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Restorative effects of ethanolic leaf extract of *Datura stramonium* against methotrexate-induced hematological impairments

Esther U. Alum[1,3*], Okechukwu P. C. Ugwu[1], Patrick M. Aja[3,4], Emmanuel I. Obeagu[1,2], Joseph E. Inya[5], Amarachi P. Onyeije[3], Esther Agu[3] and Chinaza Godswill Awuchi[6]

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*Corresponding author: Esther U. Alum, Department of Publications and Extension, Kampala International University, P.O. Box 20000 Kansanga, Kampala, Uganda
E-mail: esther.alum@kiu.ac.ug

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María Luisa Escudero Gilete, Nutrition and Bromatology, Universidad de Sevilla, Spain

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Abstract: One of the prominent complications observed in those undergoing treatment with methotrexate (MTX) is hematological profile alterations which could culminate in severe anemia. In this study, we assessed the hematological profile indices in MXT-treated rats and the effect of leaf extract of *Datura stramonium* (LEDS) supplementation in MXT-treated rats. Ethanol (98%) was the solvent used in extraction. Animals were divided at random into four groups. Animals in group 1 received normal saline (5 mg/kg) orally and feeding was limitless and did not receive MXT. Animals in group 2 were given LEDS orally (200 mg/kg body weight) for 21 days while group 3 received 20 mg/kg body weight (bw) of MXT on day 18 via the intra-peritoneum without LEDS. Rats in group 4 were given the extract (200 mg/kg bw) and also injected with 20 mg/kg bw of MXT on day 18 of the study via the intra-peritoneum. Serum levels of hemoglobin, red blood cells, packed cell volume, total white blood cells, neutrophils, lymphocytes, and platelets were determined. Rats treated with MXT had notable depletion in hemoglobin, red blood cells, packed cell volume, total white blood cells, neutrophils, and platelets, unlike the control group. Interestingly, LEDS supplementation markedly restored the altered hematological profiles. MXT injection caused hematological dysfunction while co-supplementation with LEDS restored the impaired hematological indices. Therefore, LEDS could be a promising tool in arresting hematological dysfunctions accompanying MXT chemotherapy. However, we advocate for further prospective scrutiny.

Subjects: Biochemistry; Biology; Pharmacology; Toxicology; Food Chemistry; Medicine; Allied Health

Keywords: cancer; chemotherapy; *Datura stramonium*; hematological profile; methotrexate; rheumatoid arthritis

1. Introduction

Methotrexate (MTX) is a folate analog widely effective against some diseases, especially cancers and autoimmune diseases. Folate antagonists like MXT are ancient tools in the treatment of cancer. MXT can also be used to control Rheumatoid arthritis (RA) severity (Alum et al., 2023b; Curtis et al., 2021; Hassanein et al., 2021; Weinblatt, 2013) and in the management of ectopic pregnancy (Sindiani et al., 2020; Xiao et al., 2021).

MXT is also useful in the treatment of psoriasis, systemic lupus erythematosus, inflammatory bowel disease, vasculitis, and many other connective tissue diseases (Bedoui et al., 2019). It is also useful in managing patients undergoing organ transplantation due to its anti-inflammatory and immunomodulatory properties (Chan & Cronstein, 2010). Clamping down of the generation of dihydrofolate reductase and subsequent decline in the level of tetrahydrofolate is fingered to be the primary mode of action of MXT. Dihydrofolate reductase and tetrahydrofolate are needed for the production of purine nucleotides, cell replication, and DNA synthesis (Choy et al., 2005). MTX use yields a cytotoxic effect on rapidly dividing cells like lymphocytes (Choy et al., 2005). Despite the effectiveness of MXT in cancer and RA treatment, its use still portends some deleterious effects ranging from hematological derangement, nephrotoxicity, pulmonary toxicity, teratogenesis, cirrhosis, immune suppression and increased risk of anemia (Schmiegelow, 2009; Shetty et al., 2017). High-dose MTX toxicity can be managed with leucovorin, thymidine, and glucarpidase (Lucas et al., 2019). Leucovorin is an analog of folate and hence can supplement folate and dihydrofolate deficiency. Therefore, it can downregulate MXT-induced myelosuppression, gastrointestinal toxicity, and neurotoxicity (Van der Beek et al., 2019). Glucarpidase converts MXT into 2,4-diamino-N10-methylpteroic acid (DAMPA) and glutamate. These two metabolites are nontoxic, thus enhancing MXT removal in patients with renal insufficiency. Thymidine use, though still under scrutiny, is believed to protect cells from the harmful effects of MXT (Van der Beek et al., 2019). Despite these breakthroughs in the management of MXT toxicity, an MXT-induced alteration in hematological profiles is still a prominent drawback in medical practice. There is therefore expeditious need to invent measures that would curb hematological abnormalities since this is one of the most severe adverse effects faced by RA and cancer patients using MXT. This study sought to investigate if the co-administration of LEDS with MXT could close this gap.

The use of medicinal plants in the treatment and management of diseases is as a result of some bioactive compounds inherent in them (Javaid et al., 2022; Mahmoud et al., 2019; Ugwu et al., 2023a). *Datura stramonium* is an example of such a plant. *D. stramonium* is a member *Solanaceae* family (Sayyed & Shah, 2014). It originated in America. Currently, its presence has spread to other regions of the world including Nigeria (Céspedes-Méndez et al., 2021; Melaku & Amare, 2020). Its common names are Devil's Apple, Angel's Trumpet, and Jimson Weed. In Afikpo, Southeast Nigeria, it's popularly called soldier root and its popularity among youths stems from its narcotic effects.

Several authors have reported that *D. stramonium* possesses pharmacological effects such as hepatoprotective, nephroprotective, anticancer, antioxidative, anti-inflammatory, antiasthmatic antifungal, and anticholinergic effects (Alum et al., 2023b; Nasir et al., 2022; Sharma et al., 2021). Recently, Nasir et al. (2022) reported that *D. stramonium* seed and leaves abrogated toxicant-induced oxidative inflammation and immunosuppression. *D. stramonium* possesses different chemical constituents like minerals, vitamins, and phytochemicals especially alkaloids in appreciable concentrations. These constituents are fingered to be responsible for the various pharmacological properties of *D. stramonium* (Ali & Endalew, 2021; Alum et al., 2023d). Despite the numerous medicinal uses of *D. stramonium*, its toxicity as a result of abuse is well-documented (Korkmaz et al., 2019; Ogunmoyole et al., 2019).

Fatoba et al. (2013) reported the blood-boosting effect of seed extracts of *D. stramonium* in bucks. However, there is scarce information on the assessment of hematological profile indices of MXT-treated rats with Leaf extract of *D. stramonium* (LEDS). Therefore, this study was aimed at assessing the hematological profile indices in MXT-treated rats and the effect of Leaf extract of *D. stramonium* (LEDS) supplementation in MXT-treated albino rats.

2. Materials and methods

2.1. Chemicals and reagents

Standard-grade chemicals and reagents were sourced from reputable vendors and used.

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2.2. Biological materials

Fresh leaves of *Datura stramonium* and male albino rats (Wistar) were the biological materials employed in this study. Matured leaves of *D. stramonium* were harvested in Afikpo (Amaozara precisely), Ebonyi State, Nigeria in May 2022. Mr. Nwankwo Onyebuchi, a plant Taxonomist at Ebonyi State University, Abakaliki, identified it (EBSU-H-397).

2.3. Animal care

Wistar rats (male) weighing 105–115 g were procured from the Department of Animal Science, University of Nigeria. The rats were kept for two weeks to get used to the new environment in the Animal House of the Biochemistry Department, Ebonyi State University, Abakaliki, temperature (25 ± 2 °C) and photoperiod (12 hours light and 12 hours dark). The rats had unlimited access to neat drinking water and commercial rat feed. The animals were managed in a gentle way in line with global standard (NIH Publication No. 85-23, revised 1996.

2.4. Ethical considerations

Appropriate ethical approval to embark on the study was obtained from the Research Ethics Committee of the Biochemistry Department Ebonyi State University Abakaliki, Nigeria (EBSU/BCH/ET/21/006). The guidelines are in tandem with global standards for the care and use of laboratory animals in research (NIH Publication No. 85-23, revised 1996).

2.5. Methods, evaluations, and analyses

2.5.1. Preparation of the crude ethanol leaf extract of *D. stramonium*

Extraction was carried out using the method illustrated by Ibiam et al. (2018). *D. stramonium* leaves were cleaned under a tap running water, dried under shade, ground, and sieved. About 800 g of the obtained powder was immersed in 2000 ml of 98% ethanol for 48 h at room temperature with intermittent rocking. Thereafter, it was filtered with a white clean cloth and the filtrate was heated in a water bath until the solvents dry extract was obtained. The obtained extracts were preserved in a closed box.

2.5.2. Animal grouping

When the two-weeks adaptation time elapsed, 40 animals were grouped into 4 groups comprising 10 rats ($n = 10$) as classified thus:

Group 1: Normal control (given 5 mg/kg normal saline)

Group 2: Extract (200 mg/kg Extract only)

Group 3: MXT group (20 mg/kg MXT, untreated rats)

Group 4: Positive control (200 mg/kg Extract + MXT).

Rats in group 1 received 5 mg/kg of normal saline orally, fed without restriction, and were not injected with MXT. Rats in group 2 (Extract only) received extract 200 mg/kg b.w (orally) for 21 days; rats in group 3 were injected with 20 mg/kg b.w of MXT on day 18 intraperitoneally. Lastly, rats in group 4 were given the extract at a dose of 200 mg/kg b.w all through the 21 days and methotrexate (20 mg/kg b.w) on day 18 of the study intraperitoneally (Alum et al., 2023b; Nasir et al., 2020)

2.5.3. Samples collection from the animals

On the last day of the 21 days study, rats were starved all night and anesthetized using mild diethyl ether. Whole blood was collected into serum bottles by cardiac puncture and also centrifuged to obtain the serum.

2.5.4. Assay of hematological indices

PCV, RBC, and WBC were determined by the method described by Dacie and Lewis (2000). Platelet counts were determined by the method described by Baker and Silverton (1985) while hemoglobin concentrations were determined according to the method described by Zwart et al. (1996).

2.5.5. Analysis of data

Results are indicated as mean ± standard deviation, analyzed, and compared using a one-way analysis of variance followed by Turkey's post hoc test; significance was accepted at $P < 0.05$. Graph Pad Prism version 5.00 for Windows was utilized in this statistical analysis.

3. Results and discussion

3.1. Effect of Leaf Extract of *D. Stramonium* (LEDS) on hematological indices in MTX-injected rats

Injection with MXT caused a significant ($P < 0.05$) downregulation of hemoglobin (Hb), red blood cells (RBCs), packed cell volume (PCV), white blood cells (WBCs), neutrophils, and platelets. Contrary to this observation, there was a significant ($P < 0.05$) rise in the lymphocyte levels of rats that received MXT injections. Interestingly, the administration of LEDS resulted in a significant ($P < 0.05$) rise in Hb, RBCs, PCV, WBCs, neutrophils, and platelets in the rats. More so, the administration of LEDS yielded a significant ($P < 0.05$) decline in the lymphocyte counts in the rats (Table 1).

3.2. Discussion

MXT is a folic acid antagonist commonly used in the treatment of various types of cancer. MTX inhibits dihydrofolate reductase, a key enzyme in the synthesis of DNA, RNA, and proteins (Hassanein et al., 2021). MXT is the most-preferred option for the treatment of RA, as well as other autoimmune and inflammatory disorders. MXT is the preferred choice of drug because of its cost-effectiveness and efficacy in the mitigation of RA symptoms as well as the decline in the progression of RA (Lopez-Olivo et al., 2014; Taylor et al., 2019).

Despite the widely accepted use of MXT, patients still face some adverse effects as a result of MXT use. However, these adverse effects are minimal in RA patients due to lower doses while cancer patients experience greater adverse effects since they require higher doses of MXT (Solomon et al., 2020). The commonest adverse effects of MXT use include liver damage, hematological derangement, immune system suppression, and gastrointestinal inconveniences (Mustafa et al., 2022). The severity of these adverse reactions does compel patients to withdraw treatment despite its effectiveness in chemotherapy (Romão et al., 2014).

Evaluation of the hematological profile is very crucial in the elucidation of well-being or otherwise in clinical medicine (Arika et al., 2016).

Recently (Mustafa et al., 2022), reported that the commonest hematological impairments in MXT-treated patients were anemia, thrombocytopenia, leucopenia, and pancytopenia. Most patients discontinued their treatments as a result of these adverse reactions. This report further stresses the need for an additional agent that can be supplemented with MXT so as to avert the aforementioned adverse reactions. As far as we are aware, this is the first research to probe the hematological effect of the co-administration of LEDS with MXT.

In this study, we investigated the impact of co-administration of *D. stramonium* ethanol leaf extract with MXT using a rat model. Our previous studies and other authors' reports abound suggesting various pharmacological effects of plants like anti-oxidant, anti-inflammatory, antidiabetic, anti-RA, and anti-anemic (Aja et al., 2017; Alam et al., 2020; Alum et al., 2022a; Chandan et al., 2021; Egwu et al., 2017; Ibiam et al., 2018; Ugwu et al., 2023b) but none of them reported concomitant administration of LEDS with MXT.

Table 1. Effect of leaf extract of *D. stramonium* (LEDS) on hematological indices in MTX-injected rats

Groups	PCV (%)	Hb (g/dL)	RBC (10^6/mm^3)	WBC ($\times10^3$/mm^3)	Neutrophils (%)	Lymphocytes (%)	Platelets ($10^3\times$mm^3)
Gp1	28.76 ± 0.1	8.56 ± .06	9.03 ± 0.06	9003.33 ± 5.86	34.77 ± 4.06	65.23 ± 1.00	1,101.24 ± 36
Gp2	24.56 ± 0.05*	9.35 ± .10*	9.57 ± 0.04	10020.00 ± 12.35*	32.69 ± 0.72	67.31 ± 0.9	1,201.36 ± 45
Gp3	20.86 ± 0.05*	6.58 ± .15*	7.10 ± 0.10*	9040.65 ± 16.76*	24.75 ± 1.31*	75.25 ± 1.22*	879.35 ± 42*
Gp4	27.36 ± 0.07#	8.95 ±.05#	8.95 ± 0.05#	10064.34± 22.23#	37.62 ± 0.32#	62.38 ± 0.99#	1,1683.56 ± 58#

Data are expressed as mean ± standard deviation of 10 rats in each group. "*" depicts a significant difference ($P < 0.05$) when compared to the normal control group while "#" depicts a significant difference ($P < 0.05$) difference when compared to the MXT group.
Gp1 = Control; Gp2 = 200 mg/kg LEDS; GP3 = 20 mg/kg MXT; GP4 = 200 mg/kg LEDS + MXT; LEDS= leaf extract of *Datura stramonium*

In this study, MXT injection (20 mg/kg) created hematological derangements by reducing the levels of hemoglobin, red blood cells, packed cell volume, white blood cells (Hb, RBCs, PCV, WBCs), neutrophils, and platelets. On the other hand, there was a notable rise in the lymphocyte counts of rats that received MXT injections. Interestingly, the administration of LEDS resulted in a significant ($P < 0.05$) upsurge in Hb, RBCs, PCV, WBCs, neutrophils, and platelets in the rats. Furthermore, administration of LEDS yielded a significant decline in the lymphocyte counts in the rats. The blood-boosting capacity of *D. stramonium* and other medicinal plants is well-documented (Aja et al., 2017; Fatoba et al., 2013; Syawal et al., 2021).

D. stramonium contains various phytochemicals, amino acids, vitamins, and minerals in commendable amounts (Alum et al., 2023a; Nayyar et al., 2020). Thus, the desirable effects of LEDS on the impaired hematological profile of MXT-treated rats could be a result of the phytonutrients inherent in the plant.

Our observed derangement in hematological profile as a result of MXT treatment aligns with previous authors (Hamed et al., 2022; Mustafa et al., 2022). In this study, the restorative capacity of the impaired hematological indices by the extract corroborates the blood-boosting capacity of *D. stramonium* in animals as reported by Fatoba et al. (2013). Additionally (Mhatre & Marar, 2016), reported the protective effect of *Morinda citrifolia* L. (fruit extract) on MXT-induced hematological toxicity in animals.

Anemia is the most common hematological abnormality in cancer patients undergoing treatment (Hu & Harrison, 2005). The possible mechanisms responsible for hematological abnormalities in patients undergoing chemotherapy could be cancer-related bleeding, impairment in nutrition, iron metabolism, nephron function, and compromised bone marrow capacity (Barkati et al., 2013; Candelaria et al., 2015).

PCV is a measure of the proportion of RBCs in the whole blood. It, therefore, gives an insight into the oxygen-carrying capability of the RBCs (Arika et al., 2016; Hall, 2016). Therefore, the observed decline in the PCV of MXT-treated portends anemia. Interestingly, co-administration with LEDS restored the anemic condition.

The observed decline in platelet count in MXT-treated rats could be a result of MXT-induced reactive oxygen species (ROS) generation which contributes to the untimely demise of platelets and mitochondrial dysfunction culminating in low platelet count (thrombocytopenia) (Girish et al., 2013; Paul et al., 2015; Wang et al., 2013). Previous studies have reported the use of natural products in the amelioration of MXT-induced adverse effects (Alum et al., 2022b, 2023a; Thushara et al., 2013). Thus, the discovered restoration in the platelet counts in LEDS-treated rats corroborates these previous reports.

Thrombopoietin is a hormone that regulates platelet production by the bone marrow (Arika et al., 2016). Therefore, the observed decline and rise in the platelet counts of the MXT-treated and LEDS-treated rats suggest an inhibitory and stimulatory effect on thrombopoietin, respectively.

An increase in WBC was also observed in LEDS-treated rats. This supports previous studies on the WBC-boosting potential of medicinal plants (Imoru et al., 2005; Ladokun et al., 2015). This result suggests the immune-boosting potential of *D. stramonium*. The importance of WBC in immune system protection is indisputable.

In the present study, MXT-treated rats recorded a decline in neutrophils. We noticed an opposite effect in rats that received the extract as there was an increase in neutrophil count in these rats.

According to Pamuk et al. (2013) sociocultural status, cognitive capabilities, and distress may contribute to MXT-induced neutropenia (low neutrophils count) in RA patients.

Furthermore, the observed hematological derangement in MXT-injected animals could have emanated from the nephrotoxic effect of MXT. Reports of MXT-induced renal damage abound (Alum et al., 2023b; Famurewa et al., 2017; Grönroos et al., 2006). MXT treatment may escalate the risk of pancytopenia-related death in individuals with renal insufficiency (Cheung et al., 2009).

A reduced level of Hb, RBCs, and platelets (pancytopenia) is the hallmark of hematological aberration during MXT chemotherapy (Patel et al., 2014). Pancytopenia arises when there is a low level of the three blood cell lines (red blood cells, WBCs, and platelets) (Vargas-Carretero et al., 2019). Pancytopenia could result when there is increased destruction, decreased production, or both of the blood cell lines (Gnanaraj et al., 2018). In this result, *D. stramonium* extract ameliorated the MXT-induced pancytopenia in the rats. This result gives credence to the blood cell lines-enhancing capacity of *D. stramonium* (Fatoba et al., 2013) and other plants (Dadezadeh & Nourafcan, 2018; Ekpono et al., 2019).

In the present study, MXT injection resulted to increase in lymphocytes. However, co-administration with *D. stramonium* led to a decline in lymphocyte levels. Excessive lymphocyte counts (lymphocytosis) have been reported by previous authors in MXT-treated RA and cancer patients (Mustafa et al., 2022).

MXT-induced anemia, neutropenia, and thrombocytopenia may lead to severe complications culminating in severe infections, hemorrhagic complications, and death (Lopes-Serrao et al., 2011; Noviyani et al., 2019). Therefore, the importance of finding novel adjuvant that could mitigate the severity of these hematological impairments cannot be over-emphasized.

4. Conclusion

Rats that received 20 mg/kg of MXT had derangement in their hematological profile. This finding aligns with previous research that reported toxicities of MXT including hematological toxicity. Co-administration of *D. stramonium* ethanol leaf extract with MXT restored the deranged hematological indices in MXT-treated rats. This research is the first to discover the restorative ability of co-administration of ethanol leaf extract of *D. stramonium* with MXT on hematological markers. Since there is an unmet need in the treatment of MXT-induced pancytopenia, *D. stramonium* may be considered as an adjuvant in MXT treatment since it alleviated the MXT-induced pancytopenia in rats. However, further research study is needed.

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Author details
Esther U. Alum[1,3]
E-mail: esther.alum@kiu.ac.ug
ORCID ID: http://orcid.org/0000-0003-4105-8615
Okechukwu P. C. Ugwu[1]
Patrick M. Aja[3,4]
Emmanuel I. Obeagu[1,2]
Joseph E. Inya[5]
Amarachi P. Onyeije[3]
Esther Agu[3]
Chinaza Godswill Awuchi[6]
ORCID ID: http://orcid.org/0000-0001-5071-8895
[1] Department of Publications and Extension, Kampala International University, Kampala, Uganda.
[2] Department of Hematology, Kampala International University, Kampala, Uganda.
[3] Department of Biochemistry, Faculty of Science, Ebonyi State University, Abakaliki, Nigeria.
[4] Department of Biochemistry, Faculty of Biomedical Sciences, Kampala International University, Kampala, Uganda.
[5] Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[6] School of Natural and Applied Sciences, Kampala International University, Kampala, Uganda.

Disclosure statement
No potential conflict of interest was reported by the author(s).

Authors contributions
Study conception and Design: Esther U. Alum, Patrick M. Aja, and Joseph E. Inya; Data collection: Amarachi P. Onyeije, Esther Agu, and Chinaza G. Awuchi; Analysis of Data: Okechukwu P. C. Ugwu and Emmanuel I. Obeagu; Interpretation of results: Okechukwu P. C. Ugwu, Emmanuel I. Obeagu, and Chinaza G. Awuchi; Draft manuscript preparation: Joseph E. Inya and Esther U. Alum; Editing and revision of the manuscript: Esther U. Alum, Patrick M. Aja, and Chinaza G. Awuchi. All authors reviewed the results and approved the final version of the manuscript

Data availability statement
Additional data will be made available on request.

Consent for publication
All the authors consent to the publication.

Ethical approval
The study was approved by the Ethical Committee of the Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria (EBSU/BCH/ET/21/006).

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