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# Research Article
# Ulcerogenic Potential of *Eucalyptus globulus* L. Leaf Extract in Wistar Albino Rats

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## Abstract

**Background and Objective:** *Eucalyptus globulus* (Myrtaceae) is one of the plants widely used by many traditional medicine practitioners (TMP) in Uganda and the rest of the world. Although efficacy data to support the medicinal use of this plant and its oil is widely documented, there was limited knowledge about its safety profile. This study was therefore conducted to evaluate the effect of the methanolic leaf of *E. globulus* on biochemical, heamatological and histological indices of the rats after 42 days of single daily oral dose. **Materials and Methods:** A methanolic leaf extract of *Eucalyptus globulus* was prepared and its sub-acute toxicity evaluated in Wistar albino rats. Healthy animals were administered with determined doses of the extract daily for up to 42 days. At the end of the study period, blood was collected and analyzed for biochemical and heamatological parameters while tissues from selected vital organs were microscopically examined for histopathological lesions. Numerical data was analyzed using ANOVA and mean statistical differences tested using Dunnett's test at 95% confidence. **Results:** The methanolic leaf extract of *E. globulus* significantly increased the level of neutrophils and lymphocytes but decreased the basophils. It also caused severe sloughing of the intestinal mucosa. **Conclusion:** It was concluded from current findings that the methanolic leaf extract of *E. globulus* can cause gastrointestinal ulcers in Wistar rats after subacute administration.

Key words: *Eucalyptus globulus*, biochemical, hematological Indices, histopathology, gastrointestinal ulcers, methanolic leaf extract

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The use of medicinal plants in treatment of microbial infections and other ailments continues to gain momentum due to their availability, low cost, perceived effectiveness and cultural acceptability across ethnic backgrounds as well as increasing development of resistance to existing antimicrobial drugs[1]. Herbal remedies are increasingly being used in Uganda and most parts of the world as hypolipidemics, contraceptives, abortifacients, emmenagogues, anti-hypertensives, in treatment of skin diseases, facilitating wound healing, anti-microbials and anti-diabetics[1-4].

Leaves of *E. globulus* are widely used in Uganda to prepare decoctions for treatment of a range of bacterial infections of the gastrointestinal tract, respiratory and urinary system. Other conditions treated with the plant extract include fungal infections, diabetes mellitus, rheumatism, headache, backache, sore throat, inflammations, HIV/AIDS related conditions and sepsis[5]. Apart from its ethno medicinal use, the essential oil from this plant is widely used in steam bath, body massage, insect repellant, cosmetics, air freshening and perfumes. Various scientific studies conducted have validated the claimed efficacy of various extracts from this plant[6-8]. The medicinal profile of the herb has been attributed to various phytochemicals present in the extracts which include essential oils, terpenes, tannins, aldehydes, bitter resins and polyphenolic acids[7,8]. Of these, the predominant compound responsible for the pharmacological activity of this plant being eucalyptol (cineole)[8,9].

Despite the general public belief that traditional medicines are safe since they have been used for a long time, long term use of these remedies can cause serious toxicity and even death[10]. The cause of the toxicity may be due to presence of inherent toxic chemical in the herbs, variability in active or toxic ingredients due to growing conditions, processing or preparation, misidentification of herbs and contamination or adulteration[11,12].

Whereas the herb is continuing to be used globally, its safety profile has not been fully documented. Acute toxicity studies conducted outside Africa have documented that the crude leaf extracts of *E. globulus* is safe when taken in a single oral dose within 24 h. But acute toxicity results are not evident enough to confirm toxicity of plant extracts[13]. Therefore, sub-acute and chronic studies are necessary to predict more accurately the long term toxicity on haematological, biochemical and histological parameters of animals[13,14]. The study therefore, evaluated the sub-acute toxicity profile of the *E. globulus* extract in wistar albino rats, in order to generate preclinical data regarding its safety when used as a herbal medicine.

## MATERIALS AND METHODS

**Plant collection, identification, processing and extraction:** The leaves of *E. globulus* were collected from Bushenyi district, western region of Uganda (0°32′18.8″ S and 30° 8′39.74″ E). A voucher specimen (OSB 001) was prepared and identified by Ms. Olivier Wanyana, a plant taxonomist at the herbarium of Botany Department of Makerere University. The collected samples were stored and dried in a shade in an isolated room at the Department of Pharmaceutical and Toxicology laboratory until a constant mass was obtained. The dried samples were pounded manually using a wooden mortar and pestle and then sieved to obtain a powder. A total crude methanolic extract was prepared by soaking 250 g of the powder in 1000 mL of 75% w/v of methanol in water for 3 days with occasional shaking to facilitate extraction. The extract was filtered using Whatman's filter paper No.1 and the filtrate concentrated with a rotary evaporator (CH-9230 Flawl/Schweiz, Germany) at 40°C. The concentrated extract was stored in clean labeled bottles at 0°C in a refrigerator until further use.

**Experimental animals and sub-acute toxicity testing:** Ethical approval was obtained from the School of Biomedical Sciences Research and Ethics Committee Makerere University College of Health Sciences (SBS 095) and the Uganda National Council for Science and Technology (HS1515). To evaluate the effect of *E. globulus* extract on haematological, biochemical and histopathological indices, a method of Cruz[14] was used. About 32 male and female rats of about 10 weeks old were randomly distributed in four groups (8 rats per group). The control group was orally administered with distilled water whereas the 3 experimental groups received determined doses (800, 1200 and 2000 mg $kg^{-1}$) of extract based on the median lethal dose once daily for a period of 42 days.

**Hematological and biochemical evaluation:** On 22nd day, 4 rats from each of the groups were anaesthetized with chloroform and blood (2 mL) collected by cardiac puncture using a syringe into heparinized and non-heparinized tubes. Heparinized blood samples collected was analyzed using a counter (Nihon kohden celltac F) for full blood counts. The serum obtained from the non-heparinized blood sample was

analyzed using an automated clinical chemistry analyzer (COBAS INTEGRA 6000) for levels of creatinine, urea, uric acid, total cholesterol, serum diagnostic enzymes.

**Histopathological evaluation:** On the 43rd day, the remaining four animals in each group were anaesthetized with chloroform and dissected to obtain the liver, intestine, lung and the kidney. After gross examination, tissues were isolated, fixed in 10% buffered formalin solution and processed using an automated tissue processor (Histokinette model Leica TP1020). Tissues were then sectioned with Rotary microtome (Leica RM 2235) and stained with haematoxylin and Eosin (H and E). After mounting them on slides, tissues were examined for defects using a microscope by two independent pathologists.

**Phytochemical screening:** The extract was also screened for phytochemicals as described by Tiwari *et al.*[15] to determine whether there were differences in phytochemical composition in reference to other studies.

**Statistical analysis:** The data obtained was entered in Microsoft office Excel, 2007 and exported to graph pad prism version 6.01. INC USA. The results were analyzed using One Way of Variance Analysis (ANOVA) and the mean statistical differences tested using Dunnet test at 95% confidence interval ($p<0.05$).

## RESULTS

**Biochemical and haematological analysis results:** All the rats survived up to the end of the study period and did not show any clinical toxicity signs. There were no significant differences in level of the serum liver diagnostic enzymes as well as the concentrations of urea, creatinine and total cholesterol between the treatment and control group (Table 1). Most of the haematological parameters were not significantly affected by the extract even at high doses except for neutrophils and lymphocytes that were significantly increased and basophils decreased (Table 2).

**Histopathological evaluation:** Despite the non-significant effects on the biochemical parameters, there were mild histopathological lesions in the liver, kidney and lungs but severe lesions in the intestinal lining were observed (Fig. 1-4).

Table 1: Mean values of biochemical parameters after 21 days of daily extract dosing

| | Mean values of parameter | | | |
|---|---|---|---|---|
| Parameters | Control | 800 (mg kg$^{-1}$) | 1200 (mg kg$^{-1}$) | 2000 (mg kg$^{-1}$) |
| ALT (U L$^{-1}$) | 221.60±68.56 | 155.90±17.30 | 160.80±12.96 | 140.20±16.03 |
| AST (U L$^{-1}$) | 364.90±82.97 | 332.50±39.21 | 358.70±23.25 | 235.50±20.63 |
| ALP (U L$^{-1}$) | 387.50±38.32 | 276.30±54.25 | 242.30±44.79 | 237.00±35.05 |
| Total cholesterol (mmol L$^{-1}$) | 2.32±0.210 | 2.15±0.1164 | 2.20±0.130 | 2.22±0.040 |
| Urea (mMol L$^{-1}$) | 7.50±0.420 | 7.75±0.480 | 9.23±0.580 | 7.68±0.360 |
| Creatinine (μmol L$^{-1}$) | 49.00±5.400 | 63.00±2.040 | 64.50±6.330 | 52.75±3.090 |

Values expressed as Mean±SEM, n = 4, *p<0.05, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

Table 2: Mean values for haematological parameters after 21 days of daily extract dosing

| | Mean values of parameter | | | |
|---|---|---|---|---|
| Parameters | Control | 800 (mg kg$^{-1}$) | 1200 (mg kg$^{-1}$) | 2000 (mg kg$^{-1}$) |
| WBC (10^3UL) | 8.29±3.50 | 12.23±1.43 | 11.01±2.72 | 9.48±2.201 |
| NEUT (10^3UL) | 0.04±0.00 | 1.41±0.04*** | 1.81±0.01*** | 1.93±0.03*** |
| LYMP (10^3UL) | 1.51±0.03 | 1.64±0.03 | 2.99±0.03*** | 3.32±0.09*** |
| MONO (10^3UL) | 0.71±0.20 | 0.62±0.14 | 0.76±0.23 | 0.54±0.12 |
| EO (10^3UL) | 0.16±0.06 | 0.19±0.02 | 0.14±0.05 | 0.09± 0.02 |
| BASO (10^3UL) | 0.13±0.01 | 0.10±0.01 | 0.01±0.002*** | 0.01±0.002*** |
| RBC (10^3UL) | 8.65±0.52 | 8.42±0.19 | 8.28±0.31 | 8.28±0.30 |
| HB (g dL$^{-1}$) | 14.55±1.02 | 14.48±0.22 | 14.23±0.44 | 14.53±0.29 |
| HCT (%) | 48.83±3.41 | 48.78±0.76 | 47.68±1.17 | 49.53±0.78 |
| MCV (fL) | 56.35±0.97 | 57.98±0.76 | 57.65±0.85 | 59.95±1.48 |
| MCH (pg) | 16.78±0.14 | 17.20±0.19 | 17.18±0.13 | 17.58±0.36 |
| MCHC (g dL$^{-1}$) | 29.83±0.48 | 29.68±0.13 | 29.80±0.31 | 29.33±0.13 |
| RDW-CV (%) | 21.00±1.29 | 20.30±0.25 | 20.75±0.51 | 20.33±0.70 |
| PLT (10^3UL) | 570.00±165.4 | 810.80±39.56 | 846.00±37.68 | 716.80±86.54 |
| MPV ( (fL) | 8.850±0.10 | 8.900±0.27 | 9.050±0.30 | 9.43±0.18 |

Value expressed as Mean±SEM, n = 4, *p<0.05, ***p<0.001, MONO: Monocyte, BASO: Basophils, NEUT: Neutrophils, EO: Eosinophils, LYMP: Lymphocytes, HB: Haemoglobin, MCH: Mean corpuscular haemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, PHC: Platelet count, RBC: Red blood cell, WBC: Whiteblood cell, MPV: Mean platelet volume, PLT: Platelet, RDW-CV: Red blood cell distribution width, MCHC: Mean corpuscular haemoglobin concentration, HCT: Haematocrit

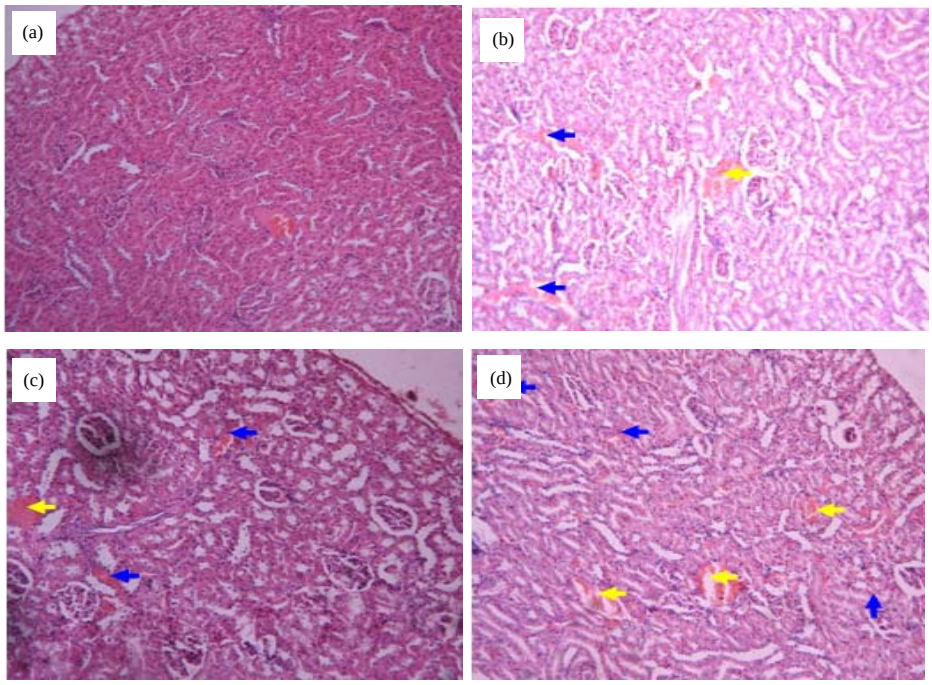


Fig. 1(a-d): Histopathology of the kidney after 42 days (a) Distilled water, No lesions, X100, 100 µm scale bar, (b) 800 mg $kg^{-1}$, congestion-blue, Hemorrhage-yellow, X100, 100 µm scale bar, (c) 1200 mg $kg^{-1}$, congestion-blue, Haemorrhage-yellow, X100, 100 µm scale bar and (d) 2000 mg $kg^{-1}$, congestion-blue, haemorrhage-yellow, X100, 100 µm scale bar

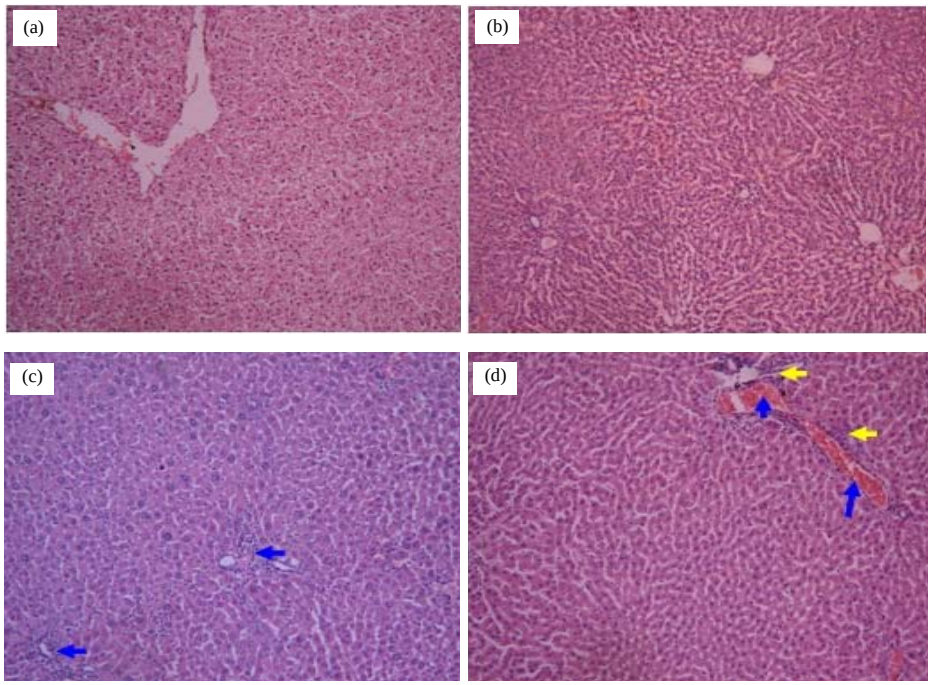


Fig. 2(a-d): Histopathology of the liver after 42 days (a) Distilled water, No lesions, X100, 100 µm scale bar, (b) 800 mg $kg^{-1}$, No lesions, X100, 100 µm scale bar, (c) 1200 mg $kg^{-1}$, perivascular degeneration-blue, X100, 100 µm scale bar and (d) 2000 mg $kg^{-1}$, congestion-blue, perivascular degeneration-yellow, X100, 100 µm scale bar

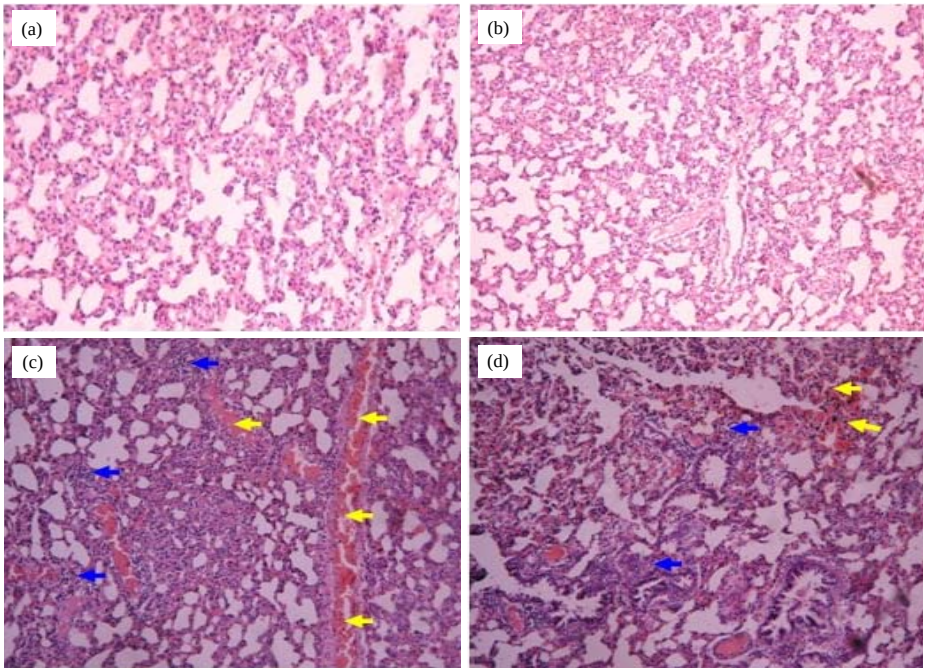


Fig. 3(a-d): Histopathology of the lungs after 42 days (a) Distilled water, No lesions, X100, 100 µm scale bar, (b) 800 mg $kg^{-1}$, No lesions, X100, 100 µm scale bar, (c) 1200 mg $kg^{-1}$, pneumonitis-blue, congestion-yellow, X100, 100 µm scale bar and (d) 2000 mg $kg^{-1}$, congestion-blue, haemorrhage-yellow, X100, 100 µm scale bar

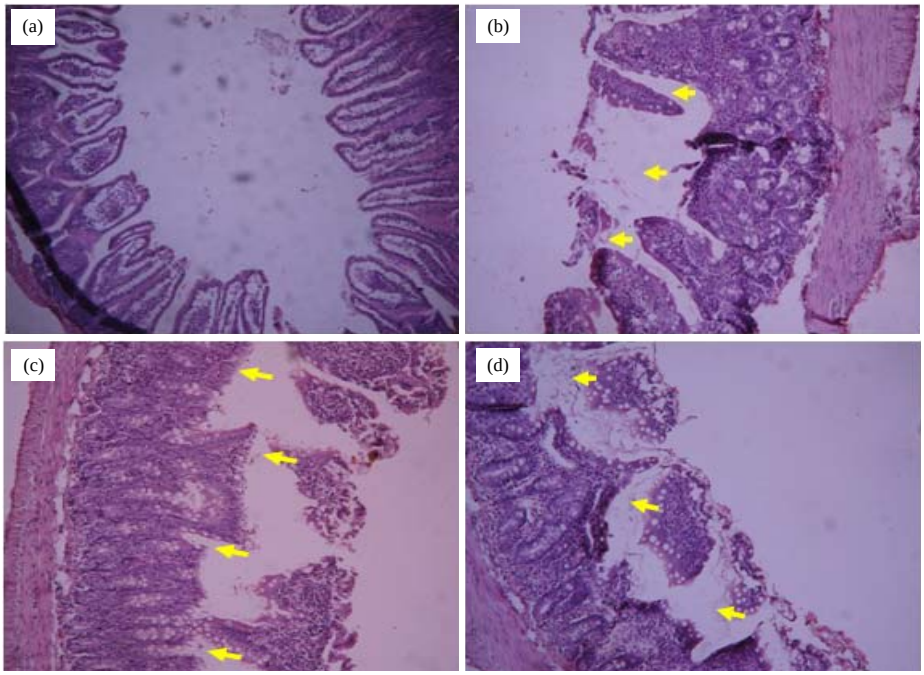


Fig. 4(a-d): Histopathology of the small intestines after 42 days (a) Distilled water, No lesions, X100, 100 µm scale bar, (b) 800 mg $kg^{-1}$, mild sloughing of the mucosa, X100, 100 µm scale bar, (c) 1200 mg $kg^{-1}$, sloughing of the mucosa, X100, 100 µm scale bar and (d) 2000 mg $kg^{-1}$, sloughing of the mucosa, X100, 100 µm scale bar

**Phytochemical screening:** The methanolic leaf extract of *E. globulus* contained saponins, tannins, glycosides, flavonoids, terpenoids, essential oils and phenols while basic alkaloids were absent.

## DISCUSSION

The significant increase in the level of neutrophils and lymphocytes in the treatment group at high doses implies that the extract stimulates the production of these components into blood or causes infection. Since histopathological findings showed mild lesions in selected organs, it is highly probable that the extract stimulates their differentiation. This may be the reason for the use of herbal remedy from this plant as an antimicrobial agent since the extract boosts the immune system of the animal. The significant decrease in the level of basophils could not be explained in the study. The non-significant difference in all other haematological indices indicated that the extract neither contains chemicals that enhance the differentiation of the hematopoietic stem cells into them nor affect their rate of removal from the circulation. The non-significant changes in total cholesterol, serum diagnostic enzymes, urea and creatinine between the treatment and the control group observed imply that the extract did not affect their normal physiology. This indicates that the extract neither affected lipid metabolism nor caused damage to the liver and kidney.

Although the results of haematological analysis obtained from this study are in agreement with those reported by Ponte *et al.*[16] they differ from those reported by Oyesomi *et al.*[17]. In this latter study, there was a general increase in almost all the haematological parameters. The explanation advanced was that it is due to the iron constituency in the essential oil of *E. globulus*. The difference in the results therefore may be explained by the fact that Oyesomi *et al.*[17] used essential oils from the *E. globulus* while this study and that of Ponte[16] used total crude extract.

The histopathological lesions observed could have been due to the inherent chemical compounds present in the extract or the products of their metabolism. However, the study could not ascertain the real compound responsible for the damages. Since congestion and haemorrhage may be due to impaired outflow of venous blood, vascular injury or depletion of the coagulation factors, it is highly probable that these effects are due to the essential oils contained in the extract. Sloughing of the intestinal mucosa is thought to be due to irritation of the gastrointestinal mucosa by the chemicals in the extract. However, the study could not identify the real chemical responsible for the severe gastrointestinal damage. Never the less, it was inferred that the mild toxicities in the vasculature associated with this plant could probably be due to essential oils present in the extract. This is because a study that used the essential oil[17] instead of the total crude extract reported higher significant differences in haematological and biochemical indices.

The phytochemicals identified in the extract have been reported to have good pharmacological activity against different microbes and other ailments as reported by Ishnava *et al.*[7]. None of them has been linked to cause toxicity within normal therapeutic doses[6-9].

## CONCLUSION

It was concluded from present study findings that the methanolic leaf extract of *E. globulus* did not cause significant damage to the liver, kidney and lungs when administered in repeated small doses. However, it caused severe sloughing of the intestinal mucosa that can results into gastrointestinal ulcers. It was recommended that more phytochemical analytical studies are conducted to identify the chemical substances responsible for the ulcerogenicity of *E. globulus*.

## SIGNIFICANCE STATEMENT

The manuscript describes the toxicological findings of a commonly used plant species in preparation of herbal remedies by many communities in Africa. The extract from *Eucalyptus globulus* causes severe sloughing of the intestinal mucosa that can result into gastro-intestinal ulcers when used in repeated doses.

The extract on the other hand increases the level of neutrophils lymphocytes which may explain its antimicrobial activity. The results show the toxicity associated with the use of *Eucalyptus globulus* in herbal medicine. So the manuscript provide knowledge regarding the toxicity of the plant.

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