ORIGINAL RESEARCH

Nephroprotective Effect of the Leaf Extract of *Ajuga* remota Benth Against Gentamicin-Induced Nephrotoxicity in Swiss Albino Mice

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Background: Drug-induced kidney injury was among the most common renal damages, from which gentamicin occupies around 25% of this injury. Gentamicin-induced renal damage is caused by increased free radicals with subsequent amplified inflammation. *Ajuga remota* leaf extract has many phytochemicals with antioxidant activities, which may improve gentamicin-induced renal damage. Thus, we aimed to investigate the nephroprotective effect of *Ajuga remota* leaf methanolic extract on gentamicin-induced nephrotoxicity in Swiss Albino Mice.

Methods: An experimental study design was used on 30 experimental mice randomly allocated in six groups: Group I, II, II, IV, and VI, among which mice were given only distilled water, only gentamicin, 600 mg/kg *Ajuga remota* leaf extract only, gentamicin along with 200 mg/kg extract, gentamicin with 400 mg/kg extract and gentamicin with 600 mg/kg extract, respectively. At the end of the experiment, the mice were sacrificed after being anaesthetized, and blood samples were collected through a cardiac puncture for renal function tests while the kidneys were removed for histopathological evaluation. The data were entered into Epidata version 4.6 and exported to SPSS version 25 for further analysis using one-way analysis of variance. Statistical significance was set at p < 0.05.

Results: Group II mice had significantly higher levels of serum creatinine and blood urea levels compared to group I and III. The body weight of the mice in group V and group VI showed a significant increase compared with Group II. Serum creatinine and blood urea levels were reduced significantly in the *Ajuga remota* leaf extract administered group of mice compared to group II. Abnormal kidney architectural changes were seen among group II mice; however, those changes were improved after administration of Ajuga remota leaf methanolic extract.

Conclusion: Methanol extract of *Ajuga remota* leaf provided effective protection against gentamicin-induced oxidative renal damage through its antioxidant effects.

Keywords: *Ajuga remota* Benth, gentamicin, nephrotoxicity, methanol

Background

Kidney disease is a major health problem worldwide.[1] One of the functions of the kidney is to eliminate toxic compounds from the body, which frequently expose the kidney to a number of poisons that culminate in the development of acute kidney injury (AKI), which in some cases progresses to chronic kidney disease (CKD).[2] In 2019, over 850 million people were affected by kidney disease, among which 463 million were diabetic and more than twenty times that of HIV/AIDS-infected individuals (37.7 million in 2020).[1,3–6] Elevated levels of serum biomarkers, such as creatinine and urea, indicate renal damage.[7]

Drug use was the factor that played the most significant role in renal damage, by which acetaminophen,[8] aminoglycoside,[9] angiotensin-converting enzyme inhibitors (ACEI),[10] cisplatin,[11] penicillin G,[12] and ranitidine contributed the most to the

development of AKI, possibly by inducing inflammation, acid–base disturbance, electrolyte abnormalities, intrarenal obstruction, and interstitial nephritis.[13] Approximately 25% of AKI cases occur due to drug toxicity, with gentamicin being the most toxic.[14–16] Nephrotoxicity occurs in 25% of the patients treated with gentamicin.[17] Gentamicin is an aminoglycoside antibiotic widely used in the treatment of severe pneumonia in children, neonatal sepsis, and complicated acute malnutrition.[18] Nevertheless, it is a major nephrotoxic drug that damages the renal tubules, deteriorates glomerular function, and increases vascular resistance, possibly through the production of reactive oxygen species, inducing inflammation within the renal tubules and activating renal cell apoptosis.[19,20] Gentamicin disrupts the normal function of the kidney by accumulating in the cell membranes of the renal tubules, resulting in altered secretion and reabsorption of electrolytes.[21] However, due to its low rate of resistance and low cost, patients still use gentamicin widely, regardless of its damaging effects on kidney function.[9,14,22,23]

Ajuga remota is a traditional medicinal plant belonging to the Lamiaceae family with more than 300 species grown in Ethiopia.[24] The therapeutic uses of *Ajuga remota* extract claimed by multiple studies were its antihyperlipidemic,[25] antimalarial,[26,27] antidiarrheal,[28,29] antidiabetic,[30] and anti-inflammatory activities.[31] In Ethiopia, this traditional medicinal plant is used to treat kidney disease, blood pressure, malaria, diarrhea, fever, stomach pain, hyperlipidemia, diabetes, and ascariasis.[25,30,32–34] The leaves of *ajuga remota* also contain secondary metabolites such as flavonoids, alkaloids, terpenoids, phenols, and steroids.[30] These active ingredients are thought to play a major role in the reduction of inflammation and oxidative stress-induced renal damage via detoxification of free radicals as well as boosting the body's antioxidant scavenging system.[35] Despite the fact that *Ajuga remota* is rich in phytochemicals with antioxidant properties that contributed to its ability to protect against oxidative-induced renal damage, no study has been conducted on its nephroprotective effect until now. Therefore, the present study aimed to investigate the nephroprotective effects of *Ajuga remota* methanol extract against gentamicin-induced nephrotoxicity in Swiss Albino Mice.

Methods and Materials
Study Area and Study Period

Phytochemical screening and extraction of *Ajuga remota* leaves were performed at the Jimma University Biochemistry Laboratory, Organic and Inorganic Chemistry Postgraduate Laboratory. Biochemical and histopathological analyses were performed at the Jimma University Medical Center. The extracted plant leaves were administered to mice to investigate their nephroprotective effects from December 25, 2021, to January 05, 2022, at the Tropical and Infectious Disease Research Center, Jimma University (TIDRC-JU), Sekoru district, Jimma, Ethiopia.

Study Design

A post-test control group experimental study design has been conducted on a mouse model to evaluate the nephroprotective effect of the leaf extract of *Ajuga remota*.

Reagents and Chemicals

In this study, multiple reagents and chemicals were used, including absolute methanol (Assay (GC) >99.9%), 10% formalin (Brand-Microxpress 500mL, Histological fixative), ketamine/xylazine intramuscular (50mg/mL) for anesthesia, acetone (ACA 280223), gentamicin (Brand name-Ruili, Model number-80mg/2mL) for induction, chloroform (EMSURE© ASC ISO), sulfuric acid (Laboratory use 98%), Mayer's reagent, ninhydrin solution, and hydrochloric acid (Grade AR).

Equipment

Eppendorf tube (L-510-GRD, Thermo Fisher Scientific KK, 1.5mL), volumetric flasks (0.25L, 0.5L, and 2L), beakers (0.08L, 0.1L, 0.5L, and 1L), funnel (75mm and 100mm), Erlenmeyer flask (conical flask), measuring cylinder (2.5mL, 5 mL, 10mL, 25mL, 50mL and 100mL), glass rood (GL160-0200), spatula (steel, 0–10mm and 6inch), automatic pipette (Brand-hangxin, Model-A3001-A3030), gavage (oral feeding syringe), syringe (3cc), metal heater, refrigerator, Whatman filter paper number-1, digital/electric balance (Wensar, Mettler, and Aczet), water bath (Doubled walled construction with

regular and GMP models), metallic stirrer, automated chemistry analyzer (analyzer URIT-810 URIT), rotary evaporator (Model-Laborota 4000; Brand-Heidolph), and plastic cage for experimental animals grouping.

Plant Collection and Authentication

Fresh leaves of *Ajuga remota* were collected from around the Jiren Mountain, Jimma town, in September 2021. The plant was identified and authenticated by a botanist, Mr Melaku Wondafrash, from the National Herbarium (ETH) of Addis Ababa University, and voucher number MA001 was given and deposited in the institute for further reference.

Preparation of Plant Crude Extract

The dried leaves of *Ajuga remota* were ground into a coarse powder using a mechanical grinder and weighed to approximately 300g using a digital balance. Two hundred and fifty gram coarse powders was measured and soaked in 2.5 L absolute methanol (Assay (GC): >99.9%) in 1:10 ratio in a flask for 72 h with periodic mechanical agitation in Jimma University Biochemistry Laboratory, Organic and Inorganic Chemistry Postgraduate Laboratory. The extracts were filtered through Whatman filter paper No. 1 and the solvent from the mixture was evaporated using a rotary evaporator (Model-Laborota 4000 and Brand-Heidolph) at 35°C. The final products were dried in a heat chamber at 25°C until all liquids were removed from the extract.

Phytochemical Analysis

The phytochemical analyses were performed using the following standard methods:

Test for alkaloid (Mayer's test): The plant extract was mixed with a few drops of Mayer's reagent and looked for white creamy precipitation, which showed the presence of alkaloids.[36–39]

Test for amino acids (Ninhydrin's test): 100mg of the plant extract was mixed well with 10mL of distilled water. The mixture was filtered using Whatman no-1 filter paper, and 2mL of the filtrate was mixed with two drops of ninhydrin solution and appeared purple.[36–39]

Test for carbohydrate (Benedict's test): each, a half milliliter of plant extract filtrate and Benedict's reagent were mixed and heated for two minutes in a boiling water bath. The formation of a colored precipitate indicated the presence of carbohydrates (sugars).[37–40]

Test for fats and oil (spot test/stain test): A small quantity of plant extract was placed on a filter paper, pressed using a second filter paper and looking for an oil stain on the paper.[37,40]

Test for anthraquinones (Borntrager's test): 1g of plant powder was dissolved in 20mL of chloroform in a test tube, and the mixture was placed in a steam bath for 5minutes. The mixture was then filtered (while hot) and allowed to cool. Equal parts of the filtrate and 10% ammonia solution were mixed and shaken well, and the presence of a pink color on the upper parts of the aqueous layer indicated anthraquinones.[41]

Test for phenolic compounds (Ferric chloride test): 50 mg of plant extract was dissolved in 5 mL of distilled water, a few drops of ferric chloride solution (5% neutral) were added, and the dark green color product was examined.[37–39,42]

Test for tannins: half a gram of plant extract and bromine water (10mL) were mixed, and the decolorization of bromine water indicated the presence of tannins.[37,43]

Test for proteins (Millon's test): 10mL of distilled water and 100mg of plant extract were mixed and filtered using Whatman no-1 filter paper. Then, 2mL of the filtrate was mixed with 2–3 drops of Million Reagent. The formation of a white precipitate indicated the presence of proteins.[37–39]

Test for saponins: 50 mg of the plant extract was dissolved in 20mL of distilled water. The mixture was shaken for 15 minutes in a cylinder, and the presence of saponins was indicated by the formation of a 2cm layer of foam.[37–40]

Test for flavonoids: The Shinoda test or alkaline reagents test was used to detect the presence of flavonoids (Mg^{2+} ribbon + concentrated HCL + crude extract → pink color or 2% of NaOH + crude extract → gives a deep yellow color + 2 drops of diluted acid → colorless).[44,45]

Salkowski's test was used to check for the presence of glycoside components (2mL of H_2SO_4 + plant crude extract → reddish-brown color).[44]

Test for terpenoids: The presence of terpenoids was detected using chloroform and sulfuric acid (2mL of chloroform, 5 mL of crude extract, and 3 mL of $H_2SO_4 \rightarrow$ gray color).[46]

Test for steroids: The presence of steroids was determined using chloroform and concentrated sulfuric acid (H_2SO_4) (2mL of chloroform + H_2SO_4 + 5 mL crude extract $\rightarrow$ red colour layer).[38,39,43]

Study Variables

Independent variable: Leaf extract of *Ajuga remota*

Dependent variables: Renal function tests, serum electrolyte level, histopathological analysis

Sample Size Determination

Sample size determination was based on Federer calculation formula, which is $(t - 1) (n - 1) \geq 15$; where "t" is the number of the groups and "n" is the experimental animal per group. $(5–1) (n - 1) \geq 15 \rightarrow n \geq 5$. According to this calculation, the minimum sample size was five experimental animals in each treatment and control group. In this study, five experimental animals were included in each treatment group and a control group, which gives a total of 30 mice used for this experimental study.

Experimental Animals

For this study, a total of thirty-five (30 males and 5 females for this experimental study and oral toxicity test, respectively) Swiss albino mice aged 6–8 weeks old (weighing 28–32 grams) and physically normal were selected from the Tropical and Infectious Diseases Research Center, Jimma University (TIDR-JU), Sekoru district, Jimma. The experimental mice were placed in plastic cages with stainless-steel covers and housed in an animal laboratory, provided with pellets and water ad libitum. The mice were maintained under natural light for 24 hrs (12-hour dark–light cycle) at a standard room temperature of 25°C and relative humidity of approximately 50% before the experimental procedures. During acclimatization for one week, the mice were randomly divided into six groups, with five experimental animals in each group. They were handled in accordance with the internationally accepted guidelines.[47–49]

Oral Acute Toxicity Test

An acute oral toxicity test was performed to determine the median lethal dose (LD_{50}) for the leaf extract of *Ajuga remota* in female Swiss albino mice, according to the OECD 425 guidelines. Prior to testing, five female Swiss albino mice weighing 28–32 grams and aged 6–8 weeks were selected randomly and caged for six days in the laboratory to become familiar with the conditions of the laboratory. The animals have an unlimited supply of drinking water. Following the measurement of the body weight of the mice after fasting for 3 hours, one mouse was selected randomly, and a 2000 mg/kg methanolic extract of *Ajuga remota* leaf was administered via stainless-steel oral gavage. The mouse was frequently observed for behavioral changes and common toxicity signs after dosing for the first 24 hours, with special consideration being given during the first 4 hours. The mouse did not develop any signs of toxicity for 24 and 14 days of observation. The other four Swiss albino mice were given comparable dose and were monitored for 14 days.[50,51]

Experimental Induction of Nephrotoxicity in Mice

Nephrotoxicity was induced by intraperitoneal (IP) injection of gentamicin at 80 mg/kg per day for consecutive 8 days. This dose caused nephrotoxicity when administered for more than 5 days based on a previous study by Huang et al, 2020.[52,53]

Experimental Design and Treatment Protocol

The six groups of experimental mice were assigned accordingly:

Group I (normal control) received distilled water (1mL) via IP for 8 days.

Group II (Gentamicin control) received gentamicin-80 mg/kg body weight via IP for 8 days.[52]

Group III (methanol extract *Ajuga remota* (MEAR) control) received 600 mg/kg per body weight of MEAR alone for 8 days, orally;

Group IV (200 MEAR) received gentamicin-80 mg/kg for 8 days and MEAR (200 mg/kg per body weight) orally for 10 days (started two days before co-administration of gentamicin).

Group V (400 MEAR) received gentamicin-80 mg/kg for 8 days and MEAR (400 mg/kg per body weight) orally for 10 days (started two days before co-administration of gentamicin).

Group VI (600 MEAR) received gentamicin-80 mg/kg for 8 days and MEAR (600 mg/kg per body weight) orally for 10 days (started two days before co-administration of gentamicin).[25]

Determination of Body Weight and Kidney Weight of Mice

The weights of the mice were measured at the beginning, middle, and at the end of the experiment before sacrifice. After scarifying by cervical dislocation, both the right and left kidneys were removed, the right kidney was weighed to obtain the absolute kidney weight of the mice, and the left kidney was placed in a screwed cup container with 10% formalin (in 1 to 10 ratios) for histopathological examination.

Sample Collection

Blood samples were collected 12 h after the last treatment by anesthetizing the mice with ketamine/xylazine (intramuscular, 500 mg/10mL (50 mg/mL)). The sample was collected via cardiac puncture (diaphragmatic approach), which was performed by inserting a 1 inch 22-gauge needle attached to a 3 mL (3cc) syringe through either notch. The collected blood samples were kept in an Eppendorf tube for approximately 30 minutes at room temperature to separate the serum from the formed elements and centrifuged at 3000 rpm for 10 min to completely separate the serum from the formed element. The serum was stored in a refrigerator (4–8°C) for blood urea, creatinine, sodium, potassium, and chloride analysis.[54,55]

Histopathological Examination of the Kidney

The animals were sacrificed using cervical dislocation techniques before awakening from anesthesia. The abdominal cavity was opened using a surgical blade, and both kidneys were removed and processed for histological examination. Kidney weight was measured using a digital/electronic balance. The kidneys were fixed in 10% formal saline 1to10 ratio for histopathological examinations.[56,57] Tissue processing involves dehydration, clearing, and paraffin impregnation. Dehydration is the process of water removal by immersing the tissue in ascending concentrations of alcohol until water is removed. Next, the alcohol was cleared using an organic solvent, xylene, and paraffin impregnation, followed by immersion of the tissue in molten wax at 60°C. Embedding was then performed using melted paraffin and a metal mold, which was achieved by assembling the metal mold and lubricating it using liquid paraffin, after which the tissue was carefully placed at the bottom of the metallic mold. The melted paraffin was allowed to harden at room temperature, after which the metallic mold was removed and the blocks were trimmed and placed in a refrigerator before sectioning. A microtome was used to cut tissue sections (4–5μm thick) with one tissue block at a time. The cut sections were floated and flattened in lukewarm water in a water bath (50–55°C), placed on a clean slide, and left to dry. The dried slides were labeled and stained in multiple steps using hematoxylin and eosin and examined by a pathologist who was blinded to the experiment via light microscopy at a magnification of 400x to assessment of the kidney sections.[58,59]

Data Analysis

Data were collected and entered into Epidata version 4.6 and analyzed using SPSS version 25. The results of various biochemical parameters were expressed as mean ± SEM and compared between the groups using analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparisons to determine the particular group showing a significant difference at $p < 0.05$. Histopathological examinations were performed under a microscope by a senior pathologist and presented using photomicrographs.

Results
Acute Oral Toxicity Test

An oral acute toxicity test indicated that the crude extract of *Ajuga remota* leaf was safe when administered orally at a dose of 2000 mg/kg. After 24 hours and 14 days of observation, the first tested mouse as well as the remaining four

mice did not show any signs of toxicity, which was evidenced by the absence of central nervous system signs such as tremors, convulsions, salivation, lethargy, and coma. In addition, none of the MEAR administered mice died. Based on these oral toxicity test results, the LD_{50} for MEAR leaf was considered to be greater than 2000 mg/kg body weight. This encouraged us to use 200, 400, and 600 mg/kg of MEAR leaf dosages for our current experimental study.

Phytochemical Screening Tests

Phytochemical screening of MEAR (Table 1) revealed the presence of glycosides, terpenoids, steroids, phenolic compounds, flavonoids, tannins, fats, oils, and saponins.

Effects of *Ajuga remota* Extract on Mouse Body Weight

As shown in Table 2, the body weights of the mice did not show a statistically significant difference among the groups at the beginning (1st day) and 5th day of the experiment. However, on the 10th day of the experiment, the body weights of mice in all groups showed a statistically significant difference. Compared to the normal control, the mean body weights of all mice in groups IV (p = 0.001, 17.57%), V (p = 0.049, 9.1%), and VI (p = 0.018, 10.52%) were significantly reduced. This study also reported that the mean body weights of mice in groups V (p = 0.001, 18.8%) and VI (p = 0.003, 16.94%) were significantly higher than those in groups II. In addition, a significant increase in the mean body weight was observed in groups I and III (30.69% and 19.29%, respectively) compared to that in groups II (p = 0.01). In general, there were reductions in body weight in all groups compared with the 1st day of body weight measurement of each group, except for the normal control group (GI).

Table 1 Results of Phytochemical Screening Test of MEAR Leaf

Test	Detection Method	Result
Alkaloids	Mayer's/Bertrand's/Valser's test	–
Steroids	Hesse's response test	++
Phenolic compounds	Gelatin test	++
Flavonoids	Conc. H_2SO_4 test	+++
Saponnins	Froth test	+
Tannins	Gelatin test	+
Fixed oils and fat	Spot test/stain test	++
Glycosides	Borntrager's test	+
Protein and amino acid	Xanthoproteic test	–
Carbohydrate		–
Terpenoids		++
Anthraquinones	Borntrager's test	–

Notes: +, presence of the expected bioactive compound; -, absence of the expected bioactive compound.
Abbreviation: H2S04, Sulfuric acid.

Table 2 Mean Body Weight of Swiss Albino Mice from the Beginning to the End of the Experimental Day

Mean b/wt. in Gram	Groups (G)					
	GI	GII	GIII	GIV	GV	GVI
1st day b/wt.	30.00 ± 0.63	29.60 ± 0.75	29.8 ± 0.58	29.60 ± 0.75	30.30 ± 0.49	29.30 ± 0.70
5th day b/wt.	31.30 ± 0.69	28.54 ± 0.77	29.00 ± 0.35	28.64 ± 0.78	29.00 ± 0.63	28.50 ± 0.59
10th day b/wt.	32.32 ± 0.68[b]	24.73 ± 0.69	29.5 ± 0.52[b]	26.60 ± 0.93[a]	29.38± 0.48[ab]	28.92±0.61[ab]

Notes: The results were presented using mean± SEM; [a]Significance difference compared to group I; [b]Significance difference compared to group II at p < 0.05.
Abbreviation: b/wt, body weight.

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Effects of *Ajuga remota* Extract on Serum Urea and Creatinine Level

As shown in Table 3, groups IV and V ($p < 0.001$) showed a statistically significant increase in serum urea levels compared to those of group I and III, while group VI ($p > 0.05$) did not show a significant difference but showed significant reductions when compared to group IV and II ($p < 0.001$, 194.55%, and 200%, respectively). This study also determined lower urea levels in groups IV, V, and VI ($p < 0.001$, 21.3%, 38.58%, and 53.09%, respectively) than in group II. A higher mean serum creatinine level was observed in group IV ($p = 0.008$, 78.46%) than in group I. The results of this study also revealed that mice in group IV ($p = 0.002$, 90.2%) and V ($p = 0.037$, 65.6%) showed a significant increase in serum creatinine levels compared with those in group III. Moreover, decreased serum creatinine levels were observed in group V ($p = 0.027$, 30.34%) and VI ($p < 0.001$, 47.59%) mice compared with group II mice. In contrast, lower mean serum creatinine levels were observed in groups I and III than in the distilled water control group.

Effects of *Ajuga remota* Extract on Serum Electrolyte Level

In this study, as shown in Table 4, there was no significant difference in serum electrolyte levels among all groups; however, there was a slight non-significant increase in serum sodium, potassium, and chloride levels among the groups and a slight non-significant decrease in serum calcium levels compared with groups I and III.

Effects of *Ajuga remota* Extract on Relative Kidney Weight of the Mice

At the end of this experiment (Table 5), the relative kidney weights of mice in group IV ($p = 0.016$, 8.83%), V ($p = 0.007$, 9.89%), and VI ($p = 0.006$, 10.25%) were significantly lower than those of group II. In contrast, there were no significant

Table 3 Effects of Methanol Extract of Ajuga Remota on Serum Creatinine and Urea Level in Gentamicin-Induced Nephrotoxicity

Groups	Variables	
	Serum Creatinine Level (mg/dl)	**Serum Urea Level (mg/dl)**
Group I	0.65 ± 0.06	22.00 ± 2.67
Group II	1.45 ± 0.15[a,d]	64.80 ± 4.92[a,d]
Group III	0.61 ± 0.03	21.6 ± 1.21
Group IV	1.16 ± 0.12[a,d]	51.00 ± 1.98[a,b,d]
Group V	1.01 ± 0.06[b,d]	39.80 ± 1.32[a,b,d]
Group VI	0.76 ± 0.02[b,c]	30.40 ± 0.68[b,c]

Notes: The results were presented using mean± SEM; [a]Significance difference compared to group I; [b]Significance difference compared to group II; [c]Significance difference compared to group IV; [d]Significance difference compared to group III and at $p < 0.05$.
Abbreviation: mg/dl, milligram per deciliter.

Table 4 Effects of Methanol Extract of Ajuga Remota on Serum Electrolyte Level on Gentamicin-Induced Nephrotoxicity in Mice

Groups (G)	Variables (mmol/L)			
	Sodium (Na$^+$)	**Potassium (K$^+$)**	**Calcium (Ca^{2+})**	**Chloride (Cl$^-$)**
Group I	140.6 ± 0.51	4.86 ± 0.05	9.76 ± 0.10	100.78 ± 0.94
Group II	142.2 ± 0.37	5.06 ± 0.05	9.38 ± 0.13	102.20 ± 0.92
Group III	140.2 ± 0.37	4.88 ± 0.04	9.86 ± 0.04	100.85 ± 0.86
Group IV	141.4 ± 0.25	5.02 ± 0.07	9.50 ± 0.18	103.52 ± 0.42
Group V	141.6 ± 0.40	5.10 ± 0.06	9.40 ± 0.18	101.54 ± 1.01
Group VI	141.2 ± 0.37	5.06 ± 0.08	9.62 ± 0.07	101.2 ± 0.66

Note: The results were presented using mean± SEM.
Abbreviation: mmol/L, millimole per liter.

Table 5 Effects of Methanol Extract of Ajuga Remota on Kidney Weight in Gentamicin-Induced Nephrotoxicity

Variable	Groups (G)					
	GI	GII	GIII	GIV	GV	GVI
Mean Kidney relative b/wt.(gram)	0.25± 0.04	0.28±0.09[a]	0.25±0.04[b]	0.26±0.03[b]	0.26±0.02[b]	0.25±0.02[b]

Notes: The results were presented using mean± SEM; [a]Significance difference compared to group I; [b]Significance difference compared to group II at $p < 0.05$.
Abbreviations: b/wt., body weight.

differences among groups I, III, IV, V, and VI. However, there was a significant increase in the mean kidney weight of mice in groups I and III (13.65% and 12.3%, respectively) compared with that of mice in group II ($p < 0.02$).

Effects of *Ajuga remota* Extract on Kidney Histopathological Changes

Microscopic examinations of the renal tissue of the mice in groups I and III showed normal glomerular structure and renal tubular interstitium with no evidence of protein casts, inflammatory infiltration, necrosis, and vacuolization. In contrast, kidney sections of the mice induced with only gentamicin (Group II) showed multiple renal histopathological abnormalities with renal tubular necrosis, inflammatory infiltration, protein casts, diminished glomeruli, and dilated as well as degenerated renal tubules. However, Group IV (200 mg/kg), group V (400 mg/kg), and group VI (600 mg/kg) of mice showed improved renal histopathological abnormalities as evidenced by reduced renal tubular necrosis, inflammatory infiltration, and diminished glomeruli compared with those of group II mice. In a dose-dependent manner, 600 mg/kg was observed to have better nephroprotective effects (see Figure 1 for further detail).

Discussion

In the present study, the nephroprotective effects of *Ajuga remota* leaf extract were examined on gentamicin-induced nephrotoxicity in Swiss albino mice. According to the findings of this study, *Ajuga remota* leaf extract reduced the deleterious effects of gentamicin, such as loss of body weight, increased relative kidney weight, and increased blood urea and serum creatinine levels. Moreover, histopathological analysis of the kidney revealed a reduced loss of normal kidney structural changes induced by gentamicin after administration of MEAR.

Various drugs cause nephrotoxicity through multiple mechanisms, such as dose, route, and time of drug exposure to the kidney, higher concentration, and accumulation of some drugs in the renal cells.[60] Gentamicin is a clinically effective drug against many severe infections caused by the gram-negative bacteria, Enterococcus and Staphylococcus.[61] Gentamicin is a well-known nephrotoxic drug that causes tubular, glomerular, and blood vessel damage, which results from the production of reactive oxygen species.[62]

The results of this study indicate that the IP administration of gentamicin (80 mg/kg) reduces kidney function, which manifests in increased serum creatinine and blood urea levels. This finding is congruent with the results of a study conducted in Ethiopia on gentamicin-induced mice.[63] Furthermore, the increased in serum creatinine and blood urea levels due to gentamicin injection was remarkably reduced by the administration of methanol leaf extract of *Ajuga remota*, which is in line with the results of previous studies of different medicinal plants performed on gentamicin-induced mice.[64–66] The current study also found significantly decreased mean serum creatinine and blood urea levels in MEAR-treated mice. These effects are expected to be due to the presence of nephroprotective bioactive molecules in *Ajuga* species. Bioactive molecules such as phytoecdysteroids can activate gene transcription and increase biosynthesis of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).[35] In addition, flavonol and iridoid glycosides extracted from *Ajuga remota* have antioxidant activities, which may attenuate oxidative stress-induced renal damage.[67,68]

In the present study, a non-significant reduction in serum calcium levels was found in the gentamicin-treated groups, which is in agreement with previous study findings.[69] The reduction in serum calcium levels occurs due to the stimulation of the entry of extracellular calcium and the inhibition of calcium ion transporter by gentamicin.[20,70,71]
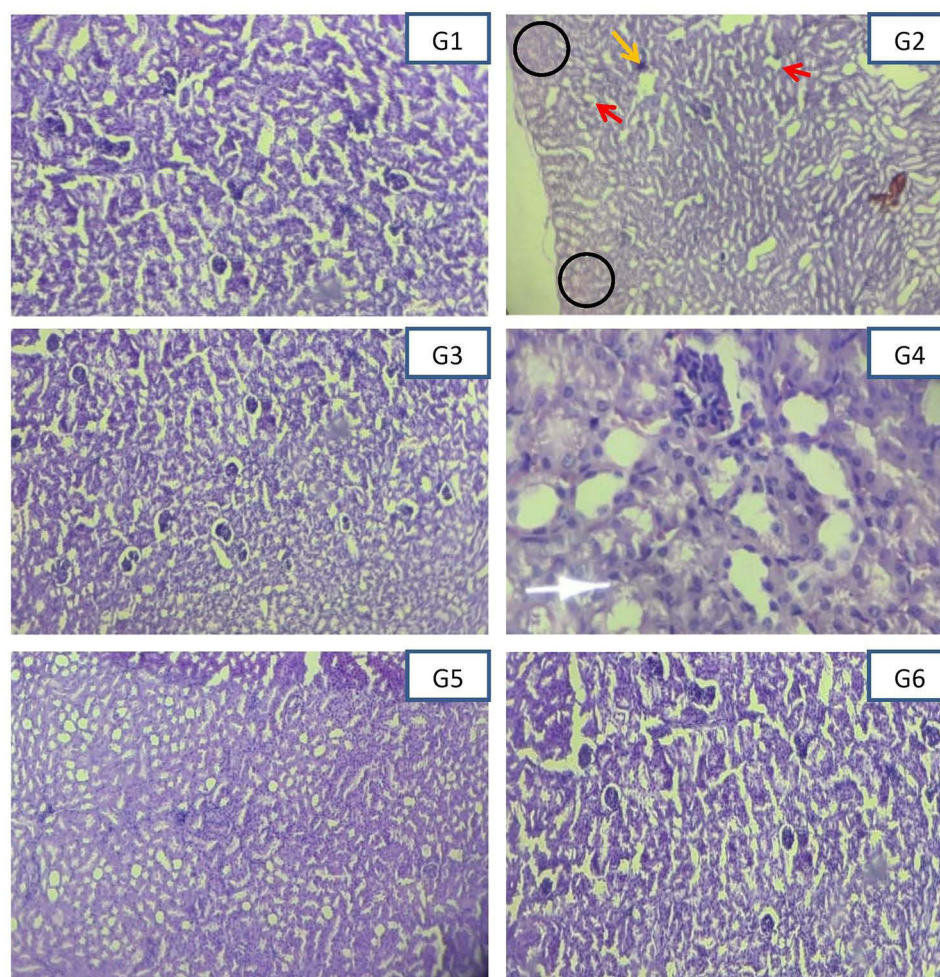
Figure 1 Photomicrograph renal tissue of the Swiss albino mice (at 400x, stained by hematoxylin and eosin).
Notes: **G1**: group I; **G2**: group II; **G3**: group III; **G4**: group IV; **G5**: group V; **G6**: group VI. Red arrow: dilated and degenerated renal tubules; orange arrow: inflammatory infiltration; black circle: necrosis; white arrow: vacuolization.

Increased phosphorus retention due to gentamicin-induced tubular damage (necrosis), leading to increased urinary excretion of calcium, is another possible reason for the decreased serum calcium level.[20,72]

However, regarding serum sodium, potassium, and chloride levels, a non-significant increase was seen in gentamicin-induced mice. This finding is consistent with study finding reported by Bouhrim et al.[69] The possible mechanisms for elevated levels of these electrolytes have been ascribed to the alteration of kidney function, or reduced glomerular filtration rate, and decreased reabsorption of water caused by gentamicin.[73,74]

Moreover, in the current study, an increase in kidney weight and a loss of body weight at the end of the experiment were observed in gentamicin-administered mice. This is in line with a previous study done on gentamicin-treated rats that reported a significant increase in kidney weight as a result of tubular necrosis, increased catabolism, and loss of appetite, which ultimately resulted in the loss of body weight.[65,74] Loss of body weight in gentamicin-induced mice has been attributed to be due to increased catabolism and renal tubular damage, with reduced water reabsorption in the renal tubules, followed by dehydration.[73,74]

Administration of MEAR for ten consecutive days resulted in reduced morphological changes compared with the gentamicin control group, which was illustrated by the significant difference in relative kidney weight among the groups. In the experimental groups, 600mg/kg and 400mg/kg MEAR administered mice showed normal renal anatomy, whereas mild-to-moderate damage to the renal tubules, glomeruli, and renal tubular cells was observed in mice administered

200mg/kg MEAR. This might be due to the antioxidant and anti-inflammatory effects of the phytochemicals present in the extract, which activate the transcription of genes encoding antioxidant enzymes and improve immune function.[35,75]

Furthermore, the current study is also designed to assess the microscopic examination of the kidney sections of the mice. We observed necrosis, inflammatory infiltration, dilated and degenerated tubules, protein casts, and vacuolization in the gentamicin control (GII) group. This finding is in agreement with those of previous studies on gentamicin-induced nephrotoxicity in mice.[20,21] A possible reason for this abnormal histopathological result in mice induced by gentamicin was gentamicin accumulation in the renal tubules (proximal convoluted tubules), which subsequently resulted in the loss of tubular integrity, necrosis, degeneration, and inflammatory infiltration within the renal cells and tissue.[17] However, improved abnormal histopathological results were observed after mice were treated with *ajuga remota* leaf extract. This finding is in agreement with that of a study conducted in Ethiopia.[63]

Limitations

We were unable to include all parts (root, flower, seed, and stem) of *Ajuga remota* to compare their nephroprotective effects. The study was also unable to identify the standard marker compounds from the extract and the effects of the individual active ingredients of *Ajuga remota* on serum creatinine, blood urea nitrogen, and kidney histopathology.

Conclusion and Recommendations

This study finding indicates that 600 MEAR leaf extract of *ajuga remota* had nephroprotective effects relative to gentamicin-induced mice, as confirmed by decreased serum creatinine and blood urea levels and improved histopatho-logical results after treatment with *ajuga remota* leaf extract. In general, mice treated with methanol extract of *ajuga remota* leaf exhibited nephroprotective effects against gentamicin-induced renal damage.

Further experimental research is needed to compare the effects of *Ajuga remota* leaf extract and standard drugs on gentamicin-induced nephrotoxicity. In addition, we would like to recommend the examination of various parts of the plant for their nephroprotective effects. Moreover, the antioxidant activities of the Ajuga remota leaf extract should also be examined in relation to its nephroprotective effects.

Abbreviations

ACEI, angiotensin-converting enzyme I, AKI; Acute kidney disease; CKD, chronic kidney disease; IP, intraperitoneal; MEAR, methanol extract of *ajuga remota*; TIDRC, Tropical Infectious Disease Research Center.

Data Sharing Statements

The corresponding author can be contacted to get all the necessary data that support the finding of this study.

Ethical Approval

This study was conducted after obtaining a letter of ethical clearance from the Institutional Review Board of Jimma University with reference number of IHRPG1/9/21. All experimental activities were conducted following the ethical declaration of national and international standards for experimental animals to protect their rights of experimental animals with treating the animals to avoid pain, suffering, injury, hunger, and thirst.[47,76,77]

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflict of interest for this work

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