ORIGINAL RESEARCH

Effect of moonseed vine (*Triclisia gilletii* Staner) on ethane-1,2-diol-induced urolithiasis and its renotoxicity in Wistar albino rats

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Abstract

Background: Moonseed vine (*Triclisia gilletii* Staner) in the family Menispermaceae is a robust creeper of up to 10 cm diameter, of the lowland dense rain forest. In Ondo State, located in the South Western part of Nigeria, the plant which is usually called Peshe is used for the management of renal-related ailments. The present study was undertaken to explore the efficacy of *Triclisia gilletii*, a folkloric therapy in the management of renal-related ailment.

Results: Phenols, steroids, saponins, and flavonoids are present in the TGME with a total antioxidant capacity of (30.36 ± 1.90 (mg GAE/g extract), LD_{50} greater than 5000 mg/kg b.w., and in vitro anti-nucleation activity ($iC_{50} = 7.09$ mg/mL). Calcium oxalate stone formation as a result of oxalate from ethane-1,2-diol was evident by hypocalcemia, and further electrolyte imbalance and decreased glomerular filtration rate. The enhanced oxidative milieu in hyperoxaluria was evident by increased MDA and PC and decreased enzymatic and non-enzymatic antioxidants as well as renal membrane enzymes activities. The renal histopathological study further emphasized oxalate-induced damage and the ameliorative potential of TGME.

Conclusion: The abnormal biochemical, redox electrolyte, membrane integrity, and histological alterations were attenuated by TGME which affirms its usage as nephroprotectant.

Keywords: Nephrolithiasis, *Triclisia gilletii*, Inflammation, Oxidative stress, Electrolyte imbalance, Nephroprotectant

1 Background

Nephrolithiasis or urinary calculi or kidney stone disease is a pathological condition (-iasis) in which stones (lith-) are formed in the kidneys (nephro-). Many people all over the globe suffer from this pathological condition, and there is an increasing preponderance from the twentieth century until today [1]. It is a common disorder estimated to occur in approximately 12% of the population with a recurrence rate of 70–80% in male and 47–60% in female [2]. In Nigeria, earlier reports on nephrolithiasis showed low prevalence until recent studies indicated a rising incidence of urolithiasis [3].

The nature and type of stones depending on the constituents of the urine depend on the nature of the diet consumed in a given population [4]. About 85% of kidney stones are caused by the aggregation of calcium oxalate or uric acid crystals in the kidney tubules [5].

Ethane-1,2-diol popularly known as ethylene glycol (EG) can induce calcium oxalate (CaOx) kidney stones, inflammation, and renal damage following oxalate saturation, supersaturation, nucleation, crystal growth, aggregation, crystal retention, and stone formation [6]. CaOx and high oxalate load also cause oxidative damage and interstitial inflammation in the kidney, via production of reactive oxygen species, such as superoxide and hydrogen peroxide [7]. This ultimately leads to alterations in renal functional capacity.

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Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 2 of 16

Surgical endoscopic stone removal and extracorporeal shock wave lithotripsy have revolutionized the treatment of urolithiasis but do not prevent the likelihood of new stone formation aside acute kidney injury as a result of shockwave [8]. Also, therapies like thiazide as diuretic and alkali citrate commonly used in preventing the recurrence of hypercalciuria and hyperoxaluria which induce calculi are less effective [2].

The raised incidence of nephrolithiasis in our world today which could be as a result of increasing trend toward western diet and the various side effects accompanied with available therapies in managing the disease has gotten many researchers to probe into the good old traditional medicinal plants to find solutions since most of these fatal modern diseases were not dominant during the ages where medicinal plants were widely used [1].

The genus *Triclisia* comprises 20 species throughout the world with 12 in tropical Africa Region. Plants belonging to this genus possess potential therapeutic values known since ancient times to cure various ailments and infectious diseases such as malaria, venereal diseases, epileptic attacks, diarrhea, stomach problems, leprosy, mental health problems, dysentery, respiratory diseases, convulsive coughing, and renal-related ailments including edema, anemia, gout, urinogenital infections, and swelling of extremities [9–12].

Moonseed vine (*Triclisia gilletii* Staner) in the family Menispermaceae is a robust creeper of up to 10 cm in diameter, of the lowland dense rain forest. In Ondo State, located in the South Western part of Nigeria, the plant which is usually called 'Peshe' in Ondo City or 'Ogbogan' in Okeluse, Ondo State, Nigeria, and Akan-Akyem Sanhoma in Ghana is used locally for the management of swelling of extremities and other kidney-related diseases or infections either singly or in combination with other condiments by traditional practitioners. Tiam et al. [12] reported its usage for the management of edema and anemia which by our bet is closest to kidney function.

Various classes of bioactive compounds have been isolated and identified [12]. These include flavonoids (2′-hydroxy-4′-methoxyochnaflavone, myricetin, quercetin, and 3-methoxyquercetin), steroids (nonacosan-10-ol, stigmasterol, 3-*O*-β-D-glucopyranosylsitosterol, and 3-*O*-β-D-glucopyranosylstigmasterol), and triterpene (oleanolic acid). These various phytoconstituents have connection with nephroprotection.

This study establishes the protective property of *Triclisia gilletii* on ethane-1,2-diol-induced urolithiasis and renotoxicity in Wistar albino rats.

2 Methods

2.1 Drugs and chemicals

Ethane-1,2-diol was purchased from Titan Biotech Limited Bhiwadi-301019, Rajasthan, India. Tamsulosin hydrochloride was purchased from Sun Pharmaceutical Ind. Ltd. Dewas, India. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, uric acid, bicarbonate, albumin, and total protein estimation kits and saponin were obtained from Sigma-Aldrich Co, St Louis, USA. Calcium, sodium, potassium, chloride, and glucose estimation kits were purchased from AGAPPE Diagnostics Switzerland GmbH. All other chemicals used in the experiment were of the highest grade commercially available.

2.2 Plant material

The leaves of *Triclisia gilletii* were collected from a location at Okeluse, Ose Local Government Area of Ondo State in May 2016. The plant was identified by Mr. Omomoh B.E., and a voucher specimen (IFE-17536) of the authenticated *Triclisia gilletii leaves* was deposited in the herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The leaves were separated for other extraneous matter and subjected to shade drying.

2.3 Preparation of aqueous methanolic leave extract of *Triclisia gilletii* (TGME)

The air-dried leaves were subjected to a coarse powder using a dry grinder. The powdered leaves were soaked in 80% methanol for 72 h and filtered using Whatman filter paper no. 1 to obtain the aqueous methanolic extract (TGME). The filtered extracts were concentrated in a rotary evaporator and further concentrated to dryness using freeze dryer. After drying, a dark brown aqueous methanolic extract (17.21% w/w) was obtained.
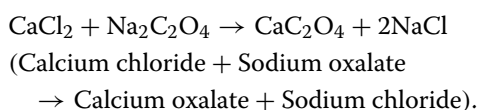
2.4 Preliminary phytochemical screening and quantitative estimation of phytoconstituents

Preliminary phytochemical screening [13] of TGME was carried out to detect the presence of phenolics, flavonoids, steroids, saponins, and tannins. The total phenolic content was determined spectrometrically [14] and expressed as milligram of gallic acid equivalents (GAEs) per gram of extract. Total flavonoid content was measured by aluminum chloride colorimetric assay [15] and expressed as milligram of quercetin equivalent per gram of extract. Total tannins content was measured according to the method described by Broadhurst et al. [16] and expressed as milligram of catechin equivalent per gram

Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 3 of 16

extract. Total saponins were determined according to the method of Brunner [17] and expressed as milligram saponin equivalent per gram sample.

2.5 Nucleation assay

The method as described by [18] was used to study oxalate crystallization. In this method, crystallization was studied without inhibitor (i.e., control) and with extract *Triclisia gilletii* (i.e., inhibitor). The result was expressed in percentage. The following reaction resulted in the growth of crystals [19]:

$$CaCl_2 + Na_2C_2O_4 \rightarrow CaC_2O_4 + 2NaCl$$

(Calcium chloride + Sodium oxalate

$$\rightarrow Calcium\ oxalate + Sodium\ chloride).$$

2.6 Animal studies

Sixty healthy male Wistar rats (age 6 weeks old; body weight 150–180 g) obtained from a private breeder in Akure metropolis of Ondo State, Nigeria, were used for the study. The animals were housed at standard housing condition (27 ± 3 °C) under 12-h light/dark cycle in polypropylene pathogen-free cages and fed standard rodent chow (Vita Feeds Nigeria Limited) and water ad libitum.

2.7 Acute toxicity study

Healthy Wistar rats, starved overnight, were subjected to acute toxicity studies to determine non-observable adverse effect dose level (NOAEL) by acute toxic class method [20]. The rats ($n = 3$) were orally administered TGME in the limit test dose of 5000 mg/kg orally and observed continuously for behavioral, neurological, and autonomic profiles for 2 h and after a period of 24 h for any lethality, moribund state, or death and approximate LD_{50} determination. Dosages between 0 and 1000 mg/kg were repeated every other day for 7 days. Animals were euthanized via cervical dislocation 24 h after the last administration, blood collected for serum biochemical analysis of AST, ALT, ALP activity, and urea, creatinine, and uric acid concentration using Randox diagnostic kits.

2.8 Grouping, treatments, and induction of urolithiasis

The rats ($n = 6$) were divided into 7 groups.

Group I Control—Animals in this group were allowed access to distilled water ad libitum and 1 ml/kg distilled water for 28 days.

Group II (Induced)—Animals in this group were allowed access to drinking water containing 1% ethane-1,2-diol ad libitum and 1 ml/kg distilled water for 28 days.

Group(s) III–V—Animals in these group(s) were allowed access to drinking water containing 1% ethane-1,2-diol ad libitum with co-administration of aqueous

methanol leaf extract of *Triclisia gilletii* (50, 100, 200 mg/kg b.w.) orally for 28 days.

Group VI—Animals in this group were allowed access to drinking water containing 1% ethane-1,2-diol ad libitum *w*ith co-administration of tamsulosin hydrochloride (standard drug) orally for 28 days.

Group VII—Animals in this group were administered the highest dosage of the aqueous methanol leaf extract of *Triclisia gilletii* (200 mg/kg b.w.) orally for 28 days.

The groups II–VI received 1% ethane-1,2-diol in drinking water ad libitum for 28 days, respectively, to induce urolithiasis and generate CaOx deposition into kidneys. The experimental model of urolithiasis utilizes the toxic mechanism of ethane-1,2-diol poisoning. Following ingestion, ethane-1,2-diol is first hepatically metabolized to glycolaldehyde by alcohol dehydrogenase. Glycolaldehyde is then oxidized to glycolic acid, glyoxylic acid, and finally oxalic acid. Ethane-1,2-diol-induced urolithiasis model mimics the human clinical condition of lithiasis, whereby oxalate produced chelate calcium ions forming insoluble CaOx and ultimately leading to nephrotoxicity and renal failure [2].

2.9 General observations

During the study period, body weight, water intake, and animal health was observed regularly, so that stressed and unhealthy animals were excluded from the study.

2.10 Collection and analysis of urine

All animals were kept in individual metabolic cages, and 24-h urine samples were collected on 0, 7, 14, 21, and 28th day of calculi induction treatment for the assessment of volume and urinalysis using urinalysis reagent strips (ACON Laboratories Inc. San Diego, USA). Urine collected on the 28th day was assessed for creatinine, urea, uric acid, electrolytes (calcium, potassium, sodium, chloride, and bicarbonate), glucose, albumin, and total protein concentrations following methods in recommended kits.

2.11 Serum analysis

Blood was collected via cardiac puncture after euthanizing with cervical dislocation; serum was separated by centrifugation at 10,000 g for 10 min and analyzed for creatinine, urea, uric acid, electrolytes (calcium, potassium, sodium, chloride, and bicarbonate), glucose, albumin, and total protein concentrations using methods recommended in the kits.

2.12 Analysis of kidney homogenate sample

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and rinsed in ice-cold physiological

Olayeriju *et al. Afr J Urol* (2020) 26:4

Page 4 of 16

saline. The kidney was finely minced, and 20% homogenate was prepared in Tris–HCl buffer (0.02 mol/L, pH 7.4). Total kidney homogenate was used for assaying antioxidant parameters such as lipid peroxidation [21], glutathione peroxidase (GPx) activity [22], superoxide dismutase (SOD) activity [23], estimation of reduced glutathione (GSH) level [24], protein carbonyl concentration [25], ferric reducing antioxidant power [26], anti-inflammation—myeloperoxidase activity [27], markers of kidney mitochondria integrity—complex I activity [28], glutamine synthetase activity [29], Na^+K^+ ATPase activity [30], lactate dehydrogenase (LDH) activity [31], and protein content [32].

2.13 Histopathological analysis
Histopathological analysis was carried out as described by Sikarwar et al. [2]. The slides were examined under a light microscope to study the light microscopic architecture of the kidney and calcium oxalate deposits using H and E (hematoxylin and eosin) staining.

2.14 Statistical analysis
The data were analyzed with one-way ANOVA followed by Duncan multiple comparison post hoc tests. A statistical difference of $p < 0.05$ was considered significant in all cases.

3 Results
3.1 Phytochemicals and antioxidant activities of the extract
The phytochemical screening of the aqueous methanolic leaf extract of *Triclisia gilletii* showed the presence of phenols (67.42 ± 0.69 mg GAE/g extract),
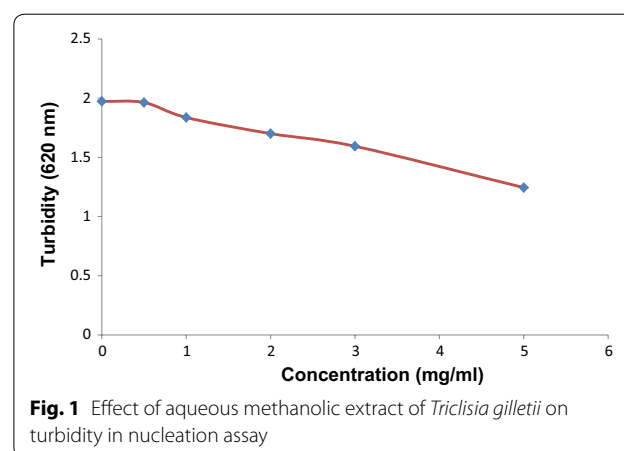
flavonoids (87.78 ± 3.85 mg QE/g extract), tannins (8.33 ± 0.95 mg CE/g extract), cardiac glycosides, saponins (73.98 ± 1.56 mg SE/g extract), and steroids with total antioxidant activity of 30.36 ± 1.90 (mg GAE/g extract) as shown in (Table 1).

3.2 Antilithiatic activity of the extract in vitro
The aqueous methanol extract of *Triclisia gilletii* leaves prevented the nucleation of calcium chloride with sodium oxalate in a concentration dependent manner (Fig. 1), with percent inhibition iC50 7.09 mg/mL (Fig. 2).
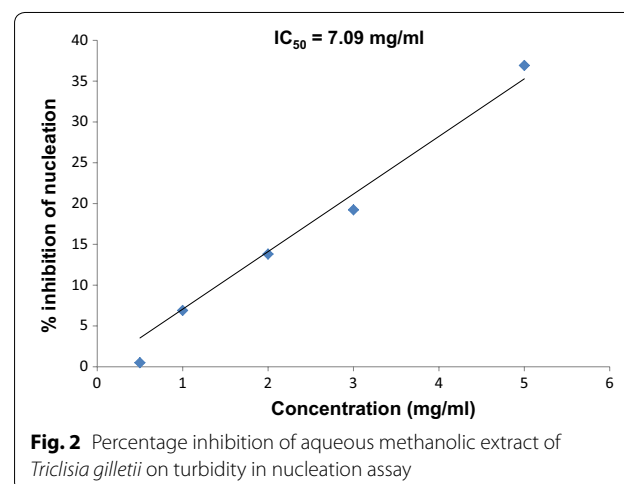
3.3 Toxicological studies
The animals did not show any sign of toxicity even after the administration of TGME at the highest dosage (5000 mg/kg) (Table 2), except weakness within the first 24 h during the experimental period. Administration of

Fig. 1 Effect of aqueous methanolic extract of *Triclisia gilletii* on turbidity in nucleation assay

Table 1 Phytochemicals present in aqueous methanolic leaf extract of TGME

Phytochemical	Amount present	Status
Alkaloids		−
Tannins	8.33 ± 0.95 (mg CE/g extract)	+
Phlobatannin		−
Phenolics	67.42 ± 0.69 (mg GAE/g extract)	+
Flavonoids	87.78 ± 3.85 (mg QE/g extract)	+
Anthraquinones		
Steroids		+
Cardiac glycosides		−
Saponins	73.98 ± 1.56 (mg SE/g extract)	+
Terpenoids		−
Total antioxidant activity	30.36 ± 1.90 (mg GAE/g extract)	+

+ Present, − absent, *CE* catechin equivalent, *GAE* gallic acid equivalent, *QE* quercetin equivalent, *SE* saponin equivalent

Fig. 2 Percentage inhibition of aqueous methanolic extract of *Triclisia gilletii* on turbidity in nucleation assay

Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 5 of 16

TGME every other day for 7 days (10, 100, and 1000 mg/kg b.w.) representing 1/500th, 1/50th, and 1/5th of the highest dosage reduced ($p < 0.05$) the biochemical enzyme activities of ALT, AST, LDH, and concentration of uric acid with a marked increase in total protein concentration in the serum of animals administered TGME in a dose-dependent manner compared with control. No significant effect was observed in the concentrations of creatinine and urea in the animals administered TGME when compared with control (Table 3).

3.4 Nephroprotective study of TGME against ethane-1,2-diol-administered rats

3.4.1 Urine dipstick analysis, body weight, and urine volume

Bilirubin, urobilinogen, ketones, leukocytes, and protein concentration increased in the urine of ethylene-glycol-administered animals when compared with control on days 7, 14, 21, and 28. There was a significant reduction in the appearance of these molecules in the urine with co-administration of *Triclisia gilletii*. The pH of the animals administered ethane-1, 2-diol was slightly alkaline, and the specific gravity was observed to be lowered compared with control. Treatment with TGME ameliorated the effect of ethane-1,2-diol on pH and specific gravity (Table 4).

EG administration also affected the body weight and urine output of the animals with a significant reduction

in weight gained and urine output on the 28th day of exposure when compared with control. Administration of varying dosages of TGME ameliorated the effect in a dose-dependent fashion (Figs. 3 and 4), respectively.

3.4.2 Urine and plasma biochemistry parameters

There was a significant ($p < 0.05$) decrease in creatinine clearance (Fig. 5) and urea clearance (Fig. 6). There was a decline in the concentrations of creatinine and urea in the urine of animals administered ethane-1,2-diol, compared with control. No significant difference was observed in uric acid concentration (Table 5). Plasma concentrations of creatinine and uric acid were significantly elevated in ethane-1,2-diol-administered animals compared with control, with no significant change in the concentration of urea (Table 6). Co-administration with TGME reversed the effect in a dose-dependent manner.

Also, administration of ethane-1,2-diol-induced electrolyte imbalance favoring nephrotoxicity and is characterized by hypocalcemia, hyponatremia, hypochloremia, and hyperkalemia in the plasma (Table 6), with concomitant hypercalciuria, hypernatruria, and hypokaluria in urine (Table 5). Treatment with TGME significantly ($p < 0.05$) modulated the observed electrolyte imbalance.

A significant decrease $p < 0.01$ in plasma concentrations of total protein and albumin (Table 6), with concomitantly increased proteinuria and albuminuria (Table 5), is observed in the animals administered ethane-1,2-diol compared with control. Co-administration with TGME significantly protected the observed proteinuria and albuminuria.

No significant difference was observed in the plasma glucose concentration of animals administered ethane-1,2-diol when compared with control (Table 6). But increased glucosuria was observed in ethane-1,2-diol-administered animals when compared with control (Table 5). TGME significantly reduced the effect in a dose-dependent manner.

There was a significant $p < 0.0001$ increase in bicarbonate concentration in the plasma of animals administered ethane-1,2-diol compared with control. Co-administration with TGME significantly brought about a decrease in bicarbonate concentration (Fig. 7).

Table 2 LD$_{50}$ determination of aqueous methanolic leaf extract of TGME in orally administered albino rats

S/N	Number of animals	TGME (mg/kg)	Mortality (x/N)	Symptoms
Stage 1	3	0	0/3	Nil
	3	10	0/3	Nil
	3	100	0/3	Nil
Stage 2	3	1000	0/3	Weakness
	3	1600	0/3	Weakness
	3	2900	0/3	Weakness
	3	5000	0/3	Weakness

LD50 greater than 5000 mg/kg b.w

TGME Triclisia gilletii aqueous methanolic extract

Table 3 Serum biochemical parameters in animals administered aqueous methanolic leaf extract of *Triclisia gilletii*

Groups	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	TP (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Control	64.49 ± 3.9[a]	29.27 ± 6.20[a]	2070.3 ± 280.64[a]	5.00 ± 0.30[b]	2.10 ± 0.56[a]	32.66 ± 3.17	0.64 ± 0.20
TGME (10 mg/kg)	55.52 ± 7.0[ab]	27.07 ± 4.24[ab]	1360.8 ± 102.34[b]	5.93 ± 0.73[a]	1.85 ± 0.64[b]	30.15 ± 8.47	0.69 ± 0.22
TGME (100 mg/kg)	51.59 ± 7.73[b]	19.80 ± 2.60[ab]	458.1 ± 66.42[c]	5.26 ± 0.20[ab]	1.76 ± 0.16[b]	35.06 ± 8.18	0.87 ± 0.10
TGME (1000 mg/kg)	37.10 ± 4.40[c]	17.67 ± 6.7[b]	520.81 ± 84.94[c]	4.94 ± 0.60[b]	1.79 ± 0.21[b]	36.06 ± 3.07	0.64 ± 0.23

Values are expressed as mean ± standard deviation ($n = 6$). Values with different superscript(s) in a column are significantly different ($p < 0.05$)

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactate dehydrogenase, *TP* total protein, *TGME Triclisia gilletii* aqueous methanolic extract

Table 4 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on urinalysis in ethane-1,2-diol-administered albino rats
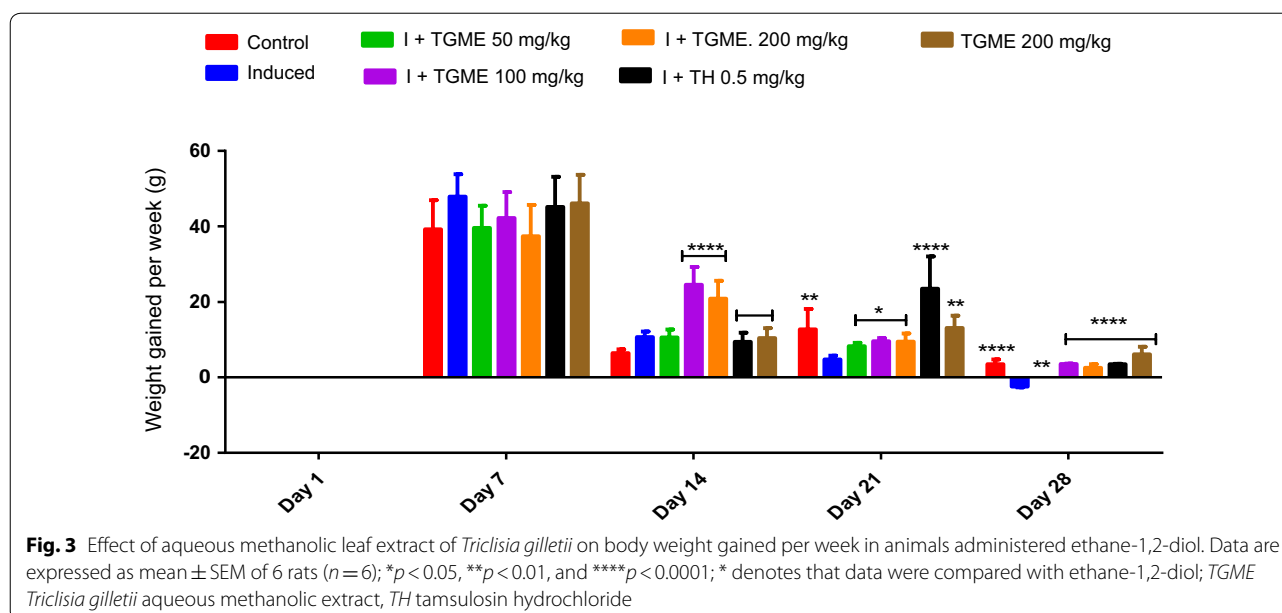
	ASC (mg/dL)	BLO (Ery/µL)	BIL (mg/dL)	URO (mg/dL)	KET (mg/dL)	GLU (mg/dL)	PRO (mg/dL)	NIT	LEU (Leu/µL)	pH	SG
Day 0											
Control	10	–	–	–	–	–	30	+	–	8	1.01
Induced	10	–	–	–	–	–	30	+	–	7.89	1.01
I+TGME (50 mg/kg)	10	–	–	–	–	–	30	+	–	8	1.01
I+TGME (100 mg/kg)	10	–	–	–	–	–	30	+	–	8	1.01
I+TGME. (200 mg/kg)	10	–	–	–	–	–	30	+	–	8.25	1.012
I+TH (5 µg/kg)	10	–	–	–	–	–	30	+	–	8	1.013
TGME (200 mg/kg)	10	–	–	–	–	–	30	+	–	8	1.01
Day 7											
Control	10	–	–	–	–	–	30	+	–	8	1.012
Induced	20	–	0.5	1	10	–	175	+	10	8	1.01
I+TGME (50 mg/kg)	20	–	–	0.2	10	–	100	+	–	8	1.023
I+TGME (100 mg/kg)	20	–	–	–	–	–	65	+	–	8.25	1.013
I+TGME (200 mg/kg)	15	–	–	–	–	–	65	+	–	8.24	1.02
I+TH (5 µg/kg)	10	–	–	–	5	–	30	+	–	7.75	1.02
TGME (200 mg/kg)	20	–	–	–	0	–	30	+	–	7.35	1.022
Day 14											
Control	10	–	–	0.1	0	–	22.5	+	–	8	1.013
Induced	15	–	1	1	10	–	100	+	10	8.5	1.005
I+TGME (50 mg/kg)	10	–	0.5	0.2	2.5	–	65	+	–	7.5	1.013
I+TGME (100 mg/kg)	10	–	–	0.1	0.1	–	22.5	+	–	7.3	1.018
I+TGME (200 mg/kg)	10	–	–	0.2	0	–	22.5	+	–	7.75	1.023
I+TH (5 µg/kg)	10	–	–	0.15	10	–	30	+	–	7.75	1.018
TGME (200 mg/kg)	15	–	–	0.2	0	–	30	+	–	7.5	1.02
Day 21											
Control	10	–	–	0.1	0	–	30	+	–	7	1.018
Induced	15	–	0.5	1	10	–	175	+	10	8.5	1.013
I+TGME (50 mg/kg)	20	–	–	1	7.5	–	100	+	7.5	7	1.013
I+TGME (100 mg/kg)	15	–	–	0.5	5	–	65	+	–	7	1.02
I+TGME. (200 mg/kg)	20	–	–	–	–	–	22.5	–	–	7.75	1.02
I+TH (5 µg/kg)	20	–	–	0.1	7.5	–	30	+	–	7.3	1.023
TGME (200 mg/kg)	10	–	–	0.2	–	–	30	–	–	7.75	1.018
Day 28											
Control	15	–	–	0.2	–	–	22.5	+	–	6.8	1.018
Induced	15	–	0.5	1.5	7.75	–	175	+	–	9	1.003

Table 4 (continued)

	ASC (mg/dL)	BLO (Ery/µL)	BIL (mg/dL)	URO (mg/dL)	KET (mg/dL)	GLU (mg/dL)	PRO (mg/dL)	NIT	LEU (Leu/µL)	pH	SG
I+TGME (50 mg/kg)	20	–	–	0.6	3	–	65	+	–	8.5	1.007
I+TGME (100 mg/kg)	20	–	–	0.2	–	–	22.5	+	–	8.3	1.015
I+TGME (200 mg/kg)	20	–	–	0.2	–	–	22.5	–	–	7.5	1.018
I+TH (5 µg/kg)	20	–	–	0.1	5	–	30	+	–	7.8	1.02
TGME (200 mg/kg)	10	–	–	0.2	–	–	22.5	–	–	8.3	1.015

Values are average of three animals

TGME Triclisia gilletii aqueous methanolic extract, *TH* tamsulosin hydrochloride, *ASC* ascorbic acid, *BLO* blood, *BIL* bilirubin, *URO* urobilinogen, *KET* ketone, *GLU* glucose, *PRO* protein, *NIT* nitrite, *LEU* leukocyte, *SG* specific gravity

Fig. 3 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on body weight gained per week in animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n = 6$); *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$; * denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

3.5 Antioxidant and markers of membrane integrity parameters

A significant increase was observed in the MDA produced (Fig. 8) and protein carbonyl concentration (Fig. 9) with a concomitant decrease in GSH (Fig. 10) and FRAP concentrations (Fig. 11) in the kidney homogenate of animals administered ethane-1,2-diol compared with control. Also, significant decreases were observed in the SOD (Fig. 12) and GPx activities (Fig. 13) in the kidney homogenate of animals administered ethane-1,2-diol when compared with control. TGME significantly brought about positive modulation in the antioxidant concentrations and antioxidant enzyme activities.

A significant increase $p < 0.0001$ was observed in the MPO activity in animals administered ethane-1,2-diol when compared with control. TGME significantly reduced the MPO activity in a dose-dependent manner (Fig. 14).

There was a significant decrease $p < 0.0001$ in complex 1 activity (Fig. 15), Na–K ATPase activity (Fig. 16), glutamine synthetase activity (Fig. 17), and LDH activity (Fig. 18) in the kidney homogenate of animals administered ethane-1,2-diol when compared with control. Co-administration with TGME significantly brought about an increase in enzyme activity when compared with induced animals (Fig. 19).

3.6 Histopathology

From the histopathology analysis, no visible lesion was seen in the control animals. Some glomeruli and tubules were degenerated along with severe congestion of the interstitium with clumps of crystal casts found in the lumen of few tubules of animals administered ethane-1,2-diol (induced group). Several degenerated tubules with cellular debris and dark staining casts in the lumen were seen in the animals co-treated with (50 mg/kg) TGME. Also, few degenerate tubules with dark staining casts were seen in the animals co-treated with (100 and 200 mg/kg) TGME. Moderate interstitial congestion was seen in animals co-treated with standard (tamsulosin hydrochloride). No visible lesion was seen in the animals treated with 200 mg/kg TGME only.
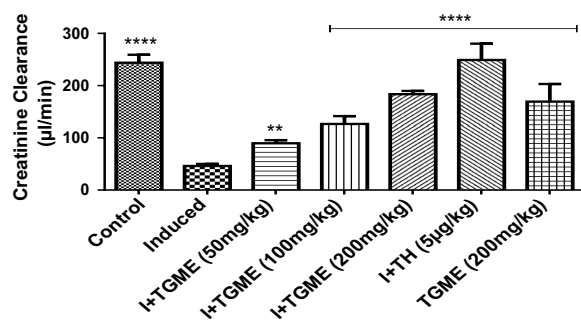
4 Discussion

The kidneys are reddish brown, paired structures that lie on either side of the vertebral column in the lumbar region of the body. The kidney carries out its homeostatic mechanisms by maintaining the overall chemical composition of the intracellular environment and regulating the quantity of water, sodium chloride, potassium, phosphate, and numerous other substances in the body [33]. It is, therefore, a vital organ, and renal problems could be highly debilitating or even fatal as a result of waste or fluid accumulation and electrolyte imbalance.

Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Previous studies indicated that 28-day administrations of 1% ethylene glycol to male albino rats resulted into the formation of renal calculi composed mainly of calcium oxalate [34–37]. Elevated urinary calcium is a favoring factor for nucleation of calcium oxalate from urine and further crystal growth basically through the sequestering power of oxalate produced from ethylene glycol metabolism and supersaturation
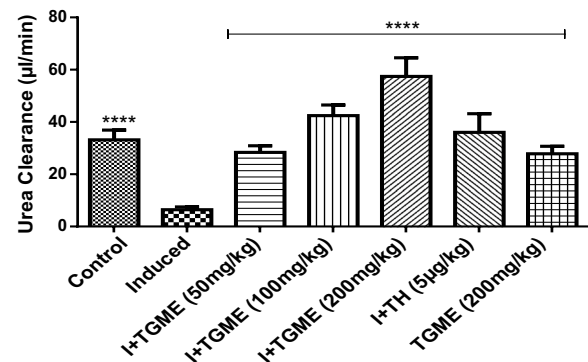
Fig. 4 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on urine volume/mL 24 h per week in animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n=6$); ****$p < 0.0001$; * denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

Fig. 5 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on creatinine clearance in animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n=6$); **$p < 0.01$, and ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

Fig. 6 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on urea clearance in animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n=6$); ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride
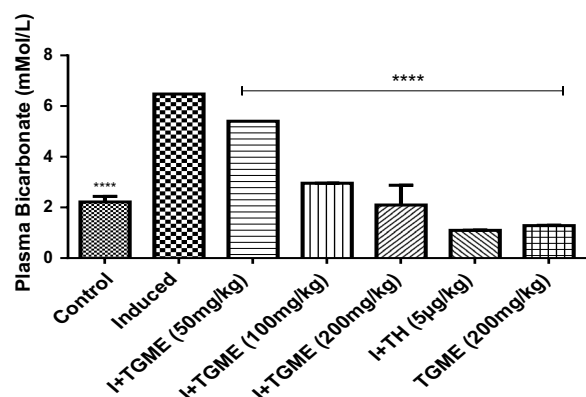
in the kidney [38]. Calcium excretion progressively increased in calculi-induced animals. This chronic hypercalciuria results in massive crystalluria and subsequently stone formation [39].

In this present study, bicarbonate concentration which is a measure of acidity or alkalinity in the blood was high in ethane-1,2-diol-administered animals. This is not consistent with ethane-1,2-diol accidental toxicity in human. A lower concentration of ethane-1,2-diol in rats has been known to favor carbon dioxide elimination, while higher concentration favors the accumulation of glycolic acid, which usually results in metabolic acidosis. The proportion eliminated as oxalate is not dose dependent [40].

Therefore, the observed damage to the kidney in the animals is as a result of oxalate or calcium oxalate [41]. In this present study, TGME showed a protective effect in the damage caused by metabolic alkalosis due to excess bicarbonate generated from low-dose ethane-1,2-diol. Electrolyte imbalance observed as a result of administration of ethane-1,2-diol was modulated by co-administration of TGME. We suggest a possible inhibition of ethane-1,2-diol metabolism by the extract which calls for further studies.

Low 24-h urine volume can change the composition of urine and promote the risk of stone formation [42].

Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 10 of 16

Table 5 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on urine biochemistry parameters in animals administered ethane-1,2-diol

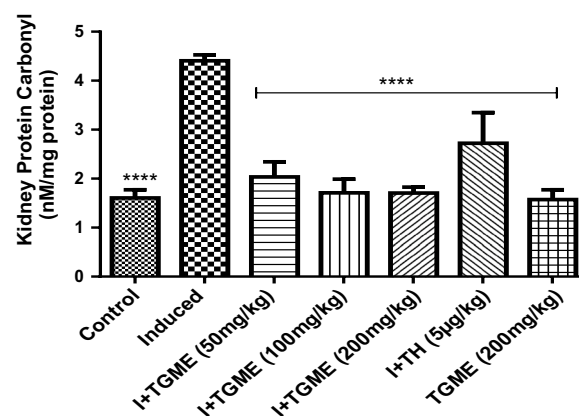| Groups	Control	Induced	I+TGME (50 mg/kg)	I+TGME (100 mg/kg)	I+TGME (200 mg/kg)	I+TH (0.5 µg/kg)	TGME (200 mg/kg)
Creatinine (mg/dL)	109.73 ± 9.34****	57.61 ± 5.03	58.00 ± 8.20	58.98 ± 4.98	85.55 ± 7.67****	99.75 ± 12.37****	72.50 ± 4.2*
Urea (mg/dL)	116.22 ± 14.85****	62.31 ± 1.25	117.55 ± 18.56****	145.40 ± 22.09****	164.25 ± 29.20****	131.22 ± 13.34****	96.70 ± 4.67*
Uric acid (mg/dL)	2.15 ± 0.18	2.27 ± 0.07	2.45 ± 0.04	2.37 ± 0.23	2.45 ± 0.26	2.88 ± 0.09****	2.77 ± 0.04****
Calcium (mg/dL)	5.61 ± 1.81****	9.57 ± 0.13	7.72 ± 0.24*	7.82 ± 0.54*	3.61 ± 0.41****	4.79 ± 0.38****	6.69 ± 1.07****
Sodium (mEq/dL)	34.27 ± 7.20****	53.31 ± 3.45	51.36 ± 5.96	37.46 ± 3.5***	38.925 ± 4.76**	31.82 ± 7.78****	33.89 ± 4.76****
Chloride (mMol/L)	45.47 ± 5.76****	35.95 ± 1.02	85.23 ± 3.50****	60.72 ± 5.35****	59.86 ± 6.51****	90.06 ± 7.89****	40.30 ± 1.23
Potasium (mEq/L)	43.17 ± 5.32****	29.49 ± 4.32	37.79 ± 3.46**	36.98 ± 3.21*	43.83 ± 2.24****	37.24 ± 4.32*	37.26 ± 2.97*
Total Protein (g/L)	6.71 ± 0.56****	12.52 ± 1.2	9.56 ± 1.81**	8.78 ± 0.42***	8.38 ± 1.81****	7.99 ± 1.53****	9.07 ± 0.28***
Albumin (g/L)	1.24 ± 0.47****	2.27 ± 0.15***	1.61 ± 0.18****	0.95 ± 0.06****	1.07 ± 0.12**	1.78 ± 0.18**	1.07 ± 0.16****
Glucose (mg/dL)	4.01 ± 0.90****	8.21 ± 0.77	7.85 ± 0.77	6.20 ± 0.52****	4.74 ± 0.52****	6.75 ± 0.26**	6.93 ± 0.52*

Data are expressed as mean ± SEM of 6 rats ($n = 6$); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

Table 6 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on plasma biochemistry parameters in animals administered ethane-1,2-diol

Groups	Control	Induced	I+TGME (50 mg/kg)	I+TGME (100 mg/kg)	I+TGME (200 mg/kg)	I+TH (0.5 µg/kg)	TGME (200 mg/kg)
Creatinine (mg/dL)	0.65 ± 0.04****	0.82 ± 0.01	0.68 ± 0.01**	0.66 ± 0.08**	0.65 ± 0.02***	0.57 ± 0.07****	0.59 ± 0.12****
Urea (mg/dL)	30.63 ± 3.74	29.63 ± 4.85	26.08 ± 2.32	28.96 ± 2.86	24.17 ± 2.85	31.64 ± 5.13	30.27 ± 2.80
Uric acid (mg/dL)	0.91 ± 0.18****	3.27 ± 0.24	2.23 ± 0.13****	1.76 ± 0.02****	0.54 ± 0.14****	2.80 ± 0.30**	0.90 ± 0.06***
Calcium (mg/dL)	10.77 ± 1.56****	3.44 ± 0.05	6.91 ± 0.40****	7.87 ± 1.52****	10.54 ± 0.99****	10.11 ± 0.21****	9.91 ± 0.79****
Sodium (mEq/dL)	157.49 ± 9.93****	116.90 ± 3.8	127.32 ± 5.53	131.15 ± 5.53*	131.98 ± 8.2**	130.18 ± 5.22*	130.36 ± 2.38*
Chloride (mMol/L)	101.32 ± 9.70	92.07 ± 10.98	107.27 ± 10.16	109.19 ± 15.80	95.25 ± 8.86	102.91 ± 13.58	103.79 ± 8.86
Potassium (mEq/L)	6.44 ± 0.63****	17.62 ± 2.87	2.95 ± 0.58****	3.50 ± 0.14****	4.40 ± 0.82****	3.23 ± 0.48****	2.95 ± 0.58****
Total Protein (g/L)	59.89 ± 1.91**	53.78 ± 1.02	64.72 ± 2.67****	59.07 ± 1.21**	61.76 ± 3.33****	59.03 ± 1.78**	59.59 ± 3.46**
Albumin (g/L)	26.06 ± 1.08**	22.51 ± 1.06	25.34 ± 1.70*	26.38 ± 1.17***	26.92 ± 1.41***	26.00 ± 1.56**	26.29 ± 1.91**
Glucose (mg/dL)	174.15 ± 15.02	182.60 ± 15.08	171.04 ± 12.01	170.74 ± 16.00	158.52 ± 13.24	179.15 ± 11.24	155.78 ± 13.24

Data are expressed as mean ± SEM of 6 rats ($n = 6$); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

However, phytochemicals have proven to be effective in reducing stone formation [43], such as steroids, terpenoids, flavonoids, polyphenols, and tannins, those present in TGME. In most cases, they are acting as diuretics as observed in our present study with TGME.
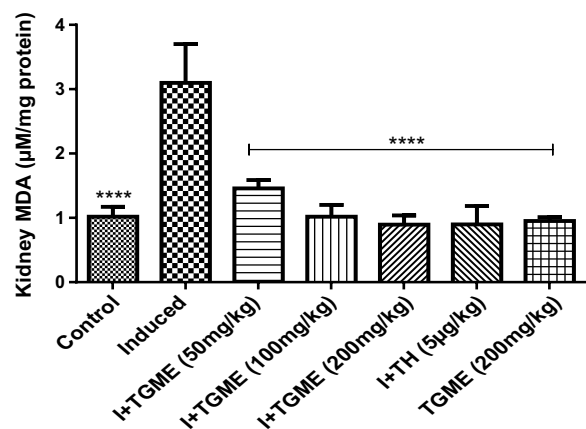
In nephrolithiasis, the glomerular filtration rate decreases due to the obstruction to the flow of urine by stones in the urinary system as seen in the present study. Due to this, the waste products, particularly nitrogenous
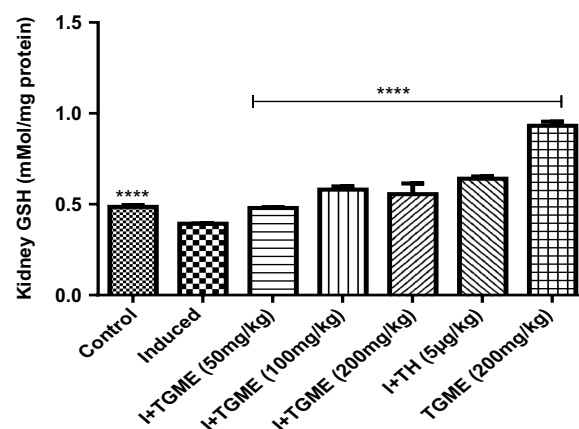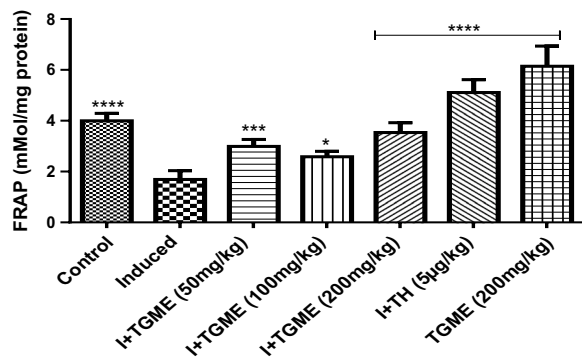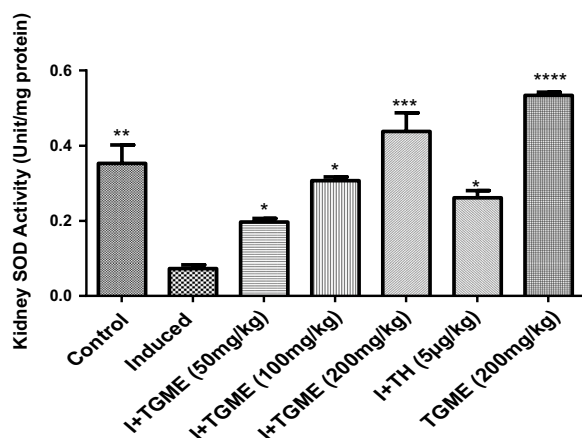
Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 11 of 16

Fig. 7 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on plasma bicarbonate concentration in animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n = 6$); ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

Fig. 9 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on protein carbonyl concentration in animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n = 6$); ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

Fig. 8 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on MDA produced in animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n = 6$); ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride, *MDA* malondyaldehyde

Fig. 10 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on concentration of GSH in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats ($n = 6$); *< 0.05, ***$p < 0.001$, and ****$p < 0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride, *GSH* reduced glutathione

substances such as urea, creatinine, and uric acid, accumulate in the blood [34].

In calculi-induced rats, marked renal damage was seen as indicated by elevated serum levels of creatinine and uric acid, which are markers of glomerular and tubular damage. Although in the present study, ad libitum exposure of animals to 1% ethane-1,2-diol did not significantly increase the plasma urea concentration when compared with control, urea clearance per 24 h revealed that there was a significant difference in the functionality rate of the
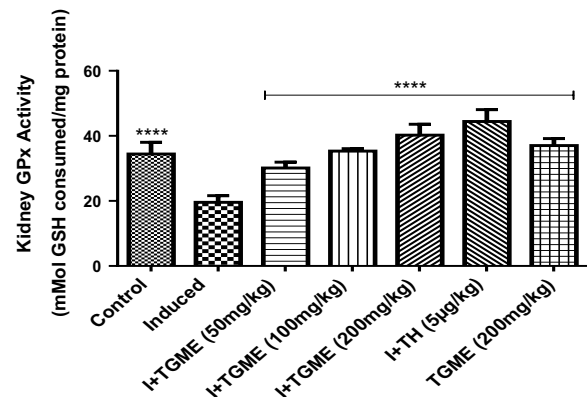
kidney in excreting urea. This suggests that accumulation of urea could be developing. Treatment with aqueous TGME showed an ameliorative effect on plasma concentrations of these markers by improving glomerular filtration. This could be ascribed possibly to the presence of phytoconstituents (saponins and flavonoids) in the TGME, as plants rich in the duo had been reported to protect against ethylene glycol-linked oxalate intoxication functioning as diuresis and possibly through an antioxidant mechanism [2].
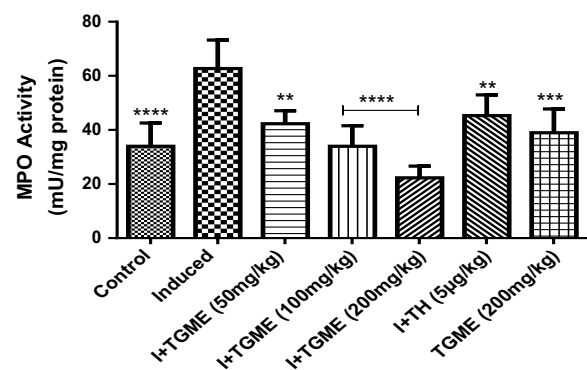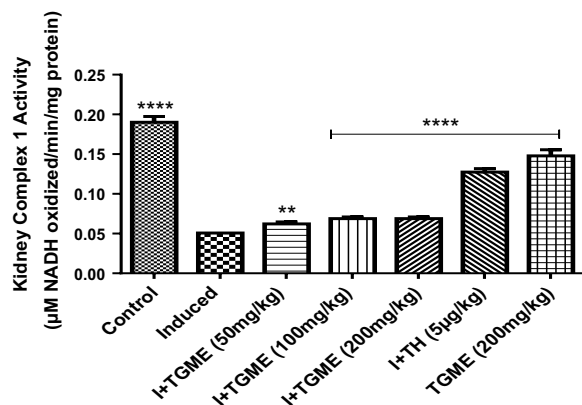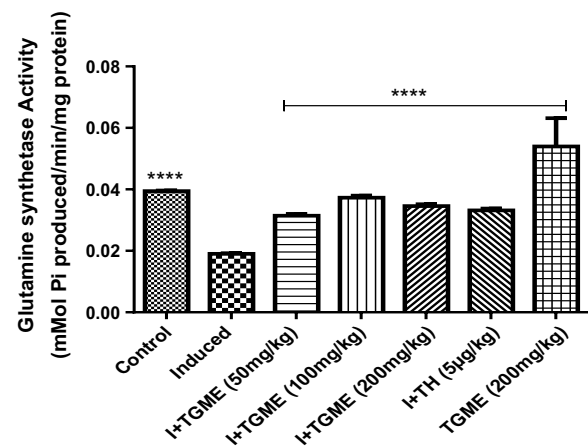
Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 12 of 16

Fig. 11 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on concentration of FRAP in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean±SEM of 6 rats ($n=6$); *<0.05, ***$p<0.001$, and ****$p<0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride, *FRAP* ferric reducing antioxidant power

Fig. 13 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on GPx activity in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean±SEM of 6 rats ($n=6$); *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride, *GPx* glutathione peroxidase

Fig. 12 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on SOD activity in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean±SEM of 6 rats ($n=6$); *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride, *SOD* superoxide dismutase

Fig. 14 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on MPO activity in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean±SEM of 6 rats ($n=6$); **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride, *MPO* myeloperoxidase

Most proteins are too large to pass through the kidney filter into the urine unless the kidney is damaged. The main protein that is mostly filtered is albumin. Increased proteinuria, albuminuria, and glucosuria in this study indicate possible glomerular and tubular damage as a result of ethane-1,2-diol administration. The protectivity of TGME on the renal epithelial could be ascribed to the antioxidant power of quercetin and oleanolic acid present in the plant [12].
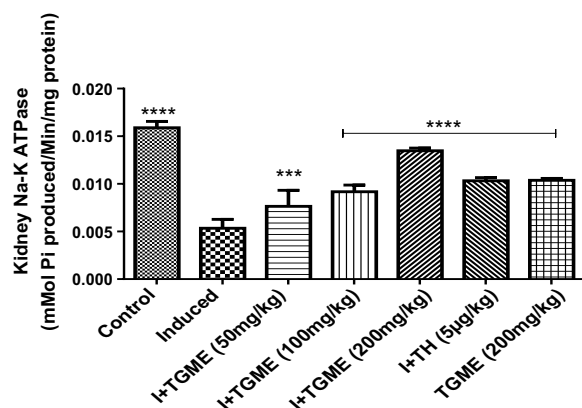
The development of tissue injury probably depends on the balance between the generation of reactive oxygen species (ROS) and the tissue antioxidant defense mechanism. Reduced tissue antioxidant enzymes activity is usually accompanied with increased free radical production in different stages of nephrolithiasis, which predispose the renal tissue under oxidative stress. The report that patients with kidney stones have less activity of antioxidant enzymes with increased lipid peroxidation further strengthened our finding [38].
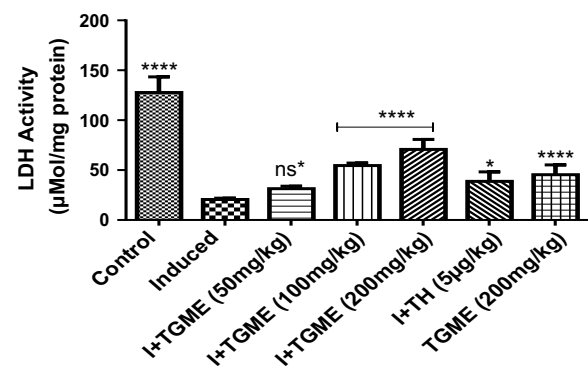
Fig. 15 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on complex 1 activity in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats (*n* = 6); **p < 0.01, and ****p < 0.0001; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

Fig. 17 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on glutamine synthetase activity in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats (*n* = 6); ns* > 0.05, *p < 0.05, and ****p < 0.0001; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

Fig. 16 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on Na–K ATPase activity in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats (*n* = 6); ***p < 0.01, and ****p < 0.0001; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride
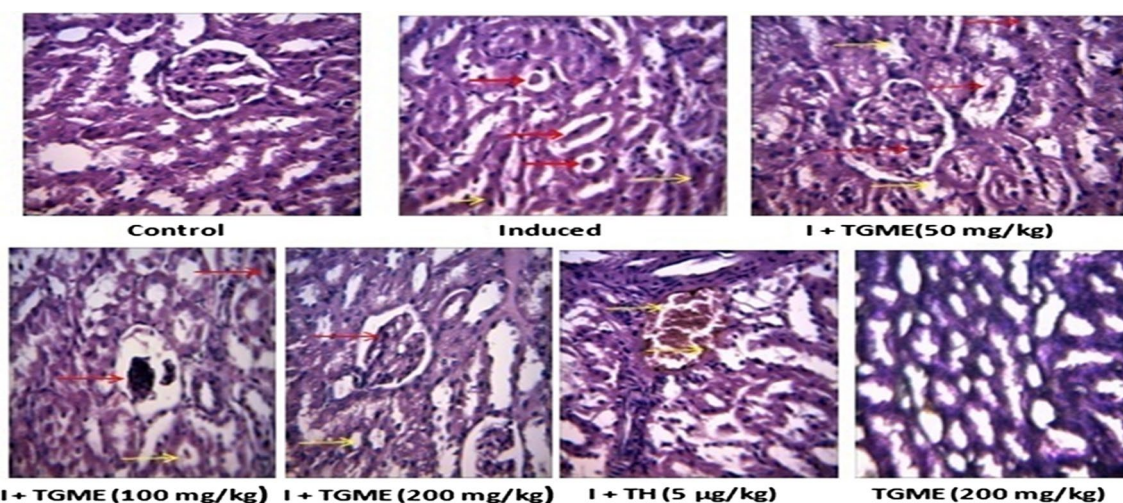

Fig. 18 Effect of aqueous methanolic leaf extract of *Triclisia gilletii* on LDH activities in the kidney of animals administered ethane-1,2-diol. Data are expressed as mean ± SEM of 6 rats (*n* = 6); ns* > 0.05, *p < 0.05, and ****p < 0.0001; *denotes that data were compared with ethane-1,2-diol; *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride, *LDH* lactate dehydrogenase

Thamilselvan and Selvam [44] showed in their study that ethane-1,2-diol down-regulates kidney antioxidant system, which was also observed in the present study with 1% ethane-1,2-diol administration in Wistar rats. Co-treatment with TGME regimen up-regulates the antioxidant parameters. Phytochemical present in the plant (oleanolic acid) which is a triterpenoid saponin is known to preserve GSH [45], β-sitosterol [46], and stigmasterol [47], possessing antioxidant activity in different models of nephrotoxicity.

Neutrophil activation in the presence of CaOx stones is usually accompanied by increased MPO activity which is a predisposing factor for inflammation-enhancing nephrolithiasis [39]. Increased MPO activity in ethane-1,2-diol-administered animals in this study proves that inflammation accompanies stone formation, and the elevation in the creatinine and urea levels could be due to damage in the kidney tubules [48] as established by the marked changes in MPO activity.

Antioxidants have been known to decrease MPO activities in tissues owing to inhibition of neutrophil activation [49]. The decreased MPO activity observed in the animals

Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 14 of 16

Fig. 19 Light microscopic architecture in the kidney section stained with hematoxylin and eosin (H&E): X100. *TGME Triclisia gilletii* aqueous methanolic extract, *TH* tamsulosin hydrochloride

co-administered with TGME cannot be farfetched from the potentials of its phytochemical constituents. Oleanolic acid is a known anti-inflammatory agent [50].

Mitochondrial function is important in regulating energy production and redox signaling which is usually impaired in patients with CaOx kidney stone disease [41, 51]. Hence, markers suggesting membrane damage were investigated in this study via complex 1 activity, Na–K ATPase activity, glutamine synthetase activity, and lactate dehydrogenase activity.

Complex 1 which is also known as NADH:ubiquinone oxidoreductase (EC 1.6.5.3) catalyzes movement of protons across the inner mitochondrial membrane which ultimately yields ATP for mitochondrial and cell function as well as movement of solute across the membrane. This enzyme (complex 1) is highly vulnerable to inactivation by free radicals and is probably the most susceptible component of the electron transport chain [52]. The increased activity of this enzyme in the kidney of animals administered TGME could be ascribed to the antioxidant potential of the extract as seen in the current study.

Also, (Na$^+$–K$^+$) adenosine triphosphatase (ATPase), which is localized to the basolateral membrane, is responsible for tubular re-absorptive process and regulated by direct interactions with membrane-associated cytoskeletal proteins that are also susceptible to oxidative damage [53]. The presence of calcium or calcium oxalate had been linked with the reorganization of the actin cytoskeleton, and it is believed to be important in the surface membrane structural, biochemical, and functional alterations in tubules [41].

Renal ammonia metabolism usually regulated by glutamine synthetase activity helps maintain acid–base balance or homeostasis [54]. Increased ammonia metabolism in the nephron is key to acid–base homeostasis in chronic kidney disease, until advanced chronic kidney disease is present [55], which is important for delaying progression of renal repair and for controlling skeletal muscle mass [56, 57].

Reduced activities of these markers of membrane stability enzymes including lactate dehydrogenase in the kidney homogenate signify progressive damage in the membrane integrity caused by oxalate or calcium oxalate stone [58, 59]. The protectivity of TGME cannot be farfetched from the antioxidant constituents in the plant.

5 Conclusion

The leaves of *Triclisia gilletii* exhibited a protective effect on experimentally induced urolithiasis and nephrotoxicity due to its effect on inhibition of crystal growth, modulation of electrolyte imbalance, and antioxidant and anti-inflammatory ability. The relatively safe property of the plant coupled with antiurolithiatic potentials possibly explains its usage as nephroprotectant in West Africa, particularly southwestern part of Nigeria (Ondo State). This investigation projects the therapeutic potential of the plant to be developed as an alternative herbal antiurolithiasis drug.

Availability of data and material
The datasets generated and analyzed during the current study are not publicly available because that is the policy of our university but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Experiments were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines after the approval of the experimental protocol by the Institutional Animals Ethics Committee of the Department of Biochemistry, the Federal University of Technology Akure. At that time, there was no number to be given to the study, but the proposal number was adopted (Proposal No. BCH/07/1376). Approved consent was granted to purchase animals from a private colony in Akure, Ondo State, Nigeria.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

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Olayeriju *et al. Afr J Urol*   (2020) 26:4

Page 16 of 16

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