

# 3<sup>RD</sup> WORLD CONGRESS ON PHARMACOLOGY & TOXICOLOGY

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## NEW PHARMACOLOGICAL APPROACH FOR INFLAMMATORY BOWEL DISEASE

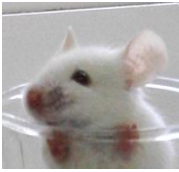
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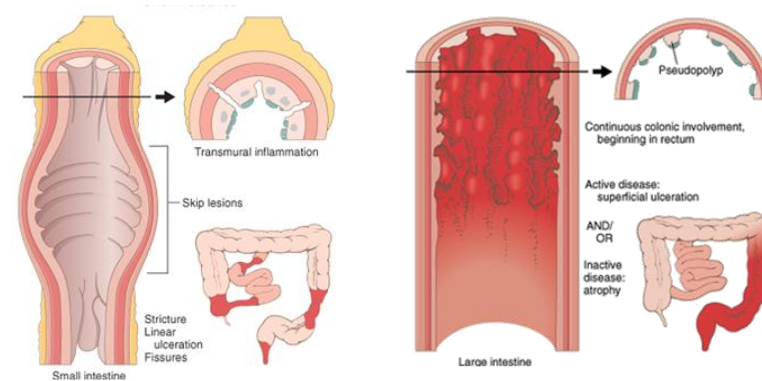


# INTRODUCTION

## INFLAMMATORY BOWEL DISEASE (IBD):

Include Crohn's disease and Ulcerative colitis

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



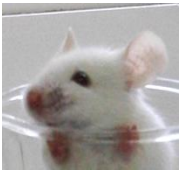
IBD affects 7–10% of people worldwide, mainly of Caucasian descent.<sup>2</sup>

IBD manifests into several intestinal and extra-intestinal symptoms, mainly related to oxidative stress, inflammation and autoimmune reaction.<sup>3</sup>

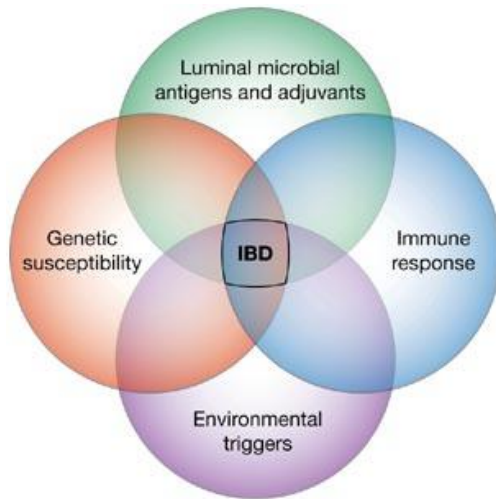
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2. Spiegel B. The burden of IBS: looking at metrics. Current Gastroenterology Reports 2009;11:265-9

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  
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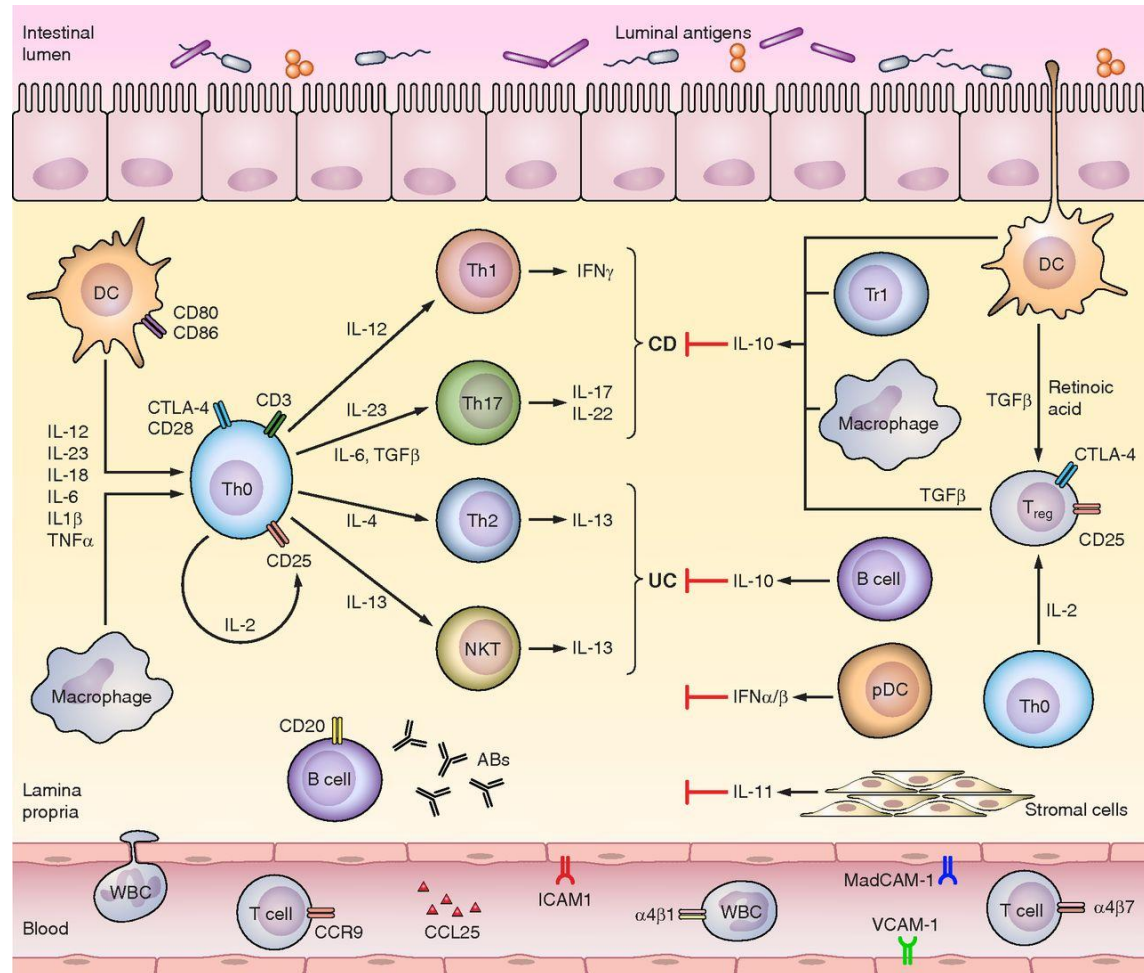


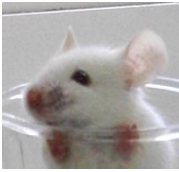
# INTRODUCTION



Chron Disease - Th<sub>1</sub> and Th<sub>17</sub> response

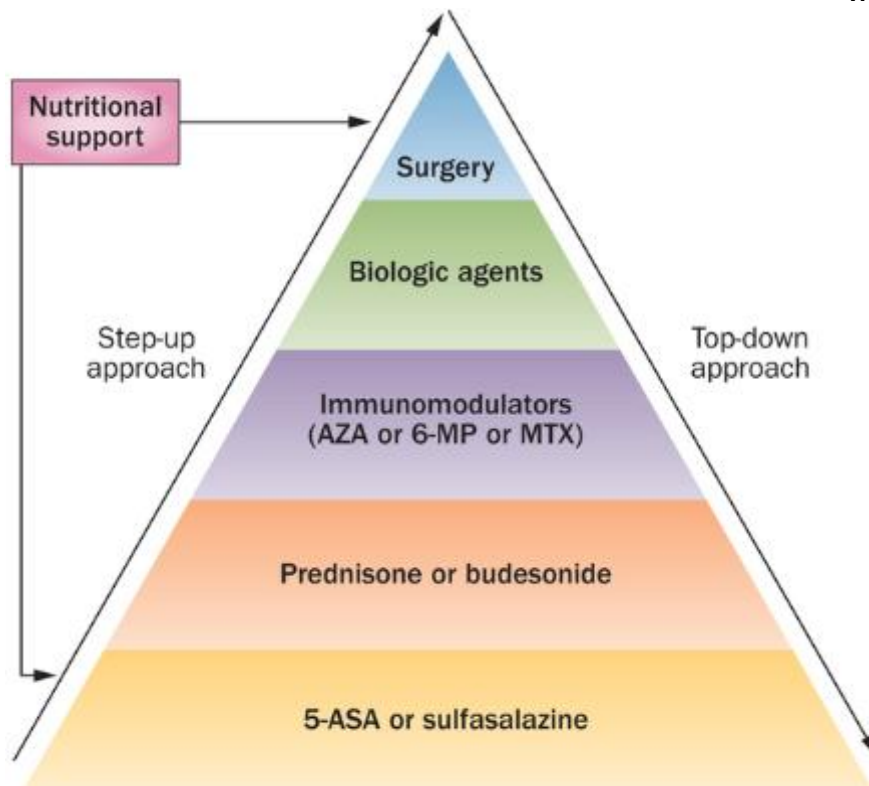
Ulcerative colitis - Th<sub>2</sub> response





# INTRODUCTION

## CURRENT PHARMACOLOGICAL APPROACHES ON IBD



Induce and maintain the patient in remission  
however no modify or reverse  
the underlying pathogenic mechanism.<sup>1</sup>



Nonclinical studies  
for  
emerging therapeutic strategies



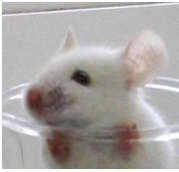
# INTRODUCTION

## 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

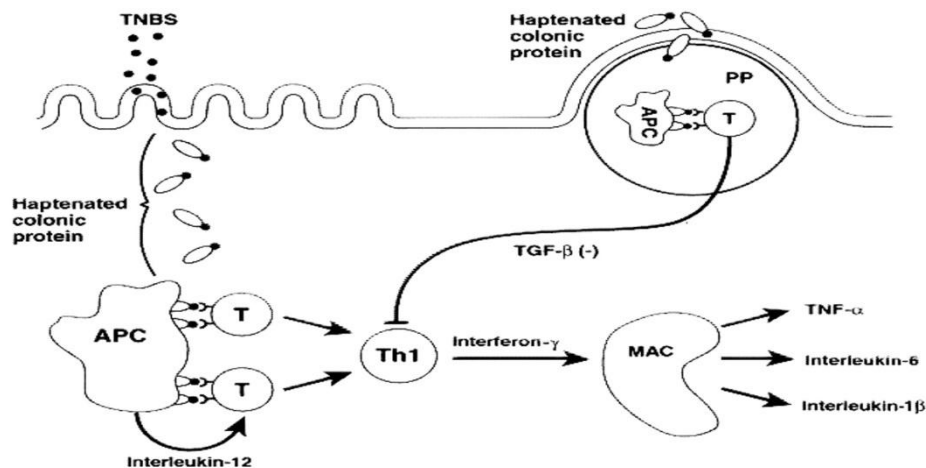


# INTRODUCTION

## Mechanism of action of TNBS / Ethanol

ETHANOL is proposed to elicit a transient increase in intestinal permeability.<sup>5</sup>

TNBS reaches the subepithelial space and haptenate tissue and microbial proteins.<sup>6</sup>



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.

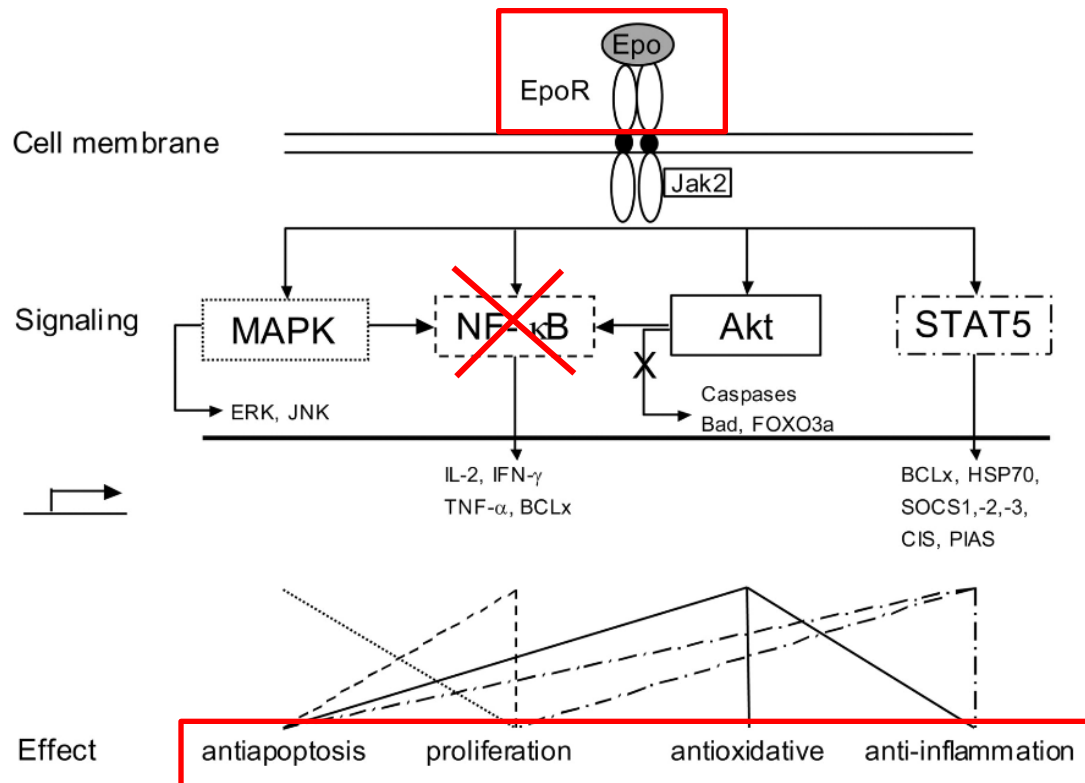
7. Sartor R. Microbial Influences in Inflammatory Bowel Diseases. *Gastroenterology*. 2008;134(2): 577–94



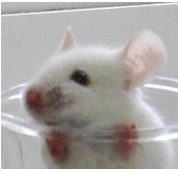
# INTRODUCTION

**Erythropoietin (EPO)** is a potent stimulator of erythroid progenitor cells.

Furthermore, EPO inhibits NF- $\kappa$ B activation and proinflammatory gene expression in the lamina propria.<sup>5,6</sup>







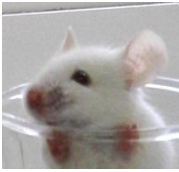
# Aim

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Evaluate the influence of a new pharmacological approach with erythropoietin in the establishment and development of inflammation associated with IBD, through of an experimental colitis model in rodents.







# MATERIALS AND METHODS

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## 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.



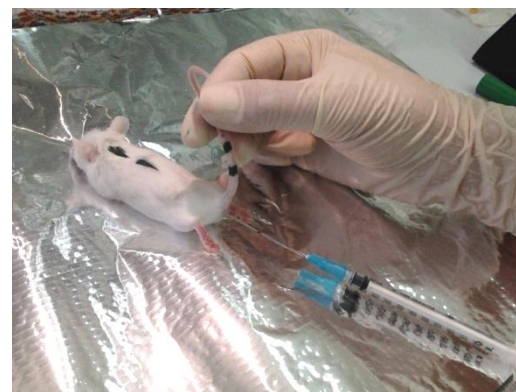


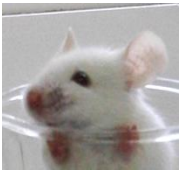
# MATERIALS AND METHODS

## TNBS-INDUCED COLITIS

According to Morris (1989)<sup>9</sup>:

- ✓ Mice were left unfed during 24h
- ✓ Mice were anesthetized with Ketamine + Xilazine IP
- ✓ 100µl of TNBS (2,5% 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
- ✓ EPO 500 – 1000 mg/Kg were daily administrated IP

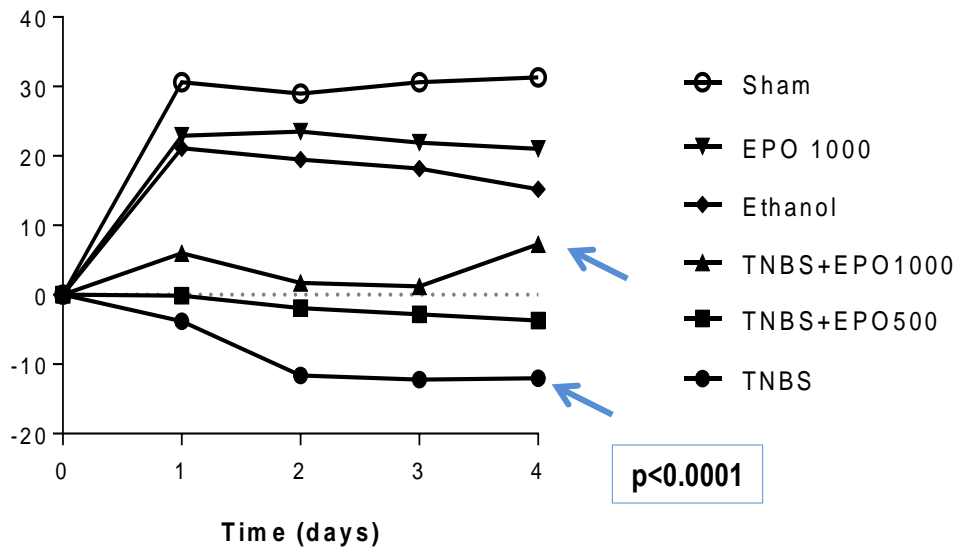




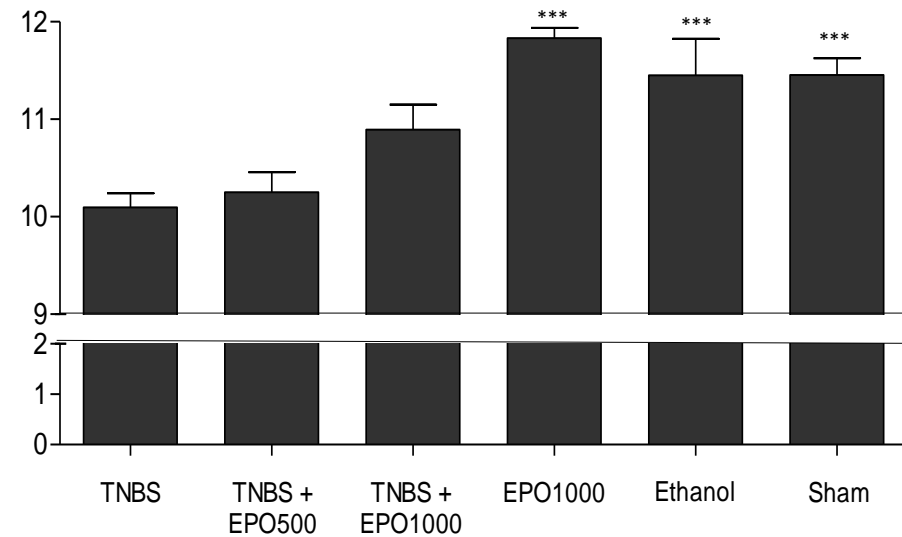
# RESULTS

## CLINICAL SIGNS AND SYMPTOMS

### CHANGE OF BODY WEIGHT (%)



### COLON LENGTH (cm)

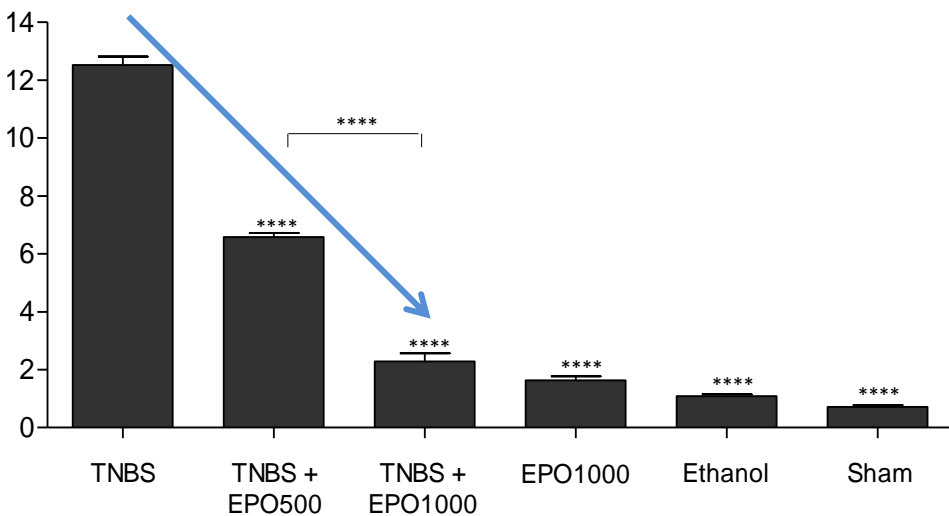




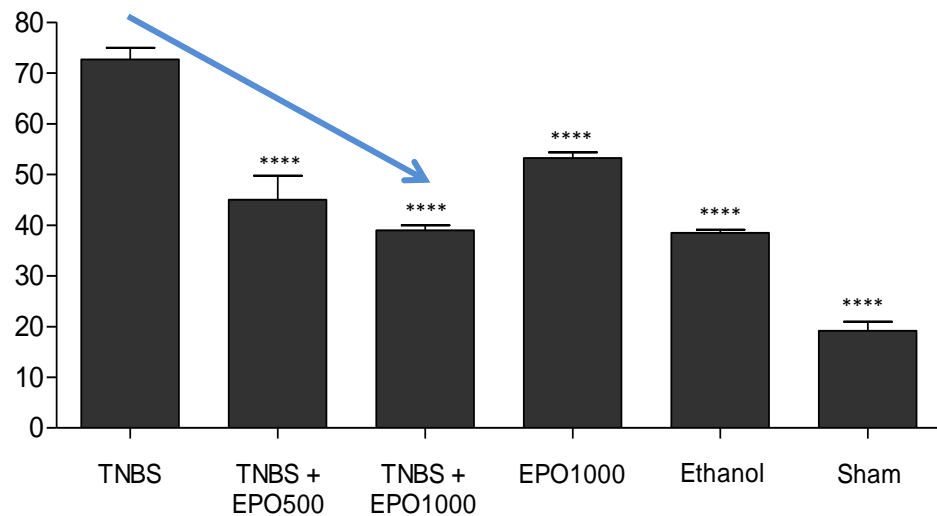
# RESULTS

## BIOCHEMICAL MARKERS

### FAECAL HAEMOGLOBIN ( $\mu\text{mol Hg/g feces}$ )

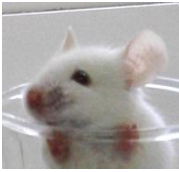


### ALP (UI/L)



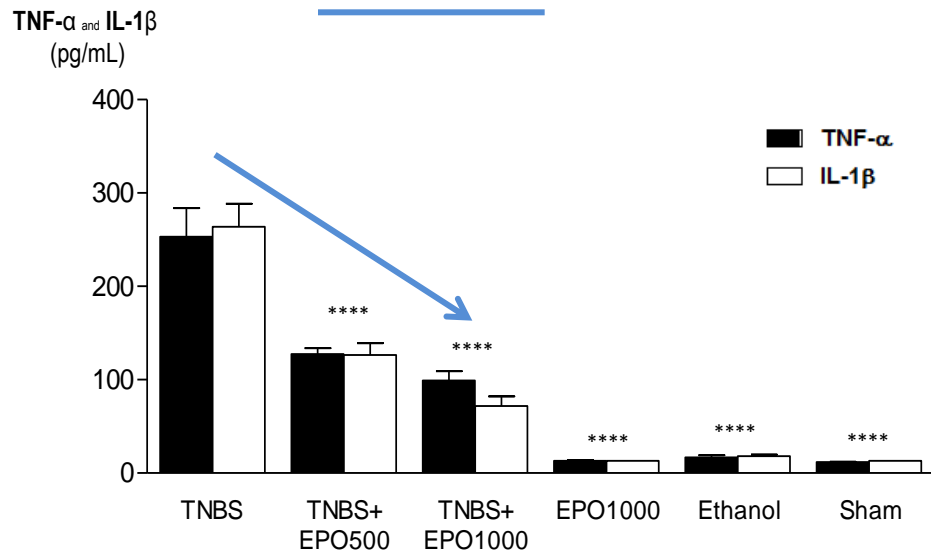
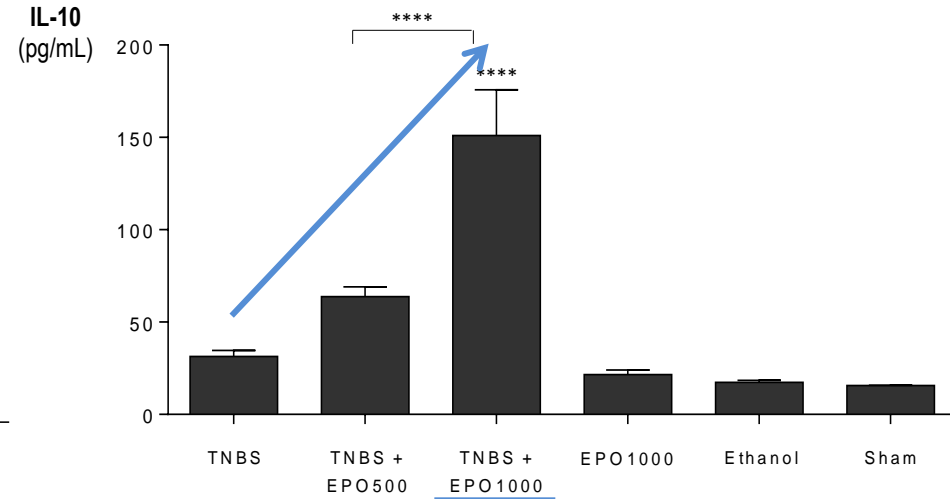
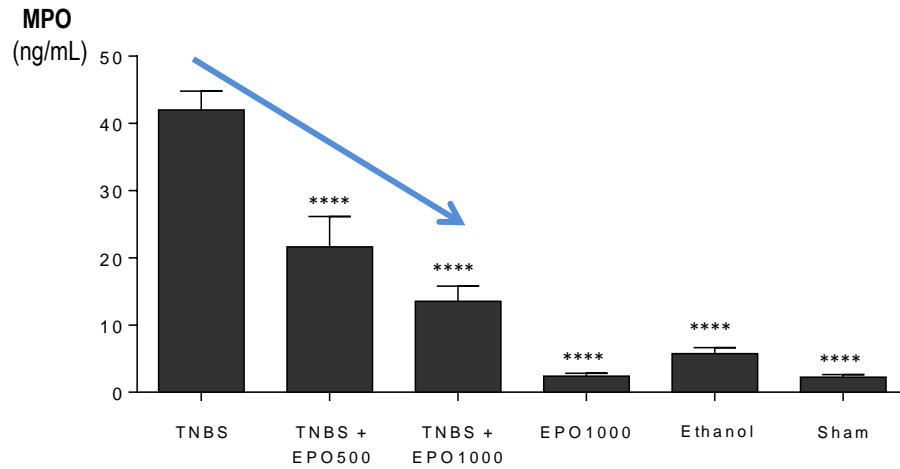
EPO decreased the intestinal bleeding

EPO seems to decrease the colonic damage



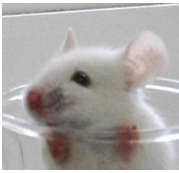
# RESULTS

## BIOCHEMICAL MARKERS



EPO  
presented an anti-inflammatory effect





# RESULTS

## HISTOLOGY

TNBS

TNBS+EPO500

TNBS+EPO1000

EPO1000

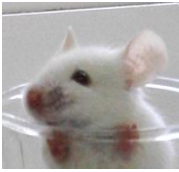
Ethanol

Sham

300µm

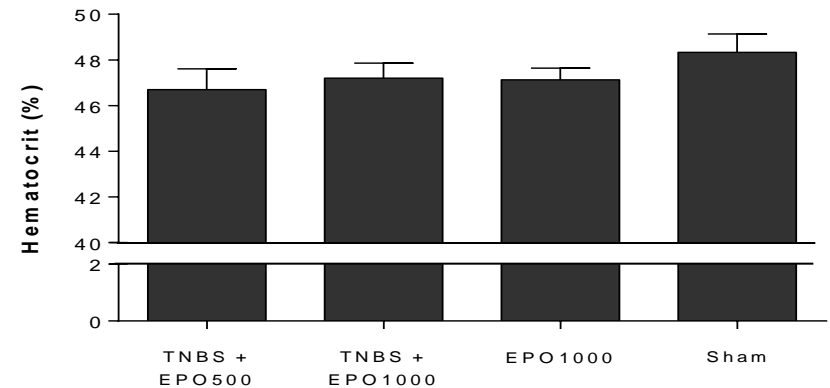
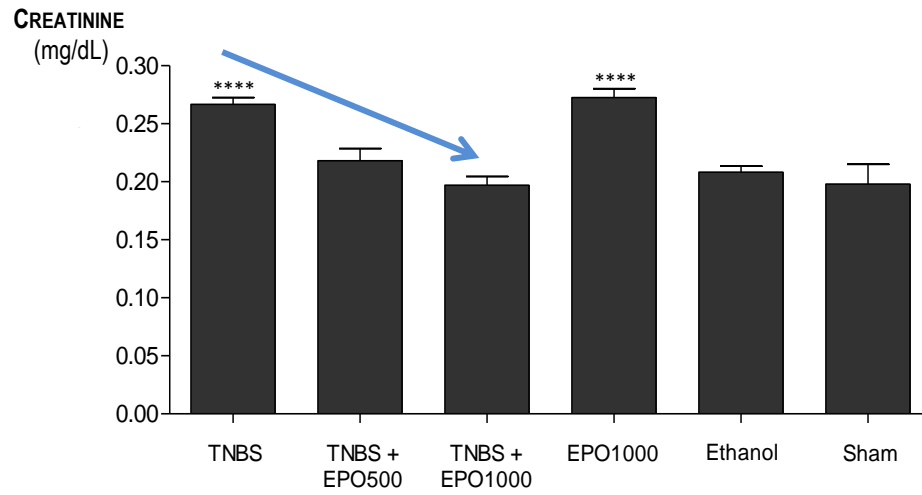
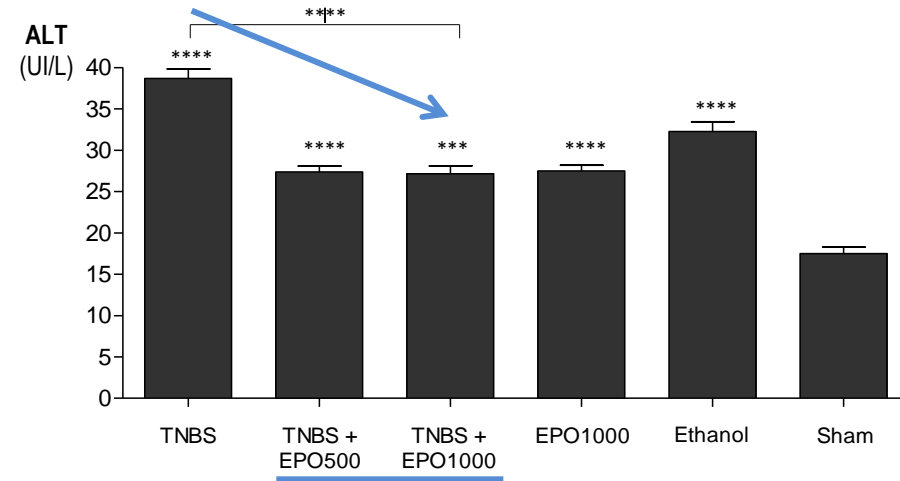
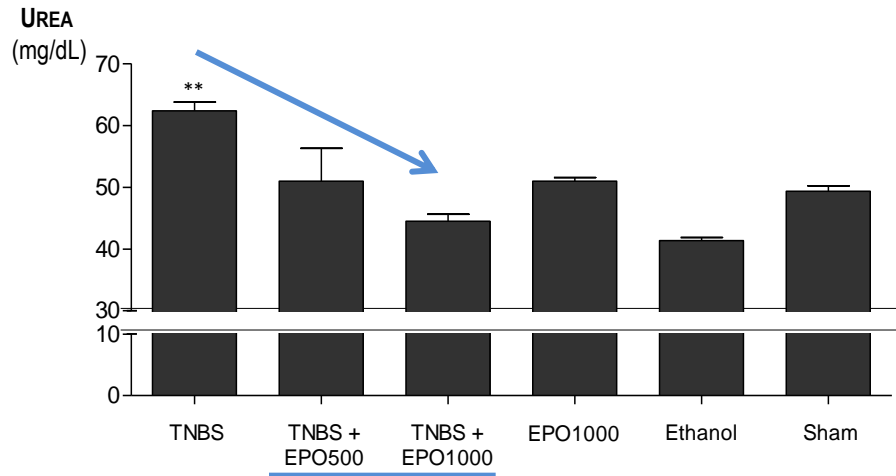
200µm

50µm

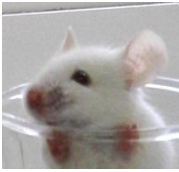


# RESULTS

## RENAL AND HEPATIC MARKERS







# DISCUSSION / CONCLUSION

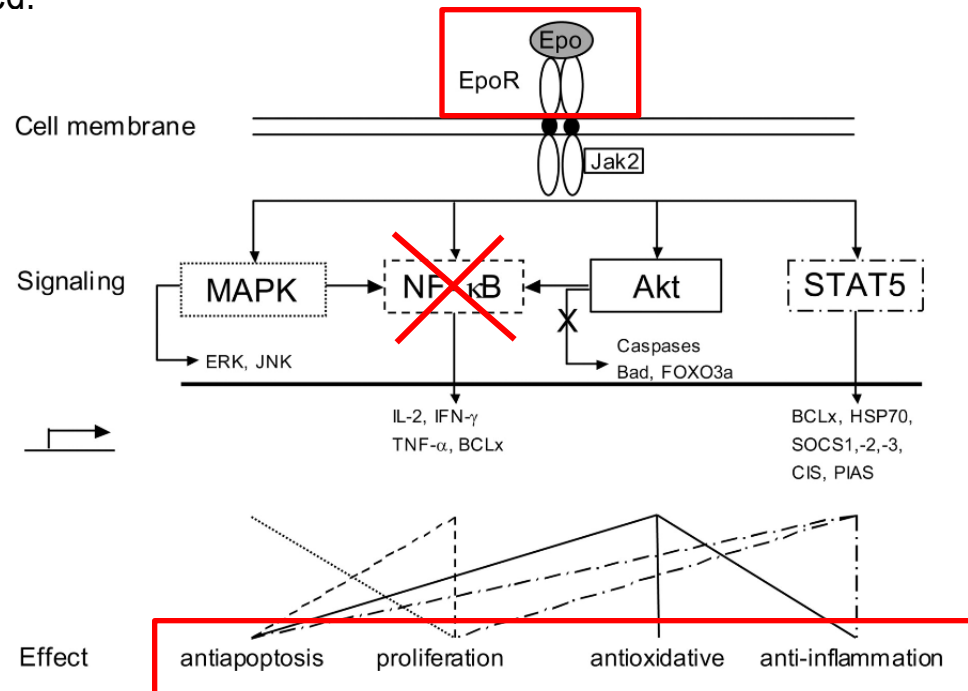
## EFFECT OF EPO ON TNBS-INDUCED COLITIS

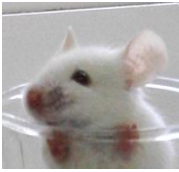
EPO promoted a beneficial effect on:

body weight, faecal haemoglobin, ALP, MPO, TNF- $\alpha$ , IL-1 $\beta$ , IL-10, urea, creatinine, ALT and histopathology

- Dose-dependent effect was observed:
- Hematocrit level was normal.

EPO had an anti-inflammatory effect  
on the TNBS-induced colitis model





## CONCLUSION

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EPO significantly inhibit the acute inflammatory response in the experimental colitis



This study may possibly contribute to the  
enrichment of the therapeutic opportunities of IBD.



# ACKNOWLEDGMENT

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**JOAQUIM  
CHAVES  
SAÚDE**

Análises  
Clínicas



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