# Synergistic Inhibitory Effect of Capsaicin and Dihydrocapsaicin on the Arachidonic Acid Pathway of Platelet Aggregation

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## BACKGROUND & AIM

- Capsaicinoids, including capsaicin (CAP) and dihydrocapsaicin (DHC), are the pungent principles of chilli peppers[1,2].
- Individually, CAP and DHC have strong inhibitory effects on arachidonic acid (AA)-induced *in vitro* platelet aggregation, and to a lesser extent with ADP-induced platelet aggregation[3]. In contrast, neither CAP nor DHC appear to have an inhibitory effect on collagen-induced aggregation[3].
- Their effects on aggregation when present in the relative proportions that they are found in different fruits, i.e., 60:40 CAP:DHC[4], are not known.
- The **aim** of this study was to determine the effects of CAP and DHC, alone and together, on platelet aggregation, platelet count, and thromboxane B2 (TXB2) formation.

## SUBJECTS & SAMPLES

- Healthy subjects (n=4) avoided aspirin and antiplatelet medications (at least 10 days), and dietary chili (at least two days), prior to collection.
- Whole blood with minimal stasis was collected to obtain platelet poor plasma (PPP) and platelet rich plasma (PRP).

## METHODS

**Platelet Aggregation**

- AA (300 µg/mL), ADP (5 µM), and collagen (4 µg/mL) were added separately to PRP (225 µL) to initiate aggregation in the presence and absence of CAP and DHC.
- CAP and DHC were investigated individually at 12.5 µM and 6.25 µM (final concentration), and in combination in a ratio of 60:40 (CAP:DHC; 7.5:5 µM and 3.75:2.5 µM).
- Aggregation was recorded for 10 min using an AggRAM aggregometer (Helena Laboratories).

**Platelet Counts**

- Platelet counts were determined using a Sysmex 1000i analyser (Roche Diagnostics) with PRP adjusted to 250 x $10^9$/L using PPP from the same subject.
- CAP, DHC (individually at 12.5 µM), and in combination (7.5 µM CAP + 5 µM DHC), were added separately to PRP, and the count measured every 15 min for 2 hours.

**Thromboxane B2**

- Thromboxane B2 release from platelets treated with AA (300 µg/mL) in the absence and presence of 12.5 and 6.25 µM of CAP and DHC and a 60:40 combination was measured using a ELISA kit according to manufacturer's instructions (Abcam).

## RESULTS

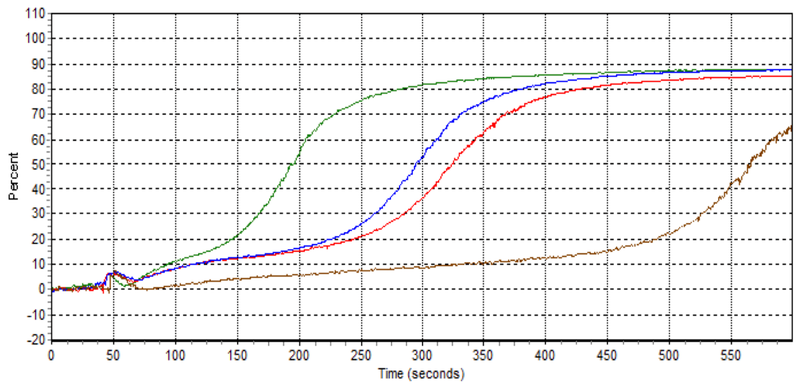


**Fig. 1. The effect of capsaicinoids on platelet aggregation induced by 300 µg/mL AA**

300 µg/mL AA
300 µg/mL AA + 12.5 µM CAP
300 µg/mL AA + 6.5 µM DHC
300 µg/mL AA + 7.5 µM CAP + 5 µM DHC

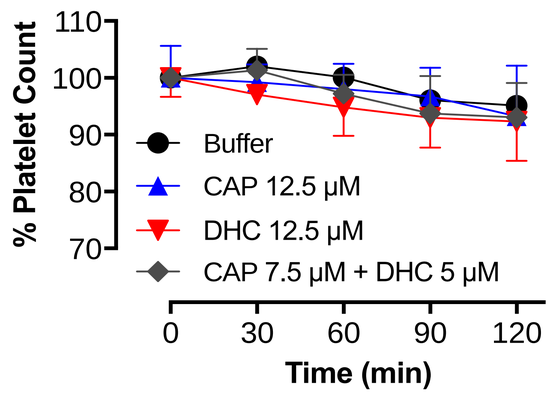


**Fig.2. Effect of CAP, DHC and their combination on platelet counts.**
12.5 µM of CAP and DHC, and the combination of CAP:DHC (7.5:5 µM), have no effect on platelet count over 2 hours of incubation. Data are presented as per cent of platelet count (normalised to platelet count at time zero). Mean ± SEM; n=4.

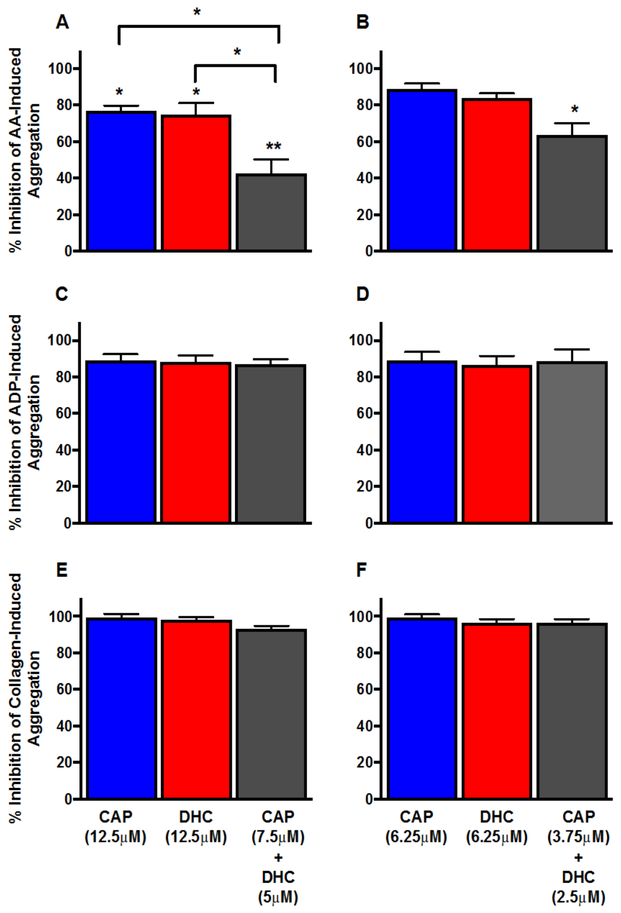


**Fig. 3. Effects of CAP, DHC and their combination on AA- (A and B), ADP- (C and D) and collagen- (E and F) induced platelet aggregation**.
Data are presented as percent area under curve normalised to aggregation in the absence of capsaicinoid (i.e., agonist only). Results are the mean ± SEM, n=4. *p<0.05, **p<0.001 compared to control (i.e., 0 µM of capsaicinoid) and to each column.

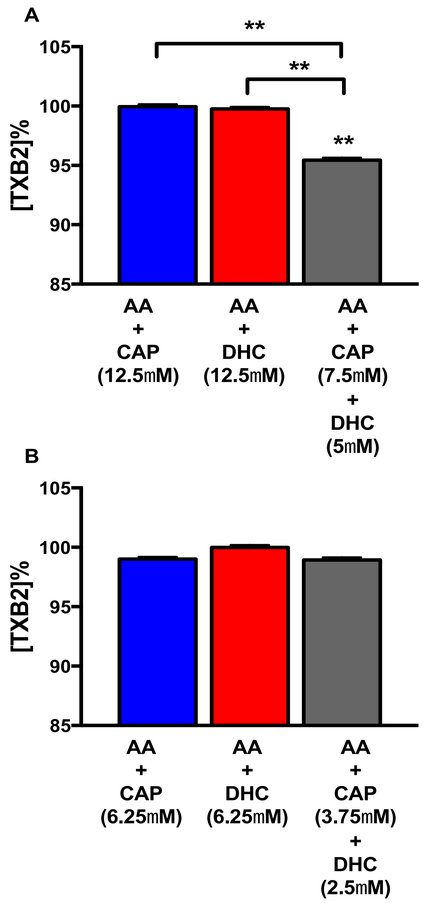


**Fig. 4. The effects of capsaicin (CAP), dihydrocapsaicin (DHC) and their combination on TXB2 formation.**
Data are presented as percent of TXB2 concentration normalised to TXB2 concentration with AA only (i.e. 0 µM capsaicinoid). Results are the mean ± SEM, n=4. **p<0.001 compared to control (i.e., 0 µM of capsaicinoid) and to each column.

## CONCLUSIONS

- The combination of CAP and DHC in the proportions they are present in chilli peppers, produces a significantly greater inhibitory effect on AA-induced (but not ADP or collagen-induced) platelet aggregation and TXB2 formation, compared to individual capsaicinoids.
- Further investigations are warranted to determine whether these capsaicinoids may be exploited for therapeutic benefit by dampening platelet activity via the arachidonic acid pathway.

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## ACKNOWLEDGEMENTS

- This study was funded by:
  - Clifford Craig Medical Research Trust, Tasmania (MJA, DPG, KDKA).
  - King Abdul Aziz University, Jeddah, Saudi Arabia (SYA).

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ISTH 2017
1295--PB
Platelet Function and Interactions
Murray Adams
DOI: 10.3252/pso.eu.ISTH2017.2017
Poster presented at ISTH2017 on:
Tuesday, July 11