EFFICACY OF *CHROMOLAENA ODORATA* SUPPLEMENTATION ON METHOTREXATE-INDUCED NEPHROPATHY AND OXIDATIVE STRESS

Usunomena Usunobun[1,*] Bridget Ikponmwosa[2], Gabriel Anyanwu[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Edo State University Uzairue, Edo State, Nigeria
[2]Department of Biochemistry, College of Natural and Applied Sciences, Bingham University, Karu, Nassarawa State, Nigeria
[*] Corresponding author e-mail: usunobun.usunomena@edouniversity.edu.ng

Abstract

We carried out this research to investigate the nephroprotective efficacy of aqueous leaf extract of *Chromolaena odorata* (AEOC) on kidney toxicity in methotrexate (MTX)-induced rats. Five groups of six albino wistar male rats each were used. Groups III-V were administered MTX at a dose of 7 mg/kg B.W. intraperitoneally from the 8th day for three days consecutively, while groups II and IV also received 250 mg/kg B.W. of AEOC orally once daily for ten days. Group I received normal saline and served as normal control. Creatinine and urea were analyzed in the serum. Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), histopathological analysis were assessed using the kidney tissues, while blood collected in EDTA bottles was used for hematology. The obtained results revealed that three days of consecutive methotrexate administration caused a significant rise in kidney function parameters as well as severe oxidative damage and oxidative stress, as shown by a significant rise in creatinine and urea, an increased level of MDA and a significant drop in SOD and CAT. However, AEOC attenuated methotrexate-induced biochemical alterations and oxidative stress in the kidney. Also, AEOC alone or in combination with methotrexate showed an increase in packed cell volume (PCV), red blood cells (RBC), hemoglobin (HB), white blood cells (WBC), and platelet levels compared to the group treated with methotrexate alone, which showed degenerative levels in the analyzed hematological parameters. Showing corroboration, histopathological analysis showed deterioration and collapse of the kidney tubules in rats treated with methotrexate alone, whereas AEOC groups showed considerable nephroprotection and restoration towards normalcy. The results of this study revealed that the AEOC has a nephroprotective efficacy against methotrexate-induced kidney toxicity and damage via scavenging of free radicals and stabilization and/or enhancement of endogenous antioxidant status.

Keywords: *Chromolaena odorata,* kidney, methotrexate, nephroprotection, oxidative stress

Introduction

In vivo exposure to toxins and poisons represents the major cause of nephropathy (Mahi-Birjand et al. 2020), which is attributed to the use of medications such as methotrexate (MTX). Methotrexate is a drug commonly employed for the treatment of cancer and inflammatory diseases such as arthritis, systemic lupus erythematosus, and dermatomyositis (Dalaklioglu et al. 2013). MTX-mediated anticancer and immunosuppressive effects are owing to its function

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as an antagonist for folic acid (competitively inhibits dihydrofolate reductase, the enzyme that catalyzes the conversion of dihydrofolate to tetrahydrofolate), which in turn prevents cellular mitosis by inhibiting thymidylate, purines, and folic acid required for DNA synthesis (Gibson et al. 2019), preventing cells from dividing (Liu et al. 2019). Prolonged MTX use generates organ toxicities and damages. Reports have previously shown that oxidative stress and the free radicals released induce hepatotoxicity (Cetinkaya et al. 2006, Usunobun et al. 2022a; Ikponmwosa and Usunobun 2022). In addition to free radicals and reactive oxygen species (ROS) generation and accumulation, an insufficient antioxidant defense status promotes hepatotoxicity (Uraz et al. 2008, Usunobun et al. 2022a, Ikponmwosa and Usunobun 2022). The kidney, a crucial organ, is responsible for filtering the blood to remove wastes, maintaining water and electrolyte homeostasis, and regulating blood pressure (Jerman and Sun 2017). Because ~90% of administered MTX is excreted unchanged through the kidney, MTX is more likely to damage the kidneys than other organs by crystal nephropathy and/or tubular toxicity as a consequence of the overwhelming production of reactive oxygen species within the kidney (Dabak and Kocaman 2015). Thus, decreasing MTX toxicities and, in turn, restricting the discontinuation of MTX therapy is a current therapeutic challenge.
Herbal medicines have been used for decades for the treatment and management of various diseases by improving immunity and general health (Babich et al. 2020, Pelvan et al. 2022). Medicinal plants are rich in phytochemicals exhibiting antioxidant activity (Iuchi et al. 2003). *Chromolaena odorata* (L.) King & Robinson originated in North America and is also very common in Nigeria. It is known as Siam Weed, Awolowo, independent weed, Christmas Bush, Devil Weed, etc. (Lalith 2009, Ngozi and Osuji 2014). The fresh leaves or decoction of *C. odorata* (L.) King & Robinson has been widely used for wound treatment and healing due to its anti-microbial effects. Numerous investigations have demonstrated its effect against dermatological problems, diarrhea, fever, toothache, diabetes, and colitis (Odugbemi 2006, Akinmoladun and Akinloye 2007). Leaves of *C. odorata* possess insecticidal properties and are used as a green manure as well as for the preservation of dead bodies (Ukwueze et al. 2013). Other pharmacological properties of *C. odorata* leaves include antimalarial (Ongkana 2003), analgesic (Chakraborty et al. 2011), antipyretic, antispasmodic (Oludare et al. 2000), anti-gonorrhoeal (Caceres et al. 1995), fungicidal, diuretic (Gopinath et al. 2009), and blood coagulating (Triaratana et al. 1991). Some specific phenolic compounds that account for medical values have been identified and isolated from leaves of *C. odorata* (Metwall and Ekejinba 1981, Usunobun and Ewere 2016). The objective of this study was to elucidate the nephroprotective efficacy of aqueous leaf extract of *Chromolaena odorata* (AEOC) against nephrotoxicity induced by methotrexate in Wistar albino male rats.

Materials and Methods

Chemicals and Reagents

MEDVALIK Pharmaceuticals Limited, Lagos, Nigeria, was the source of the used methotrexate (liquid), 50 mg/2mL (Zuvius Life Sciences, India). All the other chemicals and reagents used in this research study were of analytical grade.

Collection and Identification of plant material

C. odorata leaves were obtained from Edo State University Uzairue in Iyamho, Edo State, Nigeria, and identification was carried out at Edo State University Uzairue, Department of Plant Biology and Biotechnology Herbarium with voucher number EUH/00066.

Preparation and Extraction of plant material

The obtained *C. odorata* leaves were well rinsed, air-dried at room temperature for four weeks, pulverized, and crushed into a fine powder using an electric blender. To prepare the aqueous extract, 1 kg of the powdered plant was soaked in 5 liters of double-distilled water for 48 hours at room temperature with daily stirring for thorough extraction. Upon completion of 48 hours, filtration was carried out first with Whatman filter paper No. 42 (125 mm) and then with cotton wool. The collected filtrate was concentrated in a rotary evaporator at 40 ºC to one-tenth its original volume and then reduced in a water bath to a solid form, which was weighed and dissolved in normal saline for administration to the rats on each experiment day.

Experimental Animal and Design

A total of thirty (30) Wistar male rats with a weight ranging from 180 to 200 g were obtained from Ambrose Alli University, Ekpoma, Edo State, Nigeria, and kept in a wood-gauze cage, an adequately ventilated room, and a well-lit animal house of the Department of Biochemistry, Edo State University, Uzairue, Nigeria. All the rats were given free access to water and fed with standard laboratory pellets. The Ethics Committee of the Faculty of Basic Medical Sciences at Edo State University Uzairue gave ethical clearance and approval for this study, and guidelines for ethical conduct in the care and use of non-human animals in research were rightly respected and followed (APA 2012).

After acclimatization for seven days, the rats were weighed and distributed into groups as follows: Group I orally received normal saline daily and was the normal control. Group II received AEOC (250 mg/kg B.W.) orally once daily for ten days. Group III: received MTX (7 mg/kg B.W.) intraperitoneally from the 8^{th} day for three consecutive days. Group IV received AEOC (250 mg/kg B.W.) orally once daily for ten days and then administered MTX (7 mg/kg B.W.) intraperitoneally from the 8^{th} day for three consecutive days. Group V received Vitamin C (100 mg/kg B.W.) orally once daily for ten days and then administered MTX (7 mg/kg BW) intraperitoneally from the 8^{th} day for three consecutive days. The chosen MTX dose was based on the study of Aladaileh et al. (2019), while the chosen AEOC dose was based on the study of Ijioma et al. (2014).

Upon completion of the experiment, twenty-four (24) hours after the last administration, the rats were weighed, sacrificed, and blood taken in EDTA bottles for hematological analysis and in plain tubes to obtain serum. The blood collected in plain tubes was centrifuged at 4000 rpm for 30 min after being allowed to stand for 45 minutes to obtain serum for the determination of urea and creatinine. The kidney was carefully removed, washed in ice-cold saline, and weighed while a portion was fixed in 10% phosphate-buffered formalin for histopathological examination. The remaining portion was stored at -20 ºC and a 10 % kidney tissue homogenate was prepared using a phosphate buffer solution at pH 7.34 for the assessment of superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT).

Biochemical Parameters

Urea was assessed using the RANDOX kit (United Kingdom) following the Fawcett and Scott (1960) method, while creatinine was assessed using the Jaffe' method. Malondialdehyde (MDA) was determined as postulated by Ohkawa et al. (1979). Superoxide dismutase (SOD) activity was assessed using the Misra and Fridovich (1972) method, while the Cohen et al. (1970) method was used to assess the activity of catalase (CAT).

Hematological parameters

Hematological analyses on parameters such as hemoglobin, white blood cells, red blood cells, platelets, and packed cell volume were carried out at the hematology laboratory, Irrua Specialist Teaching Hospital, Edo Central, Edo State, Nigeria.

Histopathological studies

After the euthanasia of the rats, kidney tissues were removed and rinsed in normal saline. The removed kidneys were thereafter fixed in 10% buffered formalin and sent to the Chemical Pathology Laboratory, University of Benin Teaching Hospital, Nigeria, for histopathological examination.

Statistical Analyses

All results were expressed as mean ± standard error of the mean, and statistically, SPSS and one-way analysis of variance (ANOVA) were used, with $P<0.05$ being considered significant.

Results and discussions

Kidney Function Assessment

The efficacy of kidney function assessment as shown in Table 1 indicates a significant rise ($p<0.05$) in urea and creatinine in rats treated with 7 mg/kg MTX for three consecutive days, compared to the control group and groups treated with AEOC. However, rats given MTX and treated with AEOC or Vitamin C showed a significant drop ($p<0.05$) in urea and creatinine levels compared to rats given 7 mg/kg MTX alone for three consecutive days.

Table 1. Efficacy of aqueous leaf extract of *Chromolaena odorata* (AEOC) on kidney function assessment in methotrexate (MTX)-induced Wistar male rats

Treatment groups	Creatinine (mg/dl)	Urea (mg/dl)
Control	$0.80^{a} \pm 0.06$	$27.21^{a} \pm 2.79$
AEOC alone (250 mg/kg B.W.)	$0.81^{a} \pm 0.12$	$25.42^{a} \pm 1.70$
MTX alone (7 mg/kg B.W.)	$5.20^{b} \pm 1.03$	$89.12^{b} \pm 2.33$
MTX (7 mg/kg B.W.) + AEOC (250 mg/kg B.W.)	$1.59^{a} \pm 0.56$	$40.12^{c} \pm 1.63$
MTX (7 mg/kg B.W.) + Vitamin C (100 mg/kg B.W.)	$1.31^{a} \pm 0.19$	$38.41^{d} \pm 1.60$

Results are expressed as mean ± standard Error of the mean, n = 4. On the column, values with different superscripts (a, b, c, d) differ significantly ($p<0.05$)

Plasma infiltration and metabolic homeostasis maintenance remain important functions of the kidney. Nephrotoxicity has the capacity to retard kidney excretory activities and alter kidney physiology and structure (Zhang and Sun 2015, Shang and Falah 2019). Medicinal plants known to be easily available and cost-friendly remain best remedy for combating nephrotoxicity and hepatotoxicity caused by drug overdose (Yousuf et al. 2022). In this study, AEOC at a dose of 250 mg/kg B.W. ameliorated methotrexate (MTX)-induced kidney toxicity in Wistar male rats. As observed in this study in the group of rats administered MTX for three consecutive days, kidney impairment and damage are indicated by a buildup of creatinine and urea in the blood (Airaodion et al. 2020). The kidney toxicity could likely occur in the MTX-treatment group by precipitating MTX in kidney tubules and decreasing glomerular filtration rate. The findings of this work agree with similar reported works by Usunobun et al. 2022b, Usunobun et al. 2023). However, administration of AEOC or Vitamin C exhibited ameliorative effects, reducing the blood concentration of urea and creatinine in alignment with the works of Usunobun et al. 2022b, Usunobun et al. 2023. It is likely that the AEOC with its previously reported bioactive agents including tannins, flavonoids, alkaloids, and phenols (Usunobun and Ewere 2016) improved the cell membrane permeability and increased the clearance of creatinine and urea by the kidney.

Lipid peroxidation and antioxidant status assessment

To establish the antioxidant efficacy of AEOC, MDA concentration and SOD and CAT levels were assessed in tissue homogenates of the kidney, as shown in Table 2 below. Our study showed a significant rise *($P < 0.05$)* in MDA concentration and a significant drop in SOD and CAT levels in the kidney of the MTX group. On the other hand, administration of AEOC caused a significant drop *(P < 0.05)* in MDA concentration and noticeable improvement in levels of SOD and CAT in the tissue homogenate of the kidney.

Table 2. Efficacy of aqueous leaf extract of *Chromolaena odorata* (AEOC) on oxidative stress parameters of kidney in methotrexate (MTX)-induced Wistar male rats

Treatment groups	SOD (U/mg protein)	MDA (µmol/mg protein)	CAT (U/mg protein)
Control	$90.08^{a} \pm 3.15$	$1.83^{a} \pm 0.33$	$2.28^{a} \pm 0.20$
AEOC alone (250 mg/kg B.W.)	$96.63^{a} \pm 3.42$	$1.90^{a} \pm 0.27$	$2.42^{a} \pm 0.19$
MTX (7 mg/kg B.W.)	$45.66^{b} \pm 2.08$	$14.93^{b} \pm 2.09$	$0.50^{b} \pm 0.11$
MTX (7 mg/kg B.W.) + AEOC (250 mg/kg B.W.)	$69.89^{c} \pm 3.38$	$5.38^{c} \pm 0.99$	$1.51^{c} \pm 0.17$
MTX (7 mg/kg B.W.) + Vitamin C (100 mg/kg B.W.)	$73.55^{c} \pm 2.59$	$6.09^{c} \pm 1.05$	$1.83^{c} \pm 0.21$

Results are expressed as mean ± standard error of the mean, n = 4. On the column, values with different superscripts (a, b, c, d) differ significantly (p<0.05). SOD = superoxide dismutase; MDA = malondialdehyde; CAT = catalase

Indicating an increase in oxidative damage and oxidative stress, results from our study showed a marked decline in the levels of CAT and SOD as well as a marked rise in MDA in animals administered MTX alone compared to the animals in the control and extract groups (Table 2), similar to previous studies of Usunobun et al. 2022a, Ikponmwosa and Usunobun 2022. This could be because increased lipid peroxidation, as seen with the increased MDA, decreases membrane fluidity, thus altering the function of the plasma membrane and compromising its integrity (Wong-Ekkabut et al. 2007). However, AEOC or Vitamin C showed efficacy as treatment significantly alleviated MTX-induced oxidative damage and oxidative stress, as reflected by a reduction of MDA and enhanced endogenous SOD and CAT activities compared to the rats administered MTX alone similar to previous studies of Usunobun et al. 2022a, Ikponmwosa and Usunobun 2022). As observed in this study, AEOC enhanced endogenous antioxidant enzymes and acted as a scavenger of free radicals.

Hematological profile

The results of the efficacy of AEOC on hematological parameters on methotrexate (MTX)-induced Wistar rats are as shown in Figures 1-5 below. As observed in the figures, three days of consecutive 7 mg/kg MTX administration and subsequent toxicity showed a significant reduction in packed cell volume (PCV), red blood cell (RBC), hemoglobin (HB), white blood cells (WBCs), platelet levels when compared to the control and AEOC or Vitamin C group. However, AEOC or Vitamin C showed enhancement of hematological parameters compared to MTX degeneration.

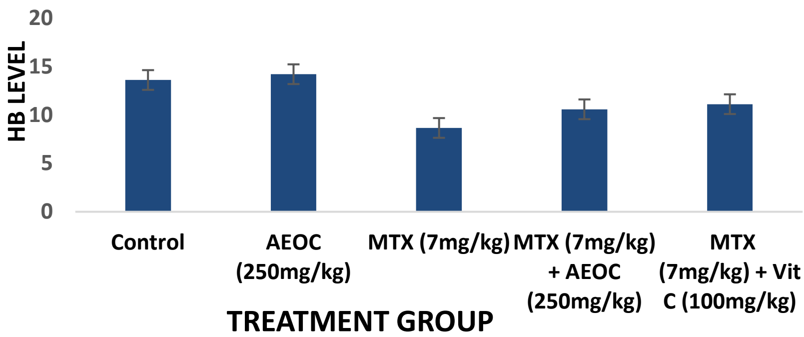

Figure A. Efficacy of the aqueous leaf extract of *C. odorata* (AEOC) on hemoglobin (HB) levels in methotrexate (MTX)-induced Wistar rats

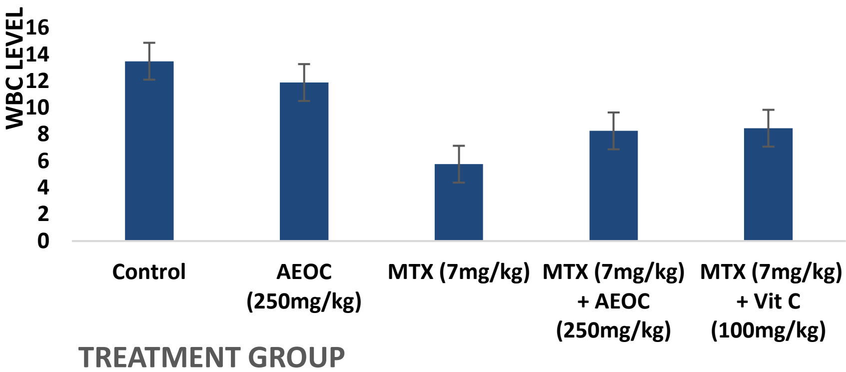

Figure B. Efficacy of the aqueous leaf extract of *C. odorata* (AEOC) on white blood cell (WBC) count in methotrexate (MTX)-induced Wistar rats

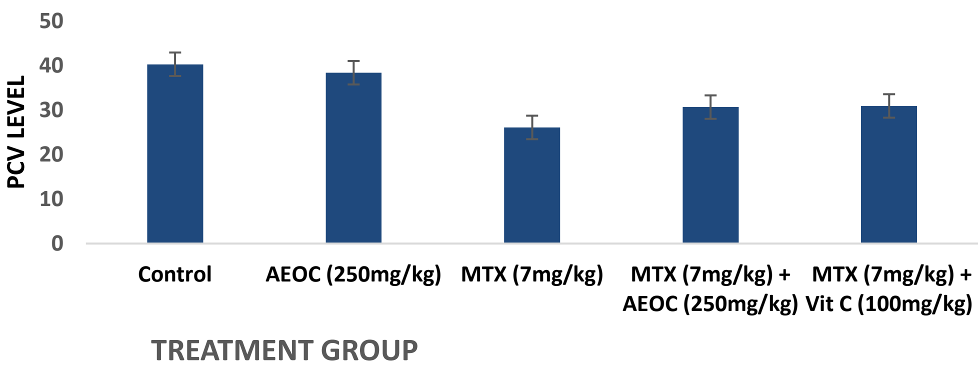

Figure C. Efficacy of the aqueous leaf extract of *C. odorata* (AEOC) on packed cell volume (PCV) in methotrexate (MTX)-induced Wistar rats

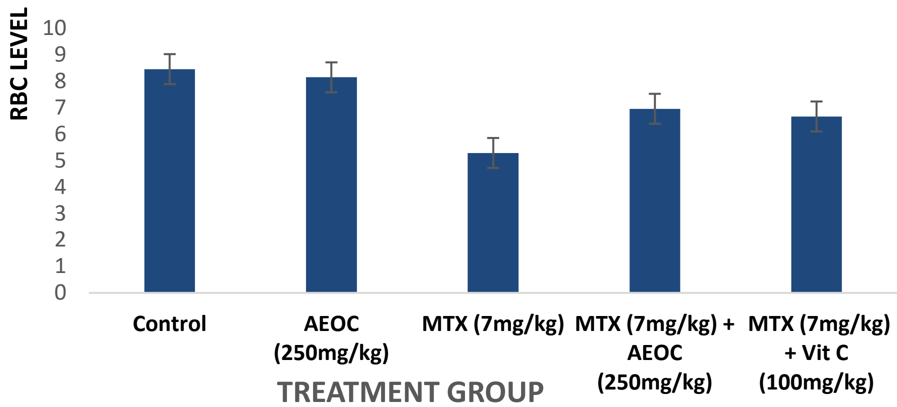

Figure D. Efficacy of the aqueous leaf extract of *C. odorata* (AEOC) on red blood cells (RBC) in methotrexate (MTX)-induced Wistar rats

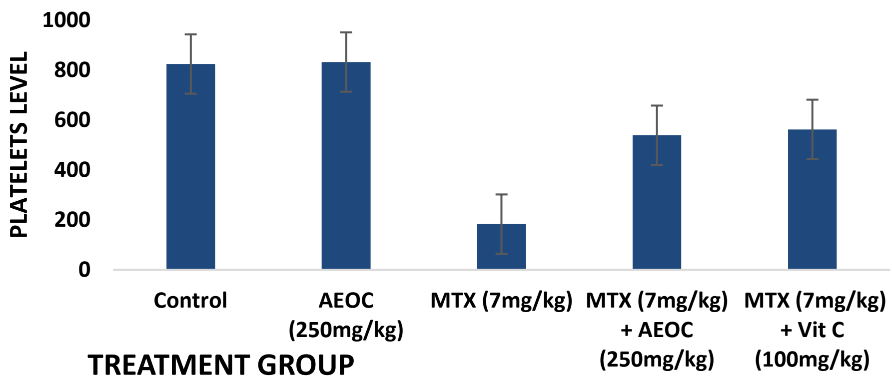

Figure E. Efficacy of the aqueous leaf extract of *C. odorata* (AEOC) on platelets (PLTS) in methotrexate (MTX)-induced Wistar rats

RBCs function in hemoglobin transport (Woo et al. 2012). As observed in this study in MTX-treated rats, a significant decline in RBC, HB, and PCV was observed, which is indicative of anemia in the rats. The observed significant decline in RBC count following MTX administration for three consecutive days imply slowed-down erythropoiesis as well as the destruction or death of mature RBCs. The trend in RBCs and WBC counts in this study aligns with Ohbayashi et al. (2010) and Rofe et al. (1994). However, administration of AEOC showed a significant increase in the aforementioned parameters, similar to those of Doğan (2018). The efficacy of AEOC on hematology can be attributable to secondary metabolites with antioxidant potential.

Histopathological assessment

The examination of the kidneys of control rats showed Malpighian corpuscles with glomeruli, which have Bowman's capsule surrounding them. The AEOC (250 mg/kg B.W.) treatment group had similar glomerular cell distribution as the control. However, the kidneys of MTX-treated rats showed congestion and necrosis in the tubules, marked glomerular collapse, and dilatation. The kidneys of rats administered AEOC and MTX showed fewer inflammatory cells around the glomerulus and improvement of the glomerular architecture. The kidneys of rats administered vitamin C and MTX also revealed massive recovery.

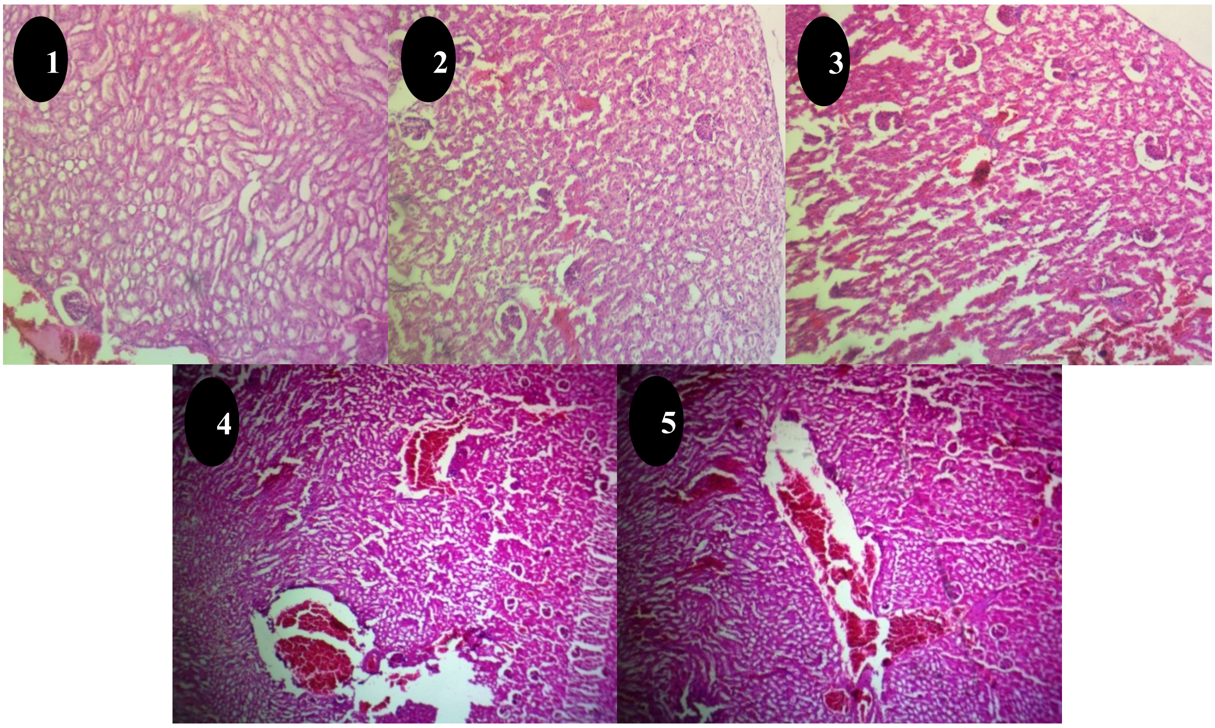

Figure 1. A photomicrograph of a rat kidney treated with normal saline solution (control) and sacrificed on the 11^{th} day, showing Malpighian corpuscles with glomeruli surrounded by Bowman's capsule.
Figure 2. A photomicrograph of a rat kidney treated with AEOC alone and sacrificed on the 11^{th} day showing normal architecture and similar glomerular cell distribution as the control.
Figure 3. A photomicrograph of a rat kidney administered methotrexate alone for three consecutive days and sacrificed on day 11 showing congestion, inflammation, marked glomerular collapse, dilatation, and necrosis in the tubules.
Figure 4. A photomicrograph of a rat kidney administered methotrexate for three consecutive days and AEOC and sacrificed on the 11^{th} day, showing fewer inflammatory cells around the glomerulus and improvement in glomerular architecture.
Figure 5. A photomicrograph of a rat kidney administered methotrexate for three consecutive days and vitamin C and sacrificed on the 11^{th} day showing reduced inflammation and massive recovery.

Our observed histological disturbances were corroborated by our observed increased serum urea and creatinine, both renal function indicators, whose above-normal increases indicate damage to the function of nephrons.

Conclusions

Bioactive agents in AEOC such as flavonoids, alkaloids, tannins, and phenols and their enhancement of endogenous enzymatic antioxidants improved AEOC potency and efficacy in reducing the effect of methotrexate-induced nephrotoxicity. This study established that AEOC possesses nephroprotective effects against methotrexate-induced kidney damage in rats via histopathology, biochemical parameters, improved endogenous enzymes (SOD and CAT), decreased oxidative stress and lipid peroxidation (MDA), improved hematopoiesis, and free radical scavenging properties. The mechanism of action of AEOC against methotrexate-induced nephrotoxicity can be attributed to the plant`s ability to fight and scavenge free radicals as well as enhance and/or maintain endogenous antioxidant status, which defends the kidneys against oxidative damage and stress tension, thus motivating kidney healing and anti-inflammatory mechanisms.

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