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PRELIMINARY STUDY TOWARDS A CHRONIC EXPERIMENTAL MODEL TO STUDY INFLAMMATORY BOWEL DISEASE

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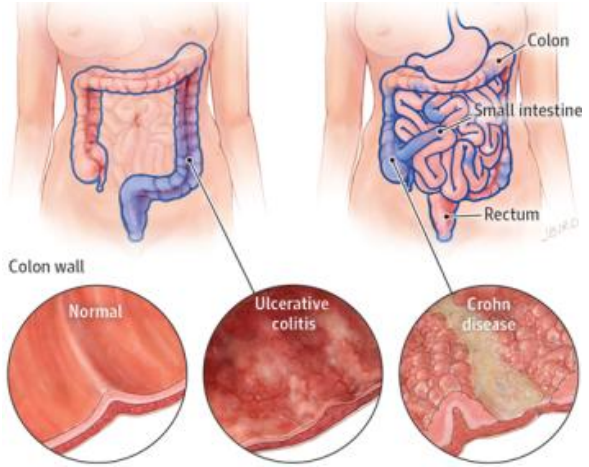
INTRODUCTION

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 westernized countries.[2]

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

1. Pithadia A & Jain S. Pharmacological Reports. 2011
2. Dias, C.C. et al. Digestive and Liver Disease. 2019
3. Mowat C, et al. Gut 2011

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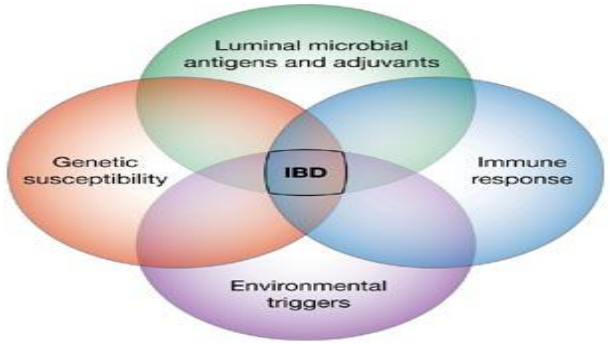
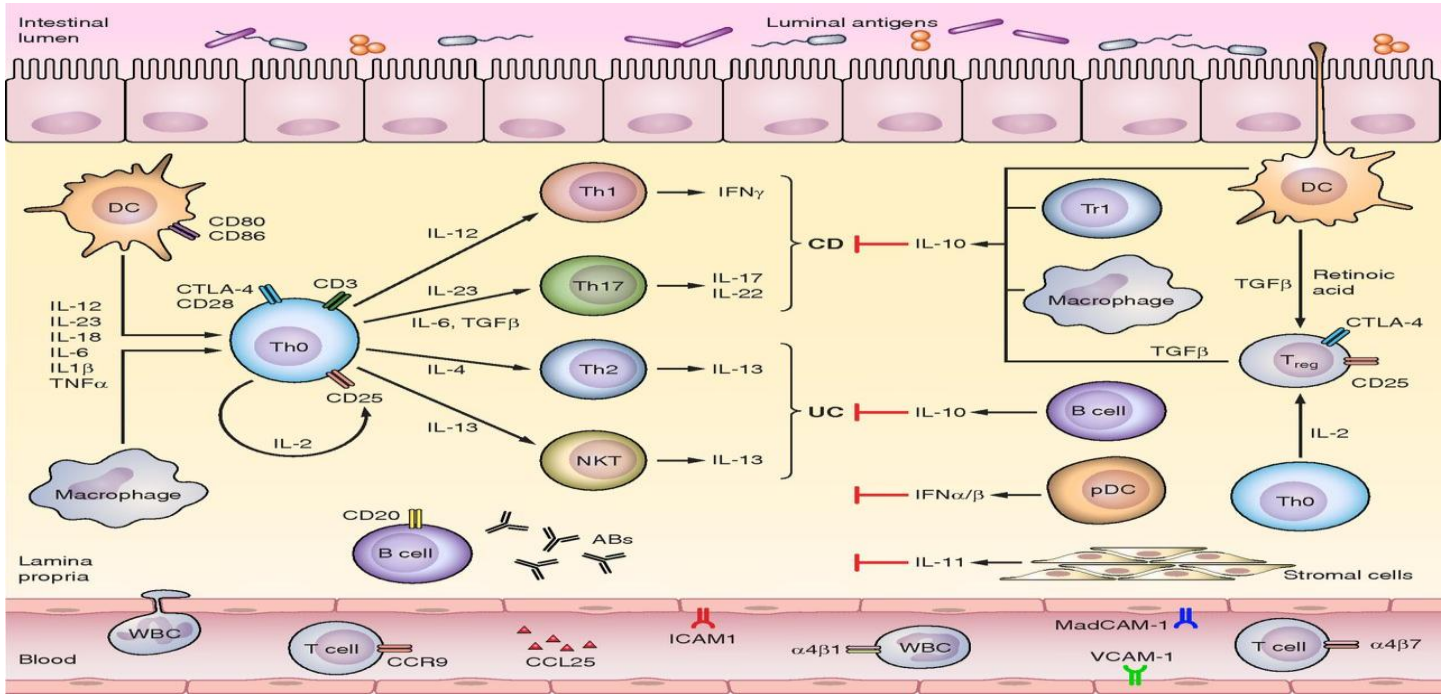
IBD includes Crohn's disease (CD) and Ulcerative colitis (UC)



Chron Disease
Th_1 and Th_{17} response

Ulcerative colitis
Th_2 response



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Valatas V, et al. Am J Physiol Gastrointest Liver Physiol. 2013
Sartor B. Nature Clinical Practice Gastroenterology & Hepatology . 2006

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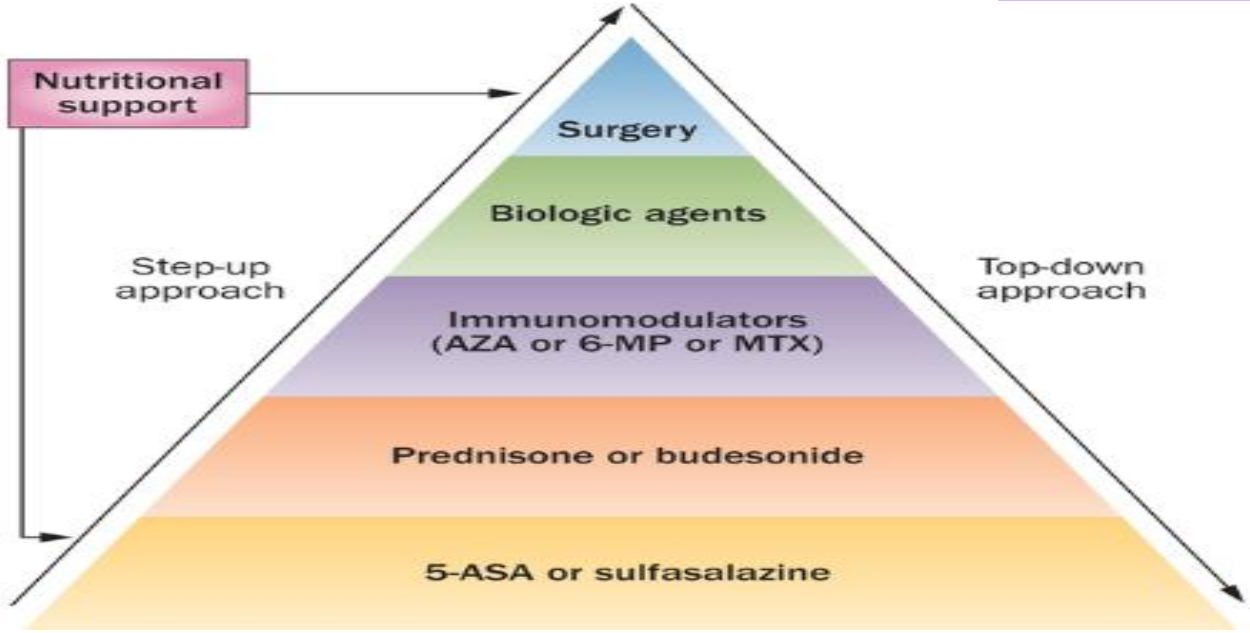
INTRODUCTION

TREATMENT OF IBD



Induce and maintain the patient in remission

However do not modify or reverse the underlying pathogenic mechanism[4]

Nonclinical studies

for emerging therapeutic strategies

4. Wilhelm, S. M., & Love, B. L. *Clinical Pharmacist 2017*
Adaptado de Katzung & Trevor's Pharmacology

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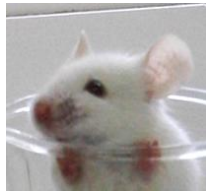

ANIMAL MODELS

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

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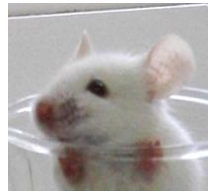
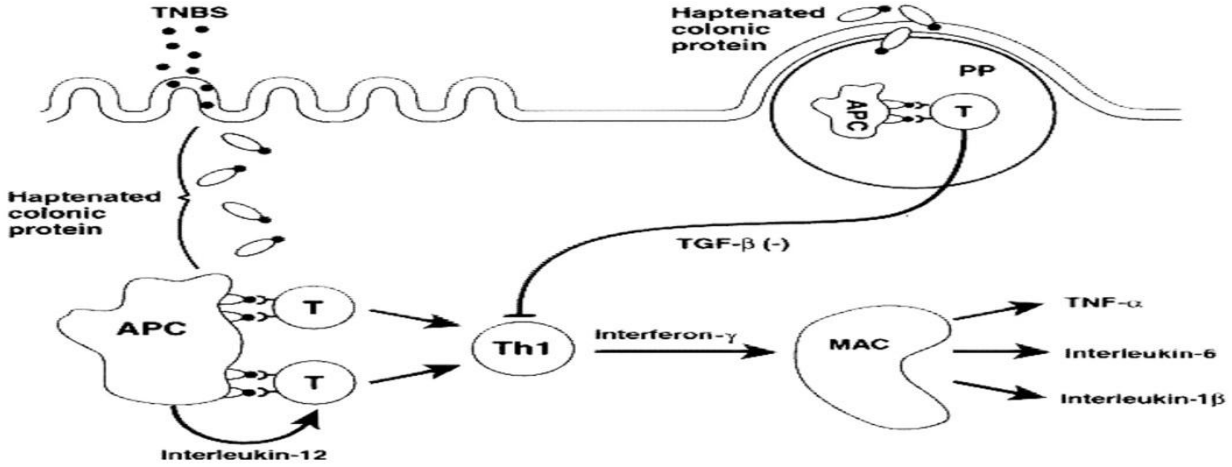


Mechanism of action of TNBS / Ethanol

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. *J Mol Neurosci* 2016
6. Wirtz, S. et al. *Nature Protocols 2017*
7. Sartor R. Gastroenterology. 2008

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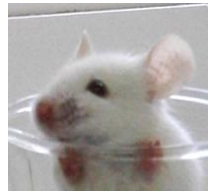

AIM

Evaluate the eficacy of new drugs in Inflammatory Bowel Disease

through an animal model of TNBS-induced chronic colitis

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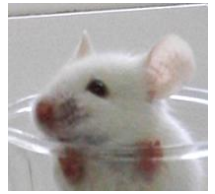
MATHERIAL AND METHODS



ANIMALS

Male CD-1 mice, 20-30g 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.

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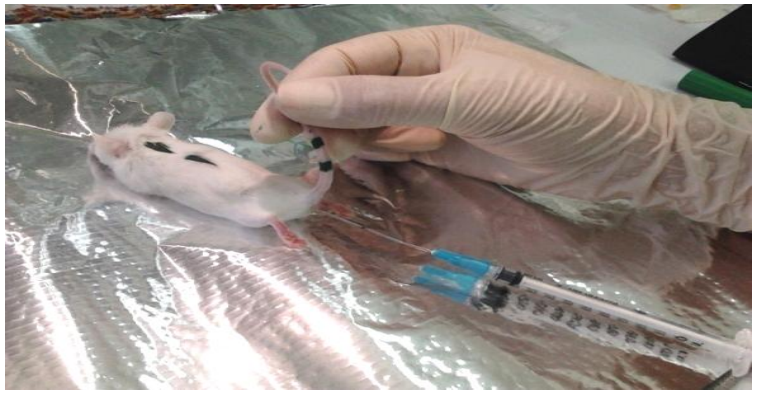
MATHERIAL AND METHODS

TNBS-INDUCED COLITIS

- Mice were left unfed during 24h
- Mice were anesthetized with Ketamine + Xilazine IP
- 100µl 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



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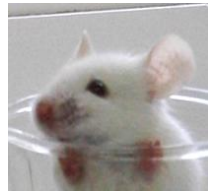
MATHERIAL AND METHODS



CLINICAL SIGNS

The animals were observed daily, monitoring body weight and mortality

BIOCHEMICAL MARKERS

There were evaluated:

Fecal Hemoglobin , Alkaline phosphatase (ALP), Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-10, Creatinine, Urea, Alanine aminotransferase (ALT)

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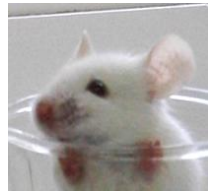
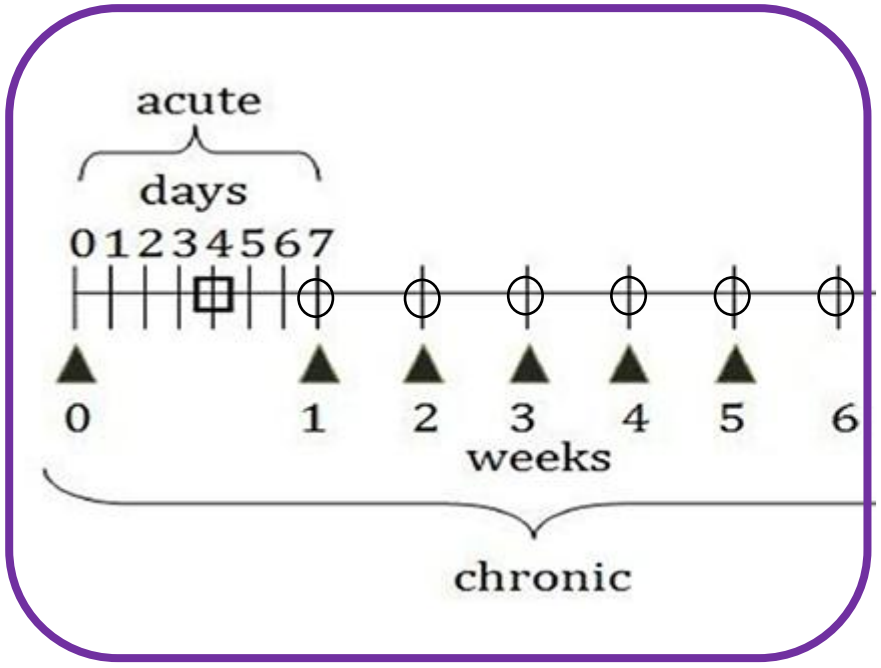
TNBS SCHEME ADMINISTRATION





▲ TNBS administration 1%

□ Sample collection (acute)

○ Sample collection (chronic)

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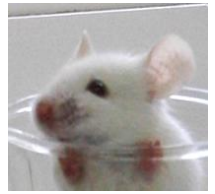
TNBS SCHEME ADMINISTRATION



TNBS GROUPS

T1 GROUP	T2 GROUP	T3 GROUP	T4 GROUP	T5 GROUP	T6 GROUP
N=15	N=15	N=15	N=15	N=15	N=15
[TNBS] 1% Euthanized day 7	[TNBS] 1% Euthanized day 14	[TNBS] 1% Euthanized day 21	[TNBS] 1% Euthanized day 28	[TNBS] 1% Euthanized day 35	[TNBS] 1% Euthanized day 42

CONTROL GROUPS

E GROUP	S GROUP
N=15	N=15
Ethanol 50%	NaCl 0.9%

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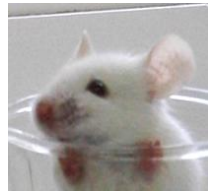
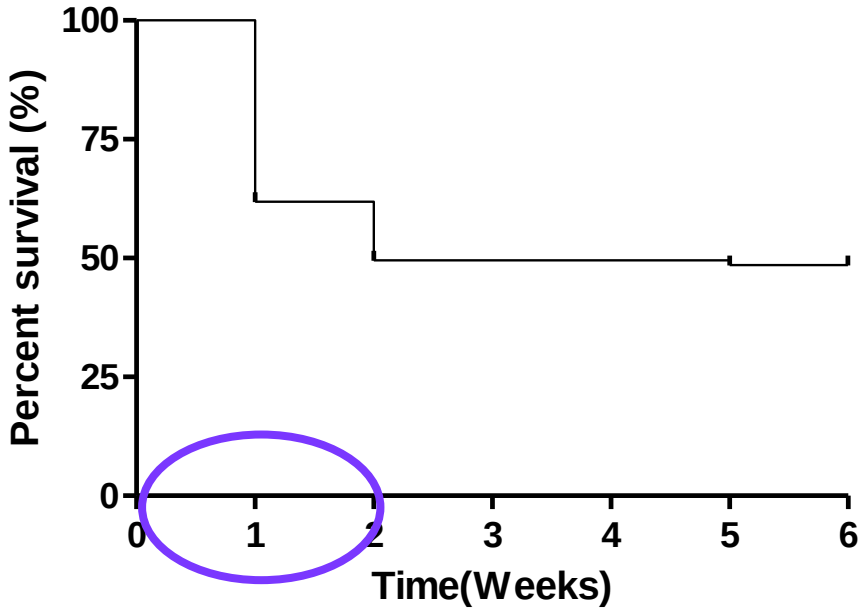
RESULTS



CLINICAL SIGNS

SURVIVAL RATE



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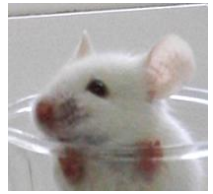
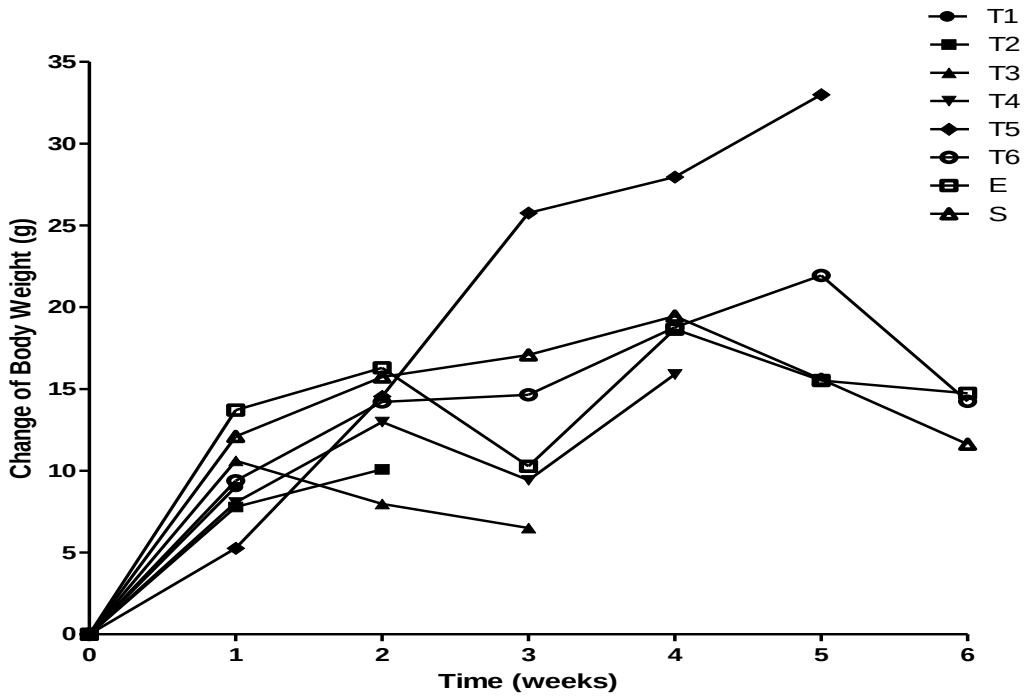
RESULTS



CLINICAL SIGNS

BODY WEIGHT



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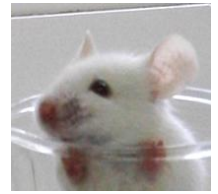
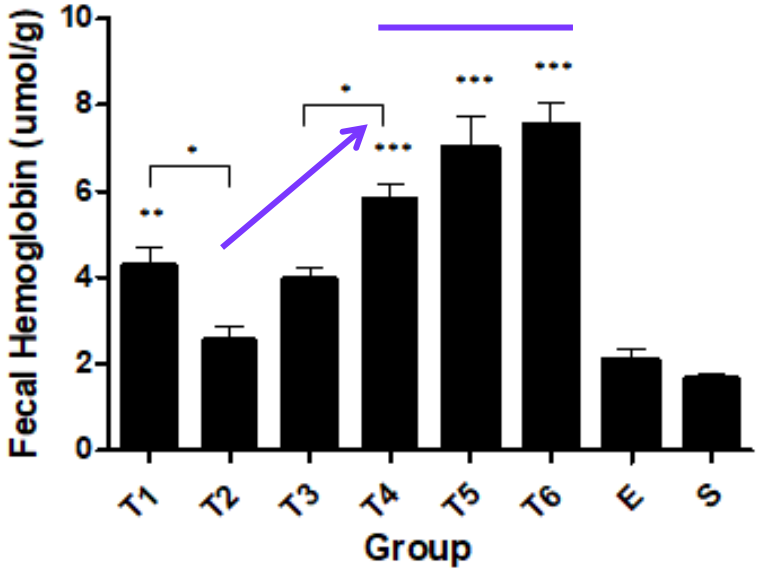
RESULTS



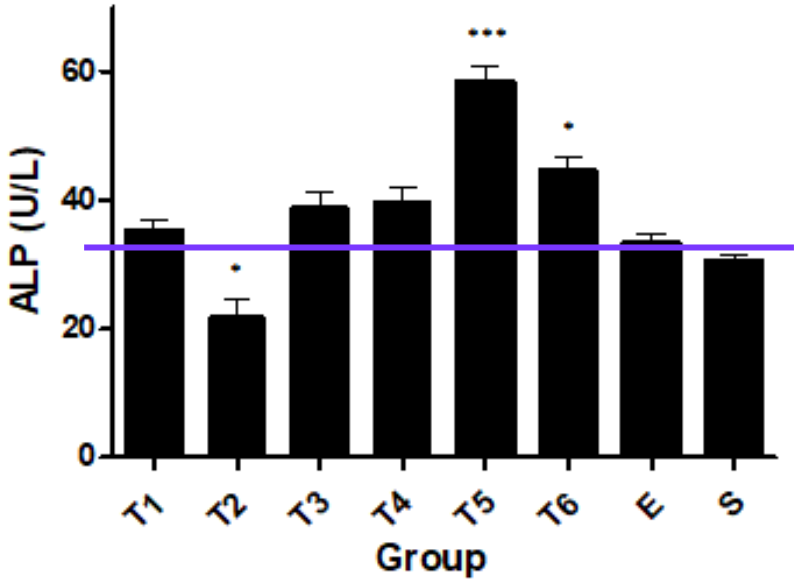
BIOCHEMICAL MARKERS

FECAL HEMOGLOBIN



Legend: One-way ANOVA and Tukey's post hoc test, * p<0.01 between groups; ** p<0.001 compared with ethanol group; *** p<0.0001 compared with ethanol group.

ALP



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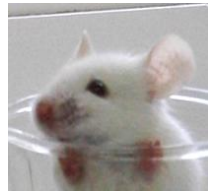
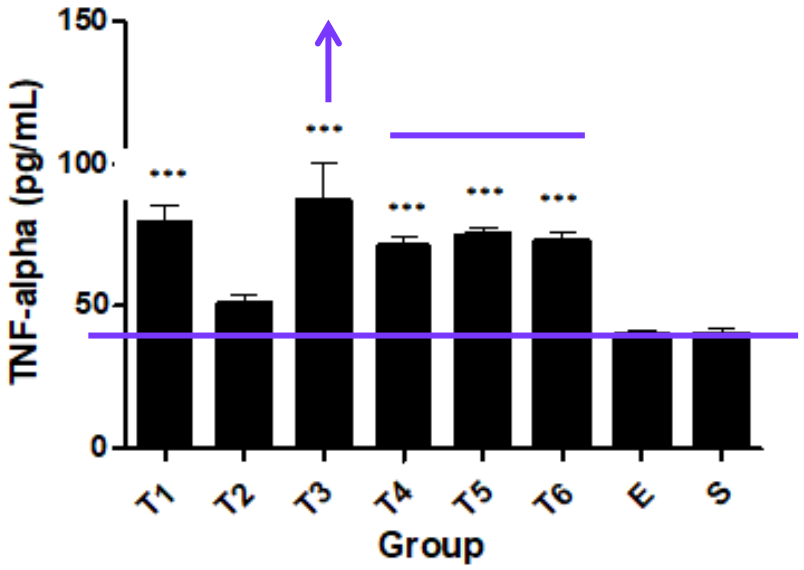
RESULTS



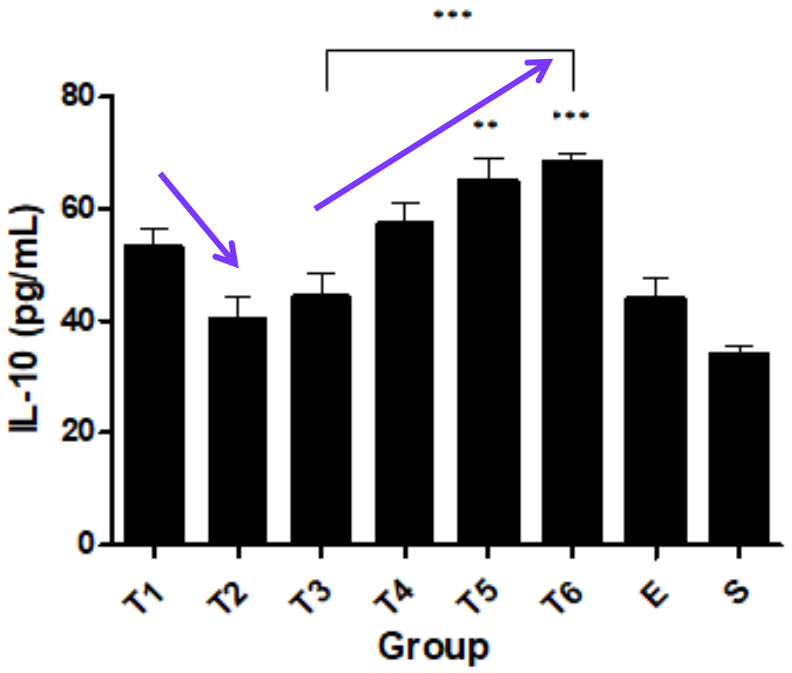
BIOCHEMICAL MARKERS

TNF - ALPHA



Legend: One-way ANOVA and Tukey's post hoc test, ***p<0.0001 compared with ethanol group or between groups.

IL-10



Legend: One-way ANOVA and Tukey's post hoc test; *** p<0.0001 compared with sham group or between groups, **p<0.001 compared with sham group.

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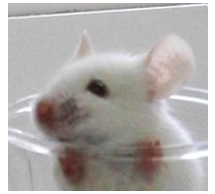
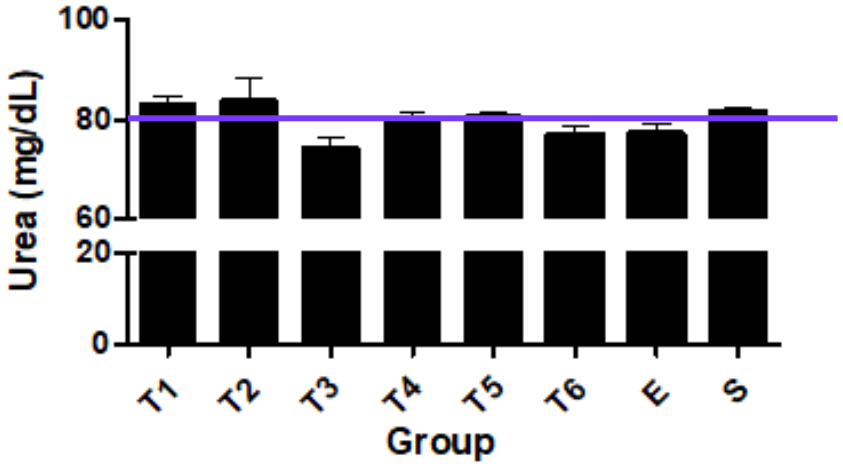
RESULTS

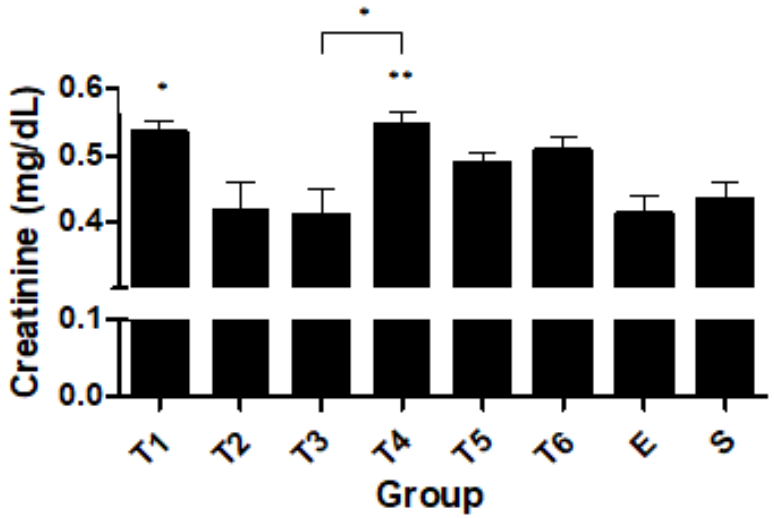


RENAL MARKERS

UREA



CREATIININE



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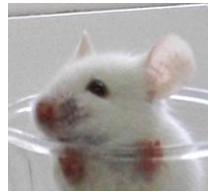
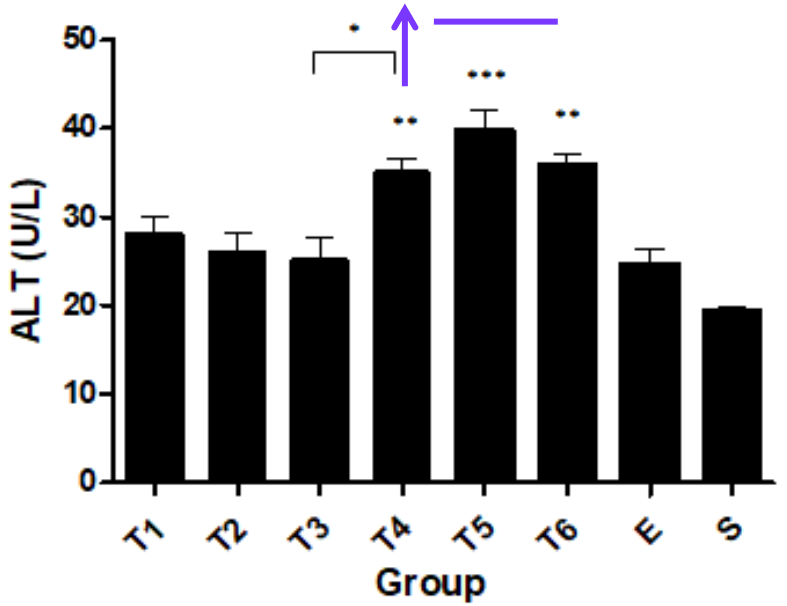
RESULTS



HEPATIC MARKER

ALT



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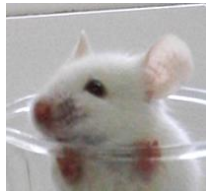
DISCUSSION / CONCLUSIONS



- Development of a TNBS-induced colitis model
  - Increased signs of the disease
  - Increased values of Fecal Hemoglobin, TNF-α and ALP in TNBS groups compared with Sham group
  - These manifestations are compatible with a correct induction of colitis[8,9]

- Acute phase of the disease in the first week
  - Peak of signs of the disease
  - Decreased of survival rate
  - Congruent with other research groups[8.10,11]

8. Morris G, et al.Gastroenterology 1989
9. Bang, B. & Lichtenberger, L.M. Curr. Protoc. Pharmacol. 2016
10. Neurath, M.F. J. Exp. Med. 1995
11. Elson, C.O, et al. J. Immunol. 1996

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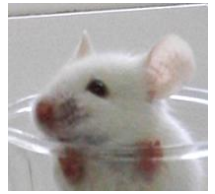
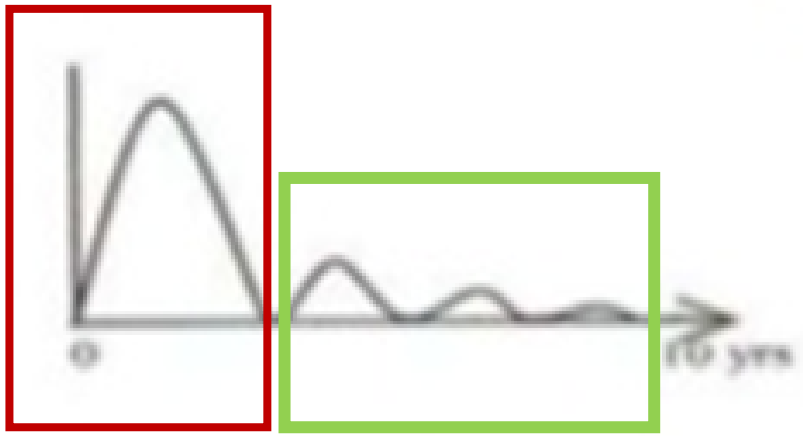
DISCUSSION / CONCLUSIONS



- Chronic phase of the disease
  - Multiple TNBS administrations[12,13]
  - Presence of signs of the disease
  - Stabilization and maintenance of values from week 4 until week



These findings seem to propose

4 administrations 1% TNBS for the induction of chronic colitis

Hystopatological analysis

12. Tomassello, G., et al. Acta Biomed. 2015
13. Elson, C.O., et al. J. Immunol. 1996

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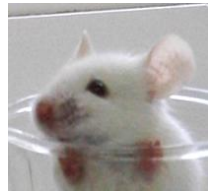
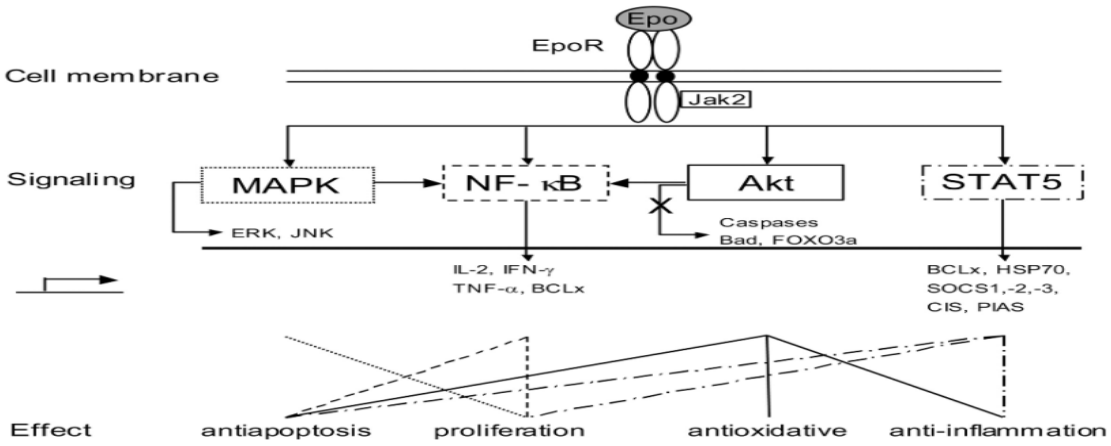
Future Prospects



Erythropoietin (EPO)



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