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جامعة الملك سعود King Saud University

IL-17/Notch1/STAT3 Pathway Contributes to 5-Fluorouracil-Induced Intestinal Mucositis in Rats: Amelioration by Thymol Treatment

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INTRODUCTION

5-Fluorouracil (5-FU) is a chemotherapeutic agent, that is considered the first line treatment for patients with colorectal cancer [1]. Cancer patients are suffering from severe adverse effects associated with chemotherapy administration. One of these effects is chemotherapy-induced intestinal mucositis (IM) which is associated with the clinical use of 5-FU that consequently can lead to its discontinuation and increased deleterious effects. it has been reported that IM was associated with a storm of oxidative stress mediators and inflammatory cytokines. The immune cell responses were found to be implicated in the pathogenesis of chemotherapy-induced IM. Activated effector T-helper cells (Th17) releases a storm of pro-inflammatory cytokines and triggers a flare-up of inflammation, causing damage to the intestinal mucosa [2]. One of these inflammatory mediators is IL-17. Notch signaling pathway regulates the differentiation and activation of various immune cell types in which activation of Notch triggers a pro-inflammatory action, and its blocking may be a promising tool for intestinal inflammation treatment [3]. Additionally, the differentiation and regulation of Th17 cells and macrophages were documented to be linked to STAT3 pathway. Therefore, STAT3 was considered a vital mediator in immune cell development and inflammation regulation. Thymol is a monoterpene phenol that was reported to possess an antioxidant and anti-inflammatory activity versus 5-FU-induced IM [5].

OBJECTIVES

Therefore our work aimed to elucidate in depth the pathophysiological mechanism of 5-FU induced-IM as well as the molecular mechanism of the immunomodulatory action of thymol against 5-FU induced-IM through the IL-17/Notch/STAT3 signaling pathway.

1. **To elucidate the contribution of Notch pathway to 5-FU-induced IM**
2. **To elucidate the correlation of Notch pathway activity and Immune cell activation**
3. **To elucidate the effect of Thymol on Notch Pathway in5-FU-induced IM**
4. **To elucidate the effect of Thymol on STAT3 in IM model.**
5. **To examine the effect of Thymol on 5-FU Cytotoxiciy**

METHODS

1. **In vivo study:** Thirty-two male Wistar rats were randomly divided into four groups and treated for 11 days as described in Table 1.

Groups	Description
Control	Rats received 0.5% dimethyl sulfoxide daily, oral gavage.
5-FU	Rats received 0.5% DMSO daily by oral gavage plus 150 mg/kg 5-fluorouracil (i.p.) on the 6th and 7th days
5-FU + Thymol 60	Rats received 60 mg/kg/day thymol n 0.5% DMSO orally plus 150 mg/kg 5-FU injected i.p. on the 6th and 7th days.
5-FU + Thymol 120	Rats received 120 mg/kg/day thymol in 0.5% DMSO orally plus 150 mg/kg 5-FU injected i.p. on the 6th and 7th days.

The following methods were used:

a. Histopathological examination using H and E

b. ELISA assessment of IL-17

c. Immunohistochemical detection of CD4, CD8, Notch, and Hes-1, expressions

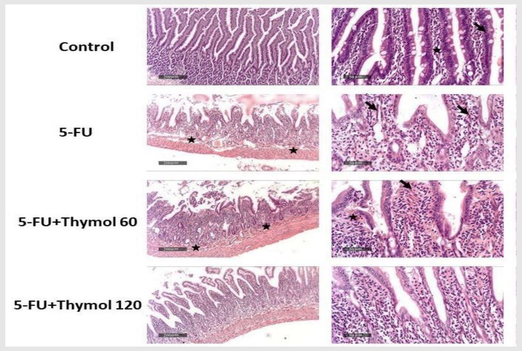
d. Immunoblot analysis of phosphorylated-STAT3/ STAT3

2. In vitro study using

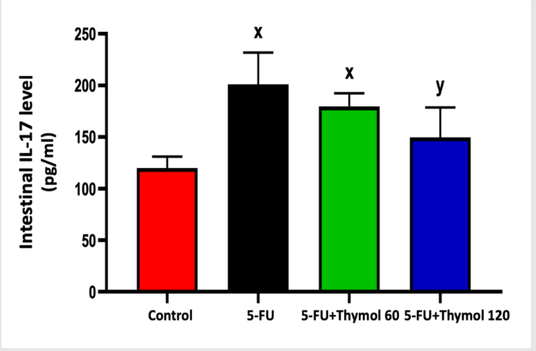
Cytotoxicity WST-1 assay was used to measure the *in vitro* cytotoxic effects of 5-FU alone or in combination with Thymol on Human breast adenocarcinoma MDA-MB-231 and colon cancer LoVo cell lines

RESULTS

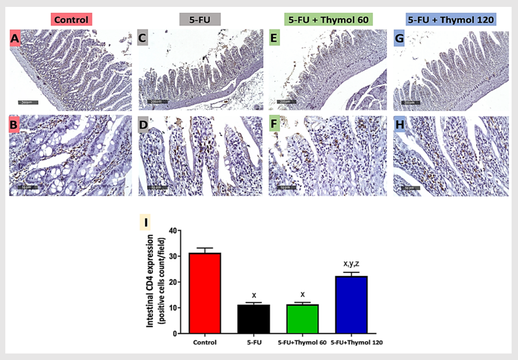
1. Impact of thymol administration on the histopathological features of intestinal samples of 5-FU-intoxicated rats



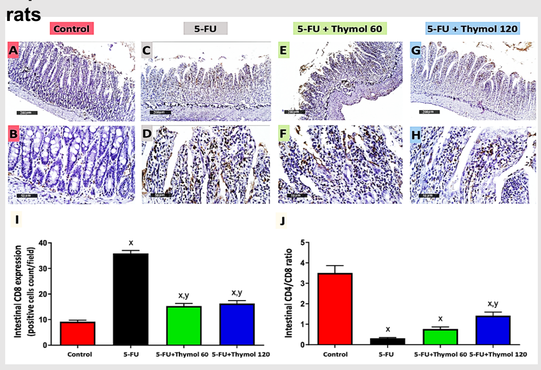
2. Thymol administration suppresses the intestinal expression of IL-17 in the 5-FU-intoxicated rats



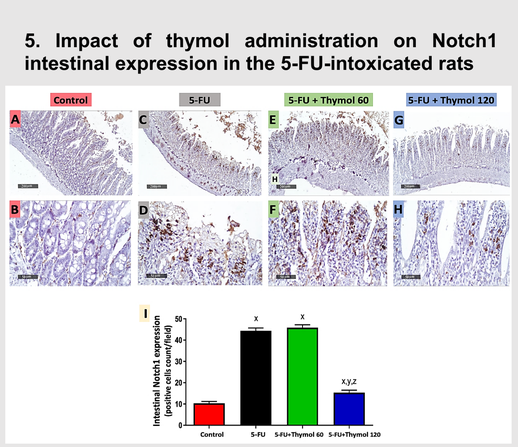
3. **Thymol administration induced CD4 intestinal expression in the 5-FU-intoxicated rats**



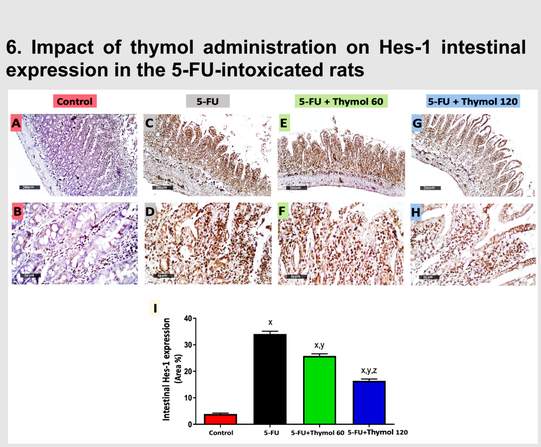
4. Impact of thymol administration on CD8 intestinal expression and CD4/CD8 ratio in the 5-FU-intoxicated rats



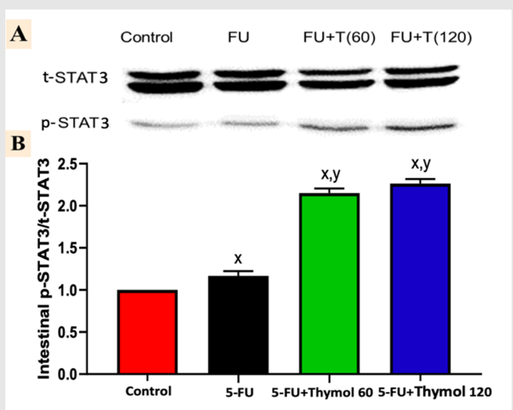
5. Impact of thymol administration on Notch1 intestinal expression in the 5-FU-intoxicated rats



6. Impact of thymol administration on Hes-1 intestinal expression in the 5-FU-intoxicated rats

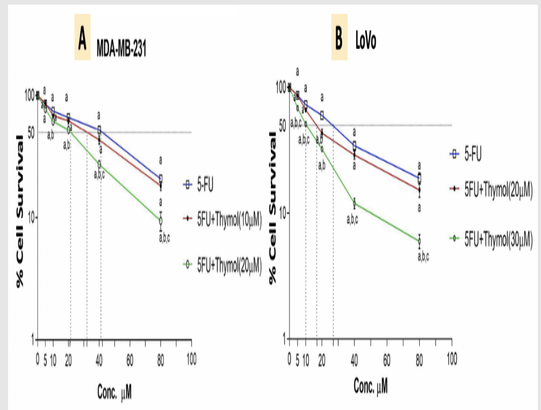


7. **Impact of thymol administration on the p-STAT3/t-STAT3 intestinal ratio in the 5-FU-intoxicated rats.**



Data are presented as mean ± SD. [x] $p < 0.05$ versus control group, [y] $p < 0.05$ versus 5-FU group.

8. Thymol augments the cytotoxic effect of 5-FU in human cancer cells



Results are means ± SD. [a] $p < 0.05$ versus untreated control cells, [b] $p < 0.05$ versus same concentration of 5-FU, [c] $p < 0.05$ versus same concentration of 5-FU and lower dose of thymol.

DISCUSSION & CONCLUSION

- Our study documented, for the first time, a marked increase in IL-17 intestinal protein level and increased p-STAT3 following a 5-FU injection
- Moreover, our results showed that 5-FU induced a marked downregulation in CD4 and a significant upregulation of CD8 associated with a decreased CD4/CD8 ratio, associated with upregulation in Notch and Hes1 intestinal expression.
- Thymol treatment relatively counteracted 5-FU induced changes, while interestingly increased p-STAT3/t-STAT3
- the cytotoxicity assay performed showed that thymol augmented the antiproliferative action of 5-FU against breast and colorectal human cancer cell lines.

REFERENCES

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4. Al-Khrashi LA, Badr AM, AL-Amin MA, Mahran YF. Thymol ameliorates 5-fluorouracil-induced intestinal mucositis:Evidence of down-regulatory effect on TGF-β/MAPK pathways through NF-κB. *Journal of Biochemical and Molecular Toxicology.* 2021:e22932.