

Effects Of Aqueous Extract Of Amblygonocarpus Andongensis (Fabaceae) Stem Bark On Acute Kidney Injury Induced By Unilateral Ureteral Obstruction In Wistar Rats

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Abstract

Objective: The aim of this study was to evaluate the mechanism of action of *Amblygonocarpus andongensis* on acute kidney injury (AKI) induced by unilateral ureteral obstruction (UO). **Methods:** 36 rats were divided into six groups of six animals each. AKI was induced by UO on all animals except those in the normal and sham control groups, under anaesthesia. The animals were treated with 5 ml/kg distilled water (normal, sham and negative controls), aqueous extract

of *A. andongensis* stem bark (AEAASB) at doses of 125, 250 and 500 mg/kg (test groups), daily for 3 days. Urinary, haematological, serum and oxidative stress parameters were assessed. Histological sections of the kidneys were taken.

Results: AEAASB caused a significant decrease in the mass of obstructed kidneys, leucocyte count ($p < 0.001$), urinary protein and serum creatinine levels and consequently increase urinary creatinine level and the value of renal creatinine clearance ($p < 0.001$). Additionally, this extract, significantly decreased malondialdehyde levels ($p < 0.05$) and increased superoxide dismutase, catalase, reduced glutathione and nitric oxide levels ($p < 0.001$). Microphotographs showed that this extract prevented renal tissue damage induced by UUO. Principal component analysis and agglomerative hierarchical clustering showed that AEAASB has therapeutic effects on AKI associated with UUO and that the 500 mg/kg dose was the most effective. **Conclusion:** AEAASB has nephroprotective effects which are thought to result from its vasodilatory and inhibitory action on the renin-angiotensin-aldosterone system, via an increase in nitric oxide production.

Keywords Amblygonocarpus andongensis, Unilateral ureteral obstruction, Nephroprotection, Antioxidant, Vasodilation.

1. INTRODUCTION

Obstructive nephropathy is a renal disorder due to a bilateral excretory tract obstruction or a unilateral single kidney obstruction [1]. It can lead to acute kidney injury (AKI) or chronic renal failure when the obstruction is not removed early [2,3]. Obstructive nephropathy accounts for approximately 10 % of all cases of both acute and chronic kidney failure [4]. Worldwide, AKI is a major cause of mortality and morbidity [5]. It is estimated that there are more than 13 million cases each year, 11 million of which occur in poor or low-income countries [6,7]. The condition is responsible for nearly 1.7 million deaths per year worldwide, with 1.4 million in low- and middle-income countries [7].

AKI secondary to obstructive nephropathy is a frequent clinical situation. It accounts for 5 to 10 % of all acute kidney injury cases [8]. In institutions with limited technical resources, particularly in developing countries, acute kidney injury linked to obstruction poses serious health problems involving the vital prognosis of those affected [9]. The most common causes of obstructive nephropathy are pelvic cancers (bladder, prostate hyperplasia and prostate cancer), urinary lithiasis, ureteral obstruction due to schistosomiasis and congenital malformations [2,8,10,11].

Renal dysfunction due to acute kidney injury is always preceded by mainly haemodynamic and inflammatory renal insults [12]. At the onset of obstruction, intra-tubular pressure increases due to urine retention. When this pressure is sufficiently high, it opposes that of glomerular filtration and leads to a decrease in glomerular filtration rate [2,3]. Depending on the severity of the blockage, renal failure can rapidly become life-threatening. Early removal of the obstruction therefore conditions the reversibility of the pathology [13]. If the obstruction continues for several days, AKI develops into tubulointerstitial fibrosis [3]. Beyond 3 weeks of total obstruction, the potential for renal parenchymal recovery is very low [14].

Symptoms commonly observed in acute kidney injury secondary to obstructive nephropathy include asthenia, anorexia, vomiting, oedema, nephrotic colic, dysuria, anuria and haematuria [10, 11]. In the case of acute complete obstruction, nephrotic or ureteral colic is frequently present due to distension of the bladder, collecting system or renal capsule. The obstruction could be incomplete, and is therefore not always associated with anuria [15]. Whatever the cause, the diagnostic and therapeutic management of AKI linked to obstruction is similar and consists of emergency drainage of the urinary tract, correction of metabolic disorders and clinical and biological monitoring [16]. To complete the renal recovery process, removal of the obstruction requires emergency surgical management [10].

Unilateral ureteral obstruction (UUO) is a widely used model to study obstructive nephropathy in order to prevent or restore renal function [3, 17]. Nowadays, several studies have shown the efficacy of medicinal plant extracts in the treatment of various diseases. *Amblygonocarpus andongensis* (Fabaceae) is a vascular plant from tropical regions of Africa, widely used for

its therapeutic properties [18]. This plant has been classified as having powerful antioxidant activity [19]. The bark of this plant is said to have anti-inflammatory, anti-ulcer, healing and cardiovascular disease properties [20, 21]. As renal failure is a multifactorial disease, the aim of this study was to elucidate the nephroprotective mechanism of the aqueous extract of *A. andongensis* stem bark on another model of AKI, namely UUO.

2 METHODS

2.1 Animal material

Adult healthy Wistar male rats strain aged 12 weeks and weighing between 180 and 220 g were used for this experiment. These animals were bred at the animal house. They were housed in wire mesh cages maintained at room temperature with a natural light/dark cycle. All the animals received a standard diet and unlimited tap water daily. Experimentation was carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Purposes [23].

2.2 Plant material

The fresh stem barks of *A. andongensis* were collected. The bark collected was weighed, thoroughly washed and dried at room temperature in the laboratory. The dry bark were ground and reduced to a fine powder. Identification and authentication of the collected plant were done by comparison with specimens N° HEFG/1736.

2.3 Preparation of aqueous extract of *A. andongensis* stem bark

A mass of 500 g of dry powder of *A. andongensis* stem bark was prepared by decoction in 3.5 L of distilled water for 15 minutes. This decoction was cooled at room temperature for 45 minutes and then filtered using Whatman N° 3 paper. The filtrate obtained was evaporated in an oven at 50 °C for 24 hours. The dry extract obtained was 65.47 g, giving a yield of 13.09 % [22].

2.4 Experimental design

Thirty-six rats were divided into 6 groups of 6 animals each, including normal, sham and negative control groups (5 mL/kg Distilled water) and three test groups (aqueous extract of *A. andongensis* stem bark 125, 250 and 500 mg/kg). Under ketamine (2.5 mg/kg, i.p)/diazepam (5 mg/kg, i.p) anaesthesia, a left flank laparotomy was performed on all animals except the normal control group. The left ureter (negative control and test groups) was ligated with 5-0 silk suture (0.1 mm suture diameter) (Agary Pharmaceutical Ltd) using two suture knots at two locations (approximately 0.5 cm apart), one proximal near the kidney and the other distal. A section was made between the knots and then the abdomen was closed by suture [3, 24]. The sham control group underwent laparotomy following the same procedure but the double ligation and section of the ureter were not performed. All the groups of animals were treated with the different solutions 30 min after surgery, once a day for three days. On the 3rd day after the different treatments, the animals were fasted and urine was collected from each animal for 24 h using individual metabolic cages. After 24 h fasting, all animals were sacrificed under ketamine (2.5 mg/kg, i.p)/diazepam (5 mg/kg, i.p) anaesthesia.

2.5 Collection of blood and organ samples

The blood of each animal was collected after rupture of the jugular vein in EDTA (Ethylene Diamine Tetra-acetic acid) tubes and dried respectively for the analysis of haematological and biochemical parameters. Kidney, liver, heart, spleen, lung, stomach and

testes from each animal were removed and weighed to determine their relative mass [25]. One kidney from each animal was used to prepare the homogenates. The second kidney was rinsed in physiological saline (0.9 %) and then preserved in 10 % formaldehyde for histological sections [26].

2.6 Determination of relative organ weight

The relative weights of the sampled organs were obtained using the formula [25]:

$$\text{Relative organ weight (\%g/g)} = \frac{\text{Absolute weight of the organ (g)}}{\text{Body weight of the animal on the day of sacrifice (g)}} 100$$

2.7 Evaluation of urinary parameters

The pH, osmolarity and protein levels were measured using urine strips (Urinalysis 11A Reagent Strips, Mission brand manufactured by ACON Laboratories, Inc). Creatininuria was assessed by the Jaffé colorimetric method cited by Delanghe & Speeckaert [27] (using the kit manufactured by SGMitalia S.r.L). Urine output (ml/min) after 24 h was calculated according to the formula [28]:

$$\text{Urine flow rate (mL/min)} = \frac{24 \text{ h urine volume (mL)}}{1440 \text{ min}}$$

Serum creatinine was assessed by the same method as creatinineuria. Serum urea was measured by the UV kinetics method, Urease-GLDH (Glutamate dehydrogenase) (using the kit manufactured by SGMitalia S.r.L).

2.8 Estimation of renal clearance

Glomerular filtration was assessed by creatinine clearance (Clcr) following the formula [28]:

$$\text{Clcr (mL/min)} = \frac{\text{Urine creatinine (mg/dL)} \times \text{Urine output (mL/min)}}{\text{Serum creatinine (mg/dL)}}$$

2.9 Analysis of haematological parameters

The levels of total white blood cells, granulocytes, monocytes, lymphocytes, red blood cells and haemoglobin of the animals were determined by blood count, using the Mindray BC-2800 haematology auto-analyser.

2.10 Determination of some parameters of oxidative stress and nitric oxide

To prepare the homogenates, 2.8 mL of phosphate buffer solution (0.2 M, pH 7.4, pKa 7.2) was added to the crushed 0.5 g of kidney in ice-cold conditions. The mixtures were homogenized and then centrifuged at 3,000 rpm for 15 minutes at 4°C. The supernatants were recovered for assays of total protein, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and nitric oxide (NO) using the methods of Biuret [29], Wilbur et al [30], Misra and Fridovich [31], Sinha [32], Ellman [33] and Montgomery & Dymock [34] respectively.

2.11 Histopathological examination

Histological analysis of kidney was carried out using the haematoxylin-eosin staining protocol described by Eroschenko [35].

2.12 Data analysis

The data were analysed by the ANOVA test followed by Tukey's post-test using Graph Pad Prism software version 8.0.1. Results were expressed as mean \pm standard error of the mean and differences between groups were considered significant at the 5 % level. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were realized with XLSTAT 2021.2.2.

3. RESULTS

3.1 Effects of aqueous extract of *A. andongensis* stem bark on changes in rat body weight

Figure 1 shows the changes in body weight of the animals over the course of the experiment. In the normal control group, an increase in body weight as a function of time was noted, followed by a decrease on day 4 after fasting. However, a decrease in body weight as a function of time was noted in all the other groups except the Sham control, in which an increase was observed on day 3. However, there were no significant differences in mean body weight between the groups.

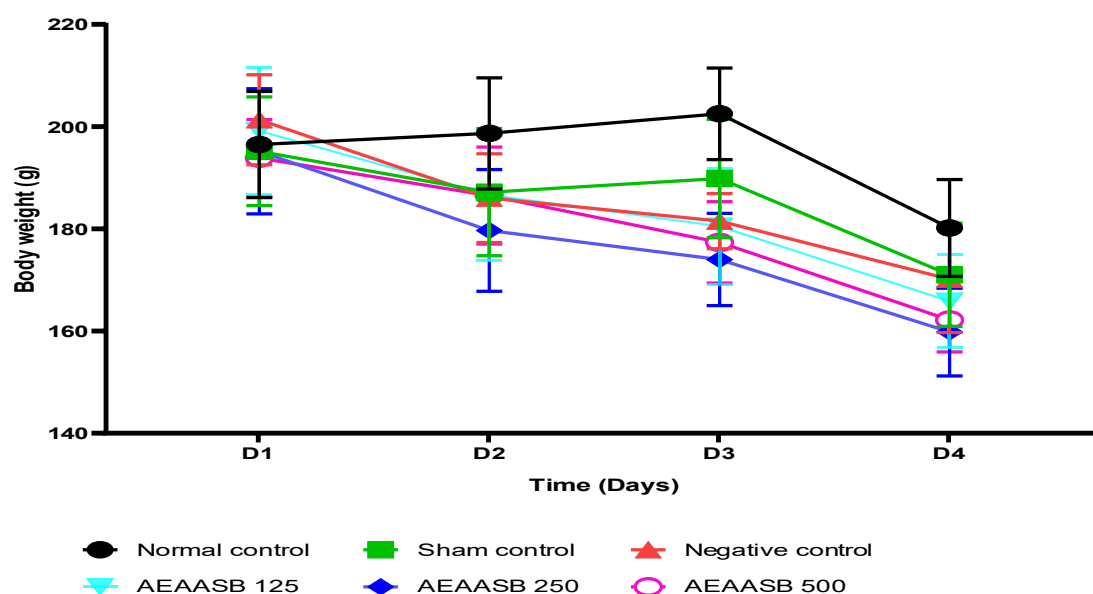


Fig. 1 Effects of aqueous extract of *A. andongensis* stem bark on body weight changes in rats. Values were expressed as Mean \pm MSE, AEAASB 125, AEAASB 250 and AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at doses of 125, 250 and 500 mg/kg respectively.

3.2 Effects of aqueous extract of *A. andongensis* stem bark on food and water intake in rats

Figures 2 and 3 show respectively, the curves representing the food and water intakes by the different groups of animals during the experiment. From day 1 to day 3, the amounts of food and water consumed by the negative control group remained low compared with the normal and sham control groups. In the groups receiving aqueous extract of *A. andongensis* stem bark (AEAASB) at the different doses, food intake did not vary significantly. However, water intake increased from day 2 to day 3, particularly with the 500 mg/kg extract dose compared with the negative control.

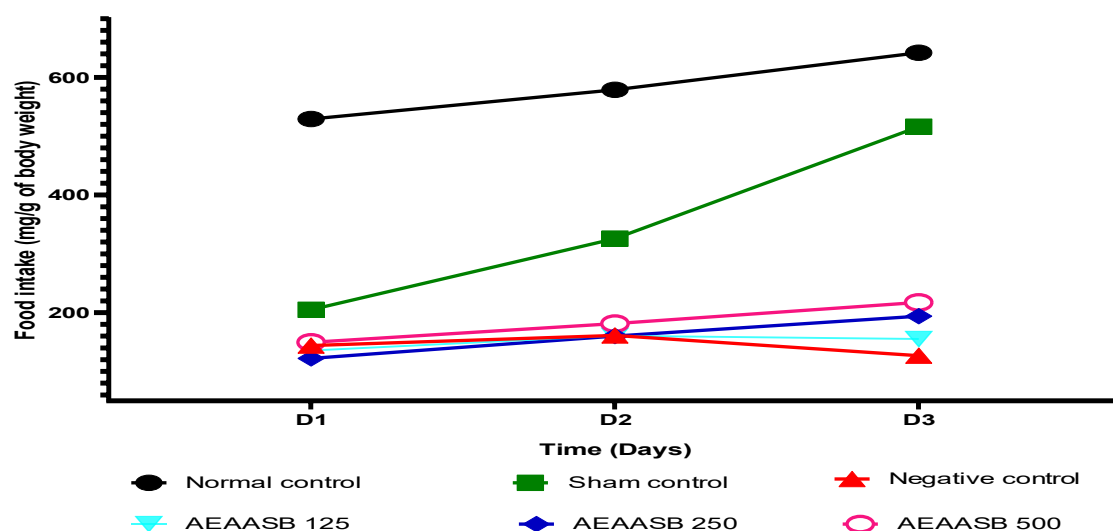


Fig 2 Effects of aqueous extract of *A. andongensis* stem bark on food intake. AEAASB 125, AEAASB 250 and AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at doses of 125, 250 and 500 mg/kg respectively.

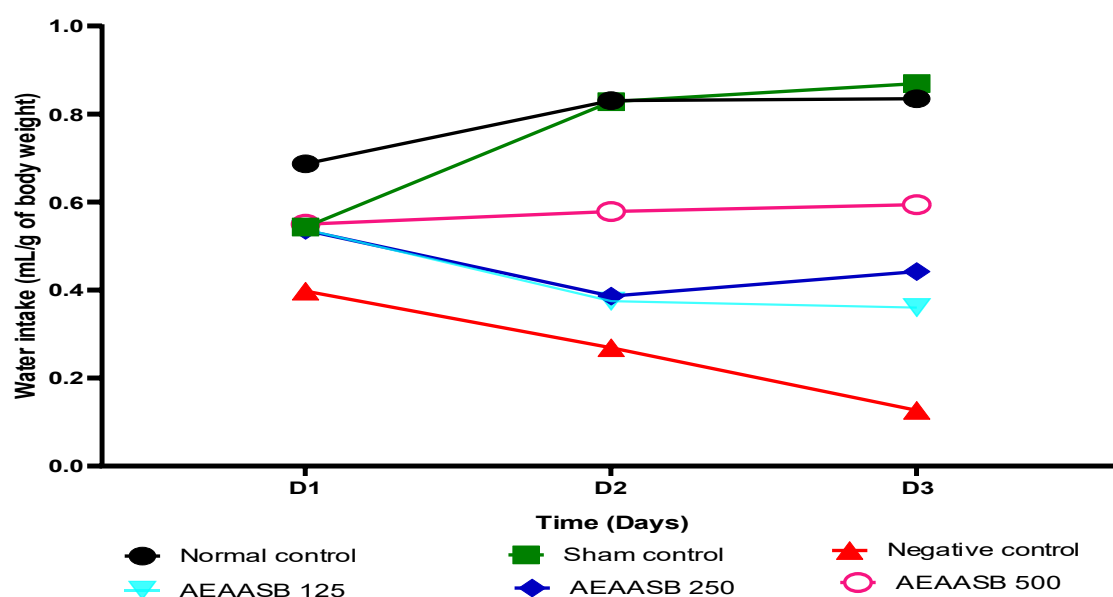


Fig 3 Effects of aqueous extract of *A. andongensis* stem bark on water intake. AEAASB 125, AEAASB 250 and AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at doses of 125, 250 and 500 mg/kg respectively.

3.3 Effects of aqueous extract of *A. andongensis* stem bark on the relative weight of some rat organs

In the negative control group, a significant increase in the relative weight of the obstructed kidney ($p < 0.001$) compared with the Sham control was noted (Table 1). However, treatment with AEAASB resulted in a significant decrease in the relative weight of the obstructed kidney, particularly at doses of 250 ($p < 0.05$) and 500 mg/kg ($p < 0.001$) compared with the negative control. There were no significant differences in the relative masses of unobstructed kidneys between the groups of animals.

A significant increase in the relative weight of the liver and a significant decrease in that of the stomach were noted in the Sham control compared with the normal control (Table 1). A significant increase in relative liver weight was also observed in the negative control compared with the Sham control. However, a significant decrease in relative liver weight accompanied by an increase in stomach weight was observed in the AEAASB 500 mg/kg group ($p < 0.001$) compared to the negative control.

Table 1. Effects of aqueous extract of *A. andongensis* stem bark on the relative weight of some organs

Groups	Kidneys (%g/g)		Heart (%g/g)	Liver (%g/g)	Lungs (%g/g)	Rate (%g/g)	Stomach (%g/g)	Testes (%g/g)
	Left	Right						
Normal control	0.31 ± 0.01	0.31 ± 0.01	0.33 ± 0.00	2.61 ± 0.04	0.78 ± 0.04	0.18 ± 0.01	1.93 ± 0.06	1.16 ± 0.07
Sham control	0.32 ± 0.01	0.34 ± 0.01	0.30 ± 0.00	2.88 ± 0.04 ^{***}	0.84 ± 0.04	0.27 ± 0.02	1.52 ± 0.06	1.32 ± 0.06 ^{***}
Negative control	0.73 ± 0.04 ^{***}	0.36 ± 0.01	0.32 ± 0.00	3.08 ± 0.02 [■]	0.82 ± 0.05	0.29 ± 0.01	1.49 ± 0.02	1.29 ± 0.05
AEAASB 125	0.64 ± 0.02	0.36 ± 0.00	0.31 ± 0.01	2.30 ± 0.05	0.76 ± 0.06	0.29 ± 0.02	1.53 ± 0.05	1.29 ± 0.08
AEAASB 250	0.61 ± 0.02 [*]	0.36 ± 0.00	0.33 ± 0.01	2.30 ± 0.04	0.85 ± 0.02	0.26 ± 0.01	1.69 ± 0.02 [*]	1.35 ± 0.05
AEAASB 500	0.56 ± 0.01 ^{***}	0.35 ± 0.00	0.30 ± 0.01	2.78 ± 0.05 ^{***}	0.77 ± 0.02	0.21 ± 0.01	1.96 ± 0.02 ^{***}	1.27 ± 0.07

Values were expressed as Mean ± MSE, AEAASB 125, AEAASB 250 and AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at doses of 125, 250 and 500 mg/kg, respectively, [■]p < 0.01, ^{***}p < 0.001 significant difference compared to the Sham control, ^{*}p < 0.05, ^{***}p < 0.001 significant difference compared to the negative control.

3.4 Effects of aqueous extract of *A. andongensis* stem bark on some urinary parameters in rats

Table 2 shows the results of the effects of AEAASB on some of the animals' urinary parameters. In the negative control group, there was a significant decrease in urine flow rate ($p < 0.01$) and a significant increase in urine protein levels ($p < 0.001$) compared with the Sham control group. However, treatment with AEAASB, particularly at doses of 250 and 500 mg/kg, resulted in a significant increase in urine output ($p < 0.01$) and a significant decrease in urine protein levels ($p < 0.001$) compared with the negative control group. OUU and treatment with AEAASB did not result in significant changes in pH and osmolarity.

Table 2 Effects of AEAASB on some urinary parameters of rats

Groups	Urine output (mL/min $\times 10^{-2}$)	Urinary creatinine (mg/dL)	Urinary pH	Urinary osmolarity	Urinary protein (mg/dL)
Normal control	0.72 ± 0.00	27.5 ± 0.33	7.00 ± 0.27	1.02 ± 0.00	100.00 ± 0.00
Sham control	0.72 ± 0.00	26.25 ± 0.08	7.20 ± 0.25	1.01 ± 0.00	100.00 ± 0.00
Negative control	$0.69 \pm 0.00^{**}$	$09.79 \pm 0.18^{***}$	7.25 ± 0.28	1.02 ± 0.00	$300.00 \pm 0.00^{***}$
AEAASB 125	$0.72 \pm 0.00^{**}$	$23.25 \pm 0.01^{***}$	7.42 ± 0.33	1.02 ± 0.00	233.30 ± 42.16
AEAASB 250	$0.72 \pm 0.00^{**}$	$26.04 \pm 0.02^{***}$	7.40 ± 0.29	1.01 ± 0.01	$140.00 \pm 40.00^{**}$
AEAASB 500	$0.72 \pm 0.00^{**}$	$27.90 \pm 0.02^{***}$	7.80 ± 0.25	1.01 ± 0.00	$100.00 \pm 0.00^{***}$

Values were expressed as Mean \pm MSE, AEAASB 125, AEAASB 250, AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at doses of 125, 250 and 500 mg/kg, respectively. $^{**}p < 0.01$, $^{***}p < 0.001$ significant difference compared to the Sham control, $^{**}p < 0.01$, $^{***}p < 0.001$ significant difference compared to the negative control.

3.5 Effects of aqueous extract of *A. andongensis* stem bark on some haematological parameters in rats

A significant increase in the total white blood cell count ($p < 0.001$) in the Sham control group compared with the normal control group was noted (Table 3). In the negative control group, there was a significant increase in the total white blood cell count ($p < 0.001$), particularly granulocytes ($p < 0.001$), monocytes ($p < 0.001$) and lymphocytes ($p < 0.05$) compared with the Sham control group. However, a significant decrease in total white blood cell count ($p < 0.001$), granulocytes ($p < 0.001$), monocytes and lymphocytes ($p < 0.01$) was observed in animals treated with AEAASB at the dose of 500 mg/kg compared to the negative control group.

Table 3. Effects of aqueous extract of *A. andongensis* stem bark on some haematological parameters of rats

Groups	Total white blood cell count (x10 ⁹ /L)	Granulocytes (%)	Monocytes (%)	Lymphocytes (%)	Red blood cell count (x10 ¹² /L)	Hemoglobin (g/dL)
Normal control	1.08 ± 0.09	4.57 ± 0.34	3.38 ± 0.29	91.23 ± 1.24	6.65 ± 0.16	13.92 ± 1.03
Sham control	1.83 ± 0.14 ^{***}	4.28 ± 0.56	3.36 ± 0.36 ^{***}	92.13 ± 2.30	5.86 ± 0.17	12.17 ± 1.05
Negative control	2.78 ± 0.09 ^{***}	7.02 ± 0.22 ^{***}	5.95 ± 0.16 ^{***}	98.53 ± 0.60 [■]	5.88 ± 0.17	12.13 ± 1.33
AEAASB 125	2.53 ± 0.13	3.82 ± 0.24 ^{***}	4.90 ± 0.20 [*]	93.92 ± 0.47	6.52 ± 0.15	13.65 ± 0.32
AEAASB 250	2.50 ± 0.06	4.46 ± 0.25 ^{***}	4.74 ± 0.08 ^{**}	91.66 ± 0.83 ^{**}	6.72 ± 0.08	13.88 ± 0.21
AEAASB 500	2.00 ± 0.04 ^{***}	4.44 ± 0.40 ^{***}	3.94 ± 0.14 ^{***}	91.58 ± 1.05 ^{**}	6.82 ± 0.11	14.18 ± 0.27

Values were expressed as Mean ± MSE, AEAASB 125, AEAASB 250, AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at 125, 250 and 500 mg/kg, respectively. ^{***}p < 0.001, significant difference compared to the normal control, [■]p < 0.001 significant difference compared to the Sham control, ^{*}p < 0.05, ^{**}p < 0.001 significant difference compared to the negative control.

3.6 Effects of aqueous extract of *A. andongensis* stem bark on some serum parameters and renal creatinine clearance

A significant increase in creatinine levels and a consequent significant decrease in renal creatinine clearance were observed in the negative control group (p < 0.001) compared with the Sham control (Table 4). In the groups treated with AEAASB at the doses of 250 and 500 mg/kg, creatinine levels were significantly decreased and renal creatinine clearance was significantly increased (p < 0.001) compared to the negative control.

Table 4. Effects of aqueous extract of *A. andongensis* stem bark on some markers of renal function

Groups	Serum creatinine (mg/dL)	Serum Urea (mg/dL)	Renal creatinine Clearance (mL/min)
Normal control	0.30 ± 0.01	59.63 ± 7.92	0.66 ± 0.02
Sham control	0.30 ± 0.02	58.50 ± 7.21	0.63 ± 0.02
Negative control	1.69 ± 0.09 ^{***}	61.62 ± 10.01	0.04 ± 0.01 ^{***}
AEAASB 125	0.93 ± 0.05 ^{**}	60.85 ± 3.68	0.18 ± 0.01 [*]
AEAASB 250	0.75 ± 0.05 ^{***}	59.57 ± 6.21	0.25 ± 0.02 ^{***}
AEAASB 500	0.49 ± 0.04 ^{***}	59.20 ± 10.00	0.41 ± 0.01 ^{***}

Values were expressed as Mean \pm MSE, AEAASB 125, AEAASB 250, AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at doses of 125, 250 and 500 mg/kg, respectively. $^{**}p < 0.01$, $^{***}p < 0.001$ significant difference compared to the Sham control, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ significant difference compared to the negative control.

3.7 Effects of aqueous extract of *A. andongensis* stem bark on levels of total protein, nitric oxide and some oxidative stress parameters in rat kidney tissue

In the negative control group, there was a significant increase in MDA levels ($p < 0.001$) in renal tissues accompanied by a significant decrease in NO and GSH levels ($p < 0.001$) compared to the Sham control (Table 5). However, administration of AEAASB, in particular at the dose of 500 mg/kg, resulted in a significant decrease in MDA levels ($p < 0.05$) associated with an increase in NO, catalase and GSH levels ($p < 0.001$) compared with the negative control. There were no significant differences in total protein levels between groups of animals.

Table 5. Effects of aqueous extract of *A. andongensis* stem bark on total protein, nitric oxide levels and some oxidative stress parameters in rats

Groups	Total Protein (mg/mL)	NO (mmol/mg of protein)	MDA (mmol/mg of protein)	SOD (U/mg of protein)	CAT (μ mol/min/mg of protein)	GSH (mmol/mg of protein)
Normal control	24.96 \pm 1.12	1.23 \pm 0.03	0.02 \pm 0.00	16.68 \pm 0.32	88.42 \pm 3.83	1.34 \pm 0.10
Sham control	25.04 \pm 0.72	1.23 \pm 0.03	0.02 \pm 0.00	16.43 \pm 0.57	85.55 \pm 2.47	1.27 \pm 0.12
Negative control	24.25 \pm 1.14	0.93 \pm 0.04 ***	0.03 \pm 0.00 ***	13.41 \pm 0.31 *	70.55 \pm 4.00	0.70 \pm 0.09 ***
AEAASB 125	23.83 \pm 1.09	1.06 \pm 0.02 *	0.02 \pm 0.00	17.38 \pm 0.93 **	80.73 \pm 6.08	1.07 \pm 0.05 *
AEAASB 250	23.83 \pm 1.56	1.10 \pm 0.02 **	0.02 \pm 0.00 *	17.35 \pm 0.81 **	92.24 \pm 2.56 *	1.20 \pm 0.05 **
AEAASB 500	25.95 \pm 1.15	1.31 \pm 0.02 ***	0.02 \pm 0.00 *	18.31 \pm 0.92 ***	104.90 \pm 4.60 ***	1.37 \pm 0.05 ***

Values were expressed as Mean \pm MSE, AEAASB 125, AEAASB 250 and AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at the doses of 125, 250 and 500 mg/kg, respectively. $^{***}p < 0.001$ significant difference compared to the Sham control, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ significant difference compared to the negative control.

3.8 Effects of aqueous extract of *A. andongensis* stem bark on histopathological changes in kidney tissue of rats subjected to OUU

Microphotographs of the kidneys of animals in the normal and Sham control groups showed an architecture consisting of glomeruli, the urinary space, and proximal and distal convoluted tubules of normal appearance (Figure 4). In the negative control group, dilatation associated with thinning of the tubule wall and a decrease in glomerular cell density were noted. In the 125 and 250 mg/kg AEAASB dose groups, persistent tubular dilatation was noted, whereas in animals treated at the dose of 500 mg/kg AEAASB, the renal microstructure was similar to that of the normal and Sham controls.

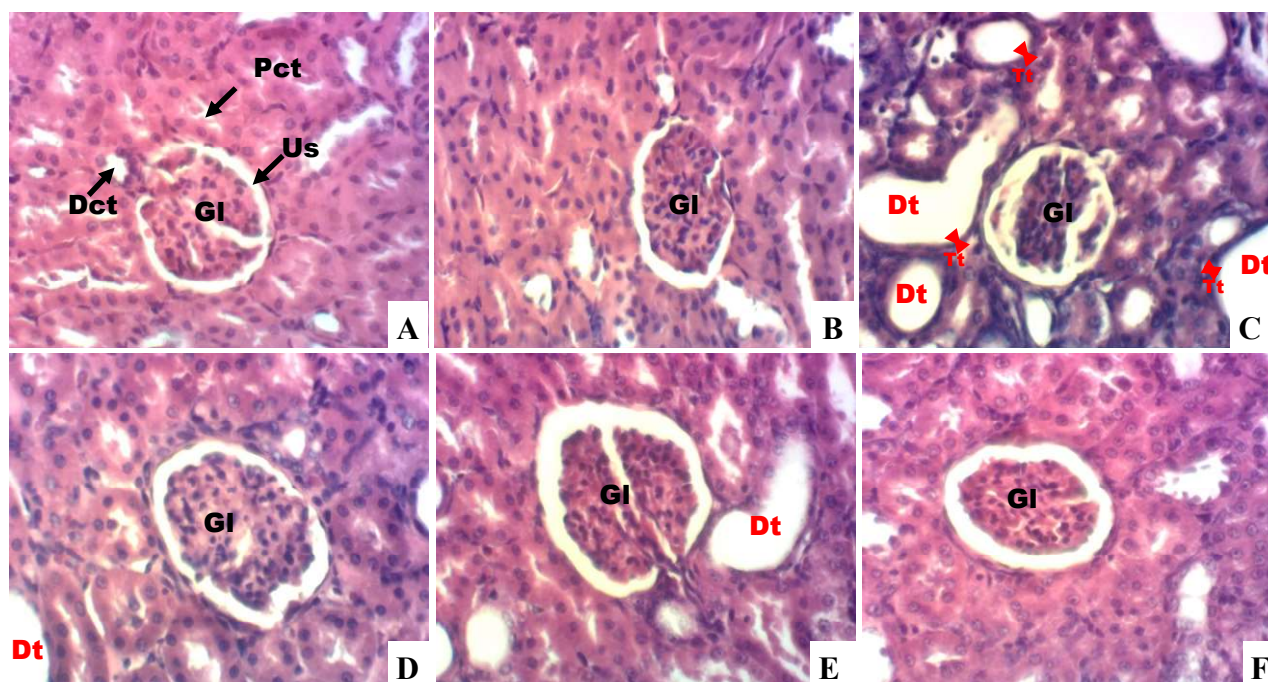


Fig 4. Microphotographs of rat kidneys (x 200, Haematoxylin-eosin). A: Normal control, B: Sham control, C: Negative control, D, E, F: groups treated with aqueous extract of *A. andongensis* stem bark at doses of 125, 250 and 500 mg/kg, respectively, Gl: Glomerulus, Us: Urinary space, Dct: Distal convoluted tubule, Pct: Proximal convoluted tubule; Dt : Dilation of the tubules; Tt: Thinning of the tubular wall.

3.9 Principal component analysis

The biplot of score and loading represents the dispersion of the six groups and the variables studied around two principal components F1 and F2 (Figure 5). Histological parameters (presence or absence of tubular dilatation and thinning of the tubule wall) were considered in this analysis as supplementary variables. The biographical graph (scores and load diagrams) resulting from the principal component analysis (PCA) illustrate the relationship between the parameters measured (urinary, haematological, serum, oxidative stress with level of NO) and represents the separation of the different groups among the first two principal components, F1 and F2. Of all the principal components, F1 and F2 accounted for 95.49 % of the total variance in the data set. F1 contributed 82.15 %, while F2 contributed 13.33 % of the total variance. The groups were successfully distinguished from each other by the principal components.

Treatments of rats subjected to OUU with AEAASB at different doses (125, 250 and 500 mg/kg) were pooled and distanced from the negative control. This may indicate that EAETAA has significant therapeutic effects against OUU in rats. Measured variables such as urinary protein levels, serum creatinine, total leukocytes, granulocytes, monocytes, lymphocytes, MDA and obstructed kidney weight) were classified into component F1 which showed an inverse correlation with component F2 variables, (urine output, urinary creatinine, SOD, GSH, NO, renal creatinine clearance). The presence of tubular dilatation and thinning of the tubular wall explained the F1 component, whereas their absence was closer to the F2 component.

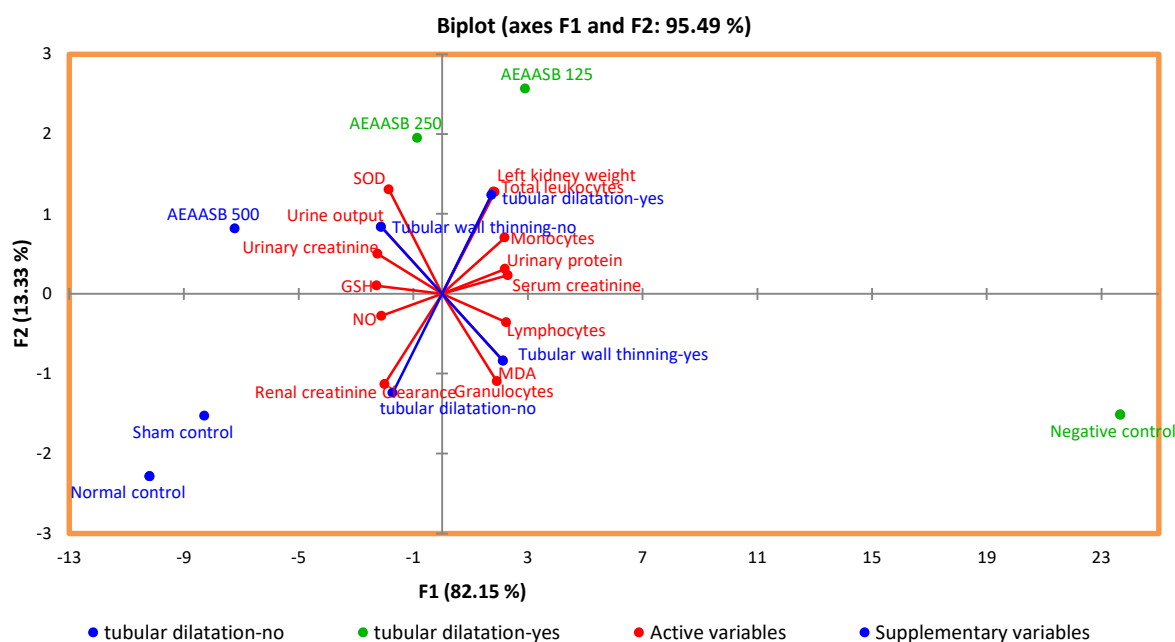


Fig 5. Biplot showing the principal component analysis score and the loadings of the different variables studied. AEAASB 125, AEAASB 250 and AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at the doses of 125, 250 and 500 mg/kg, respectively.

3.10 Agglomerative hierarchical clustering

Figure 6 shows the dendrogram presenting the similarity and distance between the different groups of rats according to the agglomerative hierarchical clustering (AHC) determined by the different parameters measured. The lowest truncation distinguishes 3 clusters: C1 (normal control, Sham control and the group given 500 mg/kg AEAASB), C2 (negative control and the group given 125 mg/kg AEAASB) and C3 (the group given 250 mg/kg AEAASB).

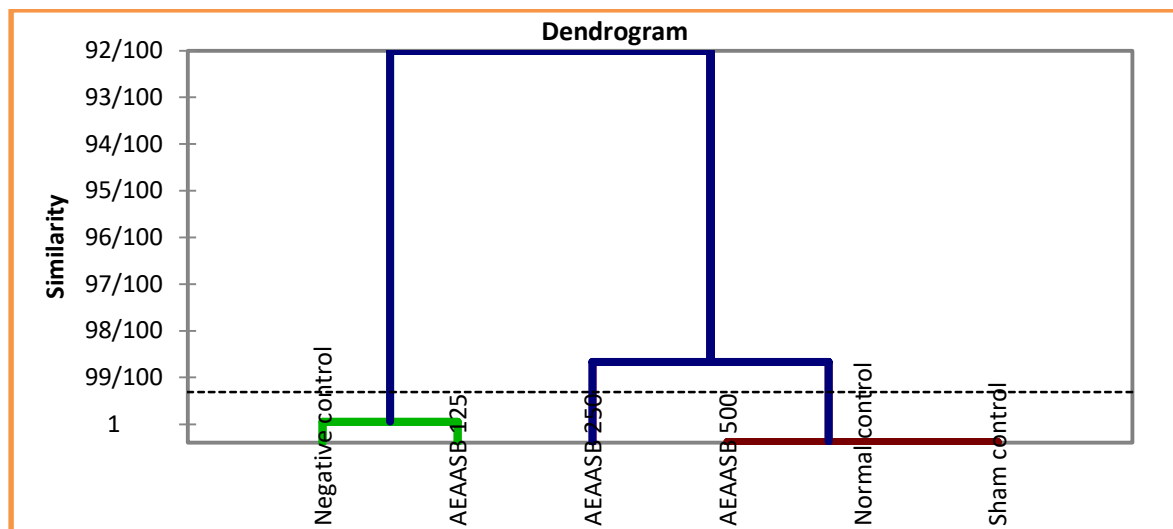


Fig 6 Dendrogram obtained by agglomerative hierarchical clustering of the different groups of rats. AEAASB 125, AEAASB 250 and AEAASB 500: groups treated with aqueous extract of *A. andongensis* stem bark at the doses of 125, 250 and 500 mg/kg, respectively.

4. Discussion

Unilateral ureteral obstruction (UUO) is a widely used experimental model to study obstructive renal failure resulting from a physical blockage of the urinary flow. To facilitate the surgical procedure, male animals are preferred because of the ease with which the ureter can be identified. The kidney that has undergone UUO is characterized by tubular dilatation, interstitial expansion, proximal tubular mass loss, hypertrophy, hydronephrosis, leukocyte infiltration, tubular epithelial cell death and fibroblast formation [3]. These alterations are the result of several molecular processes that lead to progressive tubulointerstitial fibrosis [3, 36].

It all starts with the stationary flow of urine due to the UUO, which leads to hydronephrosis by increasing hydrostatic pressure, dilating first the collecting ducts and then the distal and proximal tubules [1]. Thus, the obstructed kidney will increase in volume and therefore in weight during the acute phase. In the present study, the increase in kidney weight following UUO was observed in the negative control after three days. These results are in agreement with Nilsson et al. [37] who had shown that UUO for three days resulted in hydronephrosis characterised by a significant increase in the weight of the affected kidney. In this work, treatment with aqueous extract of *A. andongensis* at doses of 250 mg/kg and 500 mg/kg significantly decreased the weight of the obstructed kidney compared with that of the negative control, suggesting that AEAASB has hydronephrosis-limiting effects.

In the process of hydronephrosis, the increase in pressure in the proximal tubules has two effects: a decrease in glomerular filtration rate (GFR) and damage caused by mechanical stretching of the tubular epithelial cells. The decrease in GFR is related to obstruction, which leads to a progressive decrease in renal blood flow and an increase in renal vasoconstriction, which can lead to inadequate blood supply and ischaemia [1]. Indeed, estimating GFR by measuring renal creatinine clearance is a commonly used method [38]. Creatinine is a breakdown product of creatine present in the muscles, most of which is excreted in the urine. When renal function is compromised, serum creatinine levels increase [39]. Renal creatinine clearance represents the volume of blood plasma cleared of creatinine per unit time. An increase in serum creatinine and a sudden fall in GFR are signs that indicate AKI [40]. In this study, a significant increase in serum creatinine associated with a significant decrease in renal creatinine clearance was noted in the negative control group compared with the Sham control. Treatment with AEAASB, particularly at doses of 250 and 500 mg/kg, resulted in a significant fall in serum creatinine levels and a significant increase in

renal creatinine clearance compared with the negative control group. Mohammadi-Sichani et al. [41] have shown that an increase in GFR is associated with a decrease in the severity of hydronephrosis, which would favour functional recovery of the kidney.

During tubular dilatation due to UUO, increased renal vasoconstriction and hydrosodic overload are mediated by tubuloglomerular feedback and stimulation of the renin-angiotensin-aldosterone system (RAAS). The reduction in renal blood flow caused by obstruction results in tubuloglomerular feedback, the aim of which is to regulate blood flow. Due to the volume overload of the kidney, this results in an increase in NaCl supply to the cells of the *macula densa*, which leads to its increased uptake in the loop of Henle [42]. This uptake leads to the release of adenosine, which acts on A1 receptors in extraglomerular mesangial cells and triggers an increase in cytosolic calcium. This signal reaches the smooth muscle cells of the afferent arteriole which leads to vasoconstriction and a reduction in GFR [8, 43]. The reactivity of the tubuloglomerular feedback is determined by the action of angiotensin II (stimulation of the RAAS). Angiotensin II binds directly to its receptors on the smooth muscle cells of renal arterioles and stimulates contraction of these muscles [44]. It also stimulates the sympathetic nerve to promote vascular smooth muscle resistance in the kidney [45]. Angiotensin II also stimulates aldosterone secretion, which leads to Na⁺ reabsorption in the distal tubule [46]. It is important to note that in a hydronephrotic state, the increased activity of tubuloglomerular feedback is linked to reduce NO availability [42]. In this study, a significant decrease in NO levels was observed in the negative control group compared with the sham control. In animals treated with AEAASB, particularly at 500 mg/kg, there was a significant increase in NO levels in renal tissues compared with the negative control. According to Patzak et al. [47], the renal vasoconstrictor effect is inhibited by locally produced NO. Furthermore, an increase in NO concentration leads to an increase in sodium and water excretion, whereas a NO deficit leads to sodium retention [48, 49]. In the kidney, NO plays a role in regulating renal haemodynamics, maintaining medullary perfusion, mediating natriuresis, attenuating tubuloglomerular feedback, inhibiting tubular sodium reabsorption and modulating renal sympathetic neuronal activity. The net effect of NO in the kidney is to promote natriuresis and diuresis [42].

As for injury, nephron destruction during OUU is initiated by increased intratubular hydrostatic pressure causing mechanical stretching of tubular epithelial cells and secondary ischaemia [3]. Following injury, mediators (exosomes, chemokines and cytokines) are secreted by parenchymal, endothelial and tubular epithelial cells from day one and lasts throughout the OUU [50]. In response to the local expression of these mediators, leukocytes more mainly macrophages, T lymphocytes and neutrophils are recruited to the peritubular interstitium of the kidney. The leukocytes in turn amplify the inflammation, which causes further damage to other tubular cells [3]. Hence the high levels of leukocytes and in particular granulocytes, monocytes and lymphocytes observed after three days of OUU in the group of untreated animals (negative control). In animals treated with AEAASB at a dose of 500 mg/kg, leukocyte cell populations decreased significantly in number. These results are in line with those of previous studies showing that this extract attenuated the inflammation associated with paracetamol-induced nephrotoxicity in rats [22].

In the course of obstructive nephropathy, the mechanisms that induce oxidative stress are not fully understood, but oxidative stress is an important contributor to the pathogenesis of UUO [51]. During damage caused by mechanical stretching of the cells, the release of reactive oxygen species (ROS) increases in the obstructed kidney. Angiotensin II, produced as a result of continuous activation of the RAAS, contributes significantly to the release of large quantities of ROS by also activating nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX) [52]. ROS cause oxidative cell damage by reacting with various structural components of cells, resulting in lipid peroxidation. MDA is one of the major products of lipid peroxidation, as shown by Manucha et al. [53] who found high levels of MDA following OUU. In addition, Sugiyama et al. [54] reported a decrease in the activity of the antioxidant enzymes SOD, catalase and glutathione peroxidase in cases of hydronephrosis. In this study, a significant increase in MDA levels associated with a significant decrease in SOD and GSH levels was observed in untreated animals that had undergone OUU, whereas in animals treated with 500 mg/kg AEAASB, a significant decrease in MDA levels associated with a significant increase in SOD, catalase and GSH levels was noted. According to Martinez-Klimova et al. [1], antioxidant treatments could have positive effects by blocking the RAAS.

Observations of microphotographs of kidney sections from the animals involved in the present study revealed normal renal architecture in the sham group, while the negative control group showed tubular dilatation associated with thinning of the tubular wall and a reduction in glomerular cell density. Proteinuria was also noted in the latter group. Proteinuria is an indicator of

glomerular damage [55]. Jin et al. [56] showed macro and micromorphological alterations accompanied by significant renal dysfunction in the hours or days following OUU, compared with the Sham group indicates that obstructive AKI was successfully induced. In the 125 and 250 mg/kg AEAASB groups, persistence of tubular dilatation was noted, whereas in animals treated with 500 mg/kg AEAASB, renal microstructure was similar to that of normal and Sham controls. Sinha et al. [57] showed that the removal of obstruction in the AKI phase alone does not lead to resolution of this damage but that early or late addition of a RAAS blocker would prevent or allow better renal recovery.

5. Conclusion

In conclusion, AEAASB has nephroprotective effects which are thought to result, on the one hand, from its vasodilatory properties and, on the other, from its inhibition of the renin-angiotensin-aldosterone system, *via* an increase in NO production. PCA and AHC have shown that *A. andongensis* has therapeutic effects against AKI associated with OUU. The 500 mg/kg dose was the most effective, as the values of the parameters measured were very similar to those of the normal and Sham control groups.

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