Methoxy Group Number Enhances the Cytostatic and Anti-fibrotic Effects of Curcuminoids

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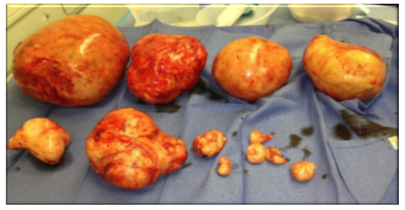
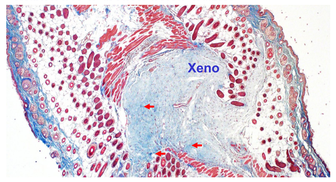
INTRODUCTION

Uterine leiomyomas (uLM) are benign tumors characterized by excessive and disorganized deposition of extracellular matrix (ECM). Key components, Including glycosylated proteoglycans and collagens, are known to be modulated by curcumin, both *in vitro* and *in vivo* (Malik et al. 2008, 2022).

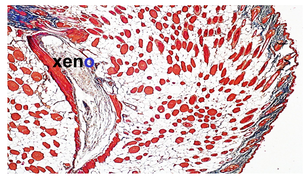


Control mouse: Masson's trichrome stain



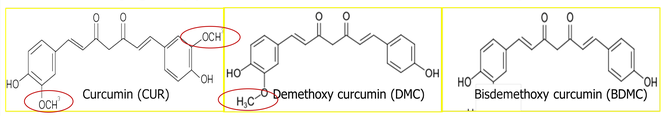
Mice on regular feed show a compact matrix compared to curcumin diet mice.

Curcumin diet mouse: Masson's trichrome stain



Mice exposed to a curcumin diet show loss of collagen protein production.

Curcumin (CUR, 2 methoxy groups), de-methoxy curcumin (DMC, 1 methoxy group) and bis-de-methoxy curcumin (BDMC; no methoxy group), form the curcuminoids in the spice turmeric.



Methoxy groups influence lipophilicity, solubility, and interaction with targets. While methoxy groups are essential for the natural potency of curcumin, replacing them with other groups may sometimes improve metabolic stability and anticancer efficacy. The methoxy group is a key site for modification to design more stable and potent curcumin-based drugs.

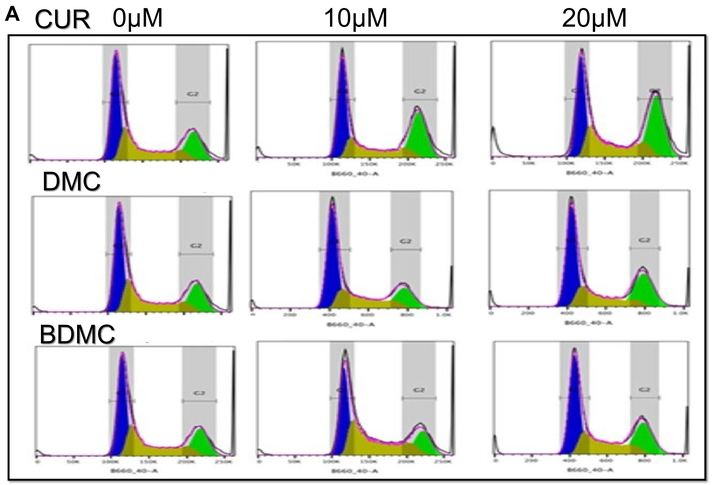
Hypothesis: The number of methoxy groups (-OCH_3) may influence the regulation of extracellular matrix protein production and the cell cycle, inducing a cytostatic effect in growth of leiomyoma cells.

MATERIALS & METHODS

Leiomyoma cells were treated with curcuminoids for 24hrs. Flow cytometry was used to study cell cycle. Western blot was used for proteomics.
CellTitre Glo assessed the efficacy of curcumin analogues against leiomyoma cells following 72hr treatment.

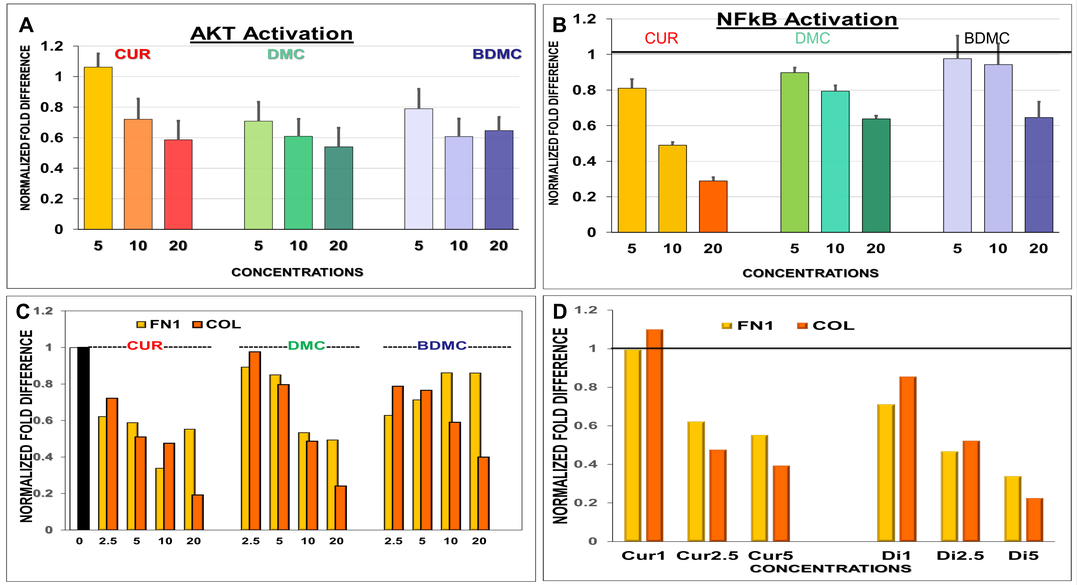
RESULTS

Figure 1. Curcumin induces a greater G2/M phase arrest compared to DMC and BDMC



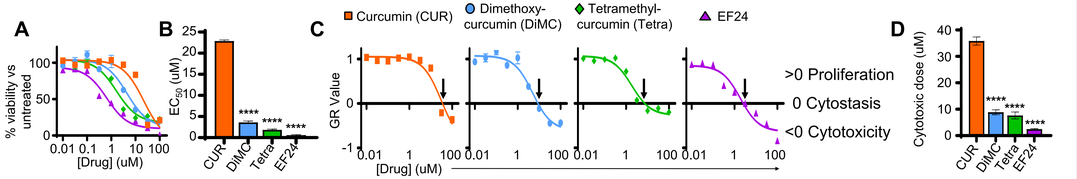
(A) Cell cycle phase distribution for uterine leiomyoma cells treated with Curcumin (CUR), Demethoxycurcumin (DMC), or Bisdemethoxycurcumin (BMDC) with the indicated concentration for 24hrs. (B) Quantification of the percentage of cells in G2/M phase from panel A. (C) Cell cycle phase diagram. (D) Western blots for uterine leiomyoma cells treated with CUR for 24hrs. Persistent upregulation of inhibitory phosphorylation (Y15) of Cdc2 prevents the cells from entering mitosis, resulting in the G2/M phase block seen in panel A.

Figure 2. Methoxy group number impacts the extent to which curcuminoids downregulate extracellular matrix production and anti-apoptotic pathways in leiomyoma cells.



(A-D) Quantification of western blot analysis of uterine leiomyoma cells treated with CUR, DMC, BDMC, or dimethoxycurcumin (Di / DiMC) for 24hrs.

Figure 3. Synthetic curcumin analogues are significantly more potent and cytotoxic than curcumin.



(A,B) Dose response curves for curcumin (CUR) versus synthetic curcumin analogues (Dimethoxycurcumin, Tetramethylcurcumin, & EF24) (A) and corresponding EC_{50} values (B). (C,D) GR values for CUR and synthetic analogues with the minimum cytotoxic dose indicated with an arrow (C) and quantified cytotoxic doses (D).

CONCLUSIONS & FUTURE DIRECTIONS

Conclusions:

- **Curcumin, with two methoxy groups, is more effective in bringing about a cytostatic effect in uterine leiomyoma cells. It also better inhibits extracellular matrix production and anti-apoptotic signaling pathways.**
- **Synthetic curcumin analogues demonstrate greater potency and cytotoxicity in leiomyoma cells compared to curcumin.**

Future Directions: We are currently assessing how curcuminoids and curcumin analogues affect myometrial cells compared to leiomyoma cells.

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This research was reviewed and approved by IACUC in accordance with all applicable Federal regulations governing the protection of animals in research.

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