**AQUEOUS LEAF EXTRACT OF *Chromolaena odorata* (**[**L.**](https://en.wikipedia.org/wiki/Carl_Linnaeus)**)** [**R.M. KING**](https://en.wikipedia.org/w/index.php?title=Robert_Merrill_King&action=edit&redlink=1) **&** [**H.ROB.**](https://en.wikipedia.org/wiki/Harold_E._Robinson)**ATTENUATES METHOTREXATE-INDUCED HEPATOTOXICITY IN WISTAR RATS**

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**Abstract**

Methotrexate (MTX) usage in orthodox treatment has increased steadily over the years due to its broad applicability for treating different ailments, including various forms of cancer. However, in the course of usage, Methotrexate causes toxicity in body organs. Certain plant species have been shown to possess therapeutic properties by offering a protective effect against drug side effects. Thus, the current study was carried out to evaluate the potential of aqueous Chromolaena odorata leaf extract (AEOC) to attenuate the effect of Methotrexate-induced hepatotoxicity in Wistar rats. The experimental design incorporated thirty (30) male Wistar rats into five groups (1-V) of six each to include: **Group I** (control), Group II (AEOC at 250mg/kg), **Group III**(Methotrexate at 7 mg/kg), Group**IV** (AEOC at 250 mg/kg + Methotrexate at 7 mg/kg), and Group**V (**Vitamin C (100 mg/kg) + Methotrexate at 7 mg/kg). *Chromolaena odorata* and Vitamin C was administered for ten consecutive days while Methotrexate was administered on day 8 for three consecutive days. Rats were sacrificed 24hrs after last administration and serum collected was used for determination of Aspartate Aminotransferase (AST), Alanine transaminase (ALT), Albumin (ALB), Total Bilirubin (TB), Total protein (TP) while liver tissue was used for assessment of Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Catalase (CAT) as well as histopathological analysis. The result showed a significant increase in the level of Superoxide dismutase (SOD), Catalase (CAT) and a significant reduction (p<0.001) in Malondialdehyde in all *Chromolaena odorata* or Vitamin C treated groups compared with the Methotrexate alone group. Also, *Chromolaena odorata* or Vitamin C significantly reduced the levels of liver function enzymes and Total Bilirubin while increasing synthetic molecules when compared with the Methotrexate alone group. *Chromolaena odorata* at 250mg/kg attenuated the toxic effect of Methotrexate, which was corroborated by histopathological analysis. In conclusion, Chromolaena odorata attenuated methotrexate induced hepatotoxicity by enhancing antioxidant status thus scavenging free radicals and reducing oxidative stress.

**KEYWORDS:** Methotrexate, Chromolaena odorata, hepatotoxicity, oxidative stress, wistar rats.

**Introduction**

Methotrexate (MTX) is an anti-metabolic agent that affects the metabolism of folic acid.1. In medical practice, Methotrexate is indicated for a plethora of clinical conditions, including Autoimmune rheumatic conditions, such as rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis, juvenile idiopathic arthritis, inflammatory myopathies, sarcoidosis, rheumatic polymyalgia, arthritis related to secondary amyloidosis and others; also other autoimmune conditions, such as Sjögren syndrome, inflammatory bowel disease, and vasculitis; and some types of neoplasms.2 The importance of Methotrexate as an effective chemotherapy agent for the treatment of cancer cannot be overemphasized as it has successfully been used in the treatment of breast cancer, leukaemia, lung cancer, lymphoma, gestational trophoblastic disease, and osteosarcoma.3 However, side effects abound**. The side effects of Methotrexate affect various systems of the body, including the Gastrointestinal System4**, Hematopoietic**System**2, **Central Nervous System**, **Respiratory System**5, **Cardiovascular System.2** Methotrexate also presents renal toxicity and decreases creatinine clearance and glomerular filtration rate.2 Also, there is evidence that Methotrexate can be oncogenic, notably in lymphomas and leukaemias.5

The use of herbal medicines has continued to gain momentum, particularly in Africa, where 70-80 % of its people depend either totally or partially on it. Certain herbs have been reported to have the potential to alleviate the side effects of most synthetic drugs.6Chromolaena odorata, a pantropic herb, possess phytochemicals and antioxidant enzymes that activate defence mechanisms and stress-sensing transcription factors to prevent oxidative damage.7 The dried leaf of Ch. odorata  contains active phytochemical substances such as flavonoid aglycones (flavanones, flavonols, flavones), including acacetin, chalcones, eupatilin, luteolin, naringenin, kaempferol, quercetin, quercetagetin, and sinensetin, terpenes and terpenoids, essential oils, alkaloids including pyrrolizidine, saponins and tannins, phenolic acids including ferulic acid, protocatechuic acid, phytoprostane compound including chromomeric acid.8,9,10 Ch. odorata have been reported to be used to treat wounds, burns, and skin infections and possess anticancer, antidiabetic, anti-inflammatory, antimicrobial, anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory, diuretic, tonic, antipyretic and heart tonic and also cough remedy.11-12] Therefore the present study evaluates the potential of Ch. odorata to reduce the effect of methotrexate-induced toxicity in rats.

**Materials and Method**

**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, 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 1000g 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 (125mm) and then cotton wool. The resultant filtrate was concentrated using a rotary evaporator set at 40oC 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 4oC. Aliquot portions of the crude plant extract residue were weighed and dissolved in normal saline on each experiment day.

**Experimental Animals and Design**

Thirty (30) male Wistar rats (180-200g) of the species- *Rattus norvegicus*  were purchased from the animal house, Department of Zoology, Ambrose Alli University, 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 hours light and 12 hours dark) in 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 was in accordance with the guidelines for ethical conduct in the care and use of nonhuman animals in research.13

After acclimatization for seven days, the rats were randomly distributed into the following groups as follows: **Group I:** Served as a control and only received normal saline orally once daily. **Group II:** Rats were given aqueous extract of *Ch. odorata*at a dose of 250 mg/kg orally once daily for ten days. **Group III**: Rats were given Methotrexate intraperitoneally at a dose of 7 mg/kg on day 8 of the experiment for three consecutive days. **Group IV:** Rats were given aqueous extract of *Ch. odorata*(250 mg/kg) orally once daily for ten days, and then Methotrexate intraperitoneally (7 mg/kg) on day 8 of the experiment for three consecutive days. **Group V:** Rats were given Vitamin C (100 mg/kg) orally once daily for ten days and then Methotrexate intraperitoneally (7 mg/kg) on day 8 for three consecutive days. Methotrexate was dissolved in saline and injected intraperitoneally (i.p.) at 7 mg/kg dose.14*Ch. odorata* at a dose of 250mg/kg was based on the study of.15

At the end of the experiment after 24hrs of last administration, the rats were sacrificed and blood was collected in plain tubes, allowed to stand for 45 minutes before being centrifuged at 4000 rpm for 25 min to obtain serum for analysis. The Serum was used for determination of Aspartate Aminotransferase (AST), Alanine transaminase (ALT), Albumin (ALB), Total Bilirubin (TB) and Total protein (TP).

The liver were immediately excised, washed in ice cold saline, weighted and a portion fixed in 10% phosphate buffered formalin for histopathological examination while the remaining portion were stored at -20oC for determination of oxidative stress and endogenous enzymes. 10 % tissue homogenate of the stored liver 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 (CAT)

**Biochemical Parameters**

**Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)** activity was determined by using the RANDOX Kit according to the manufacturer’s instructions as described by.16 Total Bilirubin was determined using the RANDOX Kit according to the manufacturer’s instructions by17. Total protein was determined by using the RANDOX Kit according to the manufacturer’s instructions as described by.18Albumin was determined by using the RANDOX Kit according to the manufacturer’s instructions based on the Bromocresol green (BCG) method as described by.19 Malondialdehyde (MDA) as indicator of lipid peroxidation was determined by reaction of thiobarbituric acid according to the method of.20 The level of Superoxide Dismutase (SOD) activity was according to the method of21 while the method of 22 was used for determination of Catalase (CAT).

**Histopathological studies**

Rats were sacrificed after and liver samples were excised, washed with normal saline (0.9% NaCl). The isolated livers were fixed in 10% buffered formalin and were further processed for

histopathological investigations. Histopathologically the liver tissues were thereafter stained with hematoxylin and eosin (H&E) stained 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 and Discussion**

The biomarkers for liver damage in Wistar rats treated with aqueous extract of *Ch. odorata* and administered with Methotrexate is presented in Table 1. Administration of Methotrexate significantly increased (p > 0.05) AST (113.67 U/L), and ALT (127.08 U/L) enzymes as well as total bilirubin (17.31 mg/dL) while total protein (3.54 g/dL) and albumin (1.56 g/dL) were reduced significantly when compared with control group and other groups in this study. However, treatment with *Ch. odorata* or Vitamin C to rats administered methotrexate significantly (p > 0.05) restored the level of AST, ALT, total Bilirubin, total protein and Albumin towards normalcy.

**Table 1: Effects of aqueous leaf extract of *Chromolaena odorata* on Liver Function and Synthetic Molecules in Methotrexate-induced Wistar rats**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment groups** | **AST (U/L)** | **ALT (U/L)** | **ALB (g/dl)** | **TBIL****(mg/dl)** | **TP****(g/dl)** |
| Control | 41.33a ± 3.33 | 36.67a ±3.17 | 7.47c ± 0.99 | 2.39a ± 0.45 | 9.65d ± 0.91 |
| *Ch. odorata* alone (250mg/kg) | 43.67a ± 3.03 | 40.70a±3.31 | 6.04c ± 0.70 | 1.25d ± 0.10 | 7.79d ± 0.93 |
| MTX alone (7mg/kg) | 113.67c ±6.67 | 127.08c±7.55 | 1.56a ± 0.32 | 17.31c ±1.71 | 3.54a ± 0.11 |
| MTX (7mg/kg) + *Ch. odorata* (250mg/kg) | 61.33b ± 4.07 | 62.08b±5.04 | 3.66b ± 0.72 | 7.23b ± 0.96 | 5.39b ± 0.14 |
| MTX (7mg/kg) + Vit. C (100mg/kg) | 56.33b ±4.88 | 73. 01b±5.57 | 3.82b ± 0.60 | 8.87b ± 0.87 | 6.07c ± 0.61 |

Values are expressed as Mean ± Standard Error of the Mean, n=6. Values with different super scripts down the column differ significantly at (p<0.05). AST-Aspartate Aminotransferase; ALT-Alanine Aminotransferase; ALB- Albumin; TBIL- Total Bilirubin; TP- Total protein; Vit. C- Vitamin C; MTX- Methotrexate.

The present study indicated a significant effect of aqueous leaf extract of Ch. odorata on liver Function enzymes, AST and ALT, in Methotrexate induced wistar rats. The administration of MTX caused significant liver toxicity marked by elevated serum levels of AST and ALT similar to previous related studies.23,24,25 Uraz et al.26 reported that such an increased level of AST indicate damage caused by methotrexate toxicity to the visceral organs. In this present study, MTX at a dose of 7mg/kg was recorded to have a higher concentration of AST and ALT in the serum, which vividly indicates hepatotoxicity as against other treatment groups which had values towards normalcy. However, administration of aqueous leaf extract of Ch. odorata or Vitamin C for ten days with intraperitoneal injection of MTX was found to improve liver functions, evidenced by the reduction in the AST and ALT values similar to previous findings of Patel et al.27. The ability of *Ch. odorata* or Vitamin C treatment groups to reduce the AST and ALT levels may result from the antioxidant activity of vitamin C or bioactive constituents of Ch. odorata leaves such as flavonoid10. Also, Xu et al.28 reported that ALT is a more specific indicator of liver damage, particularly liver inflammation, compared to AST. In the present study, MTX elevated ALT more than AST, as observed by Ozogula et al.29.

The present study also showed a significant effect of aqueous leaf extract of Ch. odorata on liver synthetic molecules, albumin (ALB), total Bilirubin (TBIL), and total protein (TP) in Methotrexate-induced rats. These parameters are used as reliable checks to indicate liver damage. From the results obtained, the low concentration of ALB in blood serum for MTX-administered rats is a clear sign of hepatic impairment compared to the control group. However, administration of aqueous leaf extract of Ch. odorata or Vitamin C for ten days with intraperitoneal injection of MTX recorded much higher values which can be attributed to the protective effect of Ch. odorata and vitamin C due to their antioxidant capacity. The findings of this study on the albumin level align with Swayeh et al.30. As opined by Swayeh et al.30, these consistent observations are probably due to an indirect effect of Methotrexate on protein synthesis by declining the amount of tetrahydrofolate. This study also recorded a reduction in Total Protein (TP) due to Methotrexate toxicity. Generally, it is expected that the body needs to be in constant production of proteins to fight infections to aid the health and growth of the body's cells and tissues amongst others. The level of total proteins shows how well the liver is working correctly to producing these proteins. Conversely, significantly lower values obtained in Methotrexate (7mg/kg) alone group further indicates the extent to which the liver is damaged and cannot properly function. The reduction in total protein and albumin could be due to damage to the liver by Methotrexate, increased intestinal protein loss, and protein-losing nephropathy.31 Similar related findings of a decrease in total protein have been previously reported by.23,32,33,34. However, administration of aqueous leaf extract of Ch. odorata or Vitamin C for ten days with intraperitoneal injection of MTX had a gradual increase in the concentration of total proteins, thus demonstrating the protective and antioxidant potential of Ch. odorata and Vitamin C.

Bilirubin analysis is done to ascertain the liver's health or monitor the progression of an affected liver. The elevated levels of Total Bilirubin recorded for the MTX (7mg/kg) administered group indicates liver damage or disease. This signifies that the ability of the liver to clear bilirubin has been impacted, hence the observed toxicity. This finding aligns with previous related studies of.35,36,37,38 However, administration of aqueous leaf extract of Ch. odorata or Vitamin C for ten days with intraperitoneal injection of MTX had a reduced concentration of Total Bilirubin compared to the value recorded by Methotrexate untreated group. This decrease can be attributed to the protection of the liver against oxidative damage caused by Methotrexate.

MDA, being an end product of lipid peroxidation, is often used as marker of lipid peroxidation. In this study, Methotrexate injection for three consecutive days at a dose of 7mg/kg resulted in a significant increase in MDA in the intoxicated group whereas treatment with *Ch. odorata* or Vitamin C significantly reduced MDA, thus hepatic damage indicating the hepatoprotection (Table 2).

Antioxidant enzymes such as SOD and CAT were estimated in the present study. There was a significant decrease in CAT content in the liver homogenates of Methotrexate-treated groups as compared to the control at 𝑃 < 0.05. The supplementation of *Ch. odorata* with Methotrexate caused a significant increase in CAT when compared with Methotrexate group (Table 2). Methotrexate at a dose of 7mg/kg for three consecutive days also decreased the activity of SOD, compared to the control group (Figure 2). However, rats that received *Ch. odorata* or Vitamin C together with Methotrexate experienced a significant increase in SOD activity compared to the Methotrexate-treated group.

**Table 2: Effect of aqueous leaf extract of Chromolaena odorata on hepatic Oxidative stress parameters in Methotrexate-induced Wistar rats**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment groups** |  **SOD****(U/mg protein)** | **MDA****(µmol/mg protein)** | **CAT****(U/mg protein)** |
| Control | 89.47a ± 3.13 | 3.29a ± 1.54 | 2.87a ± 0.21 |
| *Ch. odorata* alone (250mg/kg) | 93.59a ± 5.71 | 6.92b ± 0.85 | 3.50a ± 0.58 |
| MTX (7mg/kg) | 29.94b ± 4.79 | 13.99c ± 1.23 | 0.93b ± 0.12 |
| MTX (7mg/kg) + *Ch. odorata* (250mg/kg) | 73.22c ± 3.72 | 8.63d ± 1.37 | 1.17c ± 0.06 |
| MTX (7mg/kg) + Vit C (100mg/kg) | 56.10d ± 3.87 | 9.22d ± 0.95 | 1.42c ± 0.19 |

Values are expressed as Mean ± Standard Error of the Mean, n=3. Values with different super scripts down the column differ significantly at (p<0.05). SOD- Superoxide Dismutase

MDA- Malondialdehyde, CAT- Catalase, MTX- Methotrexate, Vit C- Vitamin C

The antioxidant defense system of the body is the primary line of defense that counteracts the deleterious effects of free radicals, oxidative damage and oxidative stress. Data from this study indicated a remarkable decrease in the activity of CAT and SOD in the group administered with MTX (7mg/kg) alone compared to the control group (Tables 2). According to Sener et al.39, oxidative stress leads to many pathological and cellular damages to liver tissues. However, administration of aqueous leaf extract of Ch. odorata or Vitamin C for ten days with intraperitoneal injection of MTX showed a significant increase in activity of CAT and SOD in the liver, a result which conforms to a previous reported study of.40-41 Unlike SOD and CAT, an increase in MDA values was observed for the group administered MTX alone. This could be because lipid peroxidation decreases membrane ﬂuidity.42 and could compromise the integrity and function of the plasma membrane, thereby leading to leakages of materials from hepatocytes into the blood. However, administration of aqueous leaf extract of Ch. odorata or Vitamin C for ten days with intraperitoneal injection of MTX had recorded a much-reduced MDA compared to the group administered MTX (7mg/kg) alone similar to previous related studies of.43,44,45,40

Histopathological analysis showed that Methotrexate (7mg/kg) caused distortion necrosis, congestion, cell infiltration, irregular loss of hepatocytes architecture with dilated central veins and dilated hepatic sinusoids as well as vacuolar degeneration (Figure 2)**.** However, administration of aqueous leaf extract of Ch. odorata or Vitamin C for ten days with intraperitoneal injection of MTX showed reduction in hepatic lesions with moderately spaced central veins surrounded by uniform hepatocytes distribution **(Figure 4 and 5).**

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**Fig. 1:** Photomicrograph ofliver of Control rats that received normal saline showing normal liver architecture. **Figs. 2:** Photomicrographof liver given Methotrexate at a dose of 7mg/kg for three consecutive days showing necrosis, cell infiltration dilation and congestion. **Fig. 3:** Photomicrograph of liver of rats that received 250mg/kg *Ch. odorata* alone for ten consecutive days showing normal liver architecture. **Fig. 4:** Photomicrograph of liver of rats given 100mg/kg Vitamin C for ten consecutive days and 7mg/kg Methotrexate for three consecutive days showing reduction in hepatic lesions. **Fig. 5:** Photomicrograph of liver of rats given 250mg/kg *Ch. odorata* for ten consecutive days and 7mg/kg Methotrexate for three consecutive days showing reduction in the histopathological lesions.

Histopathological analysis showed that Methotrexate (7mg/kg) had significant distortion on the hepatocytes as seen with observed necrosis, congestion, cell infiltration, irregular loss of hepatocytes architecture with dilated central veins and dilated hepatic sinusoids as well as vacuolar degeneration similar to findings of.46 However, administration of aqueous leaf extract of Ch. or Vitamin C for ten days with intraperitoneal injection of MTX showed reduction in hepatic lesions with moderately spaced central veins surrounded by uniform hepatocytes distribution**.**

**Conclusion**

**Put together,** Ch. odorata attenuated Methotrexate-induced hepatotoxicity by scavenging free radicals, reducing oxidative damage and oxidative stress by enhancing antioxidant status.

**Conflicts of Interest**

The authors hereby declare no conflicts of interest.

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