One Health
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Phenolic-Rich Ethanolic Extract of Kinkeliba (*Combretum micranthum*) Mitigates DSS-Induced Ulcerative Colitis in C57BL/6 Mice

Ibrahima Mamadou SALL [1]*, Dan Cristian VODNAR [2], Gheorghe Adrian MARTĂU [2], Meriem AZIEZ [3], Alina Diana HAȘAȘ [1], Dragoș Hodor [1], and Alexandru Flaviu TĂBĂRAN [1]*

[1] Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania
[2] Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.
[3] Laboratory of Plant Biotechnology and Ethnobotany, Faculty of Nature and Life Sciences, University of Be-jaia, Bejaia, Algeria
Medicine, 400372 Cluj-Napoca, Romania;
E-mail: ibrahima.sall@student.usamvcluj.ro

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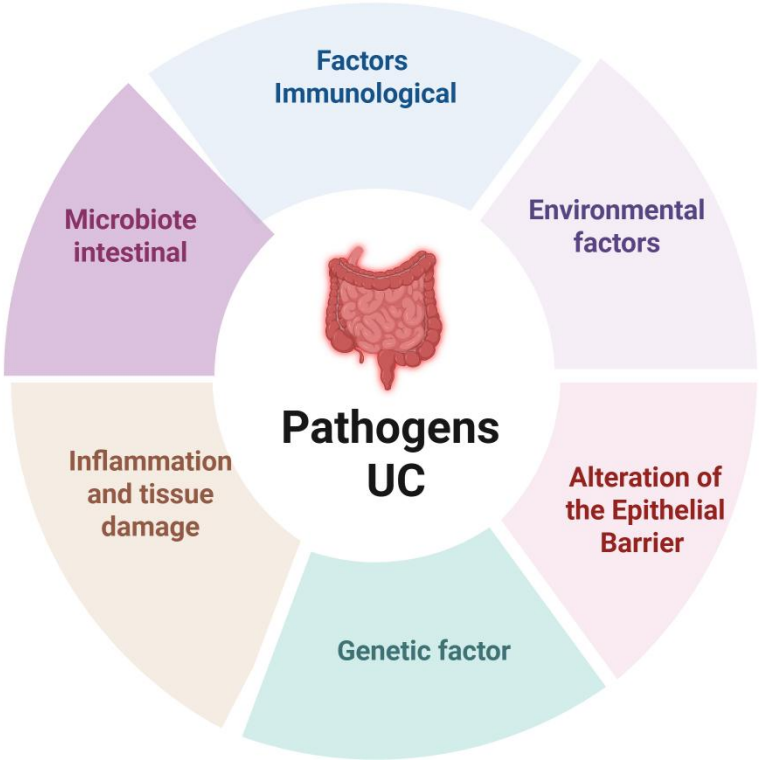
Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by chronic inflammation of the intestinal mucosa, specifically in the rectum and colon **(Kaser A *et al.*, 2010; Szigethy E et al., 2010)**

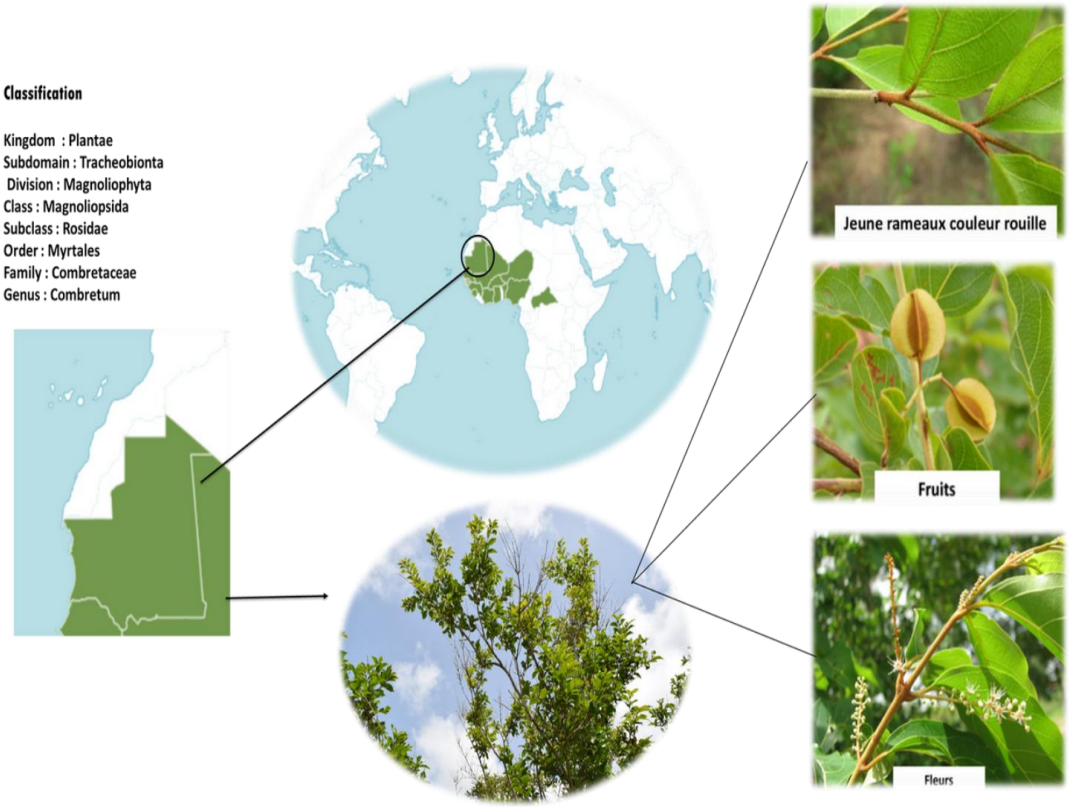
Clinically, UC presents with symptoms such as abdominal pain, diarrhea, mild fever, and weight loss **(Baumgart D C and Sandborn W J, 2007).**

The pathogenesis is multifactorial and remains uncertain, involving interactions between genetic factors, environmental factors, immune system components, and alterations of the gut microbiota **(Abraham C. and Cho J.H., 2009).**

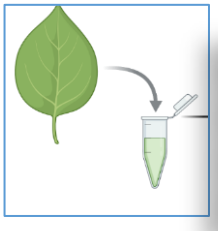


At present, there is no effective treatment without side effects for this disease. In this perspective, we propose a therapeutic alternative based on plant-derived bioactive compounds.

Combretum micranthum is well known and widely used in African traditional medicine, commonly prepared as infusions or decoctions for the treatment and prevention of various disease including diabetes, hypertension, bronchitis, inflammatory bowel disease etc. **(Welch C.R. *et al.*, 2010; Akeem A.A. *et al.*, 2012; Jean-Pierre Ngene *et al.*, 2015).**



Main objective: to determine the therapeutic effect of the ethanolic extract of *Combretum micranthum* (EECM) on ulcerative colitis.



Objective 1:
Determine the phenolic content of the ethanolic extract of *Combretum micranthum* components using an HPLC-DAD-ESI-MS analytical system.

Objective 2:
Evaluate antioxidant and antimicrobial activity of the ethanolic extract of *Combretum micranthum* .

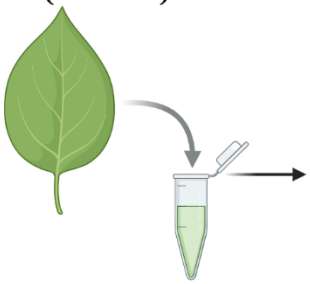
Objective 3:
To investigate the protective effect of the ethanolic extract of *Combretum micranthum*, rich in phenolic compounds, on DSS-induced ulcerative colitis in C57BL/6 mice, in relation to antioxidation and gut microbiota regulation.

Materials and methods

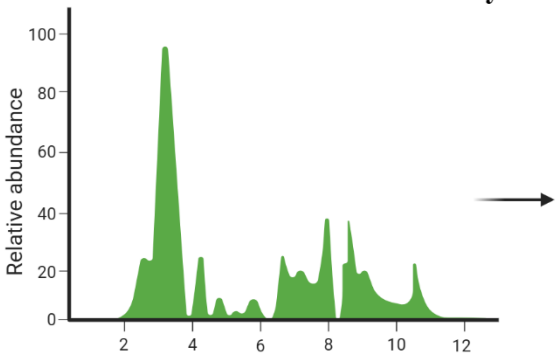
In Vitro

Combretum micranthum

Ethanol extract of *Combretum micranthum* (EECM)



Characterization of the polyphenolic composition by HPLC-DAD-ESI/MS analysis.



Biological activities

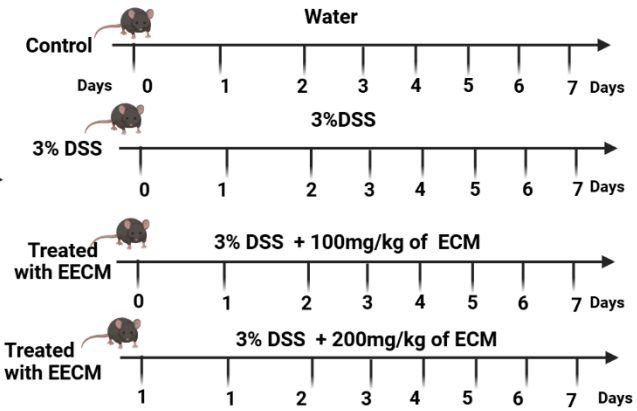
- **Antioxidant capacity**
  - DPPH
  - ABTS
  - FRAP
- **Antimicrobial activity**
  - Antibacterial activities
  - Antifungal activities

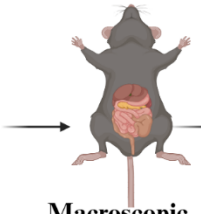
In Vivo

- 20 mices (N=10)
- C57BL/c
- Femele (N=5)
- 6-9 weeks

Groups

- 5 ♀ Control
- 5 ♀ 3% DSS
- 5 ♀ 3% DSS + 100 mg/kg
- 5 ♀ 3% DSS + 200mg/kg

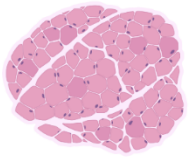




Macroscopic examination

Colon and blood collection

Biochimical and hematologica analysisl



Histological analysis

Results and discussions

1. Phenolic content of the Ethanolic Extract of *Combretum micranthum*

HPLC-DAD analysis report of the Ethanolic Extract of *Combretum micranthum*

Table 1. Phenolic structure contained in of the Ethanolic Extract of *Combretum micranthum*

	Phenolic Compounds (mg/g)	Subclass	R_t (min)	λmax (nm)	$[M+H]^+$ (m/z)	Extract
1	Gallic acid	Hydroxybenzoic acid	4.70	275	171	4.80
2	Protocatechuic acid	Hydroxybenzoic acid	9.01	280	155	14.14
3	1,6-Digalloyl-glucose	Gallotannin	13.16	280	485	5.82
4	Ellagic acid-arabinoside	Hydroxybenzoic acid	13.93	270, 360	435	14.04
5	Ellagic acid-glucoside	Hydroxybenzoic acid	14.43	270, 360	465	8.08
6	Sanguiin H-4	Ellagitannin	15.14	270, 360	635	102.56
7	Corilagin	Ellagitannin	16.22	270, 360	635	63.29
8	Ellagic acid	Hydroxybenzoic acid	16.63	270, 360	303	12.10
9	Combretastatin B1	Stilbene	22.69	275	335	68.71
	Total phenolics					**293.54**

The phenolic composition of the ethanolic extract of *Combretum micranthum* was determined, revealing the presence of nine major compounds, with a total phenolic content **of 293.54 mg/g of extract.**

Results and discussions

1. Phenolic Composition of the Ethanolic Extract of *Combretum micranthum*

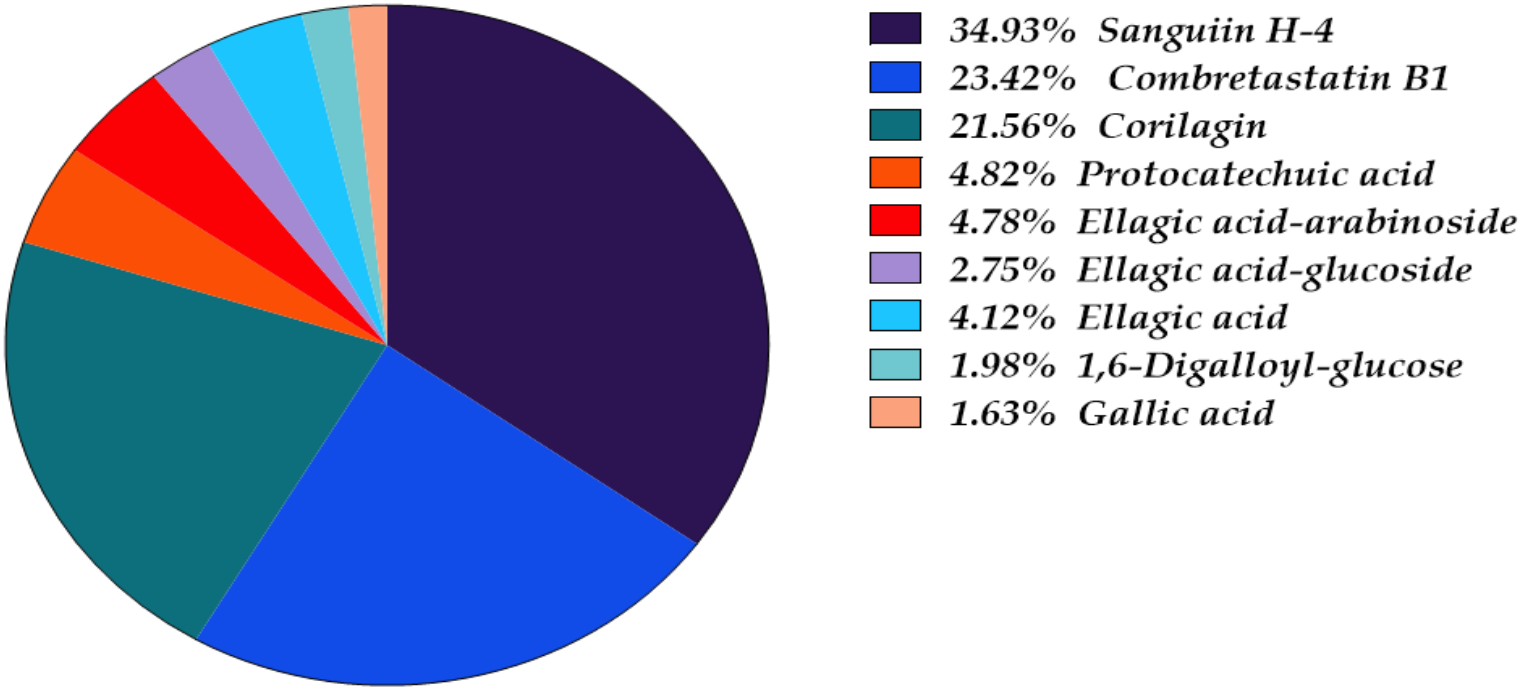


Figure 1. The phenolic compounds identified in the EECM, represented as a circular chart illustrating the proportion of major constituents (Sanguiin H-4, Corilagin, and Combretastatin B1) and minor ones (Protocatechuic acid, Ellagic acid, arabinoside acid, glucoside acid , Gallic acid, and 1,6-Digalloyl-glucose).

Results and discussions

2.Antioxidant Activity

Table 2. Values of the Ethanolic Extract of *Combretum micranthum* determined by DPPH, ABTS and FRAP radical scavenging assays at a concentration of 25 mg/Ml.

DPPH	FRAP	FRAP
135.86 ± 2.35	233.72 ± 2.81	428.86 ± 145.09

The ethanolic extract presented notable antioxidant activity, showing a DPPH radical scavenging capacity of 135.86 ± 2.35 mmol TE/g, ABTS activity of 233.72 ± 2.81 mmol TE/g, and FRAP reducing power of 428.86 ± 145.09 mmol TE/g. These results demonstrate the extract's strong ability to neutralize free radicals and reduce ferric ions at a concentration of 25 mg/mL.

Results and discussions

2.Anti-bacterial Activity

Table 3. Minimum inhibitory concentrations (MIC, µg/mL) of the ethanolic extract of *Combretum micranthum* against Gram-negative and Gram-positive bacteria.

SAMPLE	Gram-Negative Bacteria (–)			Gram-Positive Bacteria (+)			
	Escherichia coli	*Salmonella enterica*	*Pseudomonas aeruginosa*	*Bacillus subtillis*	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Enterococcus fecalis*
EECM	n.b.	1250	n.b.	12,500	12,500	25,000	n.b.
Water (C^-)	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.	n.b.
Gentamicin (C^+)	6.25	1.653	0.391	1.563	6.25	0.391	50

n.b. no bioactivity

The extract presented selective antibacterial activity: Gram-positive, ***Staphylococcus aureus*** is inhibited at 25,000 µg/mL, ***Bacillus subtilis and Streptococcus pyogenes*** are inhibited at 12,500 µg/mL, while ***Enterococcus faecalis*** was not inhibited. Gram-negative bacteria, only ***Salmonella enterica*** showed limited susceptibility 1250 µg/mL), where as ***Escherichia coli and Pseudomonas aeruginosa*** were unaffected.

Results and discussions

3. Fungi Activity

Table 4. Minimum inhibitory concentrations (MIC, µg/mL) of the ethanolic extract of *Combretum micranthum* against yeast and fungi.

SAMPLE	Yeasts			Fungi
	Sacharomyces cerevisiae var. diastaticus	*Candida parapsilosis*	*Candida albicans*	*Aspergillus brasiliensis*
EECM	n.b.	n.b.	n.b.	n.b.
Water (C^-)	n.b.	n.b.	n.b.	n.b.
Ketoconaloze (C^+)	1062.5	2125	531.25	312.5

n.b. no bioactivity

The extract presented no antifungal activity: No inhibitory effect was observed against the tested yeasts (***Saccharomyces cerevisiae, Candida parapsilosis, Candida albicans)*** or against fungi the ***Aspergillus brasiliensis***

Results and discussions

4. Clinical and Morphological Parameters of the Effect of *EECM* on the Severity of Colitis

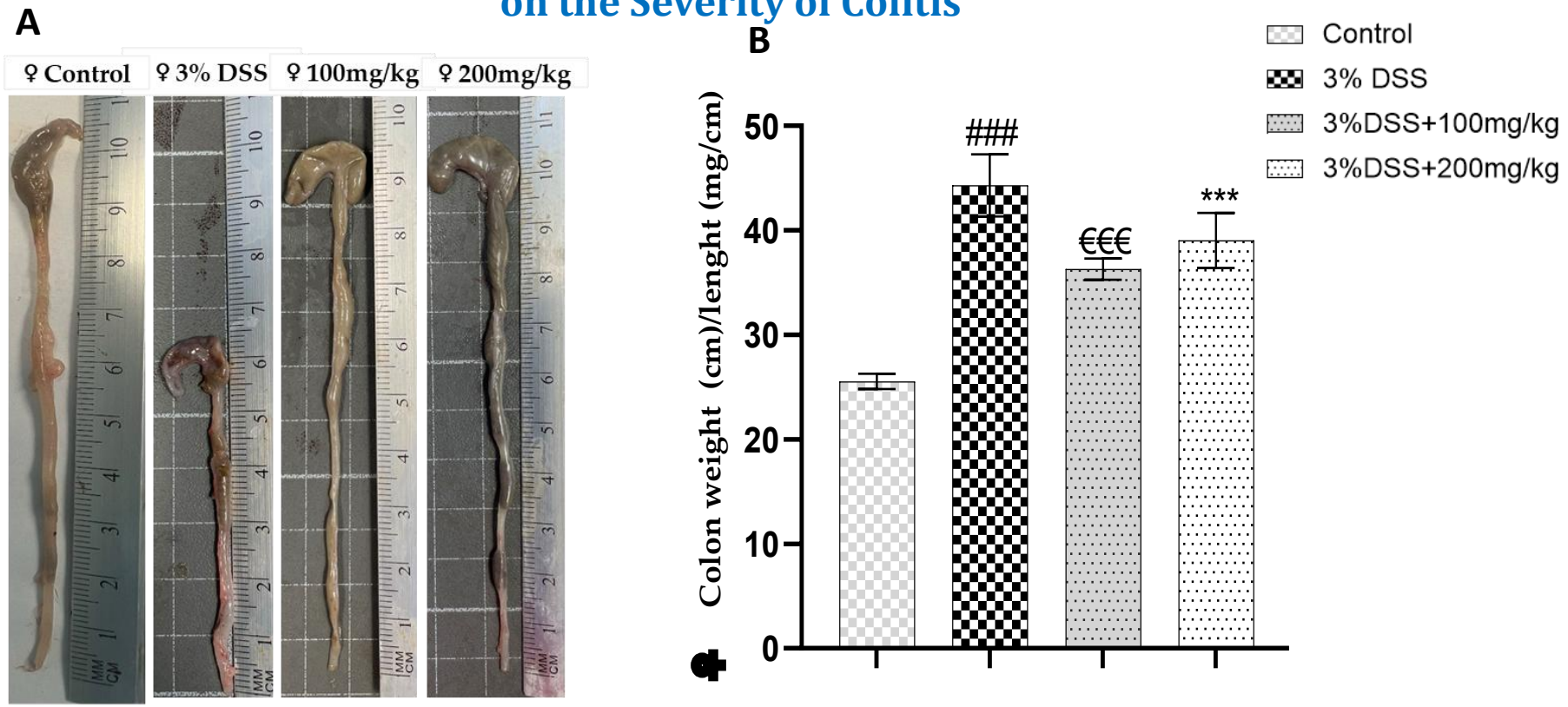


Figure 2. (A) Representative **morphological of the colons:** the colon in the DSS group was shortened compared to the control group, with treatment of EECM went to back normal length. **(B) The colon weight/length ratio,** which was elevated in the DSS group compared to the control group, and significantly reduced with treatment of EECM.

Results and discussions

4. Clinical and Morphological Parameters of the Effect of *EECM* on the Severity of Colitis

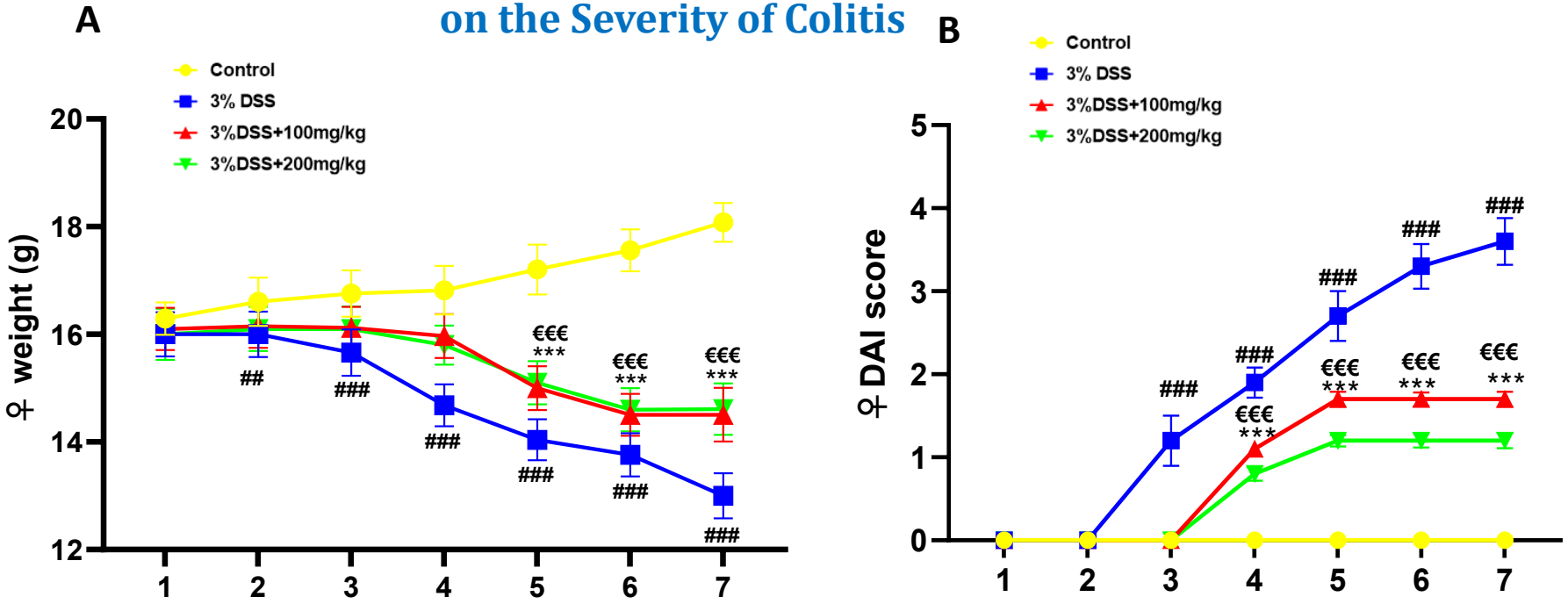


Figure 3. (**A**) **Body weight**, a significant decrease ($p < 0.001$) was observed from day 3 in the DSS group compared to the control group, progressive reduction in the groups treated with EECM. (**B**) **Disease Activity Index (DAI),** showing a progressive increase in the DSS group to the control group and a progressive reduction in the groups treated with EECM.

Results and discussions

5. Hematological Analysis

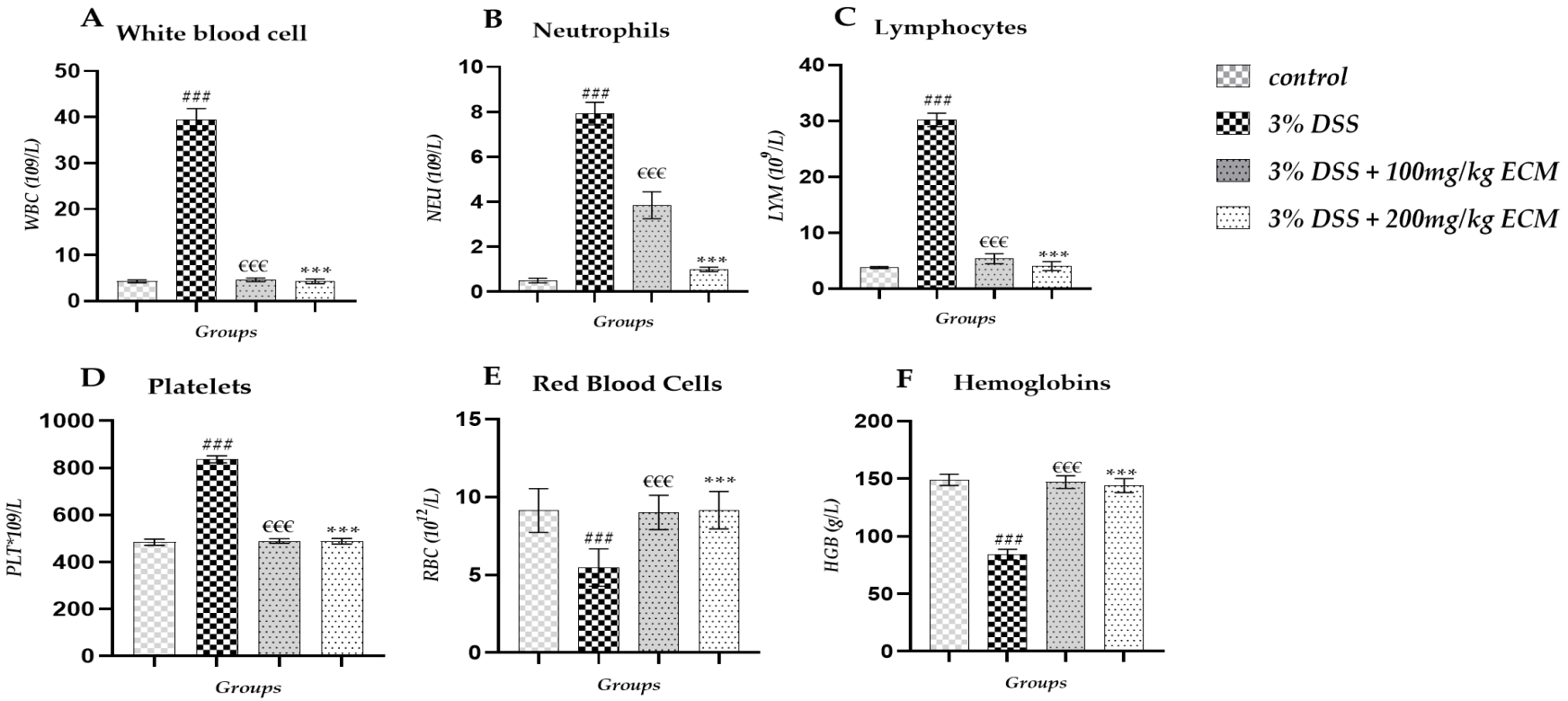


Figure 3. induction of colitis by DSS caused significant alterations in hematological parameters, with a increase in (**A**) WBC, (**B**) NEU, (**C**) LYM, and (**D**) PLT and a decrease in (**E**) RBC and (**F**) HGB compared to the control group. Administration of EECM at two doses (100 and 200 mg/kg) significantly corrected these disturbances,.

6. Biochemical Analysis

Results and discussions

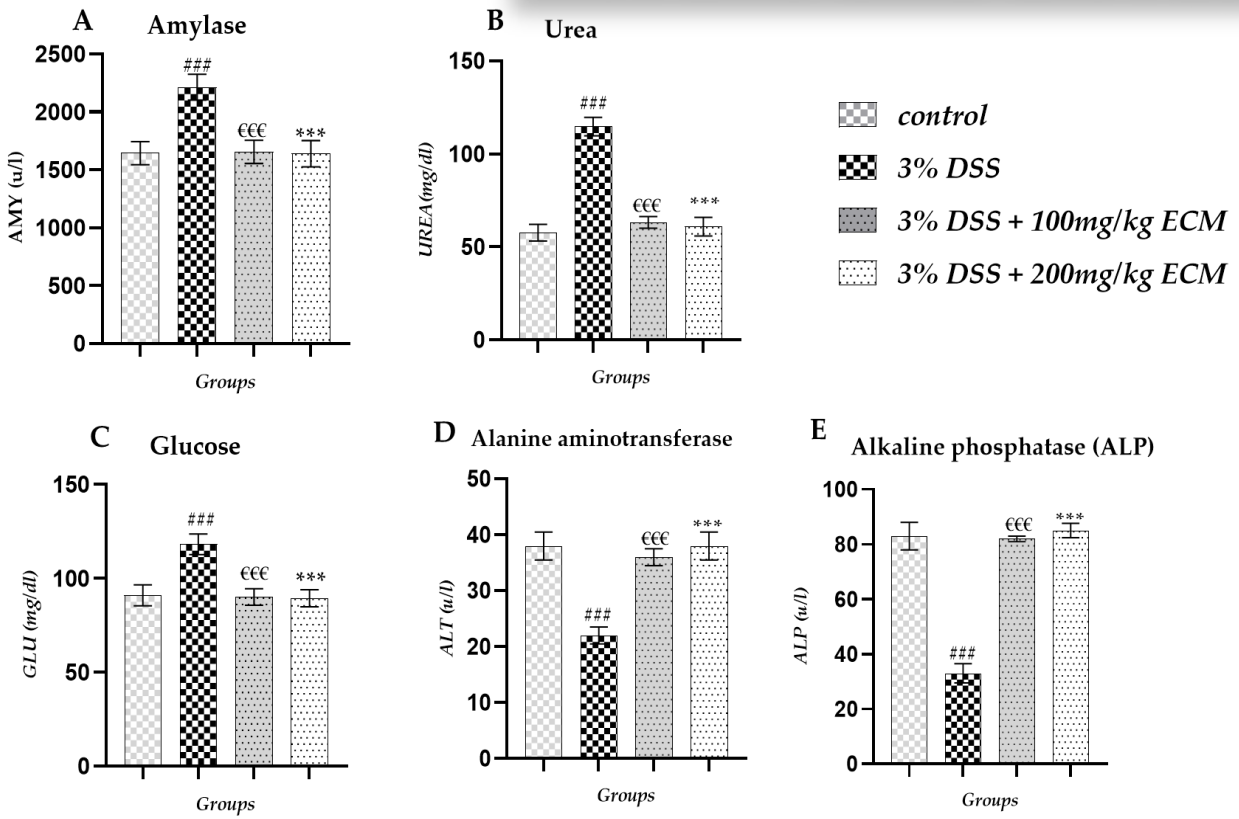


Figure 4. Induction of colitis by DSS caused significant alterations in biochemical parameters a increase in AMYLASE ,UREA, and GLUCOSE and a decrease in ALT and ALP compared to the control group. Administration of EECM at two doses (100 and 200 mg/kg) significantly corrected these disturbances.

Results and discussions

7. Histological analysis

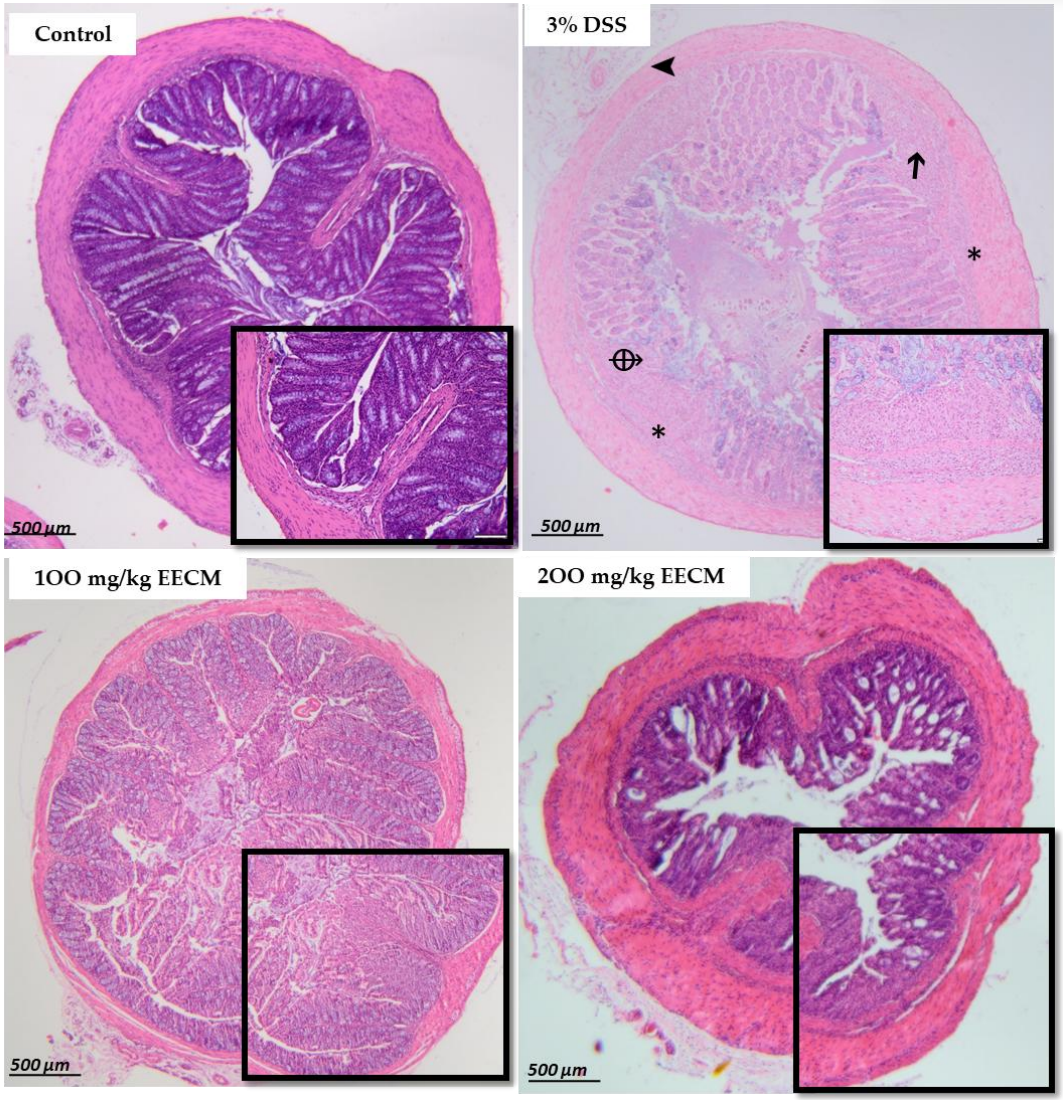


Figure 5. the **DSS group** shows severe lesions in the colon. Key: **(*)** fibrosis in the mucosa or submucosa; (➔) infiltration of polymorphonuclear and mononuclear cells in the mucosa, submucosa and muscular layer; (➤) disruption of crypt and goblet cell depletion; **(⊕)** ulceration compared to the **control group**

The groups of 100 and 200 mg/kg resulted in minor mucosal or submucosal lesions compared to **the DSS group**.

Results and discussions

7. Histological analysis

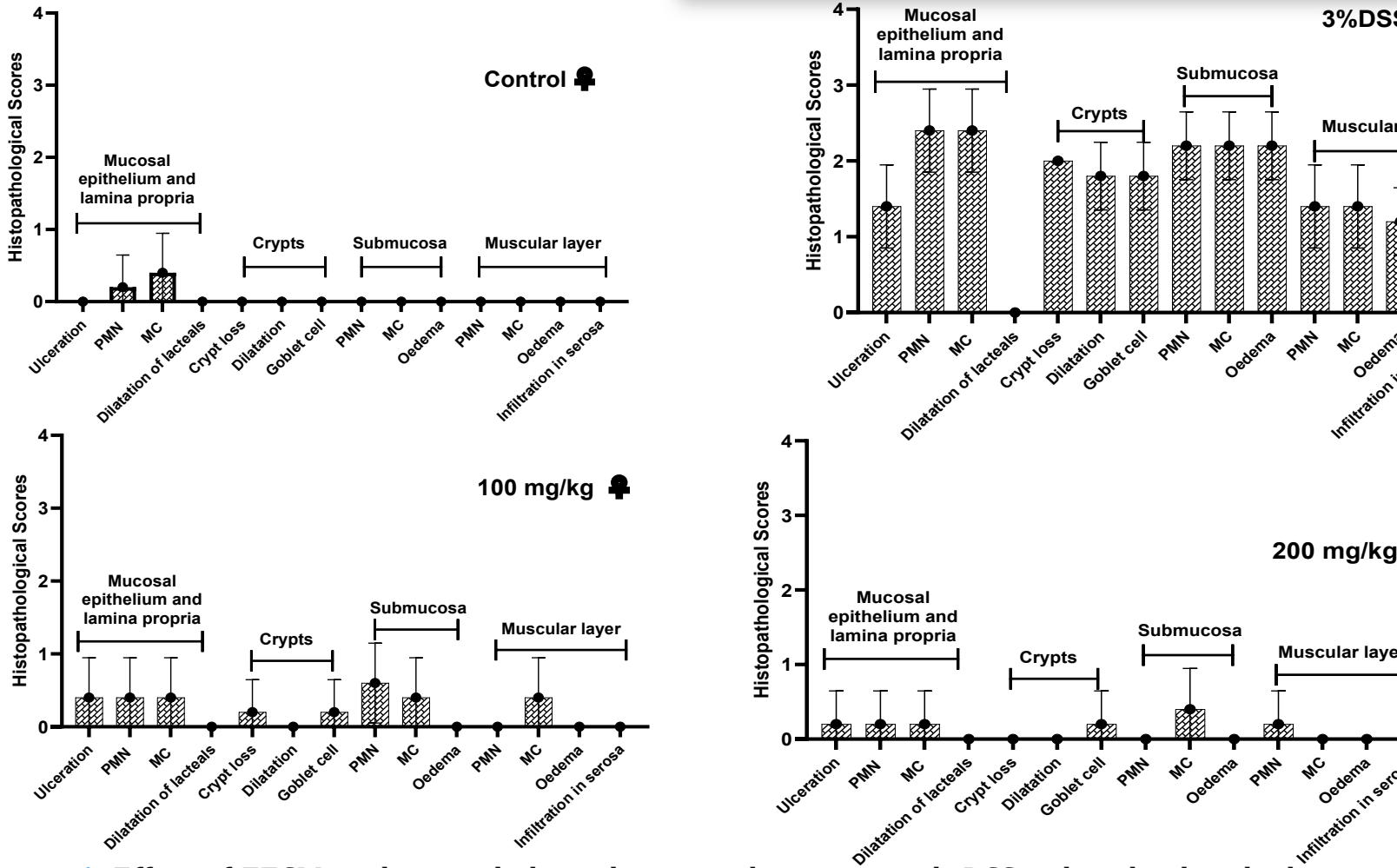


Figure 6. Effect of EECM on histopathological scores: the group with DSS-induced colitis had severe lesions, with treatment EECM resulted in notable improvements.

Conclusion

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This study highlights the therapeutic effect of the ethanolic extract of *Combretum micranthum* (EECM) in alleviating DSS-induced colitis in C57BL/6 mice, highlighting its potential as a natural agent against intestinal inflammation. This is so because richness in bioactive phenolic compounds and its anti-inflammatory, antioxidant, and antibacterial properties.

The extract's efficacy is demonstrated by histologically a reduction lesions of the colon with the treatment, and favorable modulation of biochemical and hematological parameters

Recommendations

Future research should focus on the long-term effects of EECM in the treatment of chronic colitis. These findings also highlight the potential of EECM as a basis for developing phytomedicines or dietary supplements aimed at preventing or treating inflammatory bowel diseases, while modulating the gut microbiota and minimizing the side effects of conventional therapies. These results pave the way for further preclinical and clinical studies to confirm its efficacy and safety.

References

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Thank you for your attention!

Ibrahima Mamadou SALL

Tel : 07756687744

Email : ibrahima.sall@student.usamvcluj.ro

Adress: Calea Mănăstur nr.3-5, 400372 Cluj-Napoca, Romania,

Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania