# Inhibitory effects of Curcuma longa extract on the steroid metabolizing cytochrome P450 enzymes

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## Introduction

Turmeric is a popular ingredient in the cuisine of many Asian countries. Turmeric is known for its use in Chinese and Ayurvedic medicine and comes from the roots of the *Curcuma longa*. Turmeric is rich in curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin has potent anti-inflammatory and anti-carcinogenic activities. Since many anti-cancer drugs target enzymes from the steroidogenic pathway, we tested the bioactivity of curcuminoids on cytochrome P450 CYP17A1, CYP21A2, and CYP19A1 enzyme activities. Curcuminoids were extracted from turmeric with organic solvents. We conducted a cell-based assay for CYP17A1 and CYP21A2 activities using human adrenal cell line NCI-H295R. $^3$H-pregnenolone was used for CYP17A1 assays, and $^3$H-17alpha-hydroxyprogesterone was used as a substrate for CYP21A2. For CYP19A1 activity, an in vitro assay using endoplasmic reticulum from JEG3 was used with $^3$H-androstenedione as the substrate. Curcuminoids were incubated for 1h, and the formation of $^3$H-water from the androstenedione breakdown was measured by scintillation counting. When using 10 µg/ml of curcuminoids, both the 17-hydroxylase as well as 17,20 lyase activities of CYP17A1 were reduced significantly. On the other hand, CYP21A2 activity was only reduced to ~50% control. Furthermore, CYP19A1 activity was reduced to ~20% of control when using 1-100 µg/ml of curcuminoids in a dose-dependent manner. No effect on the activity of 5alpha reductase for the metabolism of androstenedione was observed. Molecular docking studies confirmed that curcumin could dock into the active sites of CYP17A1, CYP19A1 as well as CYP21A2. In CYP17A1 and CYP19A1, curcumin docked within 2.5 Å of central heme while in CYP21A2 the distance from heme was 3.4 Å, which is still in the same range or lower than distances of bound steroid substrates. These studies show that curcuminoids may potentially cause inhibition of steroid metabolism, especially at higher dosages. The activities of CYP17A1 and CYP19A1 were inhibited by curcuminoids, which indicate potential anti-carcinogenic effects in case of prostate cancer as well as breast cancer, which can be targeted by inhibition of steroidogenesis. Also, the recent popularity of turmeric powder/curcumin as a dilatory supplement needs further evaluation for the effect of curcuminoids on steroid metabolism. Curcuminoids present in curcumin may affect activities of multiple steroid metabolizing cytochrome P450 enzymes. Computational docking suggests curcumin binds into the active sites of steroid metabolizing P450s and may serve as a model for lead discovery. Molecular structure of curcuminoids could be modified to generate better lead compounds with inhibitory effects on CYP17A1 and CYP19A1 for potential drugs against prostate cancer and breast cancer.

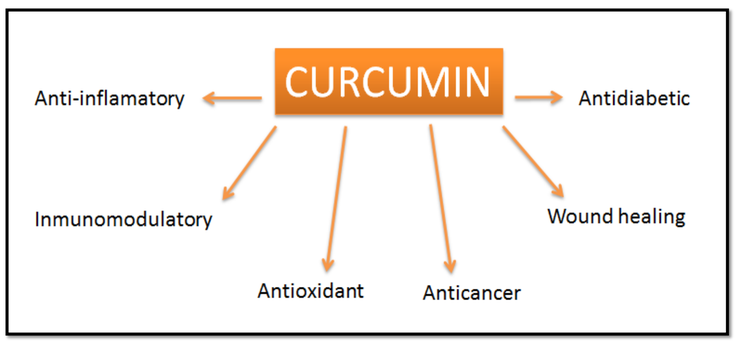


**Figure 1.** Activity of curcumin on different biological processes. Several medicinal properties have been linked to curcumin that ranges from antiproliferative activity in cancers, an antioxidant activity, anti-inflammatory, anti-bacterial, antifungal activities.

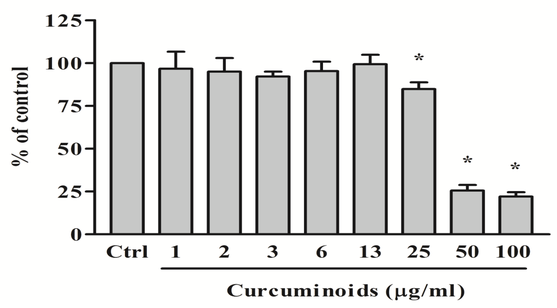


**Figure 2**: **Cell Viability Assay.** Cell toxicity and viability of human adrenal NCI-H295R cells was determined using a range of curcuminoids over 24 h. Cells were treated with varying dosages of curcumin, and cell viability was determined by MTT assay.

## Methods

Curcuminoids were extracted from a commercial turmeric supplement using organic solvents. For cell viability assays human adrenal NCI-H295R cells were seeded in 96-well culture plates at a density of 0.3x10$^6$ cells per well overnight and grown at 37°C under 5% $CO_2$, and 90% humidity. Then, the media was changed, and different concentrations of curcuminoids were added and incubated for 24h. Later, 20µl of MTT reagent (5mg/ml in PBS) was added into each well and incubated for 4h. At that point, media was removed, and 200µl of DMSO was added, and the plate was incubated 20 minutes in the dark. Finally, absorbances were measured at 570nm.

The CYP17A1 and CYP21A2 assays were performed in NCI-H295R cells using radiolabeled steroids. CYP19A1 activity was assayed with microsomes from placental JEG3 cells.

## Results

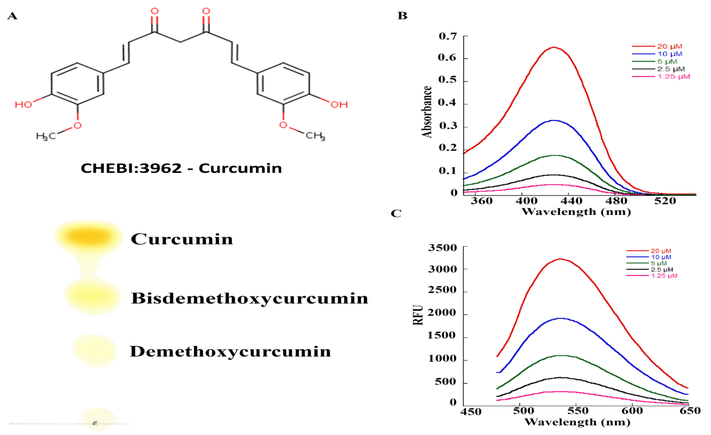


**Figure 3.** Curcuminoids, chemical structure, and separation. A thin layer chromatography separation is shown indicating the different curcuminoids present in turmeric extracts. A major component of turmeric is curcumin, desmethoxycurcumin, and bis-desmethoxycurcumin. Band C: Spectral properties of curcuminoids.

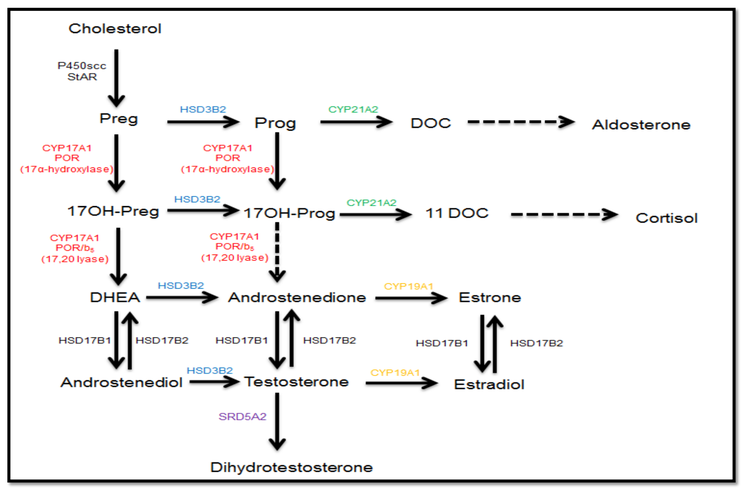


**Figure 4:** Synthesis of steroid hormones from cholesterol in humans. After entering the mitochondrion, cholesterol is converted into pregnenolone, which is used as a substrate by CYP17A1 in the endoplasmic reticulum to produce sex steroids. The CYP19A1 converts androgens into estrogens. Abbreviations: Preg=pregnenolone, Prog=progesterone, DOC=deoxycorticosterone, 11DOC=11-deoxycortisol, DHEA=dehydroepiandrosterone, HSD3B2=3β-hydroxysteroid dehydrogenase type 2, HSD17B1/2=17β-hydroxysteroid dehydrogenase type 1/2, SRD5A2=5α-reductase type 2.

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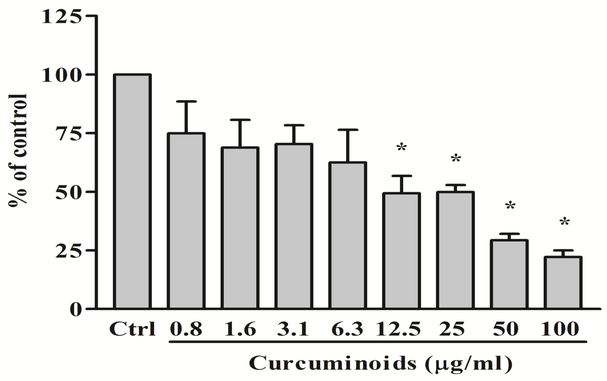
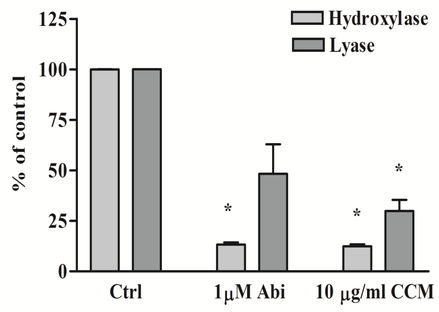



**Figure 5.** Inhibition of aromatase with different concentrations of curcuminoids in the enzyme preparation of endoplasmic reticulum obtained from placental JEG3 cells. Tritium-labeled androstenedione was used as a substrate, and product formation was monitored by quantifying the amount of tritiated water released using scintillation counting. A known inhibitor of CYP19A1, anastrozole, was used as control at a dose of 100 nM.

## Results



**Figure 6.** Effect of curcuminoids on Steroid production. **Left:** Curcuminoids inhibited 17OH-pregnenolone and DHEA production in human adrenal NCI-H295R cells. A block of DHEA production indicates that curcuminoids inhibit both the 17α-hydroxylase and 17,20 lyase activities of CYP17A1. **Right:** Curcuminoids did not have a significant effect on CYP21A2 activity.



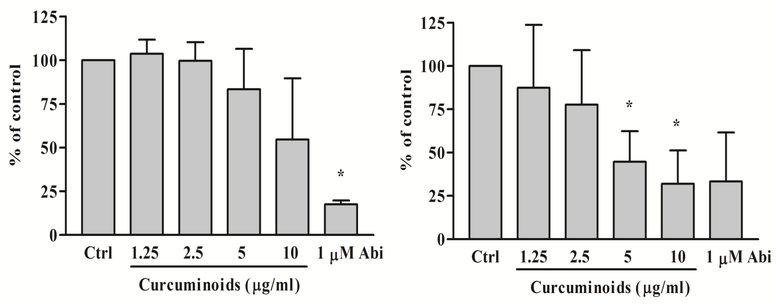




**Figure 6.** Effect of curcuminoids on CYP17A1 activities. Curcuminoids inhibited 17OH-pregnenolone and DHEA production in human adrenal NCI-H295R cells. A block of DHEA production indicates that curcuminoids inhibit both the 17α-hydroxylase and 17,20 lyase activities of CYP17A1.

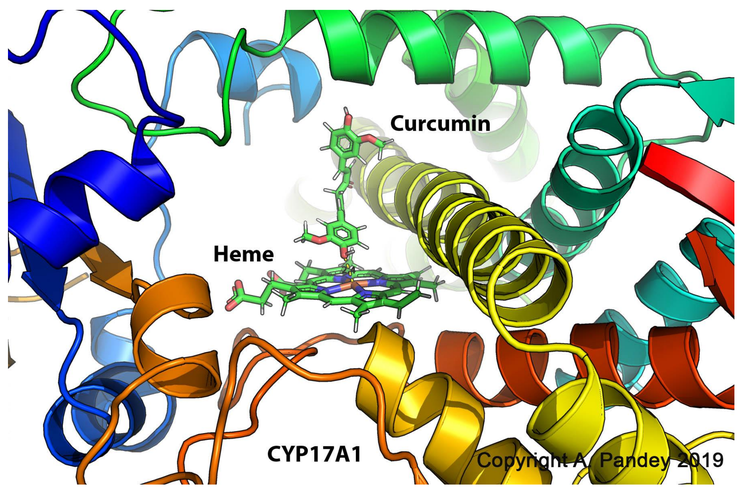



**Figure 7:** Docking of curcumin into the protein structure of CYP17A1. Published crystal structures of CYP17A1 were used for docking of curcumin by software AUTODOCK-VINA. Bound steroid ligands were removed before docking of curcumin into the active site of P450s. The poses in which curcumin docks are similar to steroid substrates, with closer fitting in case of CYP17A1 and CYP19A1 as compared to CYP21A2 (distance from heme 2.5 Å for CYP17A1/CYP19A1 versus 3.4 Å for CYP21A2).

## Conclusions

Our studies show inhibitory effects of curcumin on CYP17A1 and CYP19A1 activities. These results indicate that the steroid production in people with high amounts of Curcuma consumption may be affected not only by the inhibition of CYP17A1 but also by the CYP19A1 and no significant effect on CYP21A2. The use of curcumin in large amounts as a common over the counter health supplement requires caution. The inhibition of CYP17A1 and CYP19A1 by curcuminoids provides a template for modification to produce effective and safe compounds that can target prostate cancer as well as breast cancer.

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