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Original Article

ANTI-INFLAMMATORY ACTIVITY OF CURCUMIN AND CAPSAICIN AUGMENTED IN COMBINATION

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ABSTRACT

Objective: Dietary curcumin and capsaicin are well known for their health beneficial potencies. The current study was done to assess the anti-inflammatory activity of curcumin, capsaicin and their combination by employing *in vitro* and *in vivo* models.

Methods: We investigated the protective effect of curcumin, capsaicin and their combination using *in vitro* heat induced human red blood cell (HRBC) membrane stabilisation, *in vivo* 3% agar induced leukocyte mobilisation and acetic acid induced vascular permeability assay.

Results: Curcumin, capsaicin and their combination exhibited concentration dependent protective effect against heat-induced HRBC membrane destabilisation, while combined curcumin and capsaicin restored (87.0±0.64 %) membrane stability and it is found to be better than curcumin, capsaicin and standard diclofenac sodium (75.0±0.25. 72±0.9 and 80.0±0.31 %) protective effect. In agar suspension induced leukocyte mobilization assay, the combined curcumin and capsaicin had shown 39.5±1.58 % of inhibition compared to individual curcumin and capsaicin, which showed moderate inhibition of 16.0±3.14 and 21.6±2.17 % respectively. Besides, the combined curcumin and capsaicin had shown highly significant inhibition of acetic acid-induced vascular permeability in rats (62.0±3.14 %), whereas individual curcumin and capsaicin showed moderate inhibition of vascular permeability with 36.0±2.41 and 43.0±1.92 % respectively.

Conclusion: This study demonstrates the significant anti-inflammatory property of combined curcumin and capsaicin at half of the individual concentration of curcumin and capsaicin.

Keywords: Curcumin, Capsaicin, Leukocyte mobilization, Vascular permeability, Human red blood cell membrane

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INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli [1]. Upon contact with the stimuli, immune cells undergo activation and release inflammatory mediators such as vasoactive amines and eicosanoids to remodel the local vasculature. These mediators vasodilate and permeabilize the blood vessels causing exudation of blood plasma [2] results in enlarged and dilated blood vessels [3] thereby activation of leukocytes which play an important role in the development, propagation and maintenance of inflammation [4]. These leukocytes move towards the inflamed site or tissue to abet inflammatory response, found to be the essential step of defence against pathogens [5]. At the site of inflammation immune cells abrogate further invasion and multiplication of pathogens by phagocytosis [4] causing the production of several inflammatory mediators, including cytokines/chemokines, degrading enzymes, free radical oxygen and nitrogen species, and metalloproteases amplifying the inflammatory response and injury to surrounding tissues leading to inflammatory related diseases [6-11].

Spices are nutraceuticals which have been used as the predominant class of food adjuncts to enhance the taste, colour and flavor of foods and beverages. Curcumin, the yellow colouring principle of turmeric (*Curcuma longa*) and Capsaicin, the principle pungent component of red pepper (*Capsicum annuum*) are naturally occurring active principles. Clinical pharmacological studies on dietary curcumin and capsaicin have been proven to possesses promising health beneficial therapeutic potential such as anti-arthritic [12, 13], anti-inflammatory [14, 15], antitumor and anticancer [16-18], anti-hypoglycemic [19] and lipid-lowering activities [20]. Mechanism of action, which is responsible for the health beneficial pharmacological activity of these two commonly used curcumin and capsaicin have been extensively studied and reported [21-25]. Although these two spice principles share a considerable amount of structural homology nevertheless possesses notable differences in the mechanism of action. Hence, the protective effect of curcumin, capsaicin and their combination against copper/iron-induced low-density lipoprotein (LDL) oxidation, the toxicity of iron to liver and carrageenan-induced inflammation in rats was evaluated. The combined curcumin and capsaicin was found to be more effective than individual molecules. In addition, these molecules also shown to have decreased higher level of cholesterol and triglycerides in hypercholesterolemic and hyperlipidemic rats [23, 26]

In view of the above properties, the present study was further aimed at emphasizing the anti-inflammatory influence of combined dietary curcumin and capsaicin in *in vivo* and *in vitro* to verify, if any additive/synergistic property being exerted by them. Information on the protective effect of combined curcumin and capsaicin as anti-inflammatory agents would be more relevant in the context of the dietary source being used extensively in combination by the peoples around the world.

MATERIALS AND METHODS

Chemicals

Dietary curcumin and capsaicin were procured from Sigma-Aldrich. Agarose was procured from GeNei™ Bangalore, India. EDTA, Diclofenac sodium, Wright's stain, Evans blue, acetic acid solutions were procured from Himedia. All other chemicals used were of analytical grade.

Animals

Male Wistar rats weighing 150-200 g were housed in individual stainless steel cages, maintained on standard pellet diet with *ad libitum* water. The animals were maintained under controlled conditions of temperature with 12 h light-dark cycle. The animals were used after an acclimatization period of three days in the laboratory animal house. Animal experiments were carried out

taking appropriate measures to minimize pain or discomfort in accordance with the guidelines of the animal ethics laid down by the NIH (USA) regarding the care and use of animals for experimental procedures and with due clearance from the Institute's Animal Ethics Committee CPCSEA (Ref: NCP/IAEC/CL/14/12/2010-11).

***In vitro* anti-inflammatory assay**

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (5 ml) was collected in a heparinized tube and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and washed three times with equal volume of normal saline. The volume of blood was measured and re-constituted as 10% v/v suspension with normal saline [28, 29].

Heat-induced hemolytic assay

The reaction mixture (2 ml) consisting of 1 ml of sample/diclofenac sodium at different concentration and 1 ml of 10% RBCs suspension, parallel control was maintained under optimum conditions. The reaction mixture was incubated in water bath at 56 ˚C for 30 min. At the end of incubation, the tubes were cooled to room temperature. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was measured at 560 nm. Percent membrane stabilization activity was calculated by using the following formula [29, 30].

$$\text{Percent membrane stabilization} = \left[\frac{\text{As} - \text{Ac}}{\text{Ac}}\right] \times 100$$

Where, as and Ac represents the absorbance of sample and control, respectively.

***In vivo* anti-inflammatory assay**

Animal treatment

Male Wistar rats (6 per group) weighing 150-200 g were acclimatized for three days and then they were divided into 5 groups (A to F) as mentioned below.

Group A: Normal control (Normal saline)

Group B: Vehicle control (Olive oil)

Group C: Curcumin (100 mg/kg, p. o)

Group D: Capsaicin (30 mg/kg, p. o)

Group E: Combined curcumin and capsaicin (50 mg/kg curcumin+15 mg/kg capsaicin, p. o)

Group F: Diclofenac sodium (50 mg/kg, p. o standard drug)

The doses of curcumin and capsaicin were chosen on the basis of previous studies [31, 32].

Vascular permeability test in rats

The effect of individual and combined curcumin and capsaicin on acetic acid induced vascular permeability assay was assessed by a modified method of Whittles [33]. The animals were kept fasting for 10 h prior to the experiment and then administered suspension of normal saline, diclofenac sodium, curcumin, capsaicin and combined curcumin and capsaicin orally. After 3 h, all the groups were administered with 0.5 ml of 1% Evans blue solution (i. v), vascular permeability was induced by intraperitoneal injection of 1 ml of 0.6% acetic acid. Upon administration, the animals were left at room temperature for 20 min. Later, the animals were sacrificed under light ether anaesthesia and peritoneum was washed with 10 ml of normal saline and collected. The peritoneal fluid was centrifuged and the absorbance of the supernatant was observed at 610 nm. The percentage inhibition of vascular permeability was calculated using the formula:

$$\text{Percentage inhibition of permeability} = \left[1 - \left(\frac{\text{T}}{\text{C}}\right) \times 100\right]$$

Where T and C represent the absorbance measurements of the treated and control groups respectively.

Leukocyte mobilization test in rats

The protective effect of individual and combined curcumin and capsaicin on 3% agar induced *in vivo* leukocyte mobilization was evaluated by using the method of Rebeiro *et al.* [5]. After oral administration of normal saline, diclofenac sodium drug, curcumin, capsaicin and combined curcumin and capsaicin to respective groups, animals were left at room temperature for 3 h. Later, all the groups except parallel control were administered 0.5 ml of 3% w/v agar suspension (i. p) in normal saline. Four hours later, the animals were sacrificed under light ether anaesthesia and the peritoneal cavities were washed with 5 ml of 5% EDTA in phosphate buffered saline (PBS). The peritoneal fluid was recovered and subjected for total and differential leukocyte counts (TLC and DLC) using perfusates by following the manual cell counter after staining with Wright's stain. The percentage inhibition of leukocyte migration was calculated using the following formula:

$$\text{Percentage inhibition of leukocyte mobilization} = \left[1 - \left(\frac{\text{T}}{\text{C}}\right) \times 100\right]$$

Where T and C represent the leukocyte count of treated and control groups respectively.

Statistical analysis

All the experiments were performed in triplicate and results were recorded as mean±SD (standard deviation). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis by using Graph Pad prism 5 (San Deigo, CA). The values of $p<0.05$, $p<0.01$ were considered as statistically significant.

RESULTS AND DISCUSSION

Protective effect of dietary curcumin, capsaicin and their combination on heat induced HRBC membrane stabilization

Erythrocytes, most commonly and abundantly available cells of the human body and possess desirable physiological and morphological characteristics, hence used extensively as biological models [34]. Exposure of red blood cells (RBCs) to injurious substances results in the lysis of the membranes, accompanied by hemolysis and oxidation of hemoglobin [35]. During inflammation, lysosomes undergo lysis, as a result, they release enzymes producing a variety of implications [36]. Since lysosomal membrane components resemble human red blood cell (HRBC) membrane components [37], thereby HRBC were employed in the study.

The inhibition of heat induced human red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity of individual and combined curcumin and capsaicin. 100% lysis was observed when the HRBC membrane was heat incubated; increasing temperature could cause decreased osmotic fragility [38] and change the intracellular metabolism and perturbation of membrane structure [39]. Nevertheless, the stability of heat incubated membrane integrity was consistently maintained by curcumin, capsaicin and their combination including standard diclofenac sodium as shown in fig. 1. Curcumin, capsaicin and their combination along with standard diclofenac sodium have shown dose-dependent protective activity against heat-induced lysis of HRBC membrane. Mechanism of curcumin has indicated that it is reported to be aggregating at relatively high concentration, within the lipid bilayer of the membrane [40]. While the capsaicin was able to enter and align with the phospholipid bilayer of the membrane. Membrane stabilization activity of curcumin and capsaicin at 50 µg/ml concentration was found to be 75.0±0.25 and 72±0.9 respectively. These results are in agreement with the previous reports of Arnab *et al.* [41], where they have shown concentration dependant protective activity of curcumin against 2, 2'-azobis (2-amidinopropane) hydrochloride-induced hemolysis of HRBCs. Similarly, capsaicin has also shown a dose-dependent protective effect against the osmotic fragility of human erythrocytes, at 10^{-4} and 10^{-5} M [2]. However, combined curcumin and capsaicin have shown (25 µg/ml + 25 µg/ml) 87.0±0.64 % activity, which is more significant than

individual molecules and standard diclofenac sodium (80.0±0.31 at 50 µg/ml). Increased membrane stabilization effect of combined curcumin and capsaicin may be due to the facilitating effect of capsaicin in overcoming the mere aggregation of curcumin on the membrane surface by perturbing the packing of lipids and affecting the thermo tropic properties inside the cell.

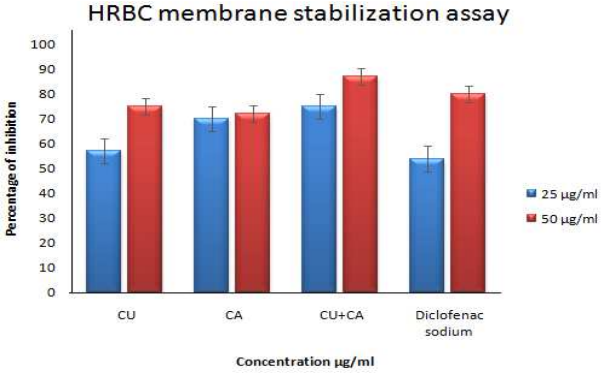

Fig. 1: Protective effect of curcumin, capsaicin and their combination on *in vitro* heat-induced lysis of human red blood cell membrane. Data is expressed as mean±SD [n=3]

Protective effect of dietary curcumin, capsaicin and their combination on acetic acid induced vascular permeability test in rats

Intraperitoneal injection of acetic acid induces dilation of blood vessels and this was evidenced by the leakage of Evans blue dye into the peritoneum cavity across the blood vessels including epithelial membrane results in the coloration of peritoneum fluid. The influence of dietary curcumin, capsaicin and their combination on vascular permeability induced by acetic acid in rats is presented in table 1. Dietary curcumin and capsaicin have shown 36.0±2.41 and 43.0±1.92 percent inhibition of acetic acid-induced vascular permeability.

A study by Saowanee *et al.* [42] reported that, oral administration of 50 and 100 mg/kg/day of curcumin for 3 w increased antioxidant and vascular protective effect on hypertension induced rats. Similarly, Jancso *et al.* [43] also reported that the local vasodilation and increased vascular permeability generated by the activation of sensory neurons was downregulated as well as reduced antidromic vasodilation in animals pretreated with capsaicin [44]. Combined curcumin and capsaicin exhibited significant inhibition of vascular permeability compared with curcumin and capsaicin, where the percentage inhibition of vascular permeability was 62.0±3.14 ($p<0.001$) and comparable with the diclofenac sodium (66.0±4.08). Specifically, an additive effect was seen when curcumin and capsaicin were fed in combination.

Table 1: Effect of dietary curcumin, capsaicin and their combination on acetic acid induced vascular permeability in rats

Treatment groups	Dose (mg/kg)	Absorbance	% inhibition
Control	-	0.77±0.09	-
Curcumin	100	0.49±0.03**	36.0±2.41
capsaicin	30	0.44±0.07***	43.0±1.92
Curcumin and capsaicin	50+15 (1: 1)	0.29±0.08***	62.0±3.14
Indomethacin	50	0.26±0.12***	66.0±4.08

Data is expressed as mean±SD [n=6], Values are performed using one-way ANOVA followed by Dunnett's multiple comparison tests. The values of *p<0.05, **p<0.01 and ***p<0.001 were considered as statistically significant.

Protective effect of dietary curcumin, capsaicin and their combination on agar induced leukocyte mobilization test in rats

The protective effect of curcumin, capsaicin and their combination on 3% agar induced leukocyte mobilization in the peritoneal cavity of rats was evaluated (table 2). Increased level of total leukocyte counts predominated by neutrophils was noticed in the peritoneum fluid collected from the agar induced rats. Whereas, curcumin, capsaicin and their combination have shown significant inhibition of leukocyte mobilization at the percentage of 16.0±3.14, 21.6±2.17 and 39.5±1.58 respectively. Nevertheless, diclofenac sodium (50 mg/kg b. w) treated group has shown 40.0±1.92 percent inhibition of leukocyte mobilization. However, half of the concentration of individual molecules in their combination has shown synergistic activity and it was almost comparable with that of the standard drug.

Our report is in agreement with Moghaddam *et al.* [45] who demonstrated that oral supplementation of dietary curcumin results in a marked reduction of total leukocyte and neutrophil counts induced by extrinsic and intrinsic inflammatory models in a dose-dependent manner in mice and Francis *et al.* [46] stated that administration of curcumin by intraperitoneal injection 3 h prior to lipopolysaccharide (LPS) injection into the air pouch cavities for 6 h resulted in the 50% inhibition of total leukocytes accumulation.

Table 2: Effect of dietary curcumin, capsaicin and their combination on agar induced leukocyte mobilization in rats

Treatment groups	Dose (mg/kg)	TLC × 10^9	% inhibition	Differential leukocyte mobilization (%)			
				Neutrophils	Lymphocytes	Eosinophils	Monocytes
Control	-	18.30	-	70.66	28.33	1.33	0.66
Curcumin	100	15.40*	16.0±3.14	59.66	38.00	1.66	0.66
Capsaicin	30	14.36**	21.6±2.17	58.00	38.66	1.33	2.0
Curcumin+capsaicin	50+15	11.08***	39.5±1.58	58.00	38.66	1.34	2.0
Diclofenac sodium	50	10.72***	40.0±1.92	60.75	36.5	1.25	1.5

Data is expressed as mean±SD [n=6], Values are performed using one-way ANOVA followed by Dunnett's multiple comparison tests. The values of *p<0.05, **p<0.01 and ***p<0.001 were considered as statistically significant.

CONCLUSION

Taken together, the present *in vitro* and *in vivo* studies demonstrate that the dietary intake of combined curcumin and capsaicin can suppress the heat induced *in vitro* HRBC membrane lysis, acetic acid-induced *in vivo* vascular permeability and agar induced *in vivo* leukocyte mobilization in a rat model. However, further biochemical and molecular studies will be necessary to reveal the molecular mechanisms by which these two commonly used combined dietary curcumin and capsaicin function *in vivo*.

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AUTHORS CONTRIBUTION

1. Thriveni Vasanthkumar: As a first author involved in designing of the work, Data collection, data analysis and data interpretation and drafting of the article.

2. Manjunatha H: As a co-author involved in the planning of the experiment, data interpretation and critical revision of the article.

3. Rajesh KP: As a co-author supported in the revision of the article.

CONFLICTS OF INTERESTS

Authors declare that there are no conflicts of interest

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