

PRELIMINARY RESULTS OF AN ANIMAL MODEL VALIDATION OF CHRONIC EXPERIMENTAL COLITIS

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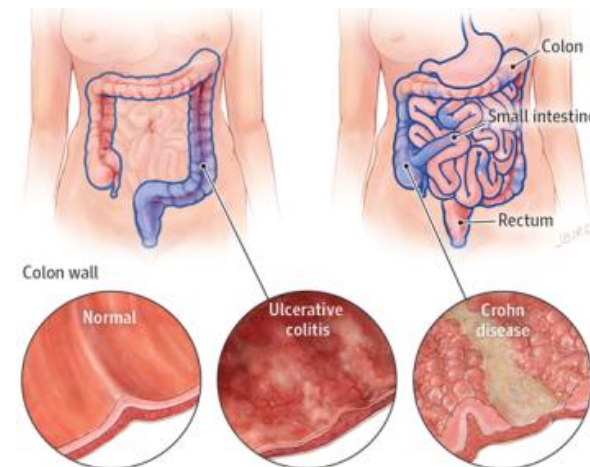
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SUMMARY

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

INFLAMMATORY BOWEL DISEASE (IBD)

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



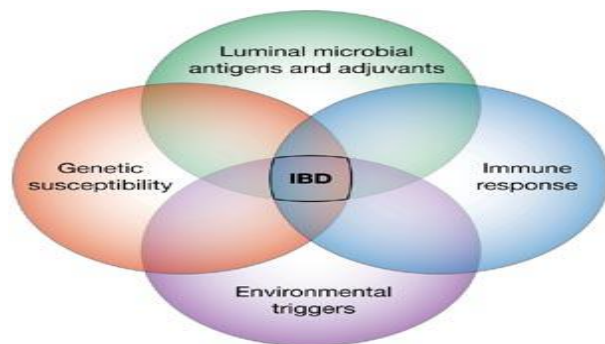
IBD prevalence exceeding 0,5% of the population in westwenized countries.²



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

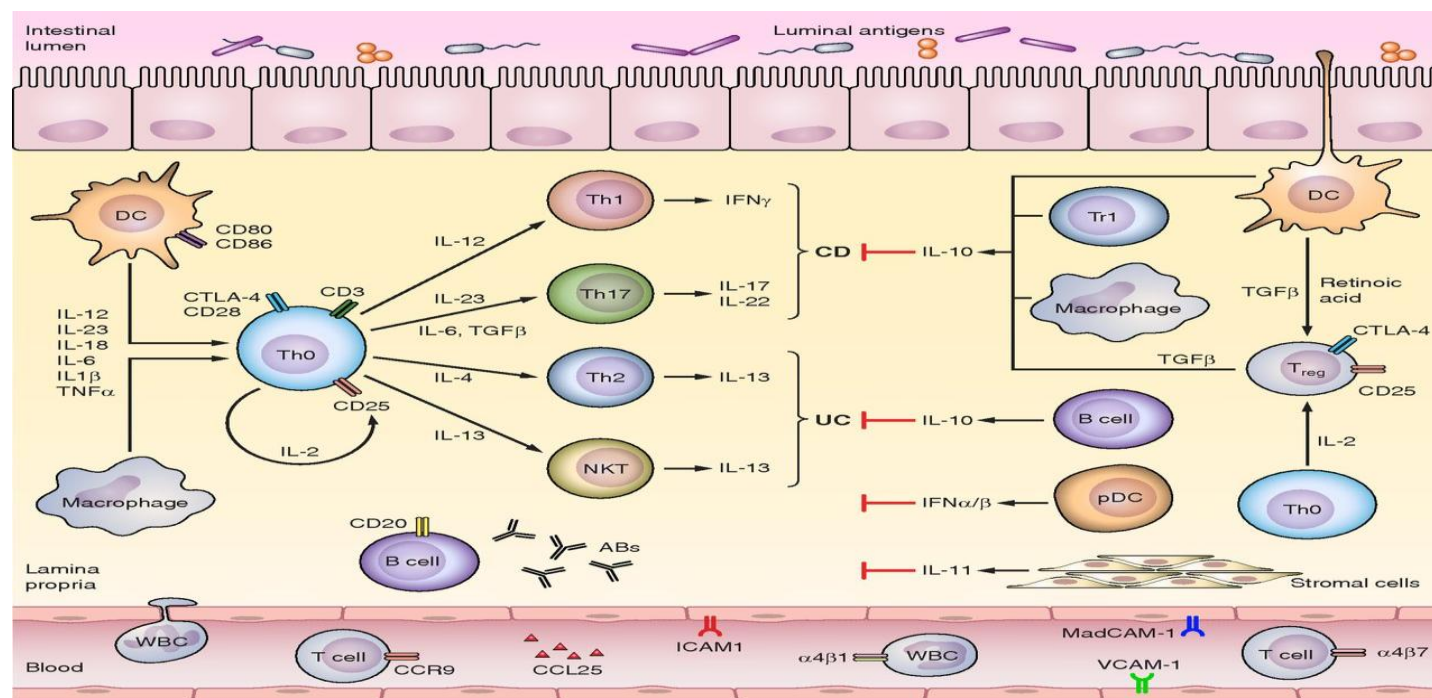
1. Pithadia A & Jain S. Pharmacological Reports. 2011
2. Dias, C.C. et al. Digestive and Liver Disease. 2019
3. Mowat C, et al. Gut 2011

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

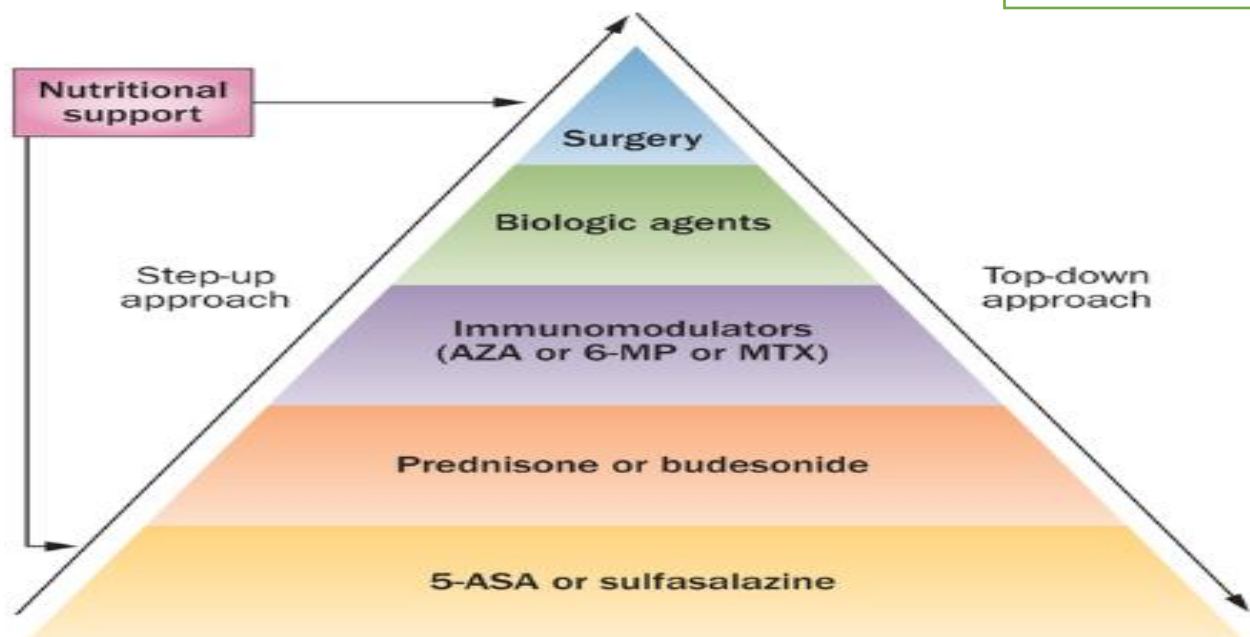


Chron Disease
Th₁ and Th₁₇ response

Ulcerative colitis
Th₂ response



TREATMENT OF IBD



Induce and maintain the patient in remission

However do not modify or reverse the underlying pathogenic mechanism⁴



Nonclinical studies
for emerging therapeutic strategies

ANIMAL MODELS

MODEL	SPECIES	METHOD OF INDUCTION	TIME COURSE	DISEASE LOCATION	TYPE OF COLITIS
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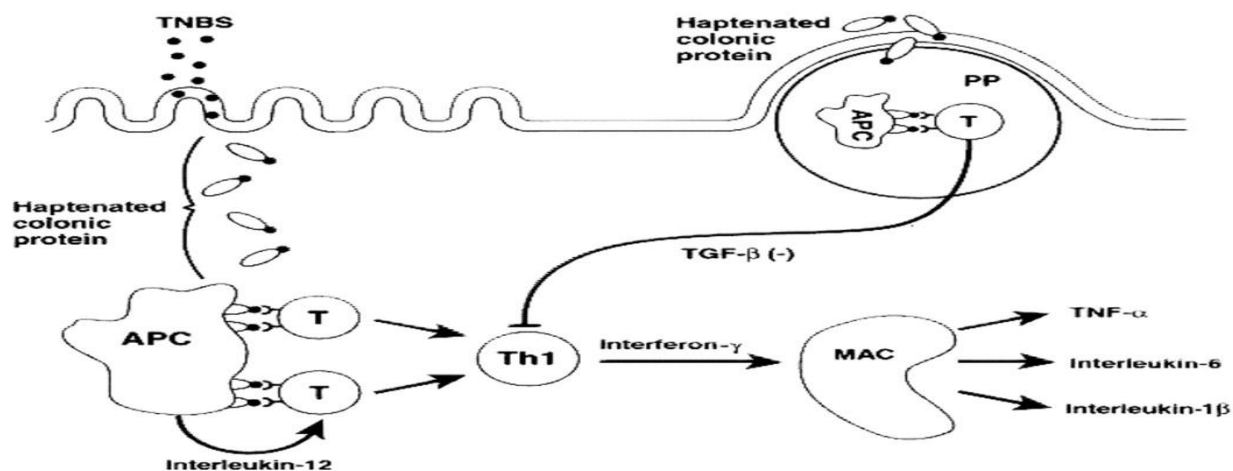
I. CHEMICALLY INDUCED MODELS

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

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

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MATERIAL AND METHODS

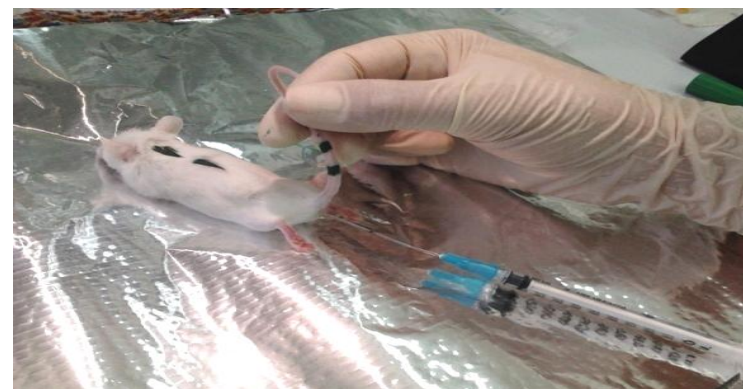
ANIMALS

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.



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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MATERIAL AND METHODS

CLINICAL SIGNS AND MACROSCOPIC ASSESSMENT OF COLITIS

The animals were observed daily, monitoring body weight and mortality, and weekly, basing on the presence of hyperemia, ulcers and its extension in the colon

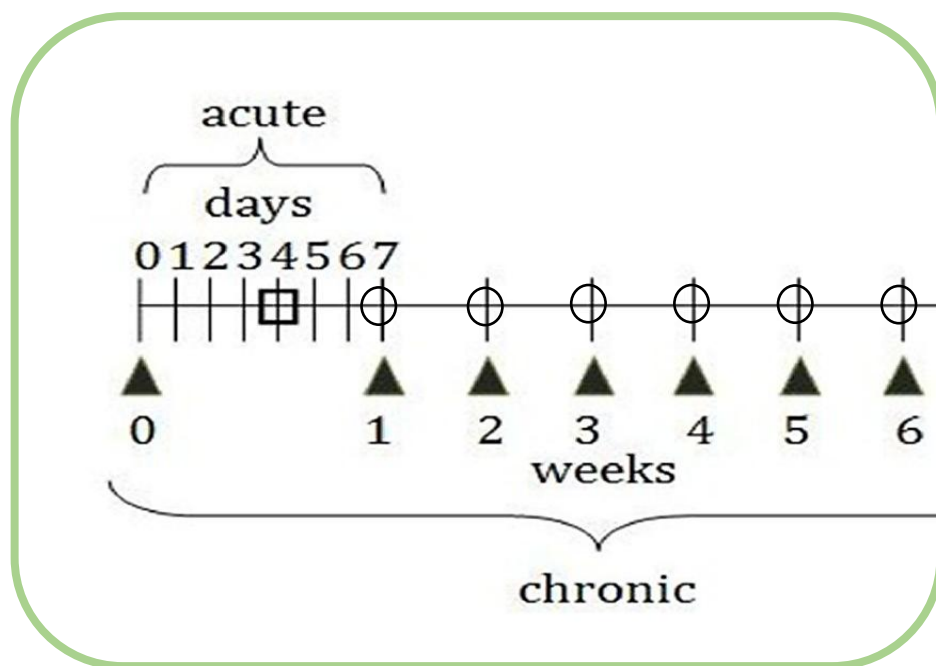
BIOCHEMICAL MARKERS

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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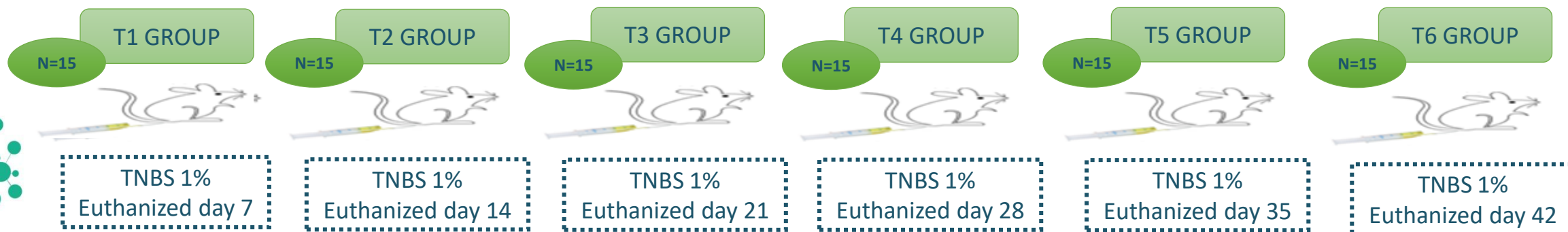
TNBS SCHEME ADMINISTRATION



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TNBS SCHEME ADMINISTRATION

TNBS GROUPS



CONTROL GROUPS

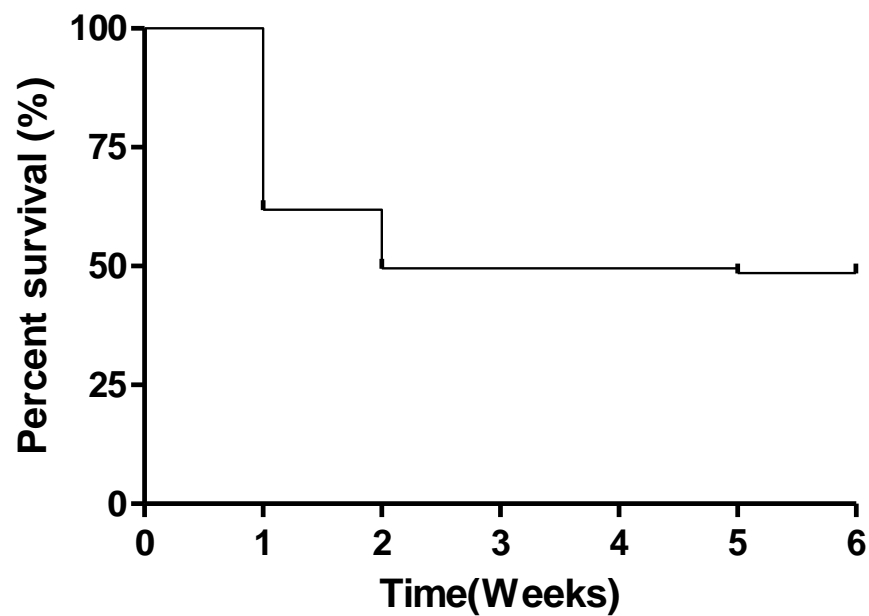


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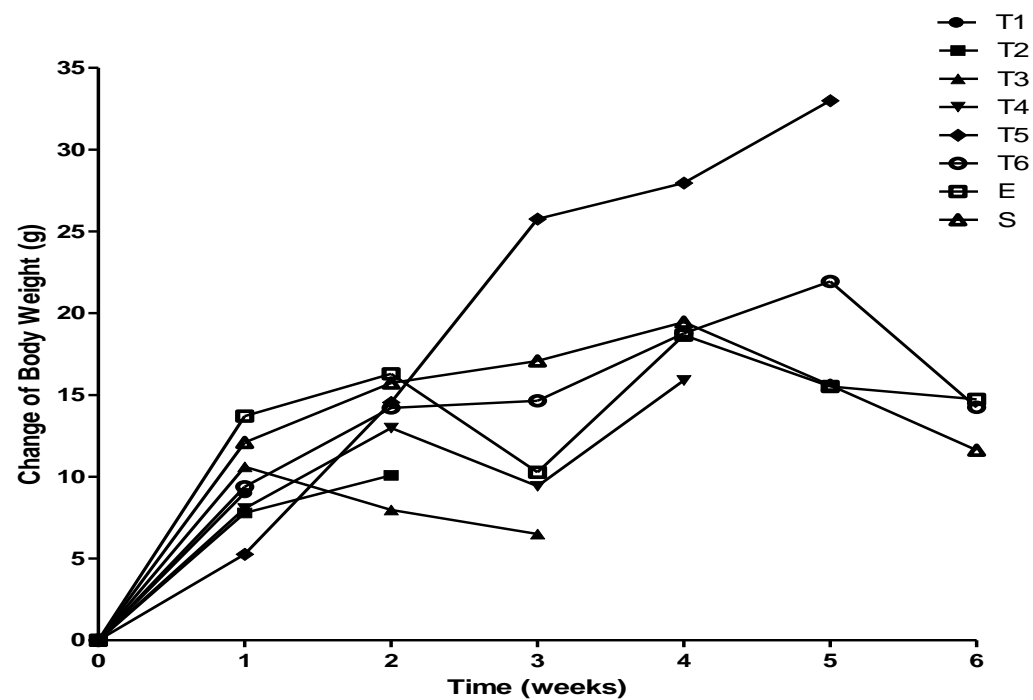
RESULTS

CLINICAL SIGNS

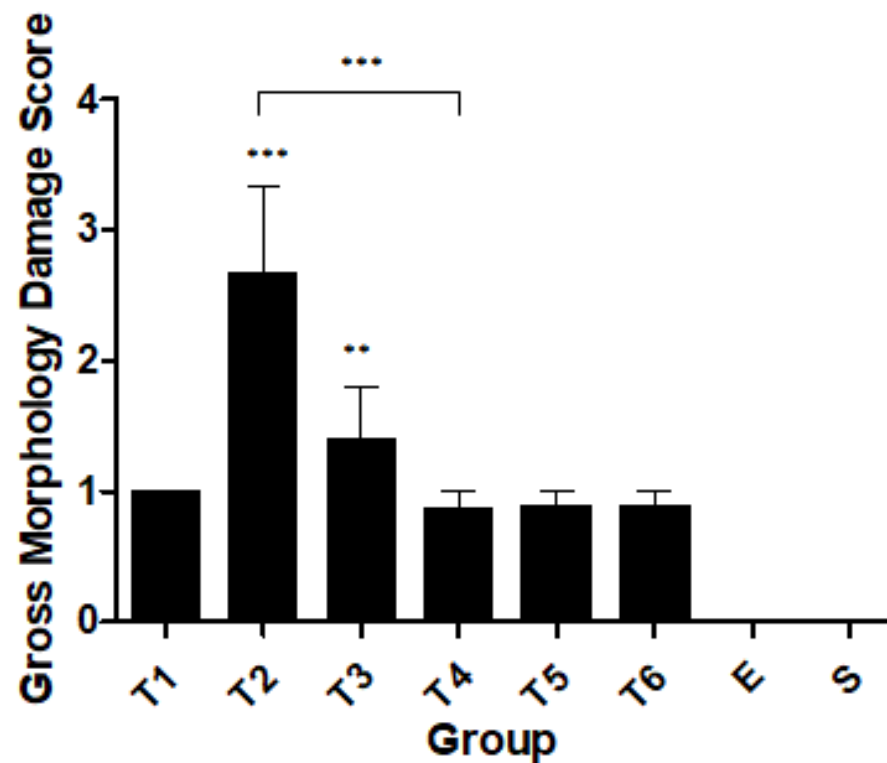
SURVIVAL RATE



BODY WEIGHT



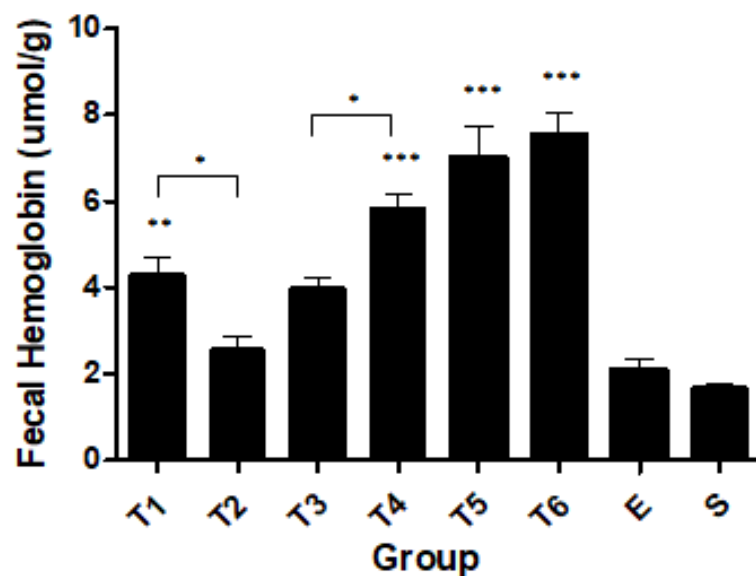
MACROSCOPIC ASSESSMENT OF COLITIS



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

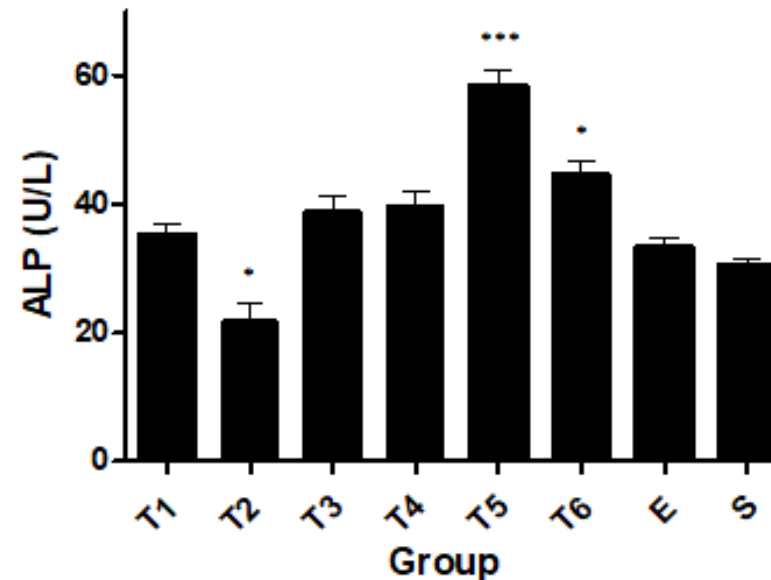
BIOCHEMICAL MARKERS

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.

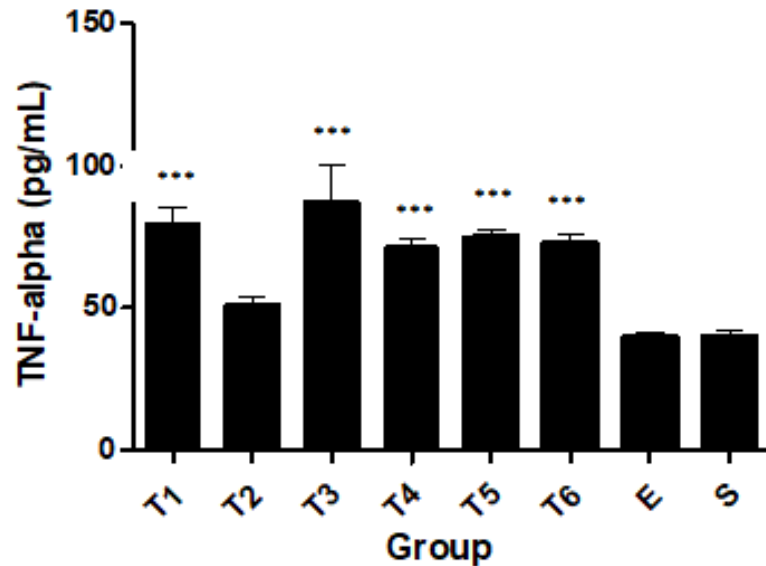
ALP



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

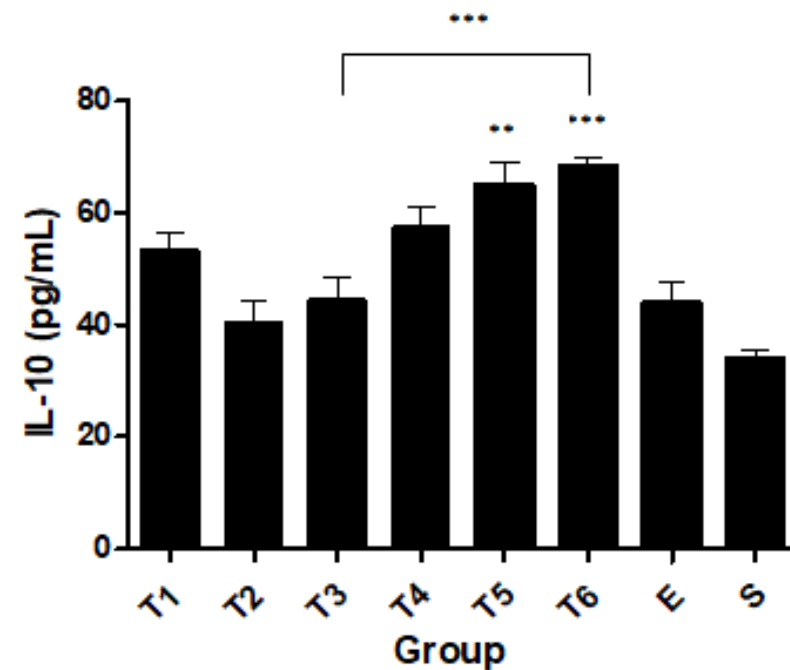
BIOCHEMICAL MARKERS

TNF - ALPHA



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

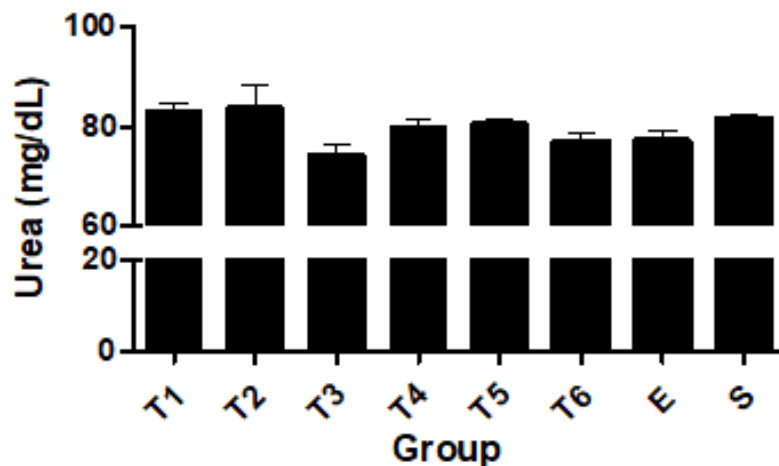
IL-10



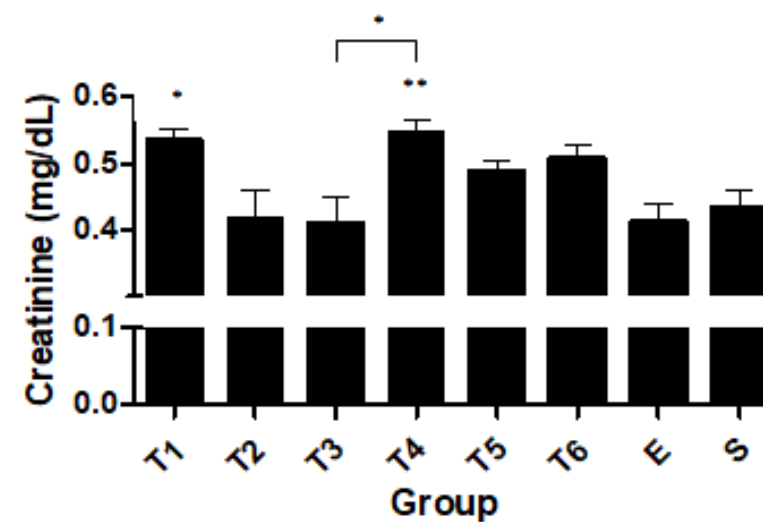
Legend: One-way ANOVA and Tukey's post hoc test; *** p<0.0001 compared with sham group or between groups, **p<0.001 compared with sham group.

RENAL MARKERS

UREA



CREATININE



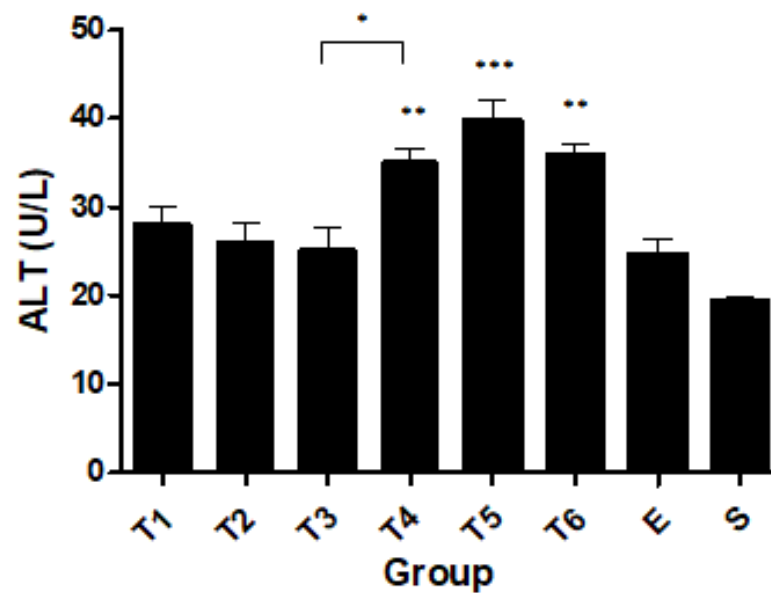
Legend: One-way ANOVA and Tukey's post hoc test, * $p < 0.01$ compared with ethanol group or between groups; ** $p < 0.001$ compared with ethanol group.

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RESULTS

HEPATIC MARKER

ALT



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.

- 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}

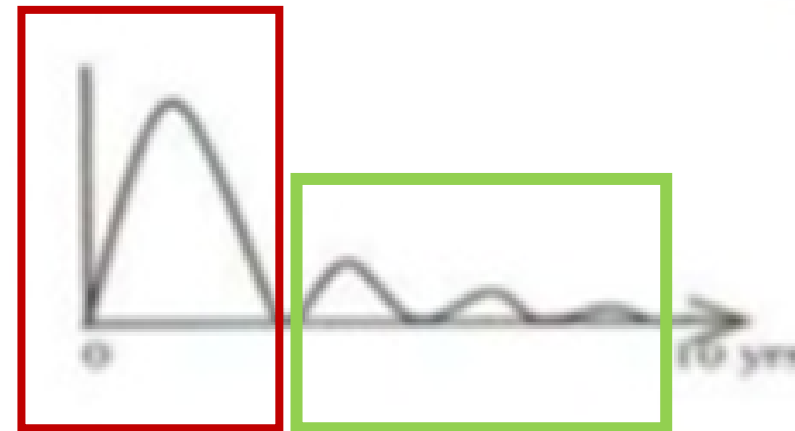
8. Morris G, et al. Gastroenterology 1989

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

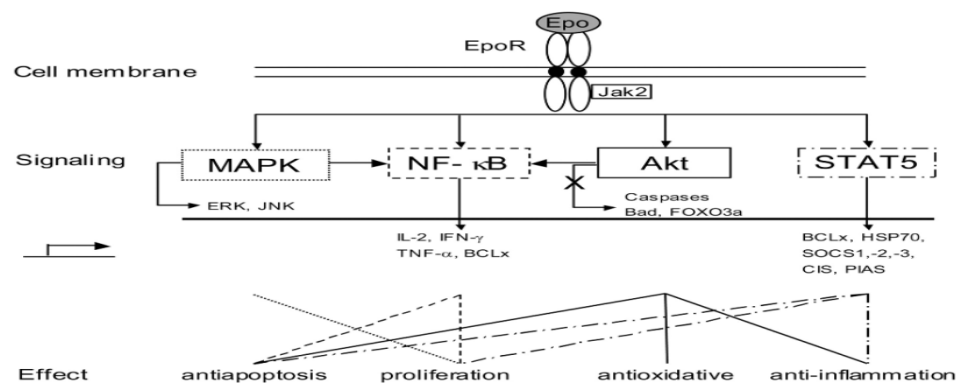
- Chronic phase of the disease
 - Multiple TNBS administrations^{12,13}
 - Presence of signs of the disease
 - Stabilization and maintenance of values from week 4 until week



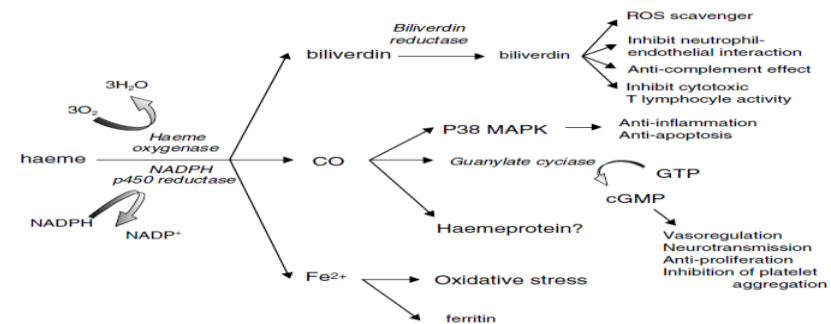
These findings seem to propose

4 administrations 1% TNBS for the induction of chronic colitis

Erythropoietin (EPO)



Hemin





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Análises
Clínicas



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