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Gastroprotective Effects of Freeze-Dried Extracts of *Erythrina Aabyssinica* Bark on Methotrexate-Induced Intestinal Damage in Rat Models

Author: Roy Olunga[1]

Co Authors: Hellen N. Kariuki[2], Teresa K. Mwendwa[3], Charles G. Githinji[4*], and Boniface M. Chege[5]

[1-4]University of Nairobi, Department of Medical Physiology, Faculty of Health Sciences, P.O Box 30197-00100, Nairobi, Kenya
[5]School of Health Sciences, Dedan Kimathi University of Technology, Private Bag-10143 Dedan Kimathi, Nyeri, Kenya

ABSTRACT

Methotrexate (MTX) medicine is used for chemotherapy and treatment of inflammatory diseases such as Rheumatoid arthritis and Psoriasis. High-dose methotrexate can cause systemic toxicity, including damage to the kidneys, liver, bone marrow, and digestive system. To manage the toxicity effects of MTX, Leucovorin (LV) and glucarpidase medications are normally used. However, these treatments also have adverse effects and are not easily accessible to individuals in low-income countries. Therefore, alternative low-cost treatments with limited or no adverse side effects for use to manage MTX adverse side effects are critical. This study aimed to investigate the protective effects of *Erythrina abyssinica* on methotrexate-induced intestinal damage rat models. 25 Sprague Dawley rats (250 – 300 g) were randomly divided into 5 groups of 5 rats each i.e., Normal control (Normal saline), positive control (5mg/kg MTX + 10mg/kg LV), negative control (5mg/kg MTX), low dose treatment group (5mg/kg + 100mg/kg *Erythrina abyssinica)* and high dose treatment group (5mg/kg + 200mg/kg *Erythrina abyssinica*). The therapeutic effects of *Erythrina abyssinica* were assessed by determining levels of glutathione (GSH), levels of malondialdehyde in intestinal tissue, catalase activity, superoxide dismutase activity in intestinal tissue and the Nitrocellulose redox permanganometry (NRP). The frequency and mass of stool were also assessed and the intestinal permeability was determined using the Evans blue staining. The data were reported as the mean ± SEM. The study showed an increase in cellular antioxidant activity in the treatment groups by increased catalase activity, superoxide dismutase (SOD) activity, and levels of reduced glutathione (GSH). The intestinal permeability was also reduced. In conclusion, the study indicate that *Erythrina abyssinica* possess protective effects to the intestines.

Key Words *Erythrina abyssinica, Gastroprotective, Methotrexate*

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RESEARCH ARTICLE

INTRODUCTION

Methotrexate (MTX) is an antimetabolite commonly used to treat cancer and inflammatory diseases such as Rheumatoid arthritis and Psoriasis[7]. Its action involves inhibiting aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase, dihydrofolate reductase, and NF-kβ[8]. Hence, it plays an essential role in managing malignancy and inflammatory diseases. High doses of methotrexate are toxic to the body, requiring the drug to be withdrawn or the dosage to be reduced, despite its extensive and beneficial use in treating inflammatory diseases and cancer[6]. High-dose methotrexate can cause bone marrow suppression, liver fibrosis, hepatitis, nephrotoxicity, pulmonary toxicity, gastrointestinal toxicity, and dermatological toxicity. Methotrexate treatment is often withdrawn due to gastrointestinal toxicity[16]. This is due to the potential for widespread intestinal mucositis caused by methotrexate, which predominantly affects the small intestine but also the digestive tract, leading to symptoms like vomiting, stomach cramps, diarrhea, and nausea[16]. Methotrexate overdose can cause tissue toxicity due to increased reactive oxygen species (ROS) and decreased antioxidants like glutathione (GSH)[26]. ROS can increase intestinal permeability and damage tight epithelial junctions[24]. Studies have shown that antioxidants can help alleviate methotrexate-induced intestinal damage. Resveratrol has been shown to help alleviate methotrexate toxicities due to its antioxidant and anti-inflammatory activities[43]. Methotrexate is a common drug used in cancer treatment and as an immunosuppressant in auto-immune diseases[17]. Systemic toxicity has been associated with its use, such as bone marrow suppression and gastrointestinal ulcers. Additionally, commonly used drugs like sulfonamides, aminoglycosides, and penicillin affect its renal elimination hence affecting its pharmacokinetics[16]. Folinic acid (Leucovorin) is commonly used to manage the toxicities of Methotrexate (MTX). However, its administration can result in various adverse effects such as gastrointestinal upset (nausea, vomiting, diarrhea), leukopenia, pruritus, skin rash, and urticarial[9]. Folinic administration is also associated with hypocalcemia with decreased levels of vitamin D in patients[14]. Also, Leucovorin is not affordable for everyone. Thus, it is necessary to find a more cost-effective alternative with few or no side effects.

Herbal remedies are ideal options since they are known to have limited side effects. Erythrina abyssinica's stem barks are most commonly used to prepare herbal remedies[28]. It treats various diseases like fungal infections, malaria, leprosy, inflammatory diseases, obesity, diabetes, and anemia[28]. Its bark treats ailments like ulcers, snake bites, and swellings. The stem barks of the plant are rich in phytochemicals such as Tannins, saponins, alkaloids, cardiac glycosides, and flavonoids. These phytochemicals give the plant its healing properties. Flavonoids possess good

RESEARCH ARTICLE

anti-inflammatory and anti-oxidant spectra, hence their use in managing inflammatory diseases like ulcers[28].

The aim of this study was to investigate the therapeutic effects of ***Erythrina abyssinica*** on methotrexate – induced intestinal damage in a rat model.

MATERIALS AND METHODOLOGY

Ethical approval for the research project was obtained from the Biosafety, Animal Use, Care and Ethics Committee of the Faculty of Veterinary Medicine, University of Nairobi. The study adhered to the ethical standards for animal research outlined in the 8th edition of the National Research Council Guide for the Care and Use of Laboratory Animals, published by the National Academies Press (updated in 2010).

ANIMALS

25 Sprague-Dawley rats weighing 200-300 g were obtained from Kabete Animal House and housed in the animal house in the Department of Human Anatomy and Medical Physiology, University of Nairobi under a 12-hour light-dark cycle and a standard room temperature**.** The animals were fed standard chow and given water *ad libitum*. The animals were acclimatized for 2 weeks before starting the experiments.

EXPERIMENTAL DESIGN

This study was a randomized experimental study. The rats were randomly assigned into five groups (n=5) as shown in figure 1.

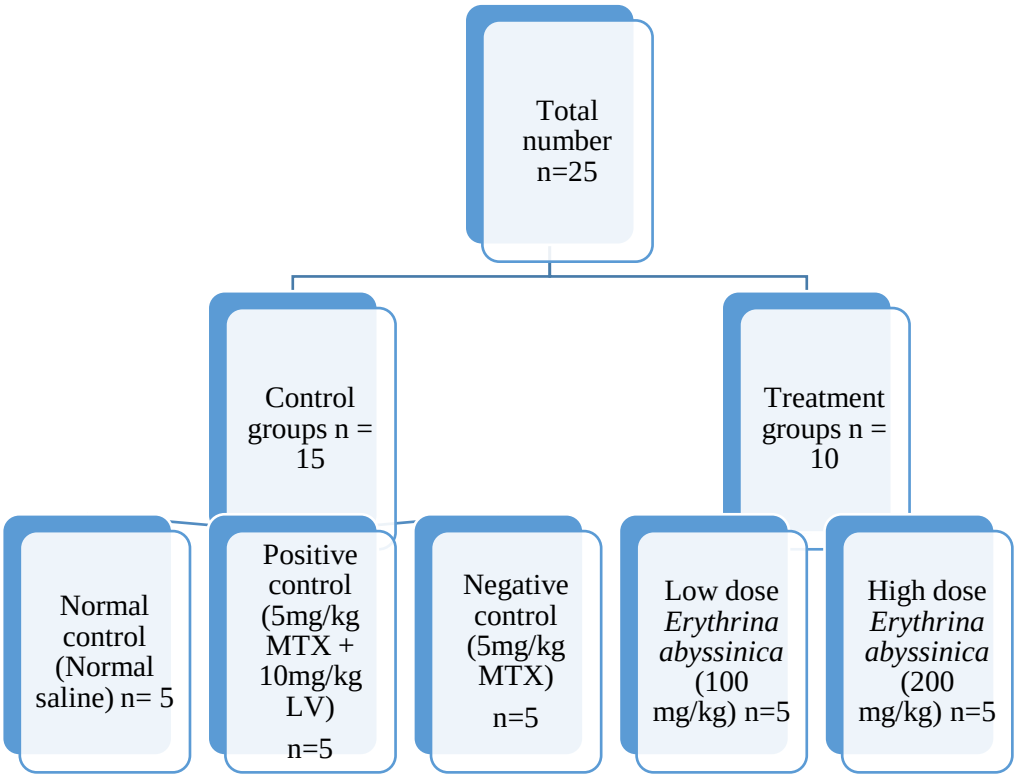

Figure 1 Illustration of study groups

RESEARCH ARTICLE

PLANT PREPARATION

Erythrina abyssinica bark was harvested in Machakos, Kenya, and identified at the University of Nairobi Herbarium. The bark was dried, and ground into powder. The powder was mixed with water in a ratio of 1:2. The solvent obtained after decanting was freeze-dried. The freeze-dried extracts were diluted to different concentrations for rat administration through intra-gastric gavage.

DRUG PREPARATION & ADMINISTRATION

Methotrexate

Methotrexate was administered intraperitoneally once daily for 7 consecutive days. The tablets were dissolved in normal saline (0.9% NaCl). A dose of 5 mg/kg was prepared based on previous studies (Li et al., 2016).

Leucovorin

Leucovorin tablets were dissolved in 0.9% NaCl and were administered orally for 7 days at a predetermined dose of 10mg/kg via gavage. (X et al., 2020)

PARAMETERS ASSESSED

BODY WEIGHT MEASUREMENT

The weights of the rats were measured using a weighing scale from the start to the end of the experiment.

MEASURING CATALASE ACTIVITY

The following were added in a test tube; 1.9 ml of 50 mM phosphate buffer, 0.1 ml of sample, and 1 ml of 30 mM hydrogen peroxide solution. The initial and final absorbance was recorded in one minute at 240nm. The catalase activity was calculated as follows:

$$Specific\ activity\ (units\ mg\ of protein/min) \frac{\Delta A\ X\ 688.073}{mg\ protein}$$

Where A= Absorbance

MEASUREMENT OF GLUTATHIONE LEVELS

Intestinal tissue was homogenized in 0.3M ice-cold phosphate buffer (pH 7.0). EDTA (0.9 ml of 0.1%) and 1.5 ml of 20% TCA were then added to 0.1 ml of the sample and mixed well. The mixture was left to stand for 5 minutes; it was then centrifuged at 3000rpm for 5 minutes. 1.8ml of DTNB was added to 0.2ml of supernatant and the solution was mixed well. The absorbance(A) of the solution was read at 412nm, 3 minutes after the addition of Dithio-nitrobenzoic acid (DTNB).

Calculation:

GSH concentration = (A × 18.38)/ C

- ✓ **C**: ml for plasma used or mg protein for tissue used in homogenate.
- ✓ 1.36×10^4 M−1 cm−1: **extinction coefficient**
- ✓ GSH concentration was expressed as mmoles /ml for plasma and as mmoles /mg protein for tissue.

MEASUREMENT OF SUPEROXIDE DISMUTASE

3.0ml of assay mixture was prepared as follows;

1ml of 50mM Tris-HCl buffer, 1.5 ml of 1mM diethylene triamine penta-acetic acid (DTPA),

RESEARCH ARTICLE

and 0.5ml of tissue homogenate were added in a test tube. The reaction was initiated by the addition of 0.5ml of 10 mmol pyrogallol solution in 10 mmol HCl. The absorbance was read at 420nm (a lag period of about 1 minute was given to allow the steady state of auto-oxidation of pyrogallol to be obtained). One unit of SOD was described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation per 3ml of assay mixture. Results were expressed as units per mg of tissue**.**

MEASUREMENT OF MALONDIALDEHYDE

The levels of MDA were determined using the method described by Ohkawa. Intestinal tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.0). 2 ml of 10% Trichloroacetic acid was added to the 1 ml sample. The mixture was then centrifuged at 5000rpm for 5 minutes. 0.5ml of 1% Thiobarbituric acid was added to 2ml of the supernatant and the mixture was heated at 95º on a water bath for 30 minutes. The solution was then cooled under running tap water. The absorbance of the resultant pink extract was measured at 532nm wavelength using a spectrophotometer. The levels of MDA were then calculated and expressed as nanomoles per gram of intestinal tissue. MDA levels were calculated as follows:

MDA concentration (C):

C = {Absorbance/ 0.156 × ml, plasma)} nmoles/ml

MEASUREMENT OF NITROSATIVE STRESS MARKER

The levels of nitric oxide were determined using the method described by Montgomery. The Griess reaction for color development was used:

The Griess reagent was prepared by reacting 0.1g N-(1-Naphthy) Ethyl—Ethylenediamine, 1g sulfanilamide, 90 ml deionized water, and 5 g phosphoric acid (in 100 ml of water) in a beaker. The solution was then stored at 4ºC. The sodium nitrite stock solution for the standard was prepared by dissolving 0.0207 g sodium nitrite in 1000 ml distilled water in a flask at room temperature. The standard curve was prepared using standard dilution and different concentrations of Sodium nitrite.

For the test samples:

The following were mixed: 100µl of Griess reagent, 300 µl of the sample, and 2.6 ml of deionized water were added to a dry test tube. The mixture was then incubated at room temperature for 30 minutes and the absorbance of the nitrite-containing sample measured at 548 nm relative to the blank.

DETERMINATION OF INTESTINAL PERMEABILITY

Evans blue stain injection, organ collection, and quantification were done to determine intestinal permeability as follows;

A 0.5% sterile solution of Evans blue stain was prepared. The solution was filtered to remove any undissolved particles. 200 µl Evans blue solution was aspirated into a syringe. The rats were placed inside a rat restrainer to immobilize them. The

RESEARCH ARTICLE

tails of the rats were dipped in warm water. The needle (small gauge, 27-30) was inserted at a 10-15-degree angle, level up, and advanced into the lateral tail vein towards the direction of the head. The needle and syringe were kept parallel to the tail. 200 µl Evans Blue solution was slowly injected into the tail veins of the rats. The rats were put back in their cages for 30 minutes.

The rats were then sacrificed through cervical dislocation to prevent interference with the vascular permeability. The rats were pinned on a board, and the thorax and abdomen cavities were incised to expose the abdominal and thoracic contents. Representative pictures of the intestines were taken to show the difference in Evans blue extravasation. The harvested intestines were weighed. 500 µl formamide was then added to each tissue sample tube. The mixture was then transferred to a water bath maintained at 55º.

The formamide/Evans Blue mixture was centrifuged at 10,000 rpm for 20 minutes to pellet any remaining tissue fragments. Absorbance was measured at 610 nm. Formamide was used as the blank. The formula: Mass (g) = concentration (M) × Volume (L) × Molecular Weight (g/mol) was used to calculate the mass of Evans blue dye for each sample. The mass was then converted to ng/g of the tissue. **(The molar absorption coefficient of Evans Blue dye is 78,100 M-1cm-1 at 626 nm).** The ng Evans Blue extravasated per g tissue was calculated from the absorbance and a graph was plotted. Statistical analysis was performed for significant differences.

NITROCELLULOSE REDOX PERMANGANOMETRY FOR REDUCTIVE CAPACITY

The method described by Homolak was used for the Nitrocellulose Redox Permanganometry:

$KMnO_4$ working solution was prepared by dissolving 0.2 g of solid $KMnO_4$ crystals in 20 mL of ddH_2O. The solution was then stirred until all crystals dissolved. 1 µL of the vortexed sample was pipetted onto the nitrocellulose membrane. This step was repeated for every sample to be analyzed. Enough space was left between the samples. The nitrocellulose membrane was left to dry out, after which it was placed in the $KMnO_4$ working solution for 30s. The nitrocellulose membrane was then removed from the $KMnO_4$ working solution and placed under running ddH_2O to terminate the reaction and increase the contrast. The membrane was again left to dry out after which it was digitalized with a camera. The images were imported into Fiji (Fiji Is Just ImageJ) software. The Fiji results were used for statistical analysis.

HISTOLOGICAL EXAMINATION

A sample of intestinal tissue from all groups was fixed in 10% buffered formalin for 24 hours. The tissues were then processed and embedded in paraffin blocks five micrometers thick. They were cut and stained using Hematoxylin and Eosin. The slides were examined microscopically for histopathological changes. Fiji software was used to determine the intestinal villi heights and crypt depths. The intestinal damage was scored as follows (0 = none, 1= mild damage, 2 =

RESEARCH ARTICLE

moderate damage, 3= severe damage. Goblet cell depletion was scored as follows: 0 = 150 – 200, 1 = 100 – 149, 2 = 50-99, 3 = 0 - 49

STATISTICAL ANALYSIS

The data was expressed as the Mean +/- SEM. The one-way ANOVA test was used to determine the groups' statistically significant differences. Post hoc statistical analysis was done using Tukey's multiple comparisons. The significance level was set at $p<0.05$.

RESULTS

BASELINE CHARACTERISTICS

There were no statistical differences in body weight between the five experimental groups on day 1 of the experimental studies: [129.8 ± 6.127 (Normal control) vs 128.6 ± 4.389 (Positive control) vs 136.8 ± 4.964 (Negative control) vs 127.0 ± 4.266 (Low dose group) vs 128.6 ± 5.938 (High dose group)].

EFFECT ON BODY MASS

There were statistical differences in the body weight (g) between the experimental groups on the seventh day of the experimental studies: [160.8 ± 3.338 (Normal control) vs 155.6 ± 2.482 (Positive control) vs 104.2 ± 3.426 (Negative control) vs 120.0 ± 4.626 (Low dose group) vs 134.6 ± 7.019 (High dose group)]. Post-hoc analysis using Tukey's multiple comparison tests showed significant differences between the normal control and positive control ($p <0.0001$), the normal control and the low dose group ($p <0.0001$), the normal control and the high dose group ($p = 0.0040$), the positive control and the negative control ($p <0.0001$), the positive control vs low dose group ($p = 0.0001$), the positive control vs high dose group ($p = 0.0249$), the negative control vs the high dose group ($p = 0.0009$). ($F (4, 20) = 28.38$: $P<0.0001$).

EFFECTS OF *Erythrina Abyssinica* ON INTESTINAL PERMEABILITY

EVANS BLUE DYE CONTENT IN INTESTINAL TISSUE

There were statistically significant differences in the intestinal tissue content of Evans blue dye on day 7 [3.028 ± 0.2483 (Normal group) vs 3.912 ± 0.4846 (Positive control group) vs 9.742 ± 0.9652 (Negative control) vs 6.044 ± 0.1873 (low dose ***Erythrina Abyssinica*** group) vs 4.368 ± 0.3029 (high dose ***Erythrina Abyssinica*** group)]. Post-hoc analysis using Tukey's multiple comparison tests showed that there were significant differences between the normal control and negative control group ($p < 0.0001$), the normal and the low dose group ($p = 0.0045$), the positive control group and the negative control group ($p < 0.001$), the negative control group and the low dose group ($p = 0.0006$) and the negative control group vs the high dose group ($p < 0.0001$).

EFFECTS OF *Erythrina Abyssinica* ON INTESTINAL TISSUE MALONDIALDEHYDE CONTENT

There were statistically significant differences in the content of Malondialdehyde in the intestinal tissue on day 7 [4.387 ± 0.2617 (Normal control vs 5.168 ± 0.5529 (Positive control) vs 14.00 ±

RESEARCH ARTICLE

1.375(Negative control) vs 10.36 ± 0.6921 (Low dose group) vs 7.224 ± 0.5925 (High dose group)].

Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative control (p <0.0001), the normal group and the low dose group (p = 0.0003), the positive control and the negative control (p <0.0001), the negative control and the low dose group (p = 0.0013), the negative control and the low dose group (p = 0.0280), the negative control and the high dose group (p <0.0001)

EFFECTS OF *Erythrina Abyssinica* ON INTESTINAL TISSUE CATALASE ACTIVITY

There were statistically significant differences in the activity of catalase in the intestinal tissue on day 7: [5.337 ± 0.1221 (Normal control) vs 5.014 ± 0.08361 (Positive control) vs 1.388 ± 0.1508 (Negative control) vs 2.155 ± 0.1578 (Low dose group) vs 4.192 ± 0.1606 (High dose group)]. Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative control (p <0.0001), the normal control and low dose group (p <0.0001), the normal control and high dose group (p <0.0001), the positive control and negative control (p <0.0001), the positive control and the low dose group (p <0.0001), the positive control and high dose group (p = 0.0035), the negative control and low dose group (p = 0.0067), negative control and high dose group (p <0.0001) and between the low dose group and high dose group (p <0.0001). (F (4, 20) = 161.8: P<0.0001).

EFFECTS OF *Erythrina Abyssinica* ON THE LEVELS OF REDUCED GLUTATHIONE IN THE INTESTINAL TISSUE

There were statistically significant differences in the levels of reduced glutathione in the intestinal tissue on day 7: [91.64 ± 1.977 (Normal control) vs 82.60 ± 3.603 (Positive control) vs 37.72 ± 2.571 (Negative control) vs 46.35 ± 2.607 (Low dose group) vs 71.68 ± 2.122 (High dose group)]. (F (4, 20) = 77.28: P<0.0001). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative control (p <0.0001), the normal control and low dose group (p <0.0001), the normal control and high dose group (p = 0.0003), the positive control and the negative control (p <0.0001), the positive control and the low dose group (p <0.0001), the negative control and the high dose group (p <0.0001) and between low dose group and high dose group (p <0.0001).

EFFECTS OF *Erythrina Abyssinica* ON THE LEVELS OF NITRIC OXIDE IN THE INTESTINAL TISSUE

There were statistically significant differences in the levels of nitric oxide in the intestinal tissue on day 7: [85.88 ± 6.536 (Normal control) vs 91.63 ± 5.761 (Positive control) vs 200.1 ± 18.51 (Negative control) vs 114.1 ± 6.885 (Low dose group) vs 100.6 ± 5.728 (High dose group). (F (4, 20) = 22.03. P<0.0001). Post-hoc analysis using Tukey's multiple comparisons showed tests that

RESEARCH ARTICLE

there were significant differences between the normal control and the negative control (p <0.0001), the positive control and the negative control (p <0.0001), the low dose group vs the negative control (p <0.0001), and the high dose group vs the negative control (p <0.0001).

NITROCELLULOSE REDOX PERMANGANOMETRY FOR REDUCTIVE CAPACITY

There were statistically significant differences in the Nitrocellulose Redox Permanganometry for reductive capacity on day 7: [22092 ± 1664 (Normal control) vs 19408 ± 1844 (Positive control) vs 9369 ± 656.1 (Negative control) vs 14524 ± 1905 (Low dose group) vs 17945 ± 1811 (High dose group). (F (4, 20) = 8.924: P=0.0003). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative control (p = 0.0002), between normal control and low dose group (p = 0.0288), between positive control and negative control (p = 0.0027) and between high dose group and negative control (p = 0.0113).

EFFECTS OF *Erythrina Abyssinica* ON SUPEROXIDE DISMUTASE ACTIVITY IN INTESTINAL TISSUE

There were statistical differences in the levels of superoxide dismutase activity among the 5 groups on day 7 of the experiment: [5.947 ± 0.1062 (Normal control) vs 5.865 ± 0.0856 (Positive control) vs 1.786 ± 0.1754 (Negative control) vs 3.153 ± 0.3621 (Low dose group) vs 5.320 ± 0.3124 (High dose group). (F (4, 20) = 61.90: P<0.0001). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative control (<0.0001), between normal control and low dose group (<0.0001), between positive control and negative control (<0.0001), between positive control and negative control (<0.0001), between negative control and low dose group (p = 0.0045), between high dose group and negative control (<0.0001) and between high dose group and low dose group (<0.0001).

EFFECT ON THE MASS OF WET UNFORMED STOOL

There were statistical differences in the masses of unformed wet stools among the five groups on day 7 of the experimental study: [0.000 ± 0.000 (Normal saline group) vs 0.2420 ± 0.2420 (MTX + LV group) vs 3.448 ± 0.1790 (MTX group) vs 2.278 ± 0.3243 (Low dose group) vs 0.5200 ± 0.2452 (High dose group)]. (F (4, 20) = 43.90: P<0.0001). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal saline group and the MTX group (p <0.0001), the Normal saline group and low dose group (p <0.0001), the positive control and MTX group (p <0.0001), the positive control and low dose group (p < 0.0001), the MTX group and low dose group (p = 0.0121), the MTX group and the high dose group (p <0.0001) and between the low dose group and high dose group (p = 0.0002).

EFFECT ON FREQUENCY OF DIARRHEA

RESEARCH ARTICLE

There were statistically significant differences in the frequency of diarrhea among the 5 groups on day 7 of the experimental studies: [0.000 ± 0.000 (Normal saline group) vs 0.4000 ± 0.4000 (Positive control group) vs 1.800 ± 0.3742 (MTX group) vs 1.400 ± 0.2449 (low dose group) vs 0.6000 ± 0.2449 (high dose group)]. (F (4, 20) = 6.524: P=0.0016). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal saline group and the MTX group (p = 0.0023), Normal saline and low dose group (p = 0.0205), and between the positive control and methotrexate group (p = 0.0205).

EFFECT ON TOTAL WATER CONTENT

There were statistically significant differences in the total water content of the stool on day 7 of the study: [0.000 ± 0.000 (Normal saline group) vs 0.1180 ± 0.1180 (positive control group) vs 2.086 ± 0.2132 (MTX group) vs 0.9160 ± 0.1666 (Low dose group) vs 0.2240 ± 0.2240 (High dose group)]. (F (4, 20) = 39.31: P<0.0001). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal saline group and the MTX group (p <0.0001), Normal saline group and low dose group (p = 0.0012), positive control and methotrexate group (p = <0.0001), positive control and low dose group (p = 0.0048), MTX group and low dose group (p = <0.0001), MTX group and high dose group (p = <0.0001) and between the high dose group and the low dose group (p = 0.0159).

HISTOPATHOLOGICAL ANALYSIS

EFFECT ON INTESTINAL TISSUE HISTOLOGY

Histopathological images of intestinal injury in the 5 groups on the 7th day (Shown in Appendix 1; Figure A: Normal control, Figure B: Positive control group, Figure C: Negative control group, Figure D: Low dose group, and Figure E: High dose group). The green arrow shows the villus. Villus atrophy and degeneration (Shown by the thin black arrow) and distorted muscularis mucosae and muscular layer (Thick arrow) are evident in the negative control. Moderate villus atrophy and muscular layer distorted (light blue star) evident in the low dose group. The normal control, positive control and high dose group show normal histology (shown in appendix 1).

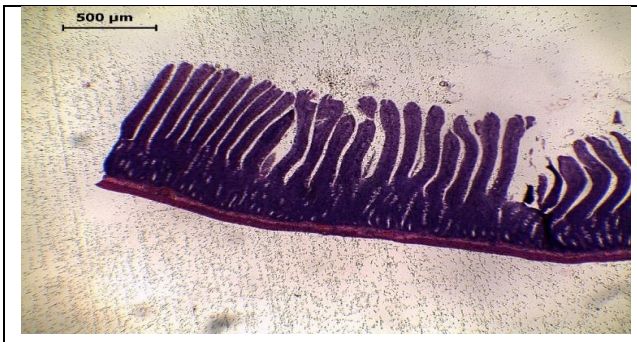

Figure A

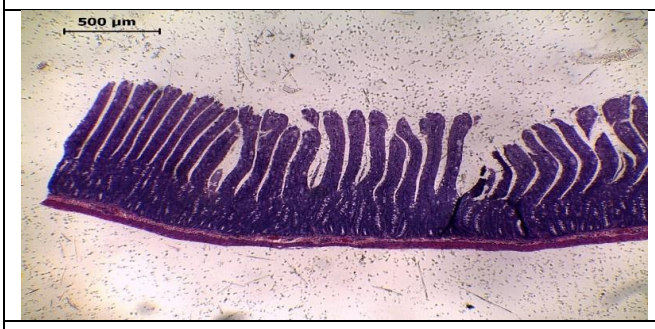

Figure B

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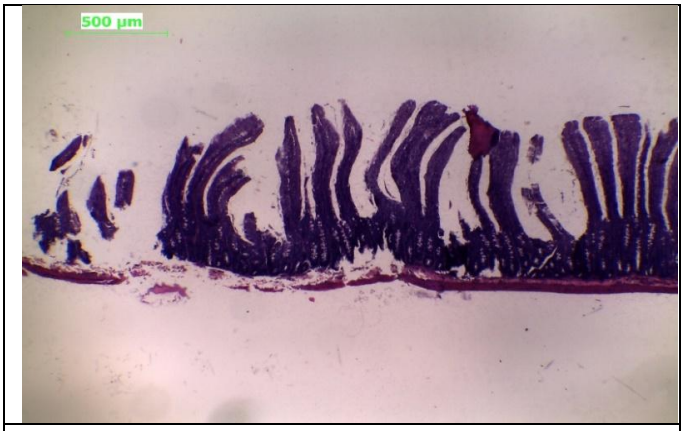

Figure C

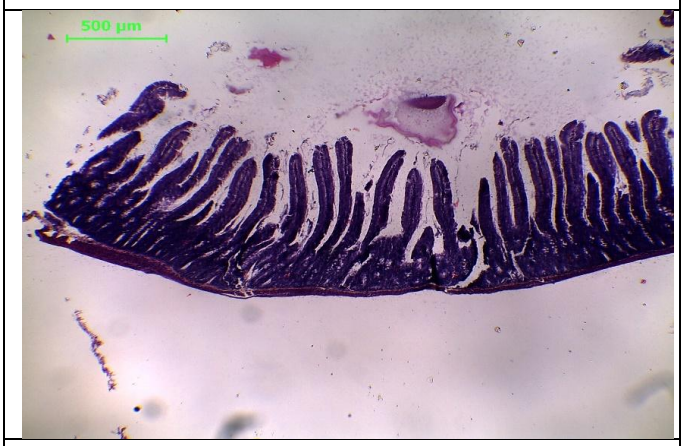

Figure D

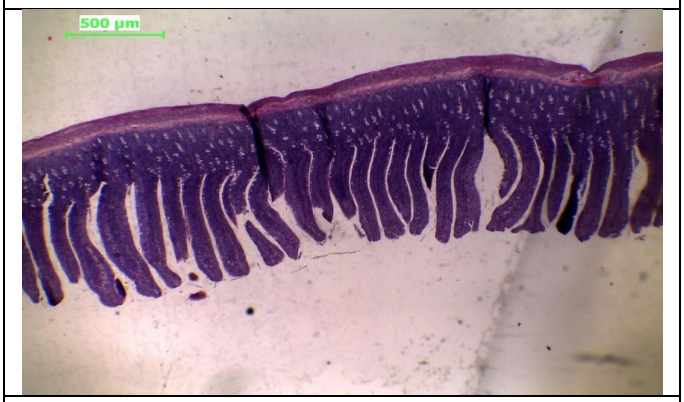

Figure E

EFFECT ON VILLI HEIGHT

There were statistically significant differences in the intestinal villi height among the groups on day 7 of the experimental study: [575.1 ± 17.74 (Normal control) vs 533.8 ± 20.88 (Positive control) vs 309.8 ± 59.12 (Negative control) vs 386.6 ± 39.96 (Low dose group) vs 463.3 ± 52.91 (High dose group). (F (4, 25) = 6.722: P=0.0008). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative group (p = 0.0011), normal control and low dose group (p = 0.0275), and between positive control and negative control (p = 0.0066).

EFFECTS ON INTESTINAL CRYPT DEPTH

There were statistically significant differences in intestinal crypt depths on day 7 of the experimental study: [365.1 ± 8.430 (Normal control) vs 315.8 ± 17.38 (Positive control) vs 146.4 ± 20.55 (Negative control) vs 191.7 ± 37.75 (Low dose group) vs 307.9 ± 30.49 (High dose group). (F (4, 25) = 13.45: P<0.0001). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative group (p <0.0001), normal control low dose group (p = 0.0004), positive control and negative control (p = 0.0006), positive control and low dose group (p = 0.0140), negative control and high dose group (p = 0.0010) and between low dose group and high dose group (p = 0.0237).

EFFECT ON INTESTINAL DAMAGE SCORES

There were statistically significant differences in the crypt damage score between the 5 groups on day 7 of the experimental study: [0.000 ± 0.000 (Normal control) vs 0.2000 ± 0.2000 (Positive control) vs 2.400 ± 0.4000 (Negative control) vs

RESEARCH ARTICLE

1.200 ± 0.2000 (Low dose group) vs 0.6000 ± 0.4000 (High dose group)]. (F (4, 20) = 11.65: P<0.0001). Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative group (p <0.0001), normal control and low dose group (p = 0.0492), positive control and negative control (p = 0.0002), negative control and low dose group (p = 0.0492), negative control and high dose group (p = 0.0018). There were statistically significant differences in the villus damage score between the 5 groups on day 7 of the experimental study: [0.000 ± 0.000 (Normal control) vs 0.4000 ± 0.2449 (Positive control) vs 2.800 ± 0.2000 (Negative control) vs 1.400 ± 0.2449 (Low dose group) vs 0.8000 ± 0.3742 (High dose). (F (4, 20) = 19.87: P<0.0001). Post-hoc analysis using Tukey's multiple comparison showed significant differences between the normal control and the negative control (p <0.0001), normal control and low dose group (p = 0.0051), positive control and negative control (p <0.0001), negative control and low dose group (p = 0.0051), negative control and high dose group (p = 0.0001).

There were statistically significant differences in goblet cell depletion score between the 5 groups on day 7 of the experimental: [0.2000 ± 0.2000 (Normal control) vs 0.6000 ± 0.2449 (Positive control) vs 2.400 ± 0.4000 (Negative control) vs 1.600 ± 0.4000 (Low dose group) vs 1.200 ± 0.4899 (High dose group). (F (4, 20) = 5.606: P=0.0034). Post-hoc analysis using Tukey's multiple comparisons showed significant differences between the normal and negative control (p = 0.0030) and positive and negative control (p = 0.0169).

EFFECTS ON TOTAL INTESTINAL DAMAGE SCORES

There were statistically significant differences in the total intestinal damage score among the 5 groups on day 7 of the experimental study: [0.2000 ± 0.2000 (Normal control) vs 1.200 ± 0.3742 (Positive control) vs 7.600 ± 0.6000 (Negative control) vs 4.400 ± 0.5099 (Low dose group) vs 2.600 ± 1.166 (High dose group)]. (F (4, 20) = 19.77: P<0.0001).

Post-hoc analysis using Tukey's multiple comparisons showed tests that there were significant differences between the normal control and the negative group (p <0.0001), normal control and low dose group (p = 0.0017), positive control vs negative control (p <0.0001), positive control vs low dose group (p = 0.0193), negative control and low dose group (p = 0.0193). negative control and high dose group (p = 0.0003).

DISCUSSION

This study offers insights into the beneficial effects of *Erythrina abyssinica* in managing intestinal damage associated with methotrexate administration. Methotrexate has multiple side effects, with gastrointestinal toxicity being the significant dose-limiting factor. Previous studies show that methotrexate causes intestinal damage by causing inflammation and oxidation and

RESEARCH ARTICLE

slowing DNA replication[17]. Leucovorin (folinic acid) is the first-line drug in managing gastrointestinal damage caused by methotrexate. However, it is relatively expensive and has various adverse drug effects, such as allergic reactions[18]. Getting a more affordable option with fewer side effects is needed. Herbal medicine offers a cheap solution with few to no side effects when managing various diseases. *Erythrina abyssinica* bark is commonly used to prepare herbal remedies[28]. It's used for treatment of various diseases like fungal infections, malaria, leprosy, inflammatory diseases, obesity, diabetes, and anemia[28]. The bark is also used to treat ailments like ulcers, snake bites, and swellings. The barks of the plant are rich in phytochemicals such as Tannins, saponins, alkaloids, cardiac glycosides, and flavonoids[28]. These phytochemicals give the plant its healing properties. Flavonoids possess good anti-inflammatory and antioxidant spectra, hence their use in managing inflammatory diseases like ulcers[28]. In this study, we investigated the protective effects of *Erythrina abyssinica* on methotrexate-induced intestinal damage. We investigated the protective effects of *Erythrina abyssinica* on intestinal parameters in a rat model.

Our findings on the tissue SOD and GSH are consistent with results from other studies investigating other herbs with similar antioxidant profiles as *Erythrina abyssinica*. Oxidative and inflammatory damage is a major cause of intestinal damage in methotrexate treatment. Reduced levels of catalase enzyme activity, superoxide dismutase, and increased MDA levels are consistent with methotrexate-induced intestinal damage. *Nigella sativa, a* herbal medicine with a similar antioxidant profile as *Erythrina abyssinica,* was shown to improve various intestinal parameters in a methotrexate rat model[11]. The activity of catalase and superoxide dismutase enzyme was significantly higher in rats treated with *Nigella sativa* than in those treated with methotrexate[11]. Similarly, in our study rats treated with *Erythrina abyssinica* had significantly higher levels of catalase and superoxide dismutase enzyme activity compared to those treated with methotrexate (See Figure 5).

SUPEROXIDE DISMUTASE ACTIVITY

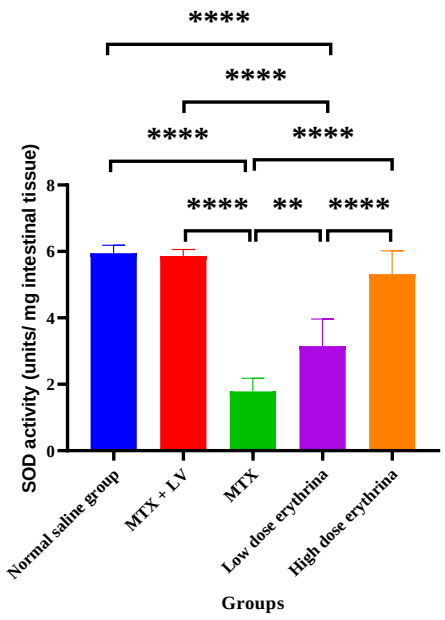

Figure 5 Mean superoxide dismutase activity **(Results are expressed as mean ± SEM. (** p < 0.01, ****p< 0.0001)**

Erythrina abyssinica protects the intestines by maintaining a high level of antioxidants (catalase

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and superoxide dismutase). Rats treated with *Erythrina abyssinica* showed increasing levels of antioxidants in a dose-dependent manner. Superoxide dismutase catalyzes the conversion of superoxide ions to hydrogen peroxide, which is less toxic and less injurious to the intestinal tissue. Additionally, the superoxide dismutase enzyme has been shown to inhibit the inactivation of NO, thus preventing peroxynitrite formation and mitochondrial dysfunction[13].

The study showed that *Erythrina abyssinica* reduced intestinal damage caused by peroxynitrite (See Figure 4).

TISSUE NITRITE CONTENT

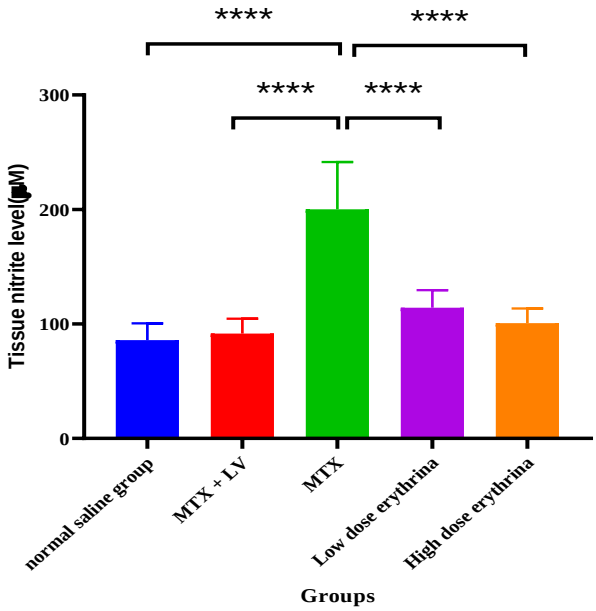

Figure 4 Mean intestinal tissue nitrite levels **(Results are expressed as mean ± SEM. (****p< 0.0001).**

The results are consistent with findings from other studies demonstrating the role of reduced tissue nitric oxide in protecting the intestines from MTX injury. Even though nitric oxide plays a regulatory role in the gut mucosal barrier, studies have shown that excessive NO production can lead to the production of peroxynitrite, which has been implicated in intestinal damage[21]. Peroxynitrite has been shown in studies to trigger apoptosis in intestinal epithelial cells. It does this by oxidizing the mitochondrial permeability pores, which initiates a process leading to cytochrome C-dependent apoptosis[32]. Then, cytochrome c activates caspase 3, an essential step in the cell death pathway. Additionally, NF-kappa B is activated over time, further regulating pro-inflammatory cytokines[32].

In this study, *Erythrina abyssinica* reduced lipid peroxidation, as shown by the decreased levels of MDA in the intestinal tissue content in both the low-dose and high-dose treatment groups (See Figure 3).

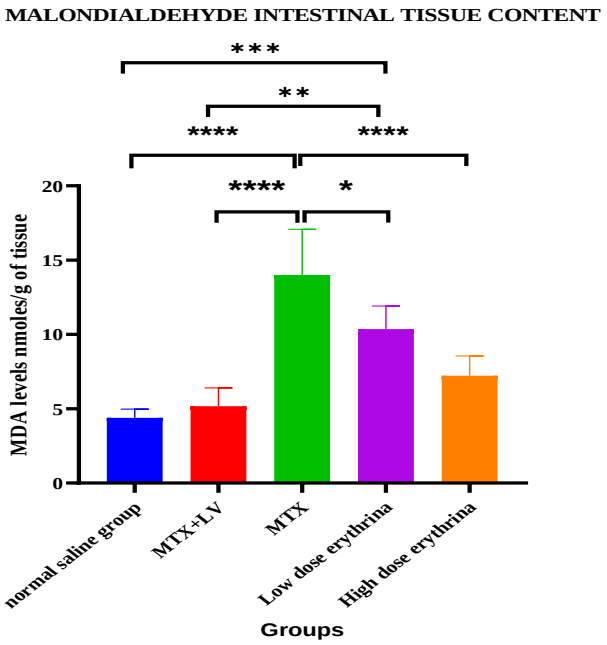

Figure 3 Mean MDA intestinal tissue content (Results are expressed as mean ± SEM. (* p < 0.05, ** p < 0.01, ***p<0.00.1, ****p< 0.0001).

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Previous studies have indicated that methotrexate-induced damage to the intestines is linked to increased levels of MDA resulting from elevated lipid peroxidation[4]. Methotrexate promotes lipid peroxidation by generating ROS. Reactive oxygen species generation can lead to the decomposition of arachidonic acid and PUFAs, thus increasing MDA levels[4]. Studies on Guarana, a natural antioxidant, showed reduced levels of MDA in a methotrexate-induced intestinal damage rat model[1], showing that natural antioxidants can reduce the rate of tissue peroxidation, hence low levels of MDA.

Methotrexate has been shown to damage the mucosal barrier and increase intestinal permeability, which allows bacteria to seep into the intestinal lumen, worsening inflammation and intestinal barrier damage. Additionally, methotrexate interferes with DNA replication, leading to breaks in DNA strands in rapidly dividing intestinal cells which compromises the intestinal barrier and further increases intestinal permeability[2]. Methotrexate (MTX) stimulates the production of reactive oxygen species and NF-kappa B, which regulates the production of pro-inflammatory cytokines. This process eventually leads to damage in the intestinal tissue. Leukocytes and other inflammatory cells can be transported to the infected cells, causing further alterations and damage to the intestinal epithelium[2]. The reactive oxygen species (ROS) increase intestinal paracellular permeability by interfering with tight intestinal junctions. This interference alters the localization of occludins and ZO-1, increasing intestinal permeability[24].

In this study, we investigated the beneficial effects of *Erythrina Abyssinica* in maintaining the integrity of the intestinal mucosal barrier. We used the spectrophotometrical approach using Evans blue stain (See Appendix 2) to assess the degree of intestinal permeability as shown in other studies[38]. The low-dose and high-dose treatment groups showed lower quantities of Evans blue dye in the intestines in a dose-dependent manner compared to the negative control group (See Figure 2).

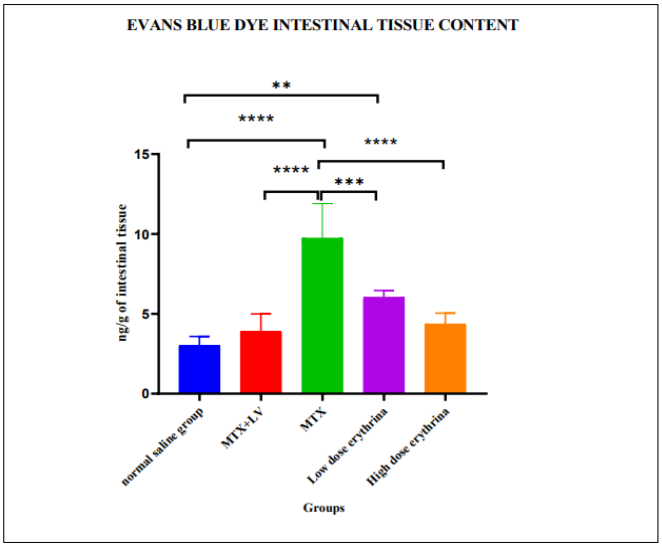

Figure 2 Mean Evans Blue Dye Intestinal tissue content (Results are expressed as mean ± SEM. (** p < 0.01, ***p<0.00.1, ****p< 0.0001).

This suggests that *Erythrina abyssinica* reduces intestinal leakage and permeability through its antioxidant and anti-inflammatory properties.

APPENDIX 2

EVANS BLUE STAINING

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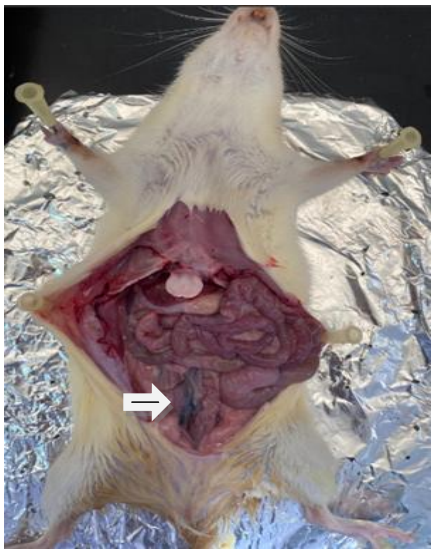

Figure A A photograph showing Evans blue staining c inferior vena cava (Shown by ⇒) in the normal control g The small intestines lack blue staining

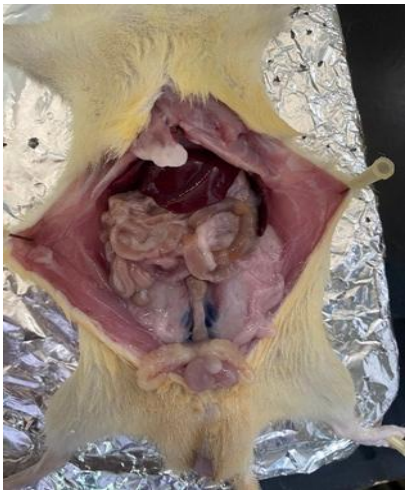

Figure B A photograph showing Evans Blue staining i positive control group. Notice the intestines aren't stained b

Figure C A photograph showing Evans Blue staining in the negative control group. The intestines are intensely stained blue **(Shown by ⟶)**

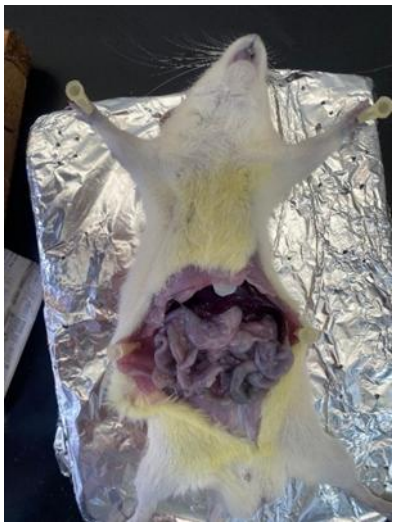

Figure D A photograph showing Evans blue staining in the low-dose group. The intestines are stained blue (The intensity of the blue staining is low compared to the negative control group)

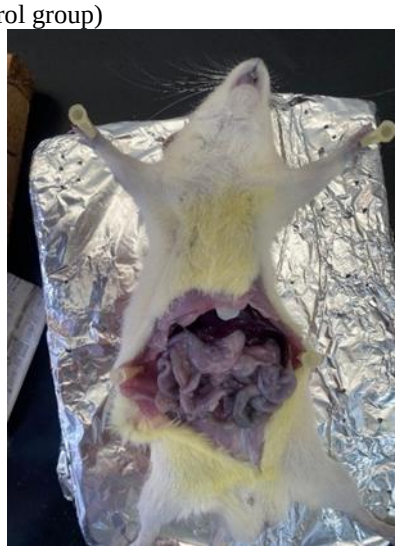

Figure E A photograph showing Evans Blue staining in the positive control group. Notice the intestines aren't stained blue

Prior research indicates that methotrexate has the potential to induce severe diarrhea. It hinders the absorption of various medications and disrupts the active transport of different substances in the intestines. Methotrexate-associated malabsorption syndrome and diarrhea have also been linked to intestinal damage and destruction of villi[40]. Severe diarrhea was noted in the negative control, which suggested malabsorption,

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possibly due to a reduction in the total intestinal absorptive capacity following gut microbiome alterations and intestinal absorptive mechanisms' destruction. The low-dose and high-dose treatment groups showed antidiarrheal properties of *Erythrina abyssinica*, as evidenced by a reduction in the frequency, water content, and weight of diarrhea in the treatment groups. This is in tandem with previous ethnobotanical studies that showed *Erythrina abyssinica* is traditionally used to manage GIT inflammatory diseases and diarrhea[28]. A study on Rwandese medicinal plants support *Erythrina abyssinica* as a potent antidiarrheal agent[25].

There were apparent differences in the histological morphologies of the intestinal epithelium among the different treatment groups. Intestinal damage scores for the negative control group aligned with previous studies[15]. The villus height and crypt depth showed significant reduction in the negative control group compared to the treatment groups (See Figure 6 and Table 1). The negative control group also showed a significant depletion of goblet cells, with marked inflammation and muscularis mucosa destruction. The low-dose and high-dose groups showed a dose-dependent difference in the histological parameters, with the high-dose group showing histological morphologies similar to those of the normal control and the positive control groups.

In conclusion, this study showed the therapeutic effects of *Erythrina abyssinica* on methotrexate-induced intestinal damage. We postulate that these effects resulted from increased levels of antioxidants and reduced glutathione. This led to decreased lipid peroxidation, as indicated by lower MDA content. Additionally, it resulted in reduced diarrhea and the preservation of intestinal integrity. *Erythrina abyssinica*, therefore, shows the potential of being an alternative for managing methotrexate-induced intestinal damage.

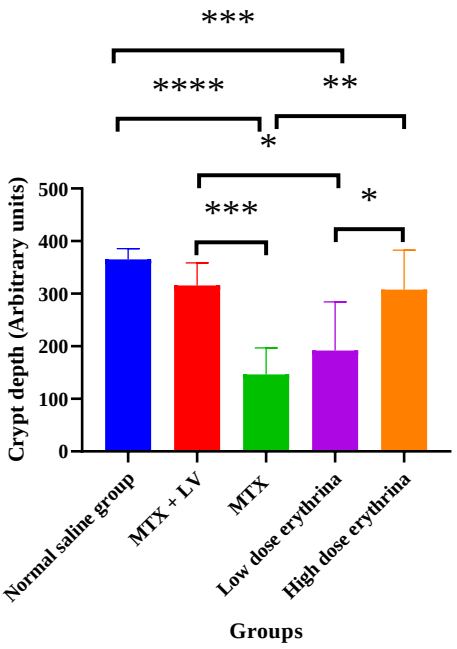

Figure 6 Intestinal crypt depth in arbitrary units **(Results are expressed as mean ± SEM. (*p<0.05, **p<0.01).**

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Table 1 A table showing crypt damage scores, villus damage scores, and goblet cell depletion *(Results expressed as Mean ± SEM)*

GROUPS	Crypt damage score	Villus damage score	Goblet cell depletion	Total intestinal damage score
Normal control	0.000 ± 0.000	0.000 ± 0.000	0.2000 ± 0.2000	0.2000 ± 0.2000
Positive control	0.2000 ± 0.2000	0.4000 ± 0.2449	0.6000 ± 0.2449	1.200 ± 0.3742
Negative control	2.400 ± 0.4000	2.800 ± 0.2000	2.400 ± 0.4000	7.600 ± 0.6000
Low dose group	1.200 ± 0.2000	1.400 ± 0.2449	1.600 ± 0.4000	4.400 ± 0.5099
High dose group	0.6000 ± 0.4000	0.8000 ± 0.3742	1.200 ± 0.4899	2.600 ± 1.166

RESEARCH ARTICLE

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DISCLOSURE

The author(s) report no conflicts of interest in this work

DATA AVAILABILITY

Data are available upon request from the corresponding author.

AUTHOR CONTRIBUTIONS.

ROO, HNK, TKM, CGG & BMC designed the study. ROO & BMC did the experimental work. ROO & BMC analysed the experimental data. ROO wrote the paper. All authors reviewed the manuscript.

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RESEARCH ARTICLE

REFERENCES

1. Aldhahrani, A. (2021). Protective effects of guarana (Paullinia cupana) against methotrexate-induced intestinal damage in mice. *Food Science & Nutrition*, *9*(7), 3397–3404. https://doi.org/10.1002/fsn3.2101

2. Ali, G. F., Hassanein, E. H. M., & Mohamed, W. R. (2024). Molecular mechanisms underlying methotrexate-induced intestinal injury and protective strategies. *Naunyn-Schmiedeberg's Archives of Pharmacology*. https://doi.org/10.1007/s00210-024-03164-x

3. Arakawa, Y., Arakawa, A., Vural, S., Mahajan, R., & Prinz, J. C. (2018). Renal clearance and intracellular half-life essentially determine methotrexate toxicity: A case series. *JAAD Case Reports*, *5*(1), 98–100. https://doi.org/10.1016/j.jdcr.2018.10.022

4. Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Medicine and Cellular Longevity*, *2014*, 360438. https://doi.org/10.1155/2014/360438

5. Borchers, A. T., Keen, C. L., Cheema, G. S., & Gershwin, M. E. (2004). The use of methotrexate in rheumatoid arthritis. *Seminars in Arthritis and Rheumatism*, *34*(1), 465–483. https://doi.org/10.1016/j.semarthrit.2003.12.003

6. Campbell, J. M., Bateman, E., Stephenson, M. D., Bowen, J. M., Keefe, D. M., & Peters, M. D. J. (2016). Methotrexate-induced toxicity pharmacogenetics: An umbrella review of systematic reviews and meta-analyses. *Cancer Chemotherapy and Pharmacology*, *78*(1), 27–39. https://doi.org/10.1007/s00280-016-3043-5

7. Chan, E. S. L., & Cronstein, B. N. (2010). Methotrexate—How does it really work? *Nature Reviews. Rheumatology*, *6*(3), 175–178. https://doi.org/10.1038/nrrheum.2010.5

8. Cronstein, B. N., & Aune, T. M. (2020). Methotrexate and its mechanisms of action in inflammatory arthritis. *Nature Reviews Rheumatology*, *16*(3), Article 3. https://doi.org/10.1038/s41584-020-0373-9

9. Damaske, A., Ma, N., & Williams, R. (2012). Leucovorin-induced hypersensitivity reaction. *Journal of Oncology Pharmacy Practice: Official Publication of the International Society of Oncology Pharmacy Practitioners*, *18*(1), 136–139. https://doi.org/10.1177/1078155210396577

10. El-Boghdady, N. A. (2011). Protective effect of ellagic acid and pumpkin seed oil against methotrexate-induced small intestine damage in rats. *INDIAN J. BIOCHEM. BIOPHYS.*, *48*.

11. El-Sheikh, A. A., Morsy, M. A., & Hamouda, A. H. (2016). Protective Mechanisms of Thymoquinone on Methotrexate-induced Intestinal Toxicity in Rats. *Pharmacognosy Magazine*, *12*(Suppl 1), S76–S81. https://doi.org/10.4103/0973-1296.176106

12. Ezhilarasan, D. (2021). Hepatotoxic potentials of methotrexate: Understanding the possible toxicological molecular mechanisms.

RESEARCH ARTICLE

Toxicology, *458*, 152840. https://doi.org/10.1016/j.tox.2021.152840

13. Fukai, T., & Ushio-Fukai, M. (2011). Superoxide Dismutases: Role in Redox Signaling, Vascular Function, and Diseases. *Antioxidants & Redox Signaling*, *15*(6), 1583. https://doi.org/10.1089/ars.2011.3999

14. Gristan, Y. D., & Moosavi, L. (2024). Folinic Acid. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK545232/

15. Gulgun, M., Erdem, O., Oztas, E., Kesik, V., Balamtekin, N., Vurucu, S., Kul, M., Kismet, E., & Koseoglu, V. (2010). Proanthocyanidin prevents methotrexate-induced intestinal damage and oxidative stress. *Experimental and Toxicologic Pathology: Official Journal of the Gesellschaft Fur Toxikologische Pathologie*, *62*(2), 109–115. https://doi.org/10.1016/j.etp.2009.02.120

16. Hamed, K. M., Dighriri, I. M., Baomar, A. F., Alharthy, B. T., Alenazi, F. E., Alali, G. H., Alenazy, R. H., Alhumaidi, N. T., Alhulayfi, D. H., Alotaibi, Y. B., Alhumaidan, S. S., Alhaddad, Z. A., Humadi, A. A., Alzahrani, S. A., & Alobaid, R. H. (n.d.). Overview of Methotrexate Toxicity: A Comprehensive Literature Review. *Cureus*, *14*(9), e29518. https://doi.org/10.7759/cureus.29518

17. Hanoodi, M., & Mittal, M. (2024). Methotrexate. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK556114/

18. Hegde, V. S., & Nagalli, S. (2024). Leucovorin. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK553114/

19. Howard, S. C., McCormick, J., Pui, C.-H., Buddington, R. K., & Harvey, R. D. (2016). Preventing and Managing Toxicities of High-Dose Methotrexate. *The Oncologist*, *21*(12), 1471–1482. https://doi.org/10.1634/theoncologist.2015-0164

20. Huang, Y., Yang, W., Liu, H., Duan, J., Zhang, Y., Liu, M., Li, H., Hou, Z., & Wu, K. K. (2012). Effect of high-dose methotrexate chemotherapy on intestinal Bifidobacteria, Lactobacillus and Escherichia coli in children with acute lymphoblastic leukemia. *Experimental Biology and Medicine*, *237*(3), 305–311. https://doi.org/10.1258/ebm.2011.011297

21. Kolli, V. K., Abraham, P., & Rabi, S. (2008). Methotrexate-induced nitrosative stress may play a critical role in small intestinal damage in the rat. *Archives of Toxicology*, *82*(10), 763–770. https://doi.org/10.1007/s00204-008-0287-9

22. Koźmiński, P., Halik, P. K., Chesori, R., & Gniazdowska, E. (2020). Overview of Dual-Acting Drug Methotrexate in Different Neurological Diseases, Autoimmune Pathologies and Cancers. *International Journal of Molecular Sciences*, *21*(10), 3483. https://doi.org/10.3390/ijms21103483

23. Levêque, D., Santucci, R., Gourieux, B., & Herbrecht, R. (2011). Pharmacokinetic drug-drug interactions with methotrexate in oncology. *Expert Review of Clinical Pharmacology*, *4*(6), 743–750. https://doi.org/10.1586/ecp.11.57

RESEARCH ARTICLE

24. Maeda, T., Miyazono, Y., Ito, K., Hamada, K., Sekine, S., & Horie, T. (2010). Oxidative stress and enhanced paracellular permeability in the small intestine of methotrexate-treated rats. *Cancer Chemotherapy and Pharmacology*, *65*(6), 1117–1123. https://doi.org/10.1007/s00280-009-1119-1

25. Maïkere-Faniyo, R., Van Puyvelde, L., Mutwewingabo, A., & Habiyaremye, F. X. (1989). Study of Rwandese medicinal plants used in the treatment of diarrhoea I. *Journal of Ethnopharmacology*, *26*(2), 101–109. https://doi.org/10.1016/0378-8741(89)90057-3

26. Miyazono, Y., Gao, F., & Horie, T. (2004). Oxidative stress contributes to methotrexate-induced small intestinal toxicity in rats. *Scandinavian Journal of Gastroenterology*, *39*(11), 1119–1127. https://doi.org/10.1080/00365520410003605

27. Natarajan, K., Abraham, P., & Kota, R. (2017). Activation of the mitochondrial apoptotic pathway contributes to methotrexate-induced small intestinal injury in rats. *Cell Biochemistry and Function*, *35*(7), 378–391. https://doi.org/10.1002/cbf.3285

28. Obakiro, S. B., Kiprop, A., Kigondu, E., K'Owino, I., Odero, M. P., Manyim, S., Omara, T., Namukobe, J., Owor, R. O., Gavamukulya, Y., & Bunalema, L. (2021a). Traditional Medicinal Uses, Phytoconstituents, Bioactivities, and Toxicities of Erythrina abyssinica Lam. ex DC. (Fabaceae): A Systematic Review. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2021*, 5513484. https://doi.org/10.1155/2021/5513484

29. Obakiro, S. B., Kiprop, A., Kigondu, E., K'Owino, I., Odero, M. P., Manyim, S., Omara, T., Namukobe, J., Owor, R. O., Gavamukulya, Y., & Bunalema, L. (2021b). Traditional Medicinal Uses, Phytoconstituents, Bioactivities, and Toxicities of Erythrina abyssinica Lam. ex DC. (Fabaceae): A Systematic Review. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2021*, 5513484. https://doi.org/10.1155/2021/5513484

30. *Optimal administration and dosage of methotrexate*. (n.d.). Clin Exp Rheumatol. Retrieved March 9, 2024, from https://www.clinexprheumatol.org/abstract.asp?a=4319

31. Papaconstantinou, H. T., Xie, C., Zhang, W., Ansari, N. H., Hellmich, M. R., Townsend, C. M., & Ko, T. C. (2001). The role of caspases in methotrexate-induced gastrointestinal toxicity. *Surgery*, *130*(5), 859–865. https://doi.org/10.1067/msy.2001.117376

32. Potoka, D. A., Upperman, J. S., Nadler, E. P., Wong, C. T., Zhou, X., Zhang, X. R., & Ford, H. R. (2002). NF-kappaB inhibition enhances peroxynitrite-induced enterocyte apoptosis. *The Journal of Surgical Research*, *106*(1), 7–14. https://doi.org/10.1006/jsre.2002.6423

33. Rattu, M. A., Shah, N., Lee, J. M., Pham, A. Q., & Marzella, N. (2013). Glucarpidase (Voraxaze), a Carboxypeptidase Enzyme for Methotrexate Toxicity. *Pharmacy and Therapeutics*, *38*(12), 732.

RESEARCH ARTICLE

34. Rtibi, K., Selmi, S., Grami, D., Sebai, H., & Marzouki, L. (2018). Methotrexate Produces Gastrointestinal Stress via Oxidative Stress-caused Acute Physiological Disruptions in Water and Electrolytes Transport in the Mucosal Intestine. *Recent Advances in Biology and Medicine*, *04*, 10. https://doi.org/10.18639/RABM.2018.04.565816

35. Spurlock, C. F., Aune, Z. T., Tossberg, J. T., Collins, P. L., Aune, J. P., Huston, J. W., Crooke, P. S., Olsen, N. J., & Aune, T. M. (2011). Increased sensitivity to apoptosis induced by methotrexate is mediated by Jun N-terminal kinase. *Arthritis and Rheumatism*, *63*(9), 2606–2616. https://doi.org/10.1002/art.30457

36. Thomas, S., Fisher, K. H., Snowden, J. A., Danson, S. J., Brown, S., & Zeidler, M. P. (2015). Methotrexate Is a JAK/STAT Pathway Inhibitor. *PLoS ONE*, *10*(7), e0130078. https://doi.org/10.1371/journal.pone.0130078

37. Uchihara, Y., Komori, R., Tago, K., Tamura, H., & Funakoshi-Tago, M. (2019). Methotrexate significantly induces apoptosis by inhibiting STAT3 activation in NPM-ALK-positive ALCL cells. *Biochemical Pharmacology*, *170*, 113666. https://doi.org/10.1016/j.bcp.2019.113666

38. Vijayaraghavareddy, P., Adhinarayanreddy, V., Vemanna, R. S., Sreeman, S., & Makarla, U. (2017). Quantification of Membrane Damage/Cell Death Using Evan's Blue Staining Technique. *Bio-Protocol*, *7*(16), e2519. https://doi.org/10.21769/BioProtoc.2519

39. X, H., Q, F., T, R., L, Z., X, Z., Z, T., L, C., & D, O. (2020). Leucovorin ameliorated methotrexate induced intestinal toxicity via modulation of the gut microbiota. *Toxicology and Applied Pharmacology*, *391*. https://doi.org/10.1016/j.taap.2020.114900

40. Yamamoto, J., Horie, T., & Awazu, S. (1997). Amelioration of methotrexate-induced malabsorption by vitamin A. *Cancer Chemotherapy and Pharmacology*, *39*(3), 239–244. https://doi.org/10.1007/s002800050567

41. Yan, H., Su, R., Xue, H., Gao, C., Li, X., & Wang, C. (2021). Pharmacomicrobiology of Methotrexate in Rheumatoid Arthritis: Gut Microbiome as Predictor of Therapeutic Response. *Frontiers in Immunology*, *12*, 789334. https://doi.org/10.3389/fimmu.2021.789334

42. Yokooji, T., Mori, N., & Murakami, T. (2011). Modulated pharmacokinetics and increased small intestinal toxicity of methotrexate in bilirubin-treated rats. *The Journal of Pharmacy and Pharmacology*, *63*(2), 206–213. https://doi.org/10.1111/j.2042-7158.2010.01213.x

43. Yulug, E., Turedi, S., Alver, A., Turedi, S., & Kahraman, C. (2015). Effects of resveratrol on methotrexate-induced intestinal injury. *Bratislavske Lekarske Listy*, *116*(11), 676–680. https://doi.org/10.4149/bll_2015_132

44. Zhang, L., & Chu, C.-Q. (2021). Gut Microbiota–Medication Interaction in Rheumatic Diseases. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.796865

45. Zhou, B., Xia, X., Wang, P., Chen, S., Yu, C., Huang, R., Zhang, R., Wang, Y., Lu, L., Yuan, F., Tian, Y., Fan, Y., Zhang, X., Shu, Y., Zhang, S., Bai, D., Wu, L., Xu, H., & Yang, L. (2018). Induction and Amelioration of Methotrexate-Induced Gastrointestinal Toxicity are Related to Immune Response and Gut Microbiota. *EBioMedicine*, *33*, 122–133. https://doi.org/10.1016/j.ebiom.2018.06.029