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# Nephroprotective Potential of Chromolaena odorata (L.) R.M. King & H. Rob. on Methotrexate-Induced Kidney Damage and Oxidative Stress

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## Abstract

***Background & Aims*:** Methotrexate despite its beneficial anti-cancer and immunosuppressant effects has continued to receive limitation in usage due to its organ toxicity. The aim of this study was to investigate the nephroprotective effect of aqueous leaf extract of Chromolaena odorata on Methotrexate-induced injury and damage on kidney in rat.

***Materials & Methods*:** The study consisted of four groups of rats: Control, Chromolaena odorata extract, Methotrexate, and Methotrexate+Chromolaena odorata extract groups. Chromolaena odorata extract was given orally (200 mg/kg) for 10 days, and Methotrexate at a single dose (20 mg/kg) was administered intraperitoneally on day 9 of the experiment. Blood and kidney were collected on day 11 to measure biochemical, hematological and oxidative stress parameters as well as histopathological analysis.

***Results*:** Methotrexate administration when compared to control and extract, treated rats decreased antioxidant agents, including catalase (CAT) and Superoxide dismutase (SOD) while it increased the Malondialdehyde level in the kidney tissues. Methotrexate also increased Urea and Creatinine in the blood samples. The result also showed that Methotrexate administration produced a significant decrease in Hemoglobin (HB), White Blood Cell (WBC), Hematocrit (HCT), Red Blood Cell (RBC), Platelets (PLTS), Lymphocyte, Basophil, and Monocyte when compared to control and all extract administered rats. The result of histopathological analysis of the kidney revealed that Methotrexate administration caused necrosis of renal tubules, renal congestion, renal tubule epithelium swelling, interstitial hemorrhage, glomerular atrophy, as well as dilatation. Chromolaena odorata extract administration significantly alleviated kidney function, improved antioxidant parameters, decreased levels of oxidative stress agents, restored the hematological parameters towards normalcy as well as resulted in noticeable improvement and attenuation toward normalcy in the kidney structure, and thus, remarkably preventing Methotrexate-induced tissue injury and damage.

***Conclusion*:** The observed data showed that Chromolaena odorata had a protective effect against Methotrexate-induced nephrotoxicity by maintaining the activity of the antioxidant defense system, which can be attributed to its bioactive constituents.

***Keywords*:** Antioxidants, Chromolaena Odorata, Kidney, Methotrexate, Oxidative Stress

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## Introduction

Undeniably and unarguably, herbs remain the foundation of conventional drugs and modern medicine. Many plants substances that have been synthesized have been found useful in the maintenance of health in the humans and other animals. Chromolaena odorata (L.) King and Robinson (formerly known as Eupatorium odoratum), known by names such as Siam Weed, Christmas Bush, Devil Weed, Camfhur Grass, and Common Floss Flower (1), although has now spread to South America, West Africa, tropical Asia, is native to North America, mostly seen in Florida and Texas to Mexico and the Caribbean (2). In Nigeria, Chromolaena odorata (Ch. odorata) is commonly known as Ewe Awolowo, Siam weed, Elizabeth weed, Obirakara, Olorohuru, and independent weed (3). Ch. odorata is popularly used for wound healing due to its antimicrobial properties (4) and its effective therapeutic effects against malaria fever, diabetes, skin diseases, dysentery, colitis, and diarrhea have been reported by several researchers (4-5). Salt mixed with leaf extracts of Ch. odorata is used as a gargle for sore throat and colds. By pounding Ch. odorata leaves till fine and appling to wounds, it can stop bleeding of wound, as during emergency, the leaves can be crushed by hand, mix with saliva and applied on wound to stop bleeding (6). In Vietnam, fresh leaves or decoction of the leaves have been used for the treatment of leech bite, soft tissue wounds, burnt wounds, skin infection, and dento-alveolitis (7-8). Usunobun and Ewere (9) previously established the presence of bioactive agents including flavonoids, saponins, alkaloids, tannins, and minerals such as calcium, sodium, potassium, magnesium, zinc, iron, etc. as well as its in vitro antioxidant property in Ch. odorata leaves.

Methotrexate (MTX), an anti-cancer drug, is known for treatment of malignancies including head and neck cancers, breast cancer, leukemia, lymphoma, osteosarcoma (10-14) as well as in the treatment of non-cancerous diseases such as rheumatoid arthritis and psoriasis (15-16). Since more than 90% of MTX is excreted unchanged via the kidneys (17), MTX treatment, particularly at high doses, may cause renal failure (13). MTX-mediated anticancer and immunosuppressive effects owes its function as an antagonist for folic acid (competitively inhibits dihydrofolate reductase, the enzyme that catalyzes conversion of dihydrofolate to tetrahydrofolate), which in turn prevents cellular mitosis by inhibiting thymidylate, purines, and folic acid required for DNA synthesis (18), preventing cells from dividing (19). Since the cytotoxic effect of MTX is not selective for the cancer cells, it can affect the normal tissues and so prolonged use of MTX has been associated with various organ toxicity (20). This study aim to ascertain nephroprotective effects of Ch. odorata leaves on Methotrexate induced nephrotoxicity and oxidative stress in Wistar rats.

## Materials & Methods

**Chemicals and Reagents:**

Methotrexate (liquid), 50 mg (Zuvius Life Sciences, India) was purchased from MEDVALIK Pharmaceuticals Limited, Lagos, Nigeria. All reagents used were of analytical grade and had the highest purity.

**Collection and identification of Plant material:**

Fresh leaves of Ch. odorata were collected from within the locality of Iyamho community, Uzairue, in Etsako Local Government Area of Edo State, Nigeria and taxonomically authenticated at the Department of Plant Biology and Biotechnology Herbarium, Edo State University, Uzairue, Edo State, Nigeria with voucher number EUH/00066.

**Preparation and Extraction of Plant Materials:**

The fresh leaves of Ch. odorata were thoroughly rinsed and air-dried at room temperature for one month, then pulverized and crushed into a fine powder using an electric blender, and weighed with an electric weighing balance. An aqueous extract of the plant was prepared by soaking 1000 g of the dried powdered plant materials in 5 liters of double-distilled water and then kept at room temperature for 48 hours to ensure a thorough extraction process. At the end of the 48 hours, the extracts were filtered first through a Whatman filter paper No. 42 (125 mm) and then through cotton wool. The resultant filtrate was concentrated using a rotary evaporator set at 40°C to one tenth of its original volume and then reduced to solid form using a water bath. The solid residue (crude extract) was stored at 4°C. Aliquot portions of the crude plant extract residue were weighed and dissolved in normal saline on each experiment day.

**Experimental Animals and Design:**

Twenty-four (24) male Wistar rats (180-200g) of the species Rattus norvegicus were purchased from the animal house, Department of Zoology, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The animals were housed in a well-lit, adequately ventilated room using a wood-gauze cage in the Animal house of the Department of Biochemistry, Edo State University Uzairue, Edo State. Standard environmental conditions were used (12-hour light and 12-hour dark) for acclimatizing the animals to the new environment. Animals were fed with standard laboratory pellets and given free access to water. This study was approved by Ethics Committee of the Faculty of Basic Medical Sciences, Edo State University Uzairue and in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research (21).

After acclimatization for seven days, the rats were randomly distributed into the following groups as follows: **Group I:** Served as control and only received normal saline orally once daily. **Group II**: Rats were given aqueous leaf extract of Ch. odorata at a dose of 200 mg/kg orally once daily for ten days. **Group III**: Rats were given Methotrexate intraperitoneally at a single dose of 20 mg/kg on day 9 of the experiment. **Group IV**: Rats were given aqueous extract of Ch. odorata (200 mg/kg) orally once daily for ten days, and then single dose of Methotrexate intraperitoneally (20 mg/kg) on day 9 of the experiment. Methotrexate was dissolved in saline and injected intraperitoneally (i.p.) at 20 mg/kg dose (22) while 200 mg/kg of Ch. odorata was chosen based on study of Ijioma et al. (2014) (23).

At the end of the experiment and after 24hrs of last administration, the rats were sacrificed and blood samples collected in EDTA tubes and plain tubes. While bloods in EDTA tubes were used for hematology, and the bloods collected in plain tubes were allowed to stand for 45 minutes before being centrifuged at 4000 rpm for 25 min to obtain serum samples which were used for determination of Creatinine and Urea.

Then, the kidneys were immediately excised, washed in ice cold saline, weighed and a portion fixed in 10% phosphate buffered formalin for histopathological examination while the remaining portion was stored at -20°C for determination of oxidative stress and endogenous enzymes. Ten percent tissue homogenate of the stored kidney tissues were prepared using phosphate buffer solution at pH 7.34. The homogenate was centrifuged at 5000 rpm for 15 minutes and a clear supernatant obtained used for determining Superoxide Dismutase (SOD), Malondialdehyde (MDA), and Catalase Activity (CAT).

**Biochemical Parameters:**

Serum urea was determined by using the RANDOX Kit (Randox Laboratories Ltd., County Antrim, UK) according to the manufacturer's instructions following the method of Fawcett (1960) (24). Serum Creatinine was determined using the RANDOX Kit (Randox Laboratories Ltd., County Antrim, UK) according to the manufacturer's instructions using Jaffe method following the method of Ghasemi (2014) (25). Malondialdehyde (MDA) content was determined by the methods of Ohkawa (1979) (26). Superoxide dismutase (SOD) and catalase (CAT) activities were assayed as described by Misra (1972) (27) and Cohen (1970) (28), respectively.

**Hematological parameters:**

The hematological parameters including Hemoglobin (HB), White Blood Cell (WBC) count, Hematocrit (HCT), Red Blood Cell (RBC), Platelets (PLTS), Lymphocyte (LYMPH), Basophil, and Monocyte were analyzed using Sysmex automated Hematology Analyser Model KX-21N.

**Histopathological studies:**

Rats were sacrificed and kidney samples excised, washed with normal saline (0.9% NaCl). The isolated kidneys were fixed in 10% buffered formalin and were further processed for histopathological investigations. Histopathologically, the kidney tissues were thereafter stained with hematoxylin and eosin (H&E) and then sections were examined under a light microscope, Leitz (Biomed), and histopathological changes were captured by a Nikon Camera, EOS700D, 18–55 lens.

**Statistical Analysis:**

All the data in the treatment groups are presented as mean ± Standard error of the mean (SEM) and statistical analysis was carried out using statistical package (SPSS) version 20, Windows 10. Mean values of the different treatment groups were compared using one-way analysis of variance (ANOVA), followed by Duncan multiple range post hoc tests. The $P<0.05$ was considered statistically significant.

## Results

The results, as shown in table 1, showed that there was significant increase in serum Urea and Creatinine in Methotrexate-treated rats as compared with control and extract treated rats. However, administration of Ch. odorata significantly reduced serum Urea and Creatinine levels compared to untreated Methotrexate-treated group (Table 1).

**Table 1.** Effects of Ch. odorata aqueous leaf extract on Kidney function parameters in Methotrexate-induced wistar rats

| Treatment groups | Creatinine (mg/dl) | Urea (mg/dl) |
|---|---|---|
| Control | $0.91\pm0.09^a$ | $33.24\pm3.37^a$ |
| Ch. odorata (200 mg/kg) | $0.84\pm0.10^a$ | $34.58\pm2.56^a$ |
| Methotrexate (20 mg/kg) | $7.84\pm1.96^b$ | $76.01\pm3.02^b$ |
| Ch. odorata (200 mg/kg) + Methotrexate (20 mg/kg | $1.63\pm0.13^c$ | $42.67\pm3.26^c$ |

Values are expressed as Mean ± Standard Error of Mean, Values with different superscripts down the column differs significantly ($p<0.05$).

The result of oxidative stress and antioxidant assessment (table 2) showed significant increase in kidney MDA level while the level of kidney CAT and SOD were found to be decreased in Methotrexate-treated rats as compared with control and extract treated rats. However, administration of Ch. odorata significantly decreased kidney MDA level in Methotrexate-induced rat and significantly increased kidney CAT and SOD activities ($P < 0.05$) (Table 2).

**Table 2.** Effects of Ch. odorata aqueous leaf extract on lipid peroxidation and antioxidant enzymes of Kidney in Methotrexate-induced wistar rats

| Treatment groups | Kidney SOD (U/mg protein) | Kidney CAT (U/mg protein) | Kidney MDA (µmol/mg protein) |
|---|---|---|---|
| Control | $93.01\pm5.98^a$ | $2.30\pm0.22^a$ | $3.53\pm0.22^a$ |
| Ch. odorata (200 mg/kg) | $87.63\pm4.06^a$ | $2.87\pm0.12^a$ | $3.03\pm0.67^a$ |
| Methotrexate (20 mg/kg) | $60.64\pm4.26^b$ | $0.43\pm0.04^b$ | $17.81\pm3.20^b$ |
| Ch. odorata (200 mg/kg) + Methotrexate (20 mg/kg | $74.30\pm2.60^c$ | $1.32\pm0.09^c$ | $5.53\pm0.43^c$ |

Values are expressed as Mean ± Standard Error of Mean, Values with different superscripts down the column differs significantly ($p<0.05$). SOD: Superoxide Dismutase; CAT: Catalase; MDA: Malondialdehyde

The result of hematological parameters is presented in tables 3 and 4. The results showed that administration of Methotrexate to rats produced a significant decrease in HB, WBC, HCT, RBC, PLTS, Lymphocyte, Basophil, and monocyte when compared to control and all extract administered rats. However, treatment with Ch. odorata significantly restored the hematology parameters towards normalcy.

**Table 3.** Effects of Ch. odorata aqueous leaf extract on HB, WBC, HCT, RBC, and PLTS in Methotrexate-induced wistar rats

| Treatment groups | HB (g/dl) | WBC($x10^3$m/L) | HCT (%) | RBC($x10^9$/L) | PLTS ($x10^3$/mm) |
|---|---|---|---|---|---|
| Control | 13.63±0.25$^a$ | 13.50±1.08$^a$ | 40.30±1.91$^a$ | 8.46±0.42$^a$ | 825.33±18.50$^a$ |
| Ch. odorata (200 mg/kg) | 13.80±0.52$^a$ | 13.23±1.21$^a$ | 38.80±1.99$^a$ | 8.33±0.19$^a$ | 842.66±22.59$^a$ |
| Methotrexate (20 mg/kg) | 9.00±0.10$^b$ | 5.66±0.49$^b$ | 24.73±1.19$^b$ | 4.80±0.52$^b$ | 256.66±18.77$^b$ |
| Ch. odorata (200 mg/kg) + Methotrexate (20 mg/kg | 10.27±0.15$^c$ | 10.40±0.50$^c$ | 30.47±0.60$^c$ | 6.88±0.32$^c$ | 471.00±21.28$^c$ |

Values are expressed as Mean ± Standard Error of Mean, Values with different superscripts down the column differs significantly ($p<0.05$). HB: Hemoglobin, WBC: White Blood Cell; HCT: Hematocrit; RBC: Red Blood Cell; PLTS: Platelets

**Table 4.** Effects of Ch. odorata aqueous leaf extract on Lymphocyte, Basophil and Monocyte in Methotrexate-induced wistar rats

| Treatment groups | Lymphocyte (%) | Basophil (%) | Monocyte (%) |
|---|---|---|---|
| Control | 66.16±3.14$^a$ | 3.13±0.21$^a$ | 39.80±3.41$^a$ |
| Ch. odorata (200 mg/kg) | 58.93±3.55$^b$ | 3.10±0.20$^a$ | 42.60±3.21$^a$ |
| Methotrexate (20 mg/kg) | 35.13±3.91$^c$ | 1.20±0.10$^b$ | 10.33±2.20$^b$ |
| Ch. odorata (200 mg/kg) + Methotrexate (20 mg/kg | 46.73±2.40$^d$ | 2.20±0.10$^c$ | 24.20±2.49$^c$ |

Values are expressed as Mean ± Standard Error of Mean, Values with different superscripts down the column differs significantly ($p<0.05$).

The results of histopathological analysis of the kidney samples revealed that the control and Ch. odorata-alone rats showed normal renal tubules, renal parenchyma, and corpuscles (Figures 1a and 1c), whereas Methotrexate administration caused multiple alterations including necrosis of renal tubules, renal congestion, renal tubule epithelium swelling, interstitial hemorrhage, glomerular atrophy, and dilatation (Figure 1b). In contrast, rats which received 200 mg/kg Ch. odorata showed noticeable improvement and attenuation toward normalcy in the kidney structure remarkably preventing Methotrexate-induced tissue injury (Figure 1d).

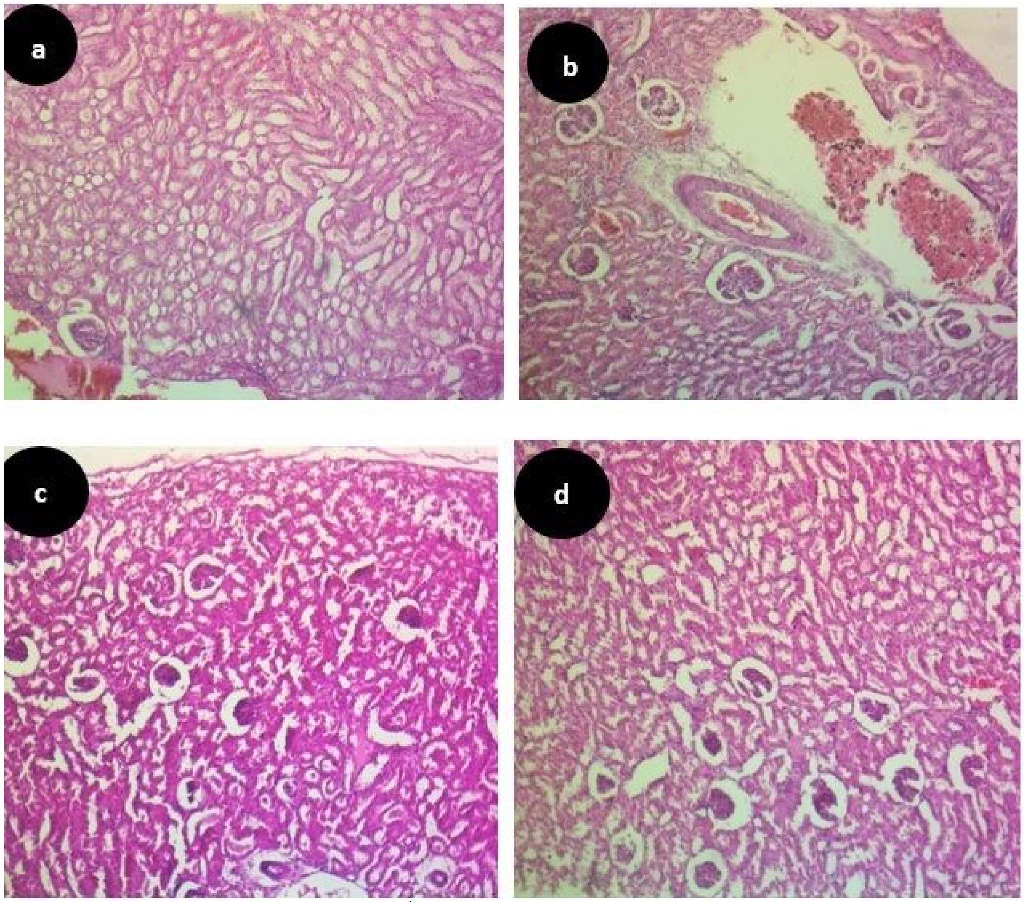


**Fig 1a**: Photomicrograph of kidney of control rats that received normal saline showing normal renal tubules and corpuscles. **Fig 1b:** Photomicrograph of kidney of rats that received Methotrexate alone at a single dose of 20 mg/kg showing necrosis of renal tubules, congestion, interstitial hemorrhage, dilatation, glomerular atrophy. **Fig 1c:** Photomicrograph of kidney of rats that received only Ch. odorata (200 mg/kg) for ten consecutive days showing normal kidney architecture. **Fig1d:** Photomicrograph of kidney of rats given Ch. odorata for ten consecutive days and single dose of 20 mg/kg Methotrexate showing reduction in the histopathological lesions.

## Discussion

Nephrotoxicity and kidney damage are commonly linked with marked elevation in the levels of Urea and Creatinine. Urea, a marker of acute renal dysfunction, is the first acute marker following renal injury while creatinine, a marker of chronic renal dysfunction, is the most dependable renal marker and increases only when the significant renal function is lost (29-30). In our study, Methotrexate administration resulted in severe nephrotoxicity as reflected by a significant increase in Urea and Creatinine levels ($p < 0.05$) as compared to the control group, similar to the results of previous studies (22, 31-33). The high levels of Creatinine and Urea are indicators of severe damage to the structural integrity of kidney nephrons. Elevation seen in Urea and Creatinine show poor clearance by kidney, indicating Methotrexate-induced damage to the renal tissues. However, the level of Urea and Creatinine significantly decreased ($p < 0.05$) towards normal levels in rats that received Ch. odorata plus Methotrexate when compared to the Methotrexate-treated group (Table 1), signaling potential for recovery from accumulative toxic effects on the kidney, similar to the results of previous studies (22, 31-33).

Antioxidant defense system, the primary line of defense against cell-damaging effects of oxidative stress, counteracts deleterious effects of free radicals. Oxidative damage to cells or tissues occurs when the concentration of ROS generated exceeds the antioxidant capability of the cell (34), and as seen in this study, the several-fold increase in MDA and the concomitant decrease in CAT and SOD following Methotrexate administration is an indication of oxidative stress, oxidative damage, and free radical generation, which resulted from Methotrexate reactive metabolites. Furthermore, the decrease in SOD and CAT activities can be attributed to either their inhibition by Methotrexate toxicity or their consumption during scavenging of excess $H_2O_2$, which must have been formed following Methotrexate metabolism and thus indicating the complete disruption of the antioxidant defense mechanism. Therefore, the significant elevation of MDA levels observed in the untreated Methotrexate-induced group is a result of the overproduction of ROS and inadequate antioxidant defense. However, Ch. odorata administration diminished the activities of renal MDA while increasing activities of SOD and CAT towards normalcy, and this may be ascribed to the plant antioxidant property that scavenges reactive free radicals, maintains the lipid structure of cell membrane, and protecting various biomolecules such as proteins and DNA in such biological systems. Administration of Ch. odorata increased the activities of kidney SOD and CAT and decreased MDA when compared to the rats treated with Methotrexate, similar to the results of previous works (22, 31, 35, 36).

The knowledge of hematology is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in the organism (37). This study showed that the aqueous leaf extract of Ch. odorata produced significant ($P<0.05$) increase in the levels of the HCT, Hemoglobin, White Blood cells, Platelets, Red blood cell, lymphocytes, basophil, and monocytes when compared with the Methotrexate alone. Hematocrit (HCT) indicates the percentage of the red cells in the total blood, and provides an indication of the oxygen-carrying capacity or efficiency of the RBC. The observed decrease seen in HCT in this study following Methotrexate administration could be an indication of clinical condition associated with abnormally low HCT, thus a confirmatory symptom of anaemia (38). The administration of leaf extract of Ch. odorata significantly ($p<0.05$) increased the levels of HCT, thus suggesting the plant potential anti-anemic properties. In a study by Mengiste et al. (39), an uncommon low hemoglobin level indicates anaemia. The result of this study showed that after treatment, the hemoglobin values of groups treated with Ch. odorata increased significantly when compared with Methotrexate alone group. The percentage increase in lymphocytes of rats that received Ch. odorata compared to rats that received Methotrexate alone may be the result of an increased ability to phagocytosize (cellular ingestion of offending agents) (40). Lymphocytes are the main-effector cells of the immune system (41). Thus administration of Ch. odorata exhibited stimulatory effect on the effectors cells of the immune system. Platelets are produced by the bone marrow through the stimulation of myeloid stem cells by thrombopoietin (38). Therefore, the observed decrease in platelets in this study, resulting from toxic effects of Methotrexate may suggest that the Methotrexate have inhibitory effect on thrombopoietin. The increases in the hematological indices observed following treatment with Ch. odorata extract might be attributed to the presence of phytochemical content and antioxidant potential of the Ch. odorata leaves as reported by Usunobun (2016) (9).

The biochemical changes shown in Methotrexate-treated rats were well corroborated with the observed histological changes seen in the kidney. The observed toxicity and damages of the kidney following Methotrexate administration could be attributed to increased production of free radicals associated with diminished antioxidant enzymes that culminate to loss of cell membrane integrity and function. However, the noticeable improvement and attenuation toward normalcy in the kidney in the Ch. odorata treated group are an indication of protection by the antioxidant compounds in Ch. odorata, thus remarkably preventing Methotrexate-induced tissue injury and damage.

In conclusion, administration of Methotrexate induced nephrotoxicity while administration of Ch. odorata protected kidney tissues from damage possibly due to the plants bioactive agents such as flavonoids, which enhance antioxidant activities and protects the kidney against oxidative stress and damage. Furthermore, hematological disturbances due to Methotrexate toxicity were successfully alleviated following Ch. odorata administration. Thus, treatment of rats with Ch. odorata leaf extract had a marked restorative effect against Methotrexate toxicity and damage. The mechanism of action of Ch. odorata against Methotrexate-induced nephrotoxicity can be attributed to its antioxidant capacity and anti-inflammatory activity as it decreased oxidative stress as well as enhanced antioxidant enzymatic and hematological parameters. As geographical and number limit are the main limitations of the current study, we recommend more studies in this field with more numbers and wider geographical distributions.

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**Conflict of interest**

The authors have no conflict of interest in this study.

**Financial support:** None

**Ethical Statement**

This study was approved by Ethics Committee of the Faculty of Basic Medical Sciences, Edo State University Uzairue and in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research.

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