistical analysis was a one-way Analysis of Variance (ANOVA) test. $^b p \leq 0.01$ highly significant change compared to the control group. $^c p \leq 0.05$ significant change compared to the diarrheal group. $^d p \leq 0.01$ highly significant change compared to the diarrheal group.

Table 3. Effect of fisetin on urinary volume and electrolyte excretion in normal and diarrheal rats.

Group	Urinary volume (mL/100 g/8 h)	Diuretic index	Urinary sod. (mmol/L)	Urinary pot. (mmol/L)	Saluretic index Sod.	Pot.	Urinary sod./pot.	Urinary chlor.
Control	0.96 ± 0.19	–	98.52 ± 6.28	56.14 ± 2.90	–	–	1.75	7.61 ± 0.45
Fisetin	0.94 ± 0.23	–	96.94 ± 5.73	55.68 ± 2.83	–	–	1.74	7.59 ± 0.58
Desmopressin drug	0.97 ± 0.18	–	97.64 ± 5.48	57.14 ± 2.69	–	–	1.70	7.60 ± 0.39
Diarrheal rats	1.30 ± 0.41^b	1.35	130.41 ± 7.20^b	75.65 ± 3.12^b	1.32	1.35	1.72	5.28 ± 0.27^b
Diarrheal rats + fisetin	0.98 ± 0.20^c	1.08	103.74 ± 5.39^c	60.13 ± 2.79^d	1.05	1.07	1.73	6.87 ± 0.59^c
Diarrheal rats + desmopressin drug	0.97 ± 0.36^c	1.05	101.26 ± 4.80^d	59.07 ± 2.24^d	1.03	1.05	1.71	6.98 ± 0.43^c

There were six rats in each group. The data are shown as mean $\pm$ SEM. Statistical analysis was a one-way ANOVA test. $^b p \leq 0.01$ highly significant change compared to the control group. $^c p \leq 0.05$ significant change compared to the diarrheal group. $^d p \leq 0.01$ highly significant change compared to the diarrheal group. Diuretic index: Urine volume of the treated group/urine volume of the control group. Saluretic sodium/potassium index: Urinary sodium/potassium of the treated group/urinary sodium/potassium of the control group. Sod., sodium; Pot., potassium; Chor., chloride.

pressin drug restored the above-mentioned body weight and organs to levels close to the control. There were no rat mortalities throughout the study's experimental period and none of the groups experienced skin patches, convulsions, or hair loss.

3.2 Kidney Function and Weight and Serum Electrolytes Results

Table 2 shows serum Na^+, K^+, and Cl^- ion levels as well as kidney function and weight in the normal and diarrheal groups. The information in the table makes it evident that the levels of serum Na^+, K^+, and Cl^- ions were significantly decreased. ($p \leq 0.01$) but serum urea and creatinine were significantly increased ($p \leq 0.01$), whereas kidney weight was significantly declined ($p \leq 0.01$) in the diarrheal group compared to the control group. All the aforementioned parameters were approached to the control levels by oral treatment of fisetin or desmopressin drug to diarrheal rats. Furthermore, normal animals administered either fisetin or desmopressin did not show any alterations in these parameters during the study's experimental period.
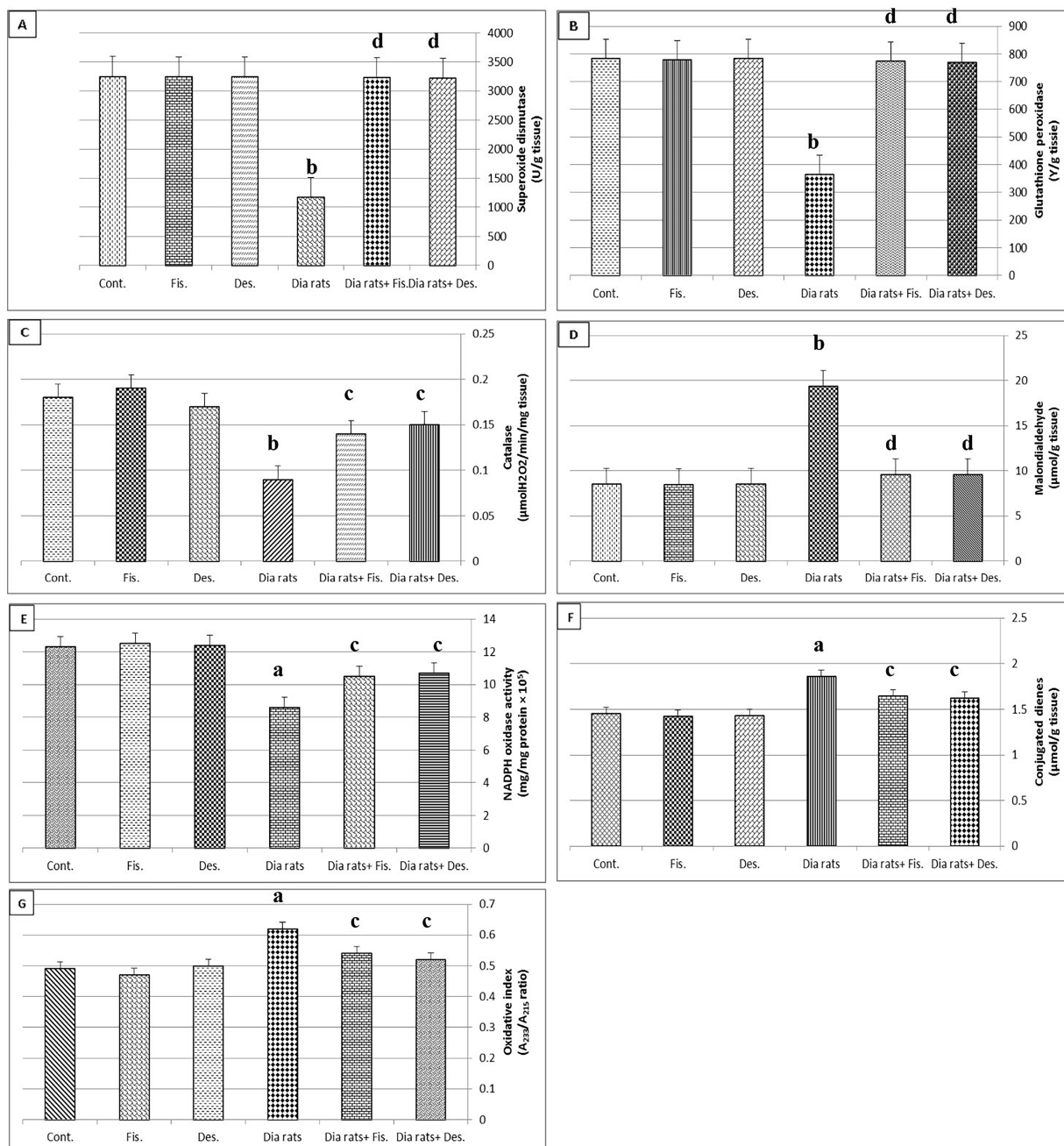
Fig. 1. Effect of fisetin on antioxidant levels in the kidneys of normal and diarrheal rats. There were six rats in each group. The data are shown as mean $\pm$ SEM. Statistical analysis was a one-way ANOVA test. (A) Superoxide dismutase (U/g tissue), (B) Glutathione peroxidase (γ/g tissue), (C) Catalase (μmolH$_2$O$_2$/min/mg tissue), (D) Malondialdehyde (μmol/g tissue), (E) Nicotinamide adenine dinucleotide phosphate (NADPH) activity (mg/mg $\times 10^5$), (F) Conjugated dienes (μmol/g tissue), and (G) Oxidative index (A$_{233}$/A$_{215}$ ratio). $^{a}p \leq 0.05$ significant change compared to the control group. $^{b}p \leq 0.01$ highly significant change compared to the control group. $^{c}p \leq 0.05$ significant change compared to the diarrheal group. $^{d}p \leq 0.01$ highly significant change compared to the diarrheal group. Cont., control; Fis., fisetin; Des., desmopressin drug; Dia rats, diarrheal rats; Dia rats + Fis., diarrheal rats + fisetin; Dia rats + Des., diarrheal rats + desmopressin drug.

3.3 Urinary Volume and Electrolytes Results

Table 3 displays urine volume and urinary Na$^+$, K$^+$, and Cl$^-$ ion levels in normal and diarrheal rats. The data refer to a significant decline ($p \leq 0.01$) in the urinary volume and urinary Na$^+$, K$^+$, and Cl$^-$ ion levels. However, a significant increase ($p \leq 0.01$) in urine was observed in the diarrheal rats compared to the control group. All the aforementioned parameters were returned to levels closer to the control values when the diarrheal rats were treated with either fisetin or the drug desmopressin orally. Addi-

tionally, normal rats treated with oral doses of either fisetin or desmopressin drug did not exhibit any changes in these parameters during the duration of the study.

3.4 Kidney Antioxidant Results

The fisetin effects on kidney antioxidants in normal and diarrheal groups are shown in Fig. 1. It is obvious that diarrhea caused, in comparison to the control group, a highly significant increase ($p \leq 0.01$) in the levels of conjugated dienes, oxidative index, and malondialdehyde, yet a highly significant decrease ($p \leq 0.01$) in the activities of superoxide dismutase, glutathione peroxidase, catalase, and NADPH oxidase. Additionally, the oral administering of either fisetin or desmopressin drug to the diarrheal group caused the results of the aforementioned antioxidant tests to approach control values. Furthermore, normal rats provided oral doses of either fisetin or the desmopressin drug showed no change in any of the antioxidants.

3.5 Kidney Inflammatory Markers Results

The impact of fisetin on the inflammatory markers in the kidneys of normal and diarrheal groups is presented in Fig. 2. Diarrhea produced a highly significant decrease ($p \leq 0.01$) in interleukin-10 levels but a highly significant increase ($p \leq 0.01$) in tumor necrosis factor-α, interleukin-1β, and interleukin-6 levels, compared to the control group. Compared to diarrheal rats, oral administration of fisetin or the desmopressin drug caused the inflammatory markers to approach control values. Additionally, neither oral administration to normal rats of the desmopressin drug nor fisetin changed any of the above markers.

3.6 Kidney Na$^+$/K$^+$-ATPase Results

The effect of fisetin on Na$^+$/K$^+$-ATPase levels in the kidneys of the normal and diarrheal groups is shown in Fig. 3. It is clear from this figure that diarrhea induced a highly significant decrease ($p \leq 0.01$) in sodium/potassium-ATPase activity compared to the control group. Furthermore, when given orally to diarrheal rats, fisetin or the desmopressin drug caused the sodium/potassium-ATPase activity indicated above to begin to approach control values, compared to the diarrheal rats. Additionally, during the study period, the normal rats that were provided fisetin or the desmopressin drug orally did not have significant changes in the sodium/potassium-ATPase activity.

3.7 Kidney DNA Damage Results

Fig. 4 shows how fisetin affected diarrheal rats by stopping DNA damage in the kidneys of the diarrheal rats. Kidney DNA from diarrheal rats was fragmentation in comparison with the control group. In diarrheal rats, fisetin therapy significantly reduced kidney DNA damage, whereas fisetin treatment in normal rats showed no differences from the control group in kidney DNA electrophoretic patterns.

3.8 Kidney Proteins Based on GC–MS Data

Fig. 5 displays the results of the GC–MS analysis for all kidney proteins. The vertical axis (pixel intensity) represents the intensity of the protein, while the horizontal axis (pixel position) represents the location of the protein. The eight kidney proteins found in this study are alpha-1 fetoprotein, alpha-2 macroglobulin, beta-2 microglobulin, transferrin, albumin, globulin, prothrombin, and fibrinogen. The control group exhibited all eight kidney-associated proteins (Fig. 5A). The prothrombin and fibrinogen protein bands disappeared in the diarrhea group, which only contained six kidney proteins (Fig. 5B). When fisetin was administered orally to diarrheal rats (Fig. 5C) at a dose of 50 mg/kg per day for four weeks, all eight kidney proteins return in the treated animals, similar to in the control group.

3.9 Histology Findings

The control group undergoes normal renal development, as shown in Fig. 6A. However, diarrhea led to a clear and widespread necrosis. Additionally, diarrhea leads to vacuolar degeneration and dilatation. Particularly in the proximal tubules of the kidneys, it caused intraluminal group creation and epithelial desquamation (Fig. 6B,C). In the diarrheal group orally treated with fisetin, the kidney tissue damage brought on by diarrhea was totally reversed, with the group resembling the control group (Fig. 6D).

4. Discussion

The mechanism of action of lactose to induce diarrhea includes intestinal disorder accompanied by renotoxicity (extra-intestinal pathology). Several studies revealed that several chemical agents (alcohol/acetic acid) have induced intestinal pathologies (gastrointestinal ulcer and intestinal colitis) and extra-intestinal alterations (hepato-nephrotoxicities). Increased tissue and plasma hydrogen peroxide, calcium, and free iron levels are indicative of alcohol- or acetic acid-induced liver and kidney injuries, as is liver and kidney lipoperoxidation and the loss of antioxidant enzyme activities, such as superoxide dismutase, catalase, and glutathione peroxidase in the liver and kidney. Increased levels of inflammatory markers and disruptions of intracellular mediators were brought on by alcohol or acetic acid. Consequently, oxidative stress and inflammation were brought on by alcohol in rat organs [57,58]. There are two potential ways that lactose causes diarrhea: (1) it directly irritates the intestinal musculature, stimulating the muscles and causing the gastrointestinal tract to contract more forcefully or frequently [59]; (2) it causes a laxative substance known as a hydragogue, which is a material that causes a watery purgation. Hydragogues work by raising the osmotic pressure of the gut above that of the blood because many of their ions or molecules stay inside the gut lumen due to their delayed absorption from the intestine. Thus, water diffuses from the circulation into the intestinal lumen. This extra
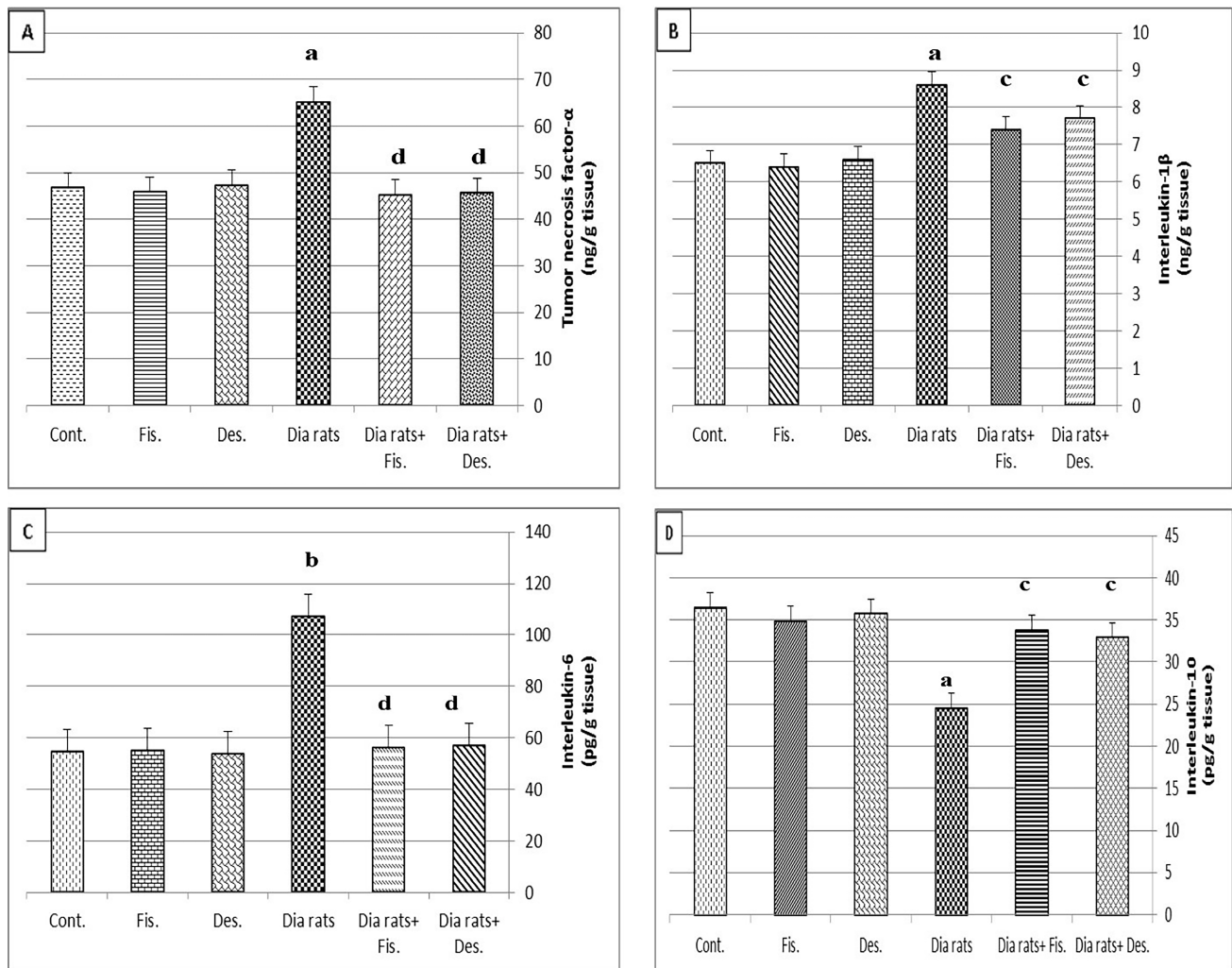
Fig. 2. Effect of fisetin on inflammatory markers in the kidneys of normal and diarrheal rats. There were six rats in each group. The data are shown as mean $\pm$ SEM. Statistical analysis was a one-way ANOVA test. (A) Tumor necrosis factor-α (ng/g tissue), (B) Interleukin 1β (ng/g tissue), (C) Interleukin-6 (pg/g tissue), and (D) Interleukin-10 (pg/g tissue). $^a p \leq 0.05$ significant change compared to the control group. $^b p \leq 0.01$ highly significant change compared to the control group. $^c p \leq 0.05$ significant change compared to the diarrheal group. $^d p \leq 0.01$ highly significant change compared to the diarrheal group. Cont., control; Fis., fisetin; Des., desmopressin drug; Dia rats, diarrheal rats; Dia rats + Fis., diarrheal rats + fisetin; Dia rats + Des., diarrheal rats + desmopressin drug.

water washes the intestinal system and dilates the intestinal walls, which causes muscle contractions [17,60].

Compared to the control group, diarrhea significantly increased kidney function levels. Moreover, urine and urinary electrolyte excretion increased significantly as a result of diarrhea, in contrast, a very noticeable drop in serum electrolytes and kidney weight following diarrhea. However, oral fisetin or desmopressin drug treatment in diarrheal rats caused the aforementioned factors to return to normal levels. This result is related to the nephroprotective properties of fisetin. Conversely, desmopressin treatment prevented chronic hyponatremia by avoiding excessive urinary water losses, meaning hyponatremia (drop in serum sodium) did not occur in the desmopressin-treated rats because desmopressin corrected the severe hypona-

tremia [61,62]. Desmopressin reduces the rate of change in the plasma sodium levels and corrects hyponatremia [63,64]. In addition, the use of fisetin reduced uricemia, declined the renal inflammatory response, and enhanced renal fibrosis [31]. Furthermore, Ren *et al*. [65] stated that fisetin inhibited the development of chronic kidney disease brought on by hyperuricemia by regulating the role of the gut microbiota in tryptophan metabolism and aryl hydrocarbon receptor activation. Furthermore, according to Prem and Kurian [66], fisetin pretreatment reduces renal injury by enhancing mitochondrial activity, enhancing renal function, lowering renal injury caused by apoptosis, and reducing free radical emission.

In the kidneys of diarrheal rats, where inflammation and oxidative state are associated with diarrhea [40], diar-
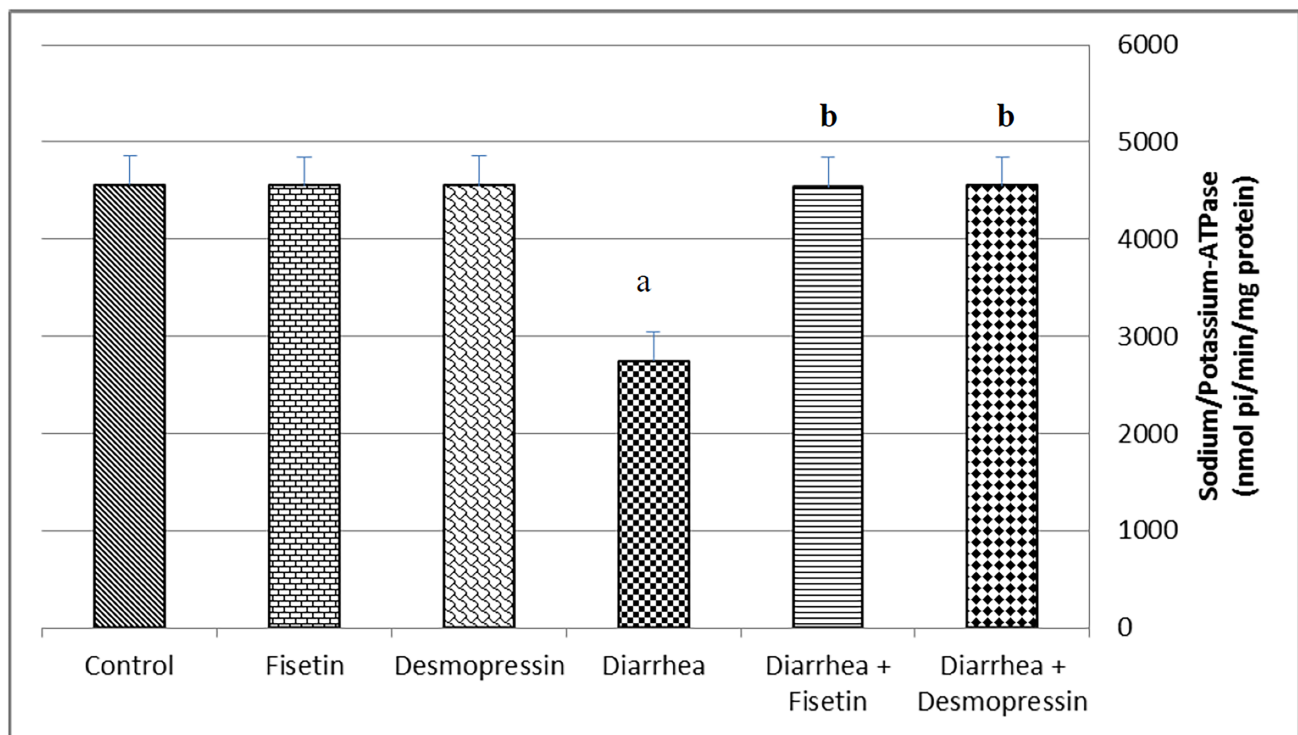
Fig. 3. Effects of desmopressin and fisetin on the sodium/potassium ATPase levels in the kidneys of rats with diarrhea and normal rats. Diarrhea + Fisetin., diarrheal rats after oral administration with fisetin; Diarrhea + Desmopressin., diarrheal rats after oral administration with desmopressin drug. There were six rats in each group. The data are shown as mean $\pm$ SEM. [a]Significant change ($p \leq 0.01$) in relation to the control. [b]Significant change ($p \leq 0.01$) from rats with diarrhea.
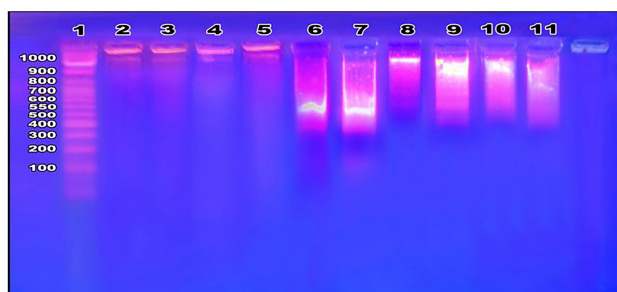

Fig. 4. Kidney DNA electrophoresis. Lane 1: DNA ladder; Lanes 2 and 3: control group; Lane 4: fisetin in normal rats; Lane 5: desmopressin drug in normal rats; Lanes 6 and 7: diarrheal group; Lanes 8 and 9: diarrheal rats + fisetin-treated group; Lanes 10 and 11: diarrheal rats + desmopressin drug-treated group.

rhea generated oxidative stress. Therefore, to restore and boost the antioxidant enzyme activity in the kidneys of diarrheal rats, fisetin was administered orally. Antioxidant and anti-inflammatory effects of fisetin are connected to this phenomenon, whereby fisetin decreases uricemia, accelerates kidney fibrosis, and controls the renal inflammatory response to guard against hyperuricemic nephropathy. Additionally, according to a number of previous studies, including Ren *et al.* [32], the antioxidant activity of fisetin was thought to be responsible for these protective effects. Fur-

thermore, fisetin was observed by Ijaz *et al.* [67] to enhance the enzymatic activity of reactive oxygen species, thiobarbituric acid reactive substance, glutathione, glutathione reductase, superoxide dismutase, and catalase. Moreover, Wang *et al.* [68] reported that fisetin had anti-inflammatory and anti-fibrotic properties, hence, fisetin can protect the kidneys from developing fibrotic disorders. Moreover, according to Zou *et al.* [69], fisetin inhibited kidney fibrosis, meaning that it is employed as a therapeutic target in the treatment of renal fibrosis. Fisetin heals renal injury brought on by atherosclerosis and diabetes. Furthermore, Ju *et al.* [70] found that fisetin is a novel therapeutic agent for obstructive nephropathy, whereby it reduces kidney fibrosis to protect against renal fibrosis. Additionally, according to Li *et al.* [71], fisetin increased tubular repair and reduced renal fibrosis, as seen by the restoration of renal function and tubular regeneration. According to Kwon [72], fisetin also has renoprotective effects and delays the course of chronic kidney disorders, including obstructive nephropathy, in addition to potential anti-inflammatory, anti-neoplastic, and antioxidant effects. Lastly, fisetin was found to reduce the levels of inflammatory markers, including nuclear factor-κB, tumor necrosis factor-α, interleukin-1β, interleukin-6, and cyclo-oxygenase-2 activity in a study by Umar *et al.* [73].
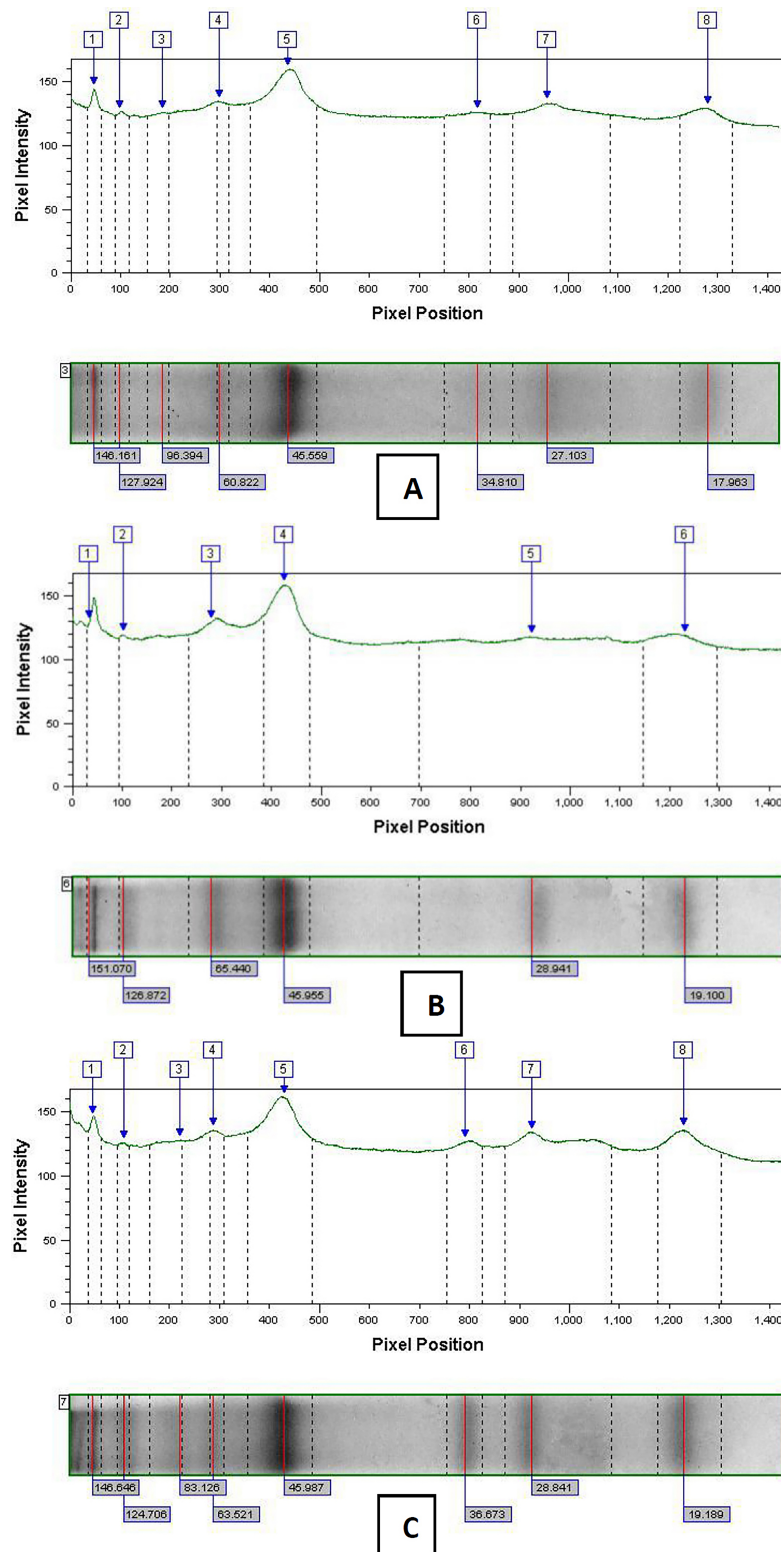
Fig. 5. Gas chromatography–mass spectra analysis of kidney proteins. (A) Eight kidney proteins were identified in the control group (alpha-1 fetoprotein, alpha-2 macroglobulin, beta-2 microglobulin, transferrin, albumin, globulin, prothrombin, and fibrinogen). (B) Six kidney proteins were found in the diarrheal group (alpha-1 fetoprotein, alpha-2 macroglobulin, beta-2 microglobulin, transferrin, albumin, and globulin), where prothrombin and fibrinogen protein bands disappeared. (C) Eight kidney proteins were found in the diarrheal group orally administrated with fisetin (alpha-1 fetoprotein, alpha-2 macroglobulin, beta-2 microglobulin, transferrin, albumin, globulin, prothrombin, and fibrinogen).
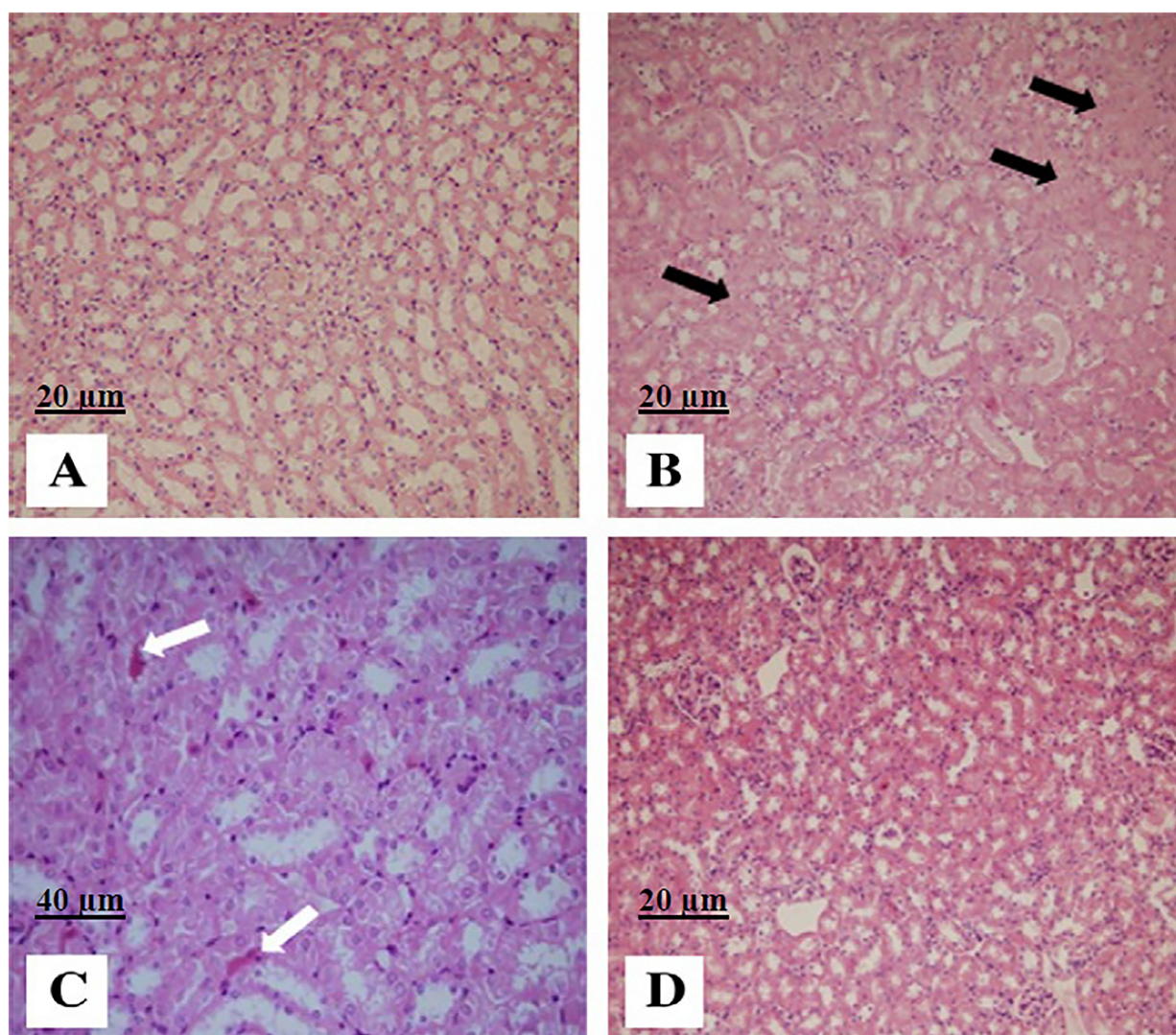
Fig. 6. Effect of fisetin on kidney tissue in normal and diarrheal groups. A photomicrograph of rat nephrotic tissue (H&E): (A, H & E × 200). The control group reveals normal kidney construction; (B, H & E × 200) and (C, H & E × 400) the diarrheal group exhibits large-scale coagulative necrosis (B, black arrows), including intraluminal cast development, vacuolar degeneration, dilatation, and desquamation of the epithelium (C, H & E × 400 white arrows) within the proximal tubules; (D, H & E × 200) After oral administration with fisetin, the diarrheal group shows an obvious improvement in the histological construction and appears similar to the control group. Scale bar = 20 μm of (A,B,D). Scale bar = 40 μm of (C).

The sodium/potassium pump was out of balance because diarrhea increased the excretion of Na^+, K^+, and Cl^- ions in urine while depleting these ions in serum. As a result, diarrhea decreased Na^+/K^+-ATPase levels in the kidneys of diarrheal rats. The Na^+/K^+-ATPase pump controls the balance between sodium and potassium by pumping sodium out of the cells, while potassium moves into the cells [74]. The primary enzyme that propels transepithelial ionic transport is the sodium/potassium-ATPase, and diarrhea lowers this enzyme's activity [75]. After receiving fisetin orally, rats with diarrhea had restored sodium/potassium-ATPase activity in their kidneys. This conclusion is connected to the observation that fisetin has anti-inflammatory, anti-neoplastic, and antioxidant proper-ties through scavenging the superoxide and hydroxyl radicals [72]. Additionally, fisetin changed the Na^+/K^+-ATPase, calcium-ATPase, and sodium hydrogen exchanger membrane transporters during senescence-induced naturally occurring aging, according to Singh *et al*. [76]. In older rats, oxidative changes were also prevented by fisetin. As a result, fisetin supplementation enhances ionic homeostasis, which causes age-related disorders. Furthermore, Das *et al*. [77] reported that pretreatment with fisetin reduced lipid peroxides, while maintaining sodium/potassium-ATPase function, which was observed to be changed in mice with epilepsy.

A change in the state of DNA and proteins would arise from free radical activity caused by diarrhea or a deficiency

in antioxidant levels. These data suggest that kidney DNA damage occurs due to diarrhea. The idea is that the base-pairing characteristics of the kidney DNA may change as a result of oxidative stress caused by diarrhea, which would result in the loss of the kidney protein bands. Many research studies, such as He et al. [78], Luo et al. [79], and Abolarinwa et al. [80] have found a connection between diarrhea and DNA damage. In contrast, administering diarrheal rats fisetin reduced DNA damage; this action is related to fisetin's antiproliferative and antioxidant properties. Numerous researchers have confirmed this finding, including Pal et al. [81], who noted that fisetin protects against DNA damage and cutaneous inflammation caused by ultraviolet B radiation. Fisetin also possesses antiproliferative and apoptotic properties, according to Rais et al. [82], hence, it functions as a chemopreventive agent to stop DNA damage. Furthermore, Kubina et al. [83] found that fisetin inhibits chemical alterations by inducing phase II enzymes, scavenging reactive oxygen radicals, enhancing DNA repair, and altering signal transduction pathways. These actions all work together to prevent DNA damage.

Using GC–MS analysis, the proteins in the kidneys of rats with diarrhea were investigated. The findings demonstrated that the prothrombin and fibrinogen protein bands disappeared as a result of diarrhea. However, after receiving a daily oral dose of fisetin for four weeks, the prothrombin and fibrinogen protein bands in the diarrheal rats reverted to resemble those in the control group. The liver produces thrombin, a protein known for its ability to aid in blood clotting. Furthermore, a separate protein known as fibrinogen is produced by the liver and delivered into the bloodstream, where it is transformed into fibrin by the enzyme thrombin. Blood clots are caused by this fibrin protein. For blood to coagulate, proteins known as fibrinogen and prothrombin are required. The liver is damaged and tainted by diarrhea, which inhibits the liver's ability to produce prothrombin and fibrinogen. According to Koriem et al. [84], there was an increase in the breakdown of serum proteins in rats with diarrhea, which led to an increase in the amount of protein being expelled in the urine. The liver can once again synthesize these two essential blood clotting proteins after treating diarrheal rats with fisetin, which prevents diarrhea-induced liver damage and toxicity from leading to hepatotoxicity and liver fibrosis [85–87]. Many scientists, such as Zhou et al. [88], suggested fisetin may reduce the alcohol-induced damage in the liver by impeding the ability of hepatic stellate cells to activate. Fisetin also demonstrated protective effects against hepatic ischemia injury by reducing inflammatory responses, according to Pu et al. [85]. Fisetin has the potential to be applied as a therapeutic treatment for alcoholic liver disease, according to Sun et al. [89].

Fisetin had a cytotherapy impact on the kidneys in diarrheal rats, according to histopathological analysis. This effect was connected to the antioxidant and anti-inflammatory properties of fisetin and according to Ren et al. [32], fisetin decreased uricemia, reduced renal inflammatory response, and improved kidney fibrosis. Moreover, according to Li et al. [71], fisetin was effective at improving kidney repair as well as the prevention and treatment of cisplatin-induced chronic kidney disease. Additionally, Chenxu et al. [90] found that fisetin inhibited oxidative stress and inflammation to protect against high-fat diet-induced nephropathy. Lastly, according to Umar et al. [73] and Koriem et al. [91], fisetin reversed hepatic tissue damage that showed hepatotoxicity in arsenic and maleate toxicity.

The oral dose (50 mg/kg) of fisetin administered here did not show any cytotoxic effects because fisetin passes rapidly through the intestine and liver, in addition to its presence of the parent compound in serum. Fisetin was swiftly and extensively metabolized to sulfates and glucuronides, as evidenced by the 2.2-fold increase in fisetin sulfates/glucuronides levels compared to fisetin glucuronides. As a result, enterocytes have less sulfation than hepatocytes because fisetin sulfates and glucuronides have excess phenolic groups from conjugation metabolism, meaning they continue to have free-radical scavenging activity [92]. After fisetin was administered intraperitoneally to mice at a dose of 223 mg/kg, the concentration of fisetin in the plasma rapidly decreased, with a maximum half-life of 3.1 hours and a rapid half-life of 0.09 hours [93]. These results help to explain why fisetin is important for biological responses and anticancer activities.

5. Conclusions

This research reveals that fisetin is a suitable therapeutic agent for treating diarrhea in rats. It performs its action by improving kidney function, urinary volume, kidney weight, and balancing electrolytes in the serum and urine, while also restoring kidney antioxidants, inflammatory markers, Na^+/K^+-ATPase level, apoptosis, and protein content in the diarrheal group. This research study establishes a basis to aid in fisetin use for treating human diarrhea, thereby representing the second step in this research to eventually use it more widely to treat diarrhea in humans.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

KMMK selected the topic and created the research plan. The experiment for the study was conducted by KMMK. KMMK carried out the study's statistical analysis and collected the outcome data. YKOF performed the histology section of the study. The initial and final forms of the work were written by KMMK. Both authors contributed to

editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

This study and all experimental methods were authorized by the National Research Centre Institutional animal care and use committee; the approval code was 12032627. This study was conducted at the National Research Centre, Egypt which adhered to strict guidelines for the housing of animals and all other experimental study procedures, particularly the protocol for the care and use of animals.

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Conflict of Interest

The authors declare no conflict of interest.

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