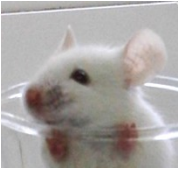


TNBS-INDUCED COLITIS IN RODENTS: PRELIMINARY RESULTS OF A CHRONIC MODEL

Silva, Inês^{1,2}; Mateus, V.^{1,2}, Pinto, R.^{2,3}

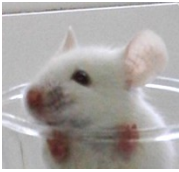
- (1) H&TRC–Health and Technology Research Center, ESTeSL–Lisbon School of Health Technology, Instituto Politécnico de Lisboa, Lisbon, Portugal
- (2) Pharmacology and Translational Research Group, iMED/FFUL, U.Lisboa
- (3) Joaquim Chaves Saúde, Dr Joaquim Chaves Lab Analises Clínicas, Algés, Portugal

Lisbon, 15 July 2019



SUMMARY

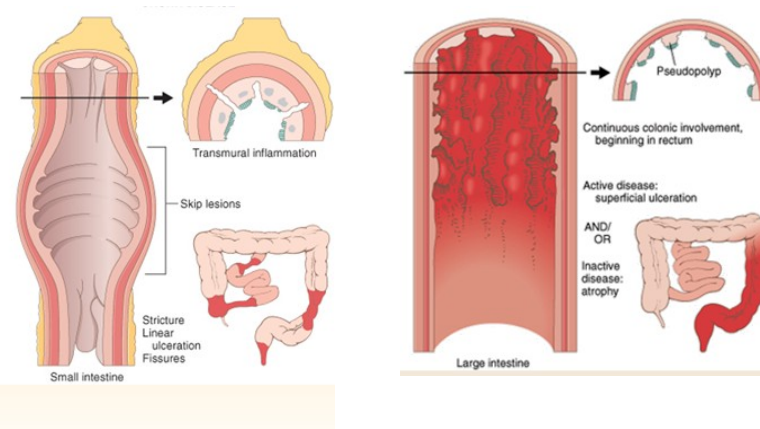
- ✓ Introduction
- ✓ Aim
- ✓ Materials and Methods
- ✓ Results
- ✓ Discussion
- ✓ Conclusion and Future Prospects



INTRODUCTION

INFLAMMATORY BOWEL DISEASE (IBD)

Chronic inflammatory disease of the gastrointestinal (GI) tract characterized by recurrent ulceration.¹



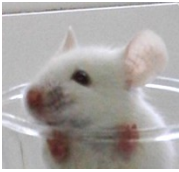
IBD prevalence exceeding 0,5% of the population in westwenized countries.²

IBD manifests into several intestinal and extra-intestinal symptoms,
mainly related to inflammation.³

1. Pithadia A & Jain S. Treatment of inflammatory bowel disease (IBD). Pharmacological Reports 2011;63:629-42.

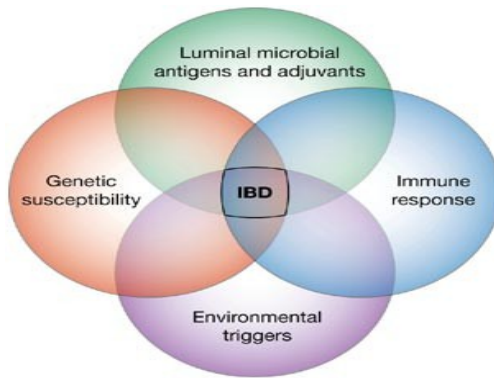
2. Dias, C.C. et al. Hospitalization trends of the Inflammatory Bowel Disease landscape: A nationwide overview of 16 years. Digestive and Liver Disease 2019.

3. Mowat C, et al. Guidelines for the management of inflammatory bowel disease in adults - On behalf of the IBD Section of the British Society of Gastroenterology. Gut 2011.



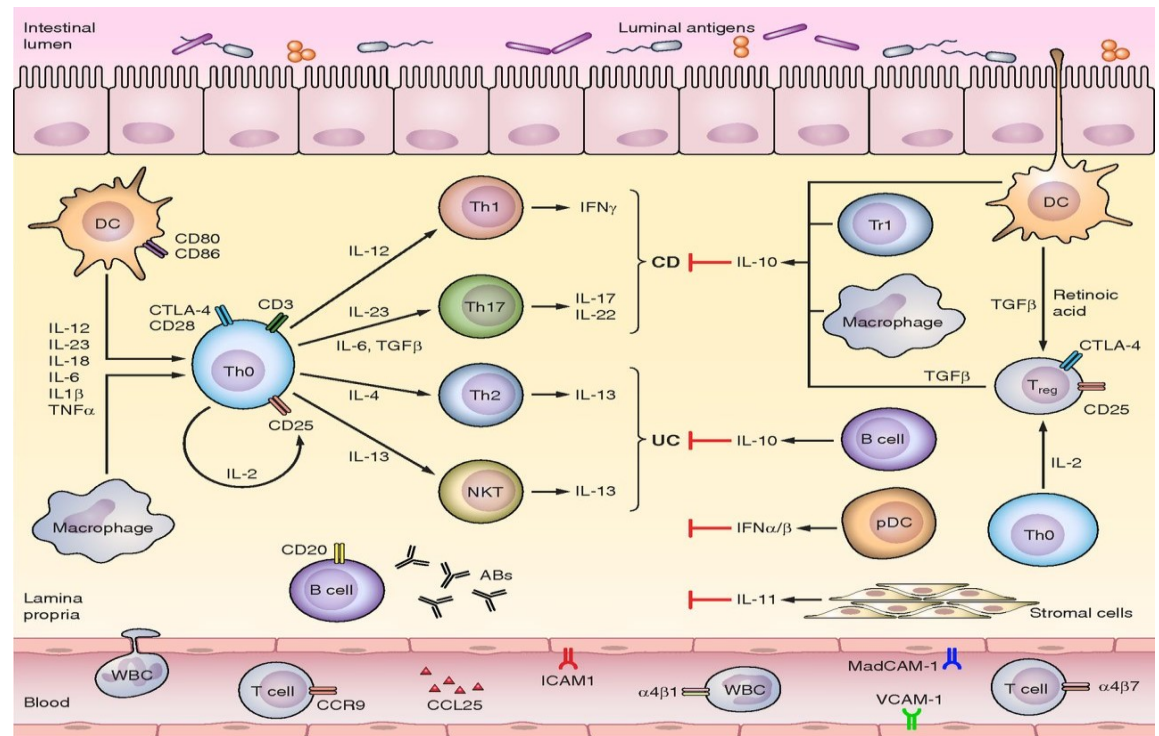
INTRODUCTION

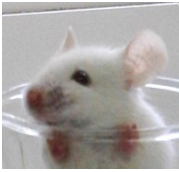
IBD includes Crohn's disease (CD) and Ulcerative colitis (UC)



Crohn Disease
Th₁ and Th₁₇ response

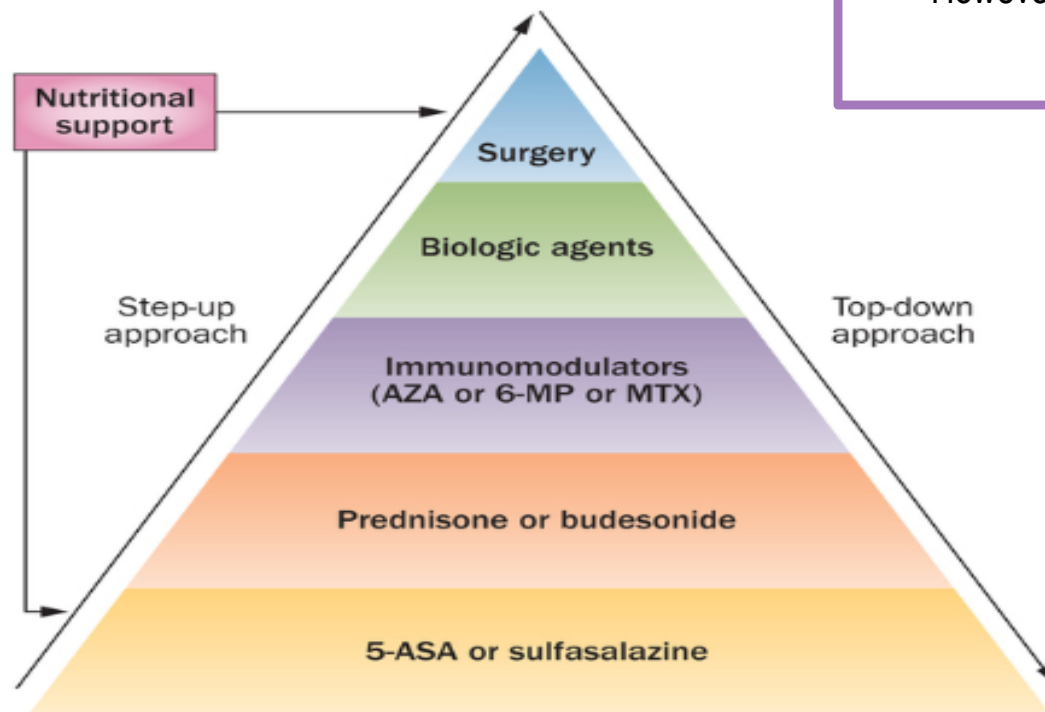
Ulcerative colitis
Th₂ response





INTRODUCTION

TREATMENT OF IBD



Induce and maintain the patient in remission

However no modify or reverse the underlying pathogenic mechanism⁴



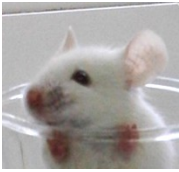
Nonclinical studies
for emerging therapeutic strategies



INTRODUCTION

ANIMAL MODELS

MODEL	SPECIES	METHOD OF INDUCTION	TIME COURSE	DISEASE LOCATION	TYPE OF COLITIS
I. CHEMICALLY INDUCED MODELS					
TNBS	Rats, mice and rabbits	TNBS enema (20-30mg in 30-50% EtOH)	3 days – 8 weeks	Small intestine or colon	Acute and chronic
DSS	Hamsters, mice and rats	2 - 10% DSS feeding	5 days – 15 weeks/	Colon	Acute and chronic
Acetic acid	Rats	1 – 10% acetic acid enema	1 day – 3 weeks	Colon	Acute
Carrageenan	Rats, guinea, pigs and rabbits	Variable oral dosing	1 – 4 weeks	Cecum and colon	Acute and chronic
Indomethacin	Rats	Oral or SC once or twice	< 1 - 8 days	Small intestine	Acute
Oxazalone	Mice and rats	Intracolonic	Rapid	Colon	Acute

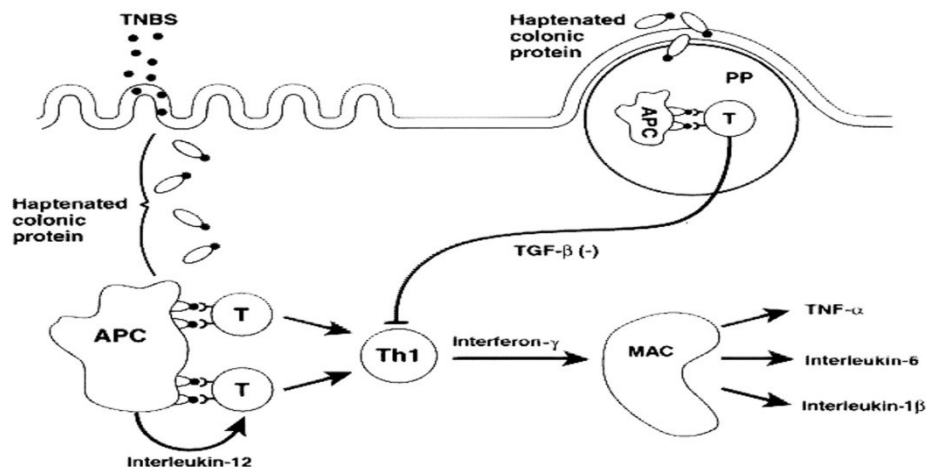


INTRODUCTION

Mechanism of action of TNBS / Ethanol

ETHANOL is proposed to elicit a transient increase in intestinal permeability.⁵

TNBS reaches the subepithelial space and haptenate tissue and microbial proteins.⁶



5. Padua, D. et al. The Role of Neuropeptides in Mouse Models of Colitis. *J Mol Neurosci* 2016, 59(2), 203–210.

6. Wirtz, S. et al. Chemically induced mouse models of acute and chronic intestinal inflammation. *Nature Protocols* 2017, 12(7), 1295–1309.

7. Sartor R. Microbial Influences in Inflammatory Bowel Diseases. *Gastroenterology*. 2008;134(2): 577–94



Aim

Evaluate the efficacy of new drugs in Inflammatory Bowel Disease
through an animal model of TNBS-induced chronic colitis

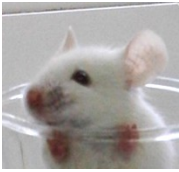


MATERIALS AND METHODS

ANIMALS

Male CD-1 mice, 30-40g in weight and 6-10 weeks of age, were housed in standard polypropylene cages with *ad libitum* access to food and water in the Faculty of Pharmacy Central Animal Facility in the University of Lisbon.

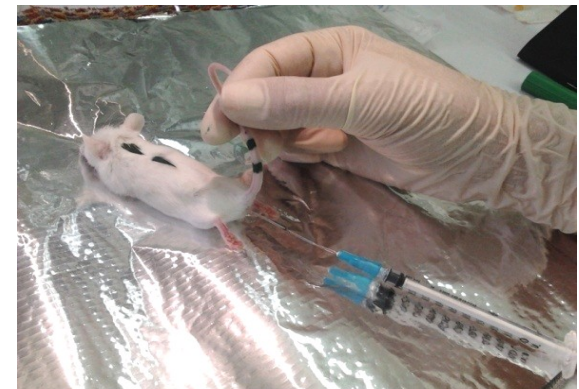


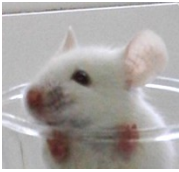


MATERIALS AND METHODS

TNBS-INDUCED COLITIS

- ✓ Mice were left unfed during 24h
- ✓ Mice were anesthetized with Ketamine + Xilazine IP
- ✓ 100 μ l of TNBS (in 50% ethanol) was administered through a catheter inserted into the rectum
- ✓ Mice were kept for 1 min in a Tredelenburg position to avoid reflux





MATERIALS AND METHODS

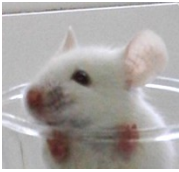
CLINICAL SIGNS AND SYMPTOMS

The animals were observed daily,
monitoring body weight, morbidity, stool consistency and anus appearance.

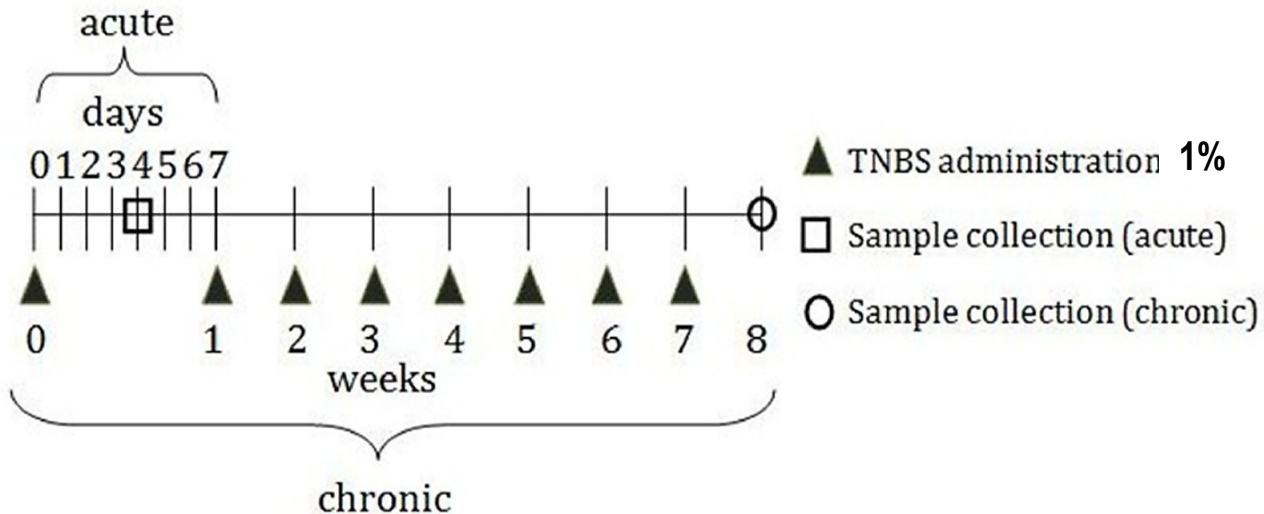
BIOCHEMICAL MARKERS

There were evaluated:

Alkaline phosphatase (ALP), Creatinine, Urea,
Alanine aminotransferase (ALT), Fecal Hemoglobin, Tumor Necrosis Factor (TNF- α),



TNBS SCHEME ADMINISTRATION



PRELIMINARY RESULTS



TNBS SCHEME ADMINISTRATION

EXPERIENCE 1

TNBS 1%



Day 0

Day 1

Day 2

Day 3

Day 4

Day 5

Day 6

Day 7

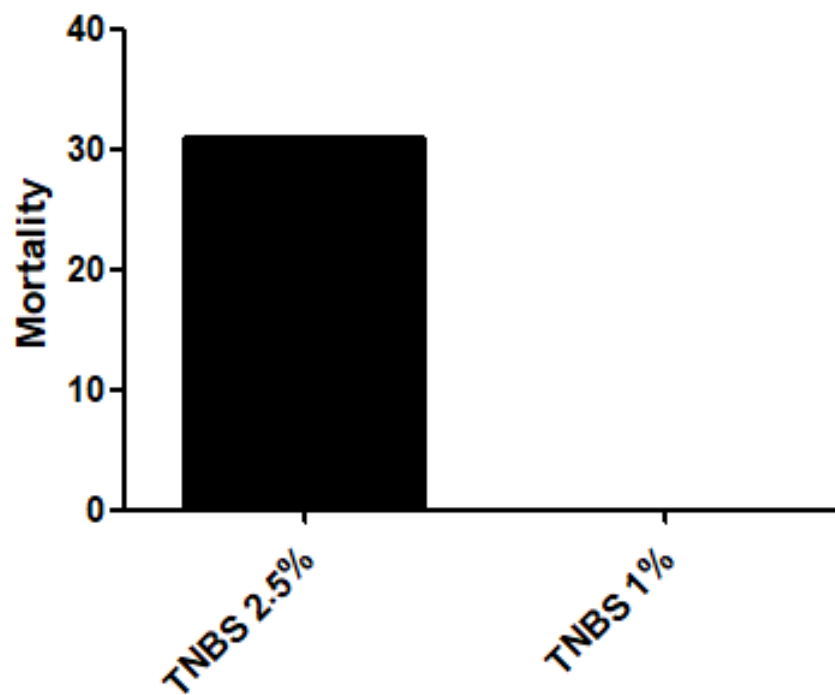


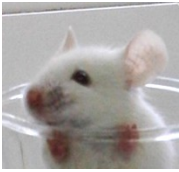
Euthanasia



RESULTS EXPERIENCE 1

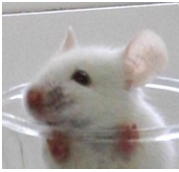
MORTALITY RATE





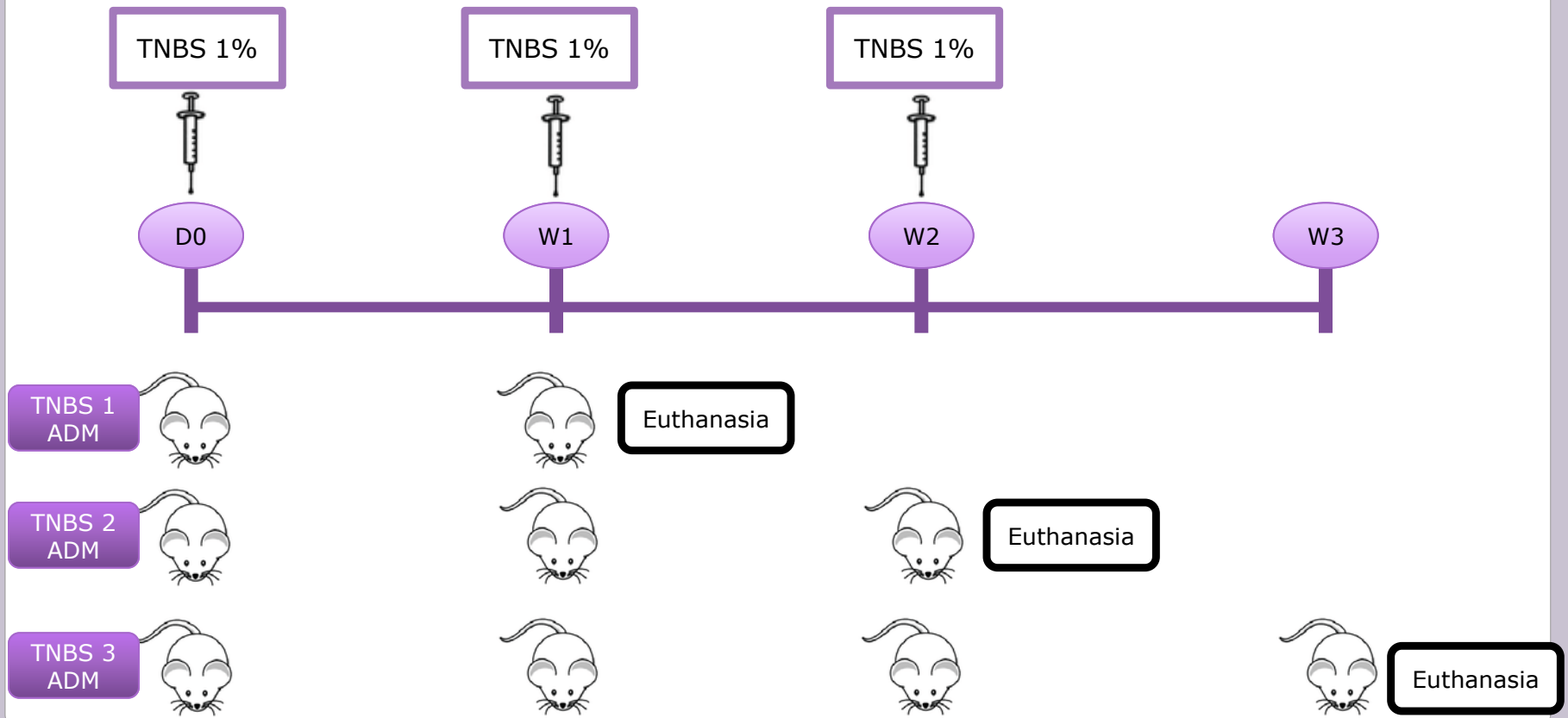
DISCUSSION EXPERIENCE 1

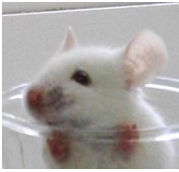
- Increased mortality in TNBS 2,5%
- 0% mortality in TNBS 1%
- Proceed the experimental colitis induction model with TNBS 1% in order to achieve a scheme of multiple administrations with low mortality



TNBS SCHEME ADMINISTRATION

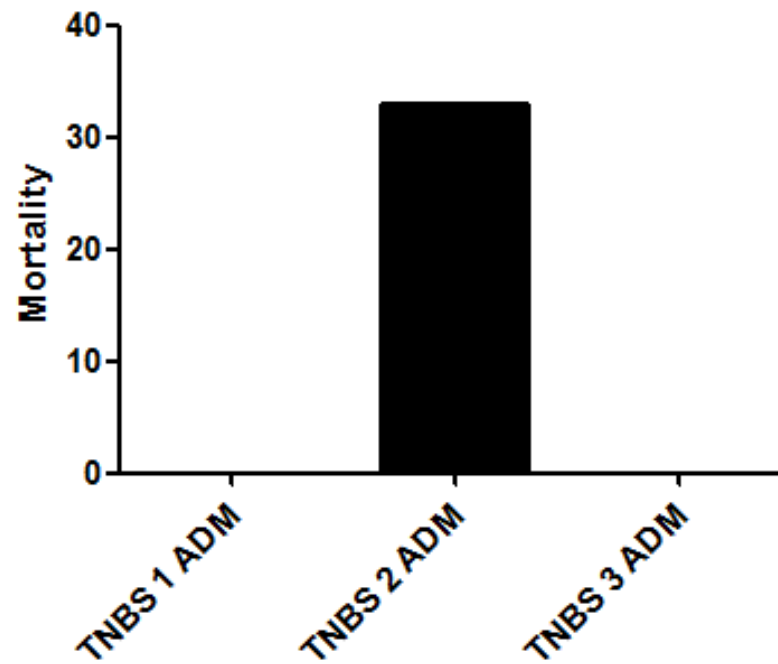
EXPERIENCE 2





RESULTS EXPERIENCE 2

MORTALITY RATE



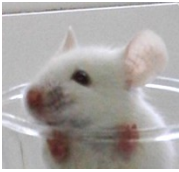


RESULTS EXPERIENCE 2

CLINICAL SIGNS AND SYMPTOMS

MORBIDITY AND STOOL CONSISTENCY

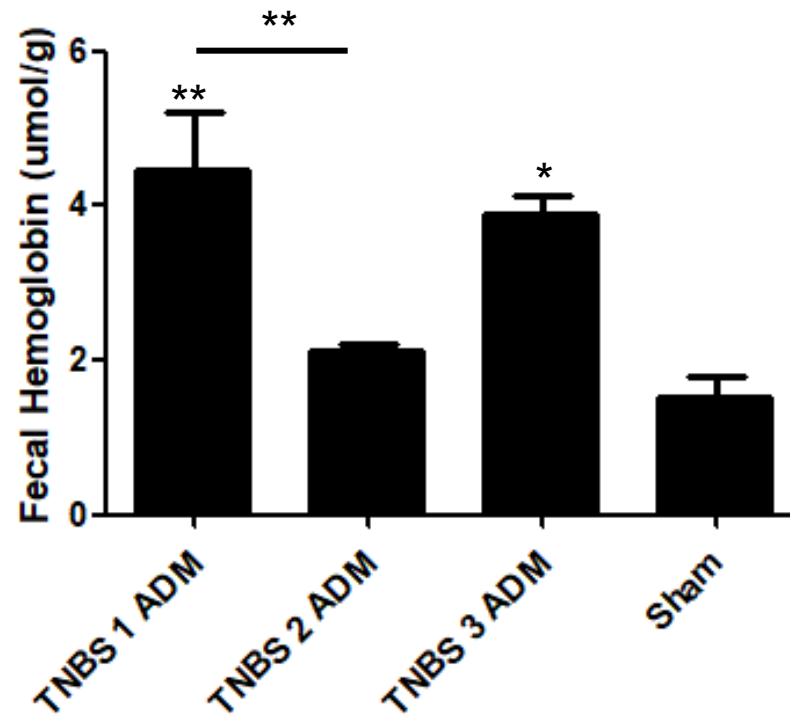
GROUPS	MORBIDITY	STOOL CONSISTENCY
TNBS 1 ADM	Mild morbidity	Soft stools
TNBS 2 ADM	High morbidity	Soft stools
TNBS 3 ADM	Soft morbidity	Soft stools
SHAM GROUP	No alterations	



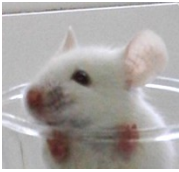
RESULTS EXPERIENCE 2

BIOCHEMICAL MARKER

FECAL HEMOGLOBIN



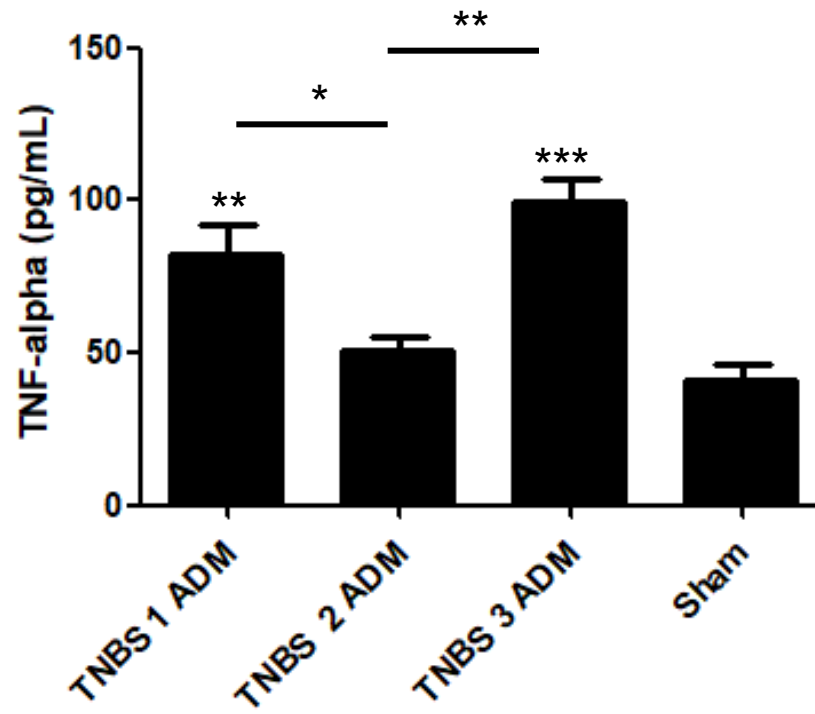
Legend: * $p < 0.05$; ** $p < 0.01$;



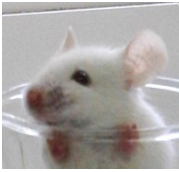
RESULTS EXPERIENCE 2

BIOCHEMICAL MARKER

TNF - ALPHA



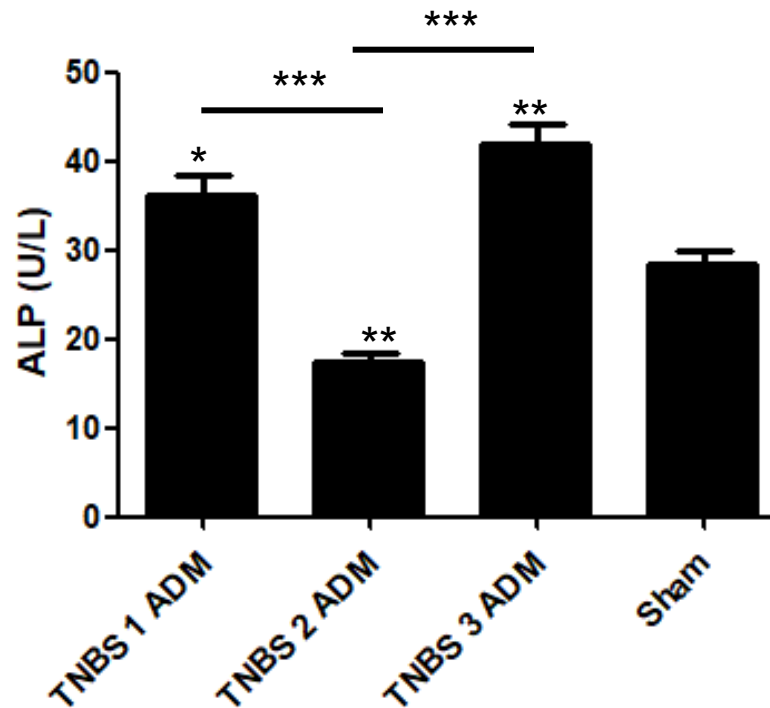
Legend: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$



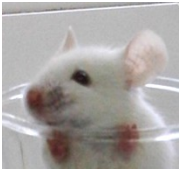
RESULTS EXPERIENCE 2

BIOCHEMICAL MARKER

ALP



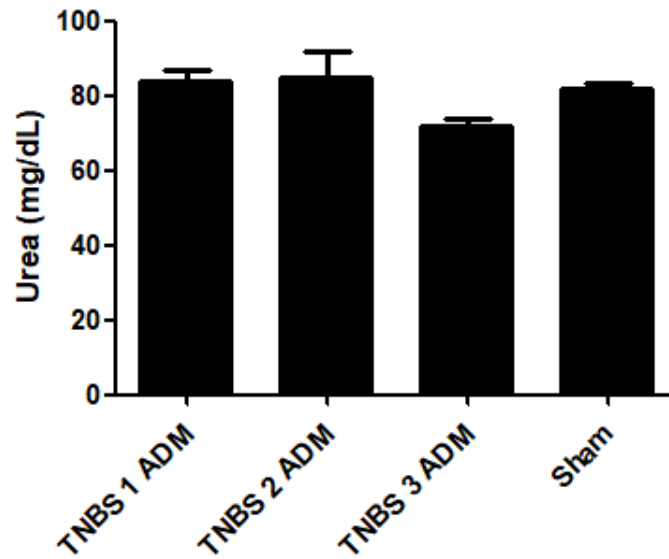
Legend: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$



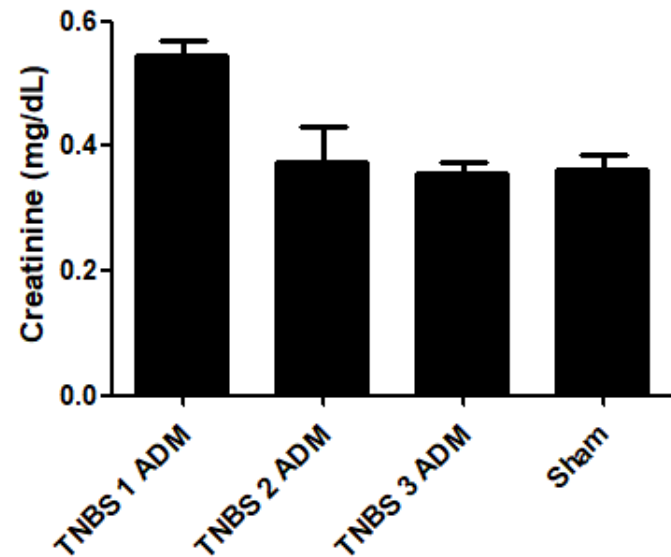
RESULTS EXPERIENCE 2

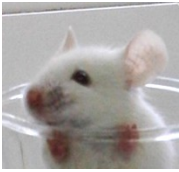
RENAL MARKERS

UREA



CREATININE

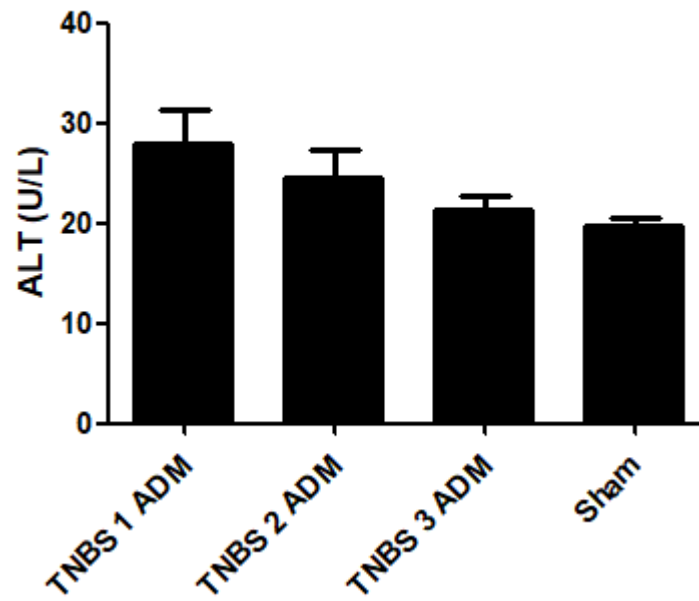


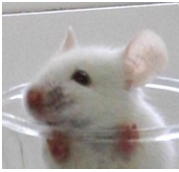


RESULTS EXPERIENCE 2

HEPATIC MARKER

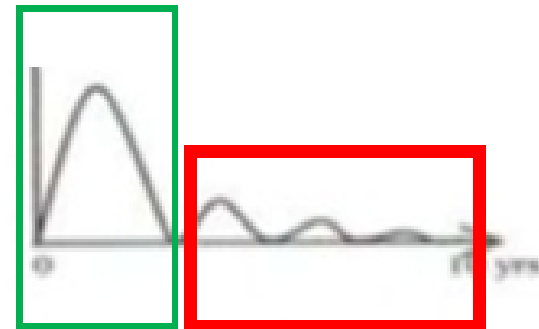
ALT

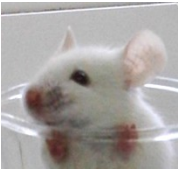




DISCUSSION EXPERIENCE 2

- Increased signs and symptoms of the disease, values of Fecal Hemoglobin, TNF-alpha and ALP in TNBS 1 ADM e 3 ADM groups compared with Sham group
 - These manifestations are compatible with a correct induction of colitis⁸
- Presence of marked morbidity and mortality in TNBS 2 ADM
- TNBS 3 ADM mice have a healthy appearance and simultaneously presence of inflammatory biomarkers





CONCLUSION

These findings seem to propose

3 administrations 1% TNBS for the induction of chronic colitis

Increase

frequency of administrations

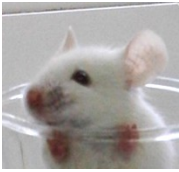
&

number of animals per group



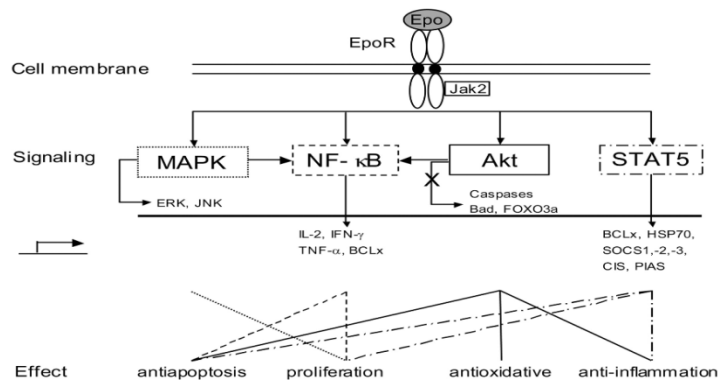
to confirm our preliminary data



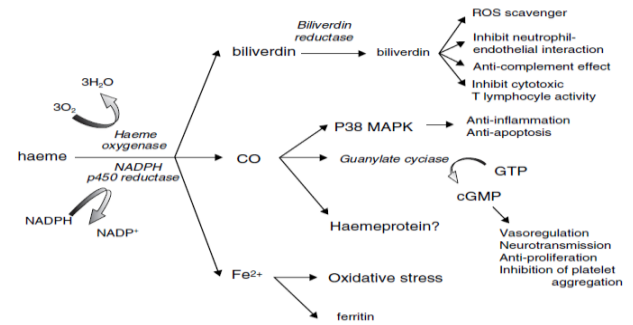


FUTURE PROSPECTS

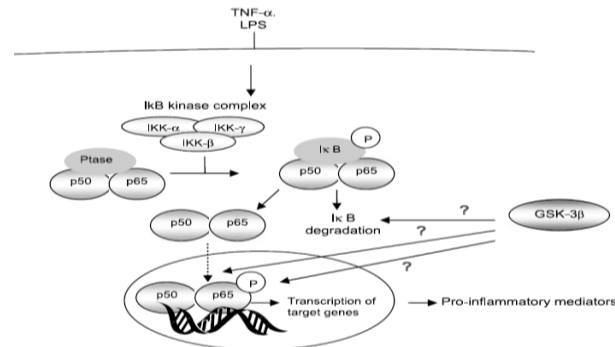
Erythropoietin (EPO)



Hemin



Thiadiazolidinone-8 (TDZD-8)



THANK YOU!



Acknowledgments:

**JOAQUIM
CHAVES**
SAÚDE **Análises
Clínicas**



Grant: IDI&CA COLITIS 2019 - Pharmacological modulation of inflammation associated with inflammatory bowel disease: a model of chronic experimental colitis