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RESEARCH ARTICLE

# Comparing the immunomodulatory and anti-inflammatory effect of curcumin and capsaicin on chronic stress-induced albino rats

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## ABSTRACT

**Background:** Stress is considered as a risk factor for many diseases. It is mainly because stress can suppress the immune system in our body by different mechanisms and also progress the inflammatory reactions. **Aim and Objectives**: In this study, we compared the immunomodulatory and anti-inflammatory effect of two commonly used spices: Curcumin and capsaicin. **Materials and Methods:** Forty-two male albino rats were examined in this study. Rats were divided into seven groups as control, acute stress, chronic stress, acute stress, and chronic stress with curcumin and acute and chronic stress with capsaicin. Blood samples were collected and analyzed for the inflammatory and immunity markers. **Results:** Results showed that exposure of chronic stress shows a significant change in CD4 and CD8 percentages and the selected inflammatory markers. Myeloperoxidase and C-reactive protein level increased significantly in the same group. Treatment with curcumin and capsaicin reverts the markers toward control group, but the change was not significant. **Conclusion:** The results also show that there is no significant difference between the activity of curcumin and capsaicin. Acute stress showed immunomodulatory effects.

**KEY WORDS:** Stress; Immunity; Curcumin; Capsaicin; C-reactive Protein; Myeloperoxidase; CD4; CD8

## INTRODUCTION

Hans Selye, Einstein of the medical research introduced the term stress to the medical research world.[1] He explained the stress as a syndrome produced by nocuous agents in 1936. In that, he also explained about the three pathological triads such as adrenal enlargement, gastrointestinal ulceration, and thymicolymphaticus involution caused by stress. Although he explained much about stress in this article, he used the term "stress" in 1950 on his first monograph, in which he defined stress as the non-specific response of the body to any demand on it. Later on, he stated stress as the effect of anything that threatens homeostasis. Numerous recent studies report data against this non-specificity theory of Selye. Latest definition of stress is the process of altered biochemical homeostasis produced by psychological, physiological, or environmental stress.[2] Stressors are the substance which cause stress and lead to the activation of neuroendocrine pathway mainly hypothalamic–pituitary–adrenal axis (HPA) and sympathetic nervous system (SNS) to cope the stress. Body's reactions to stress are collectively called stress response or general adaptation syndrome (GAS). Hans Selye explained GAS in three stages: Stage of alarm reaction, stage of resistance, and finally, the stage of exhaustion. The primary aim of GAS is to protect the host from stress caused negative effects. Withdrawal of the stressor brings the normal body function back and maintains the milieu interior within a short period of time. It is well-known fact that all physiological activities depend on keeping constant internal environment.[3] If any

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stimulus alters the internal environment of the body, it tries to maintain through specific neuroendocrine mechanism, and this is called as homeostasis. However, prolonged exposure to stress leads to challenging non-reversible changes, resulting in pathological cause for many diseases.

In this article, we tried to compare the effect of acute and chronic stress on immunity and the immunomodulatory effect of commonly used dietary spices such as curcumin and capsaicin. Psychoneuroimmunology is the field of science dealing with the interaction among CNS, endocrine system, and the immune system. It is well-documented fact that chronic stress adversely affects the immune system and this stress-related immune modulation is through HPA and sympathetic-adrenal-medullary axes. Both these axes provide an interface between stress and immune system. The paraventricular nucleus (PVN), the integrative center of the hypothalamus, receives the impulses through the sense organs and other neural and humoral pathways. PVN also receives the impulse from the amygdala, limbic forebrain, and brainstem. Activated PVN secretes corticotropin-releasing hormone (CRH) through glutaminergic neurons. This CRH secreted from PVN reaches the anterior pituitary through hypothalamo-hypophyseal portal blood circulation and subsequently stimulates the secretion of adrenocorticotropic hormone (ACTH) and released to the peripheral circulation. ACTH then reaches the adrenal gland and stimulates the production of glucocorticoids (GC). Acute secretion of GC considered as the "core response of the stress," and it stimulates the glucose metabolism and provides energy for "flight-or-fight" response of stress. Stress also causes the activation of SNS, and it causes the increased secretion of catecholamines. All these hormones including CRH, ACTH, GC, and catecholamines have their receptors on immune-mediated cells such as lymphocytes and monocytes. Reports show that lymphoid organs are also innervated by sympathetic nerves.[4] These data show the relation between the stress and immune system. Recent research reported the longitudinal correlation between the oxidative stress and inflammatory response.[5]

In this study, we evaluated the immunomodulatory and anti-inflammatory effect of commonly used dietary spices such as curcumin and capsaicin. Nowadays, there is a growing interest in research to find the beneficial effect of plant extracts as an antioxidant and anti-inflammatory agents. Flavonoids and phytochemicals present in the plants were believed to show the potential activities. Curcumin is a yellow-colored compound present in turmeric. Hundreds of studies are reported its anti-inflammatory property, and it is considered as a nonsteroidal anti-inflammatory drug. Chemically, it is 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione or diferuloylmethane. Effect of curcumin as an antioxidant, immunomodulatory, and hepatoprotective has been reported.[6,7] Its role against neurological and autoimmune disease is also been proved. It is suggested that curcumin acts as a natural chemotherapeutic agent, which kills only the cancer cell without damaging the neighboring normal cells.[8] Capsaicin, another spice, is our interest. It shows structural similarities with the curcumin. Its chemical name is 8-methyl-N-vanillyl-6-nonenamide. It is a pungent compound present in pepper, chili, and capsicum. Like curcumin, it also shows that wide range of pharmacological properties includes antioxidant, anti-inflammatory, anti-carcinogenic, and anti-inflammatory. As stated above, there are several studies explained about the beneficial effect of these spices, but there are few studies which compared these two. In this study, we are comparing the immunomodulatory and anti-inflammatory effect of these two spices.

Selected parameters in this study are CD4, CD8 count, myeloperoxidase (MPO), and C-reactive protein estimation (CRP). MPO is an antimicrobial heme enzyme (MW = 144 kDa) present in the azurophilic granules of neutrophils. It has pro-oxidative and pro-inflammatory properties. Any stimulus to the neutrophil activation increases the secretion of the same. It is also considered as the hallmark enzyme of the myeloid lineage. CRP is another ideal inflammatory marker. CRP has been proved to promote the symptoms of metabolic syndrome.[9]

## MATERIALS AND METHODS

### Animals

Forty-two male albino rats 6–8 weeks old weighing 180–200 g were used for the experiment. The rats were divided into seven groups of six rats. Animals were caged individually and maintained in an ideal laboratory condition room temperature at 22–24°C and 12-h light and dark cycle. The rats were fed with standard pellet and water *ad libitum*. All studies were conducted in accordance with the *Committee for the Purpose of Control and Supervision of Experiments on Animal*s, India guidelines. The study protocol was approved by the Institutional Animal Ethics Committee.

### Experimental Design

The animals were divided into seven groups as follows:
Group I - Normal control.
Group II - Exposed to acute water immersion stress.
Group III - Exposed to chronic water immersion stress.
Group IV - Acute water immersion + curcumin administration.
Group V - Acute water immersion + capsaicin administration.
Group VI - Chronic water immersion + curcumin administration.
Group VII - Chronic water immersion + capsaicin administration.

Rats assigned to control group were kept in a standard laboratory condition without being exposed to any type of stress. Rats selected for the stress groups were exposed to forced swimming stress for 1 h in a glass water tank, 1 day for acute group, and 7 days for chronic group. The

temperature of the water was maintained at 28°C. Capsaicin and curcumin were purchased from the Arjuna Chemicals. Spices were given orally with a concentration of 30 mg/Kg to the respective group for 10 days. Rats were sacrificed at the end of experiment, and blood was collected from the retro-orbital venous sinus. Fresh blood samples are used for the CD4 and CD8 estimation and total white blood cell (WBC) count. Remaining sample centrifuged at 4°C, and the plasma used for the estimation of MPO and CRP.

### Estimation of CD4 and CD8

Percentage of CD4+ and CD8+ cells was measured by flow cytometry (BD FACSCalibur). Antibodies were purchased from BD Scientific. 2 ml of CD8 and 1 ml CD4 antibodies mixed with 50 ml of whole blood and incubated for 15 min in the dark at room temperature. 450 ml of ×1 FACS lysing solution added to the blood antibody mixture and incubated in the dark for 15 min at room temperature and analyzed. Lymphocytes are selected by gating the appropriate population from SSC-H versus FSC-H plot. Cells are then analyzed from FL1-H versus FL2-H plot.

### Estimation of MPO and CRP

We had used spectrophotometric method for the estimation. 25 ml of plasma mixed with 50 ml of water and 25 ml of 50 mM phosphate buffer. 100 ml of reaction mix containing O-Dianisidine, hydrogen peroxide, and buffer added to the plasma mixture. Enzyme kinetics was measured at 450 nm for 5 min. CRP level in plasma was estimated using automated immune turbi metric method.

## RESULT

### CRP Concentration

Plasma CRP level was increased in stress-induced rats. Its concentration in chronic stress-induced group was 4 times more than the control group, and it showed a strong significant change, but change in CRP level was not significant in acute stressed group when compared with the control group. However, CRP level showed a marked recovery in response to curcumin and capsaicin treatment to chronic stress-induced rats compared to untreated stressed rats.

### MPO Activity

MPO activity showed a drastic increase in chronic stress-induced rats but not in acute group. Treatment with curcumin and capsaicin reversed the stress-induced effects into the normal range.

### CD4 and CD8 Count

Percentage of circulating CD4 and CD8 concentration decreased in chronic stress-induced groups. Whereas the levels tend to regained to normal range in curcumin- and capsaicin-treated groups, it was not significant. Acute stressed group showed a drastic increase in both CD4 and CD8 percentages.

Findings of our study are depicted in Table 1.

## DISCUSSION

It is universally accepted fact that stress suppresses the immunity and increases the chance for infectious diseases. In the present study, we compared the immunomodulatory and anti-inflammatory effects of curcumin and capsaicin in stress-induced rats.

It is a well-known phenomenon that stress alters the immune system and causes inflammation. Oxidative stress is the word used to express the effect of stress at molecular level. Free radicals generated during stress cause the inflammatory reaction. In our study, chronic stress-induced group showed a significant inflammatory response. Both the inflammatory markers, CRP and MPO, showed a significant increase when compared with the control group. It is a clear evidence that chronic stress induces the inflammation. The changes were not significant in curcumin- and capsaicin-treated groups, and there is no significant difference between these groups. Findings of our study showing that exposure of chronic stress decreases the percentage of CD4 and CD8 level. The previous studies reported the decreased concentration of circulating CD4 and CD8 level after exposure of chronic stress.[10] It also revealed that chronic stress suppresses the natural killer cell activity and mitogen-stimulated lymphocyte proliferation. In contrast to the effect of chronic stress, our result reported that acute exposure of stress enhances the immunity and increases

**Table 1:** Inflammatory and immunity markers in different experimental groups

| Markers | control | Acute stress | Chronic stress | Acute stress+curcumin | Acute stress+capsaicin | Chronic stress+curcumin | Chronic stress+capsaicin |
|---|---|---|---|---|---|---|---|
| CRP (mg/L of plasma) | 5.5±1.7 | 7.6±1.7 | 16.8±6 | 7±2.6 | 9.1±3.3 | 9.1±3.5 | 9.3±3.9 |
| MPO activity (U/ml of plasma) | 3.75±1.3 | 5.3±1.2 | 9.6±1.1 | 4.1±0.9 | 5.3±1.4 | 4.5±1.1 | 5.2±1.4 |
| CD4% | 12.9±2.4 | 22.97±3 | 7.6±2.5 | 26±7.7 | 9±2.7 | 22.5±7.2 | 8.6±2.3 |
| CD8% | 23.3±2.8 | 31.9±2.7 | 11.1±2.3 | 38.4±3.3 | 17.8±4.3 | 36.6±4.9 | 16.5±7.5 |

the concentration of CD8 and CD4. *In vitro* functioning of lymphocyte also increased by the induction of acute stress.[11] In our study, both the curcumin and the capsaicin in chronic stress-induced groups increases the concentration of CD4 and CD8, but the changes were not significant.

Reports are showing that free radicles activate all transcription factors that induce inflammation. Important transcription factors activated by free radicles are nuclear factor kappa B, activator protein 1, and NF-E2 related factor. Both the spices selected in our study were reported that they suppress NF-kB group of eukaryotic transcription factor responsible for the inflammation.[12,13] Recent studies are suggesting that they inhibit the activation of NF-kB by suppressing the degradation of its inhibiting factor IkBa and increasing the stability of the same.. Capsaicin also suppresses the TPA-induced activation of NF-kB.[14] They also inhibit the synthesis of other inflammatory markers such as COX, LOX, and iNOS and regulate the signaling pathways such as JAK-STAT and MAP kinase that are responsible for the inflammatory reactions.[11,15] A recent study reported that both curcumin and capsaicin lowered the inflammation induced by carrageenan and iron-induced hepatotoxicity.[16]Anti -inflammatory effect of curcumin on LPS-induced peritonitis was reported recently.[17] Our previous study reported that chronic exposure stress causes Kupffer cell hyperplasia, an inflammatory marker in liver tissue.[18] In contrast to the effect of chronic stress, acute group does not show any significant change in inflammatory markers level. It shows that acute stress has immunomodulatory and chronic stress has immunosuppressive effects.

The reason behind the bidirectional action of stress on immunity is hypothesized that exposure of acute stress causes the activation of SNS and increases the circulating WBC level by the action of norepinephrine. Sustained exposure of stress activates HPA and results in decreased level of leucocytes. HPA also causes the secretion of cortisol, which increases the migration of WBC from circulation to affected site.

A recent study reported that chronic exposure of ultraviolet (UV) radiation decreased the concentration of CD4 and CD8 around the tumor tissue.[19] The study also suggested that chronic exposure of stress increased the chance for squamous cell carcinoma induced by UV radiation. Another report by Greenberg is pointing that CD4 plays a crucial role in controlling tumor growth.[20] Several other studies also reported the direct correlation between chronic stress and cancer. In the light of our findings and previous reports, we can reach a conclusion that chronic stress can suppress the immunity and become a causative for cancer.

The great interest of research world is now to find the natural anticarcinogenic and chemotherapeutic agent. Both these spices are showing satisfactory results for this issue, mainly in *in vitro* studies. We are still far from the molecular targets of the actual mechanism of this spice, and we recommend for the molecular level researches. Several studies have shown the anti-inflammatory and immunomodulatory effects of curcumin but not much about the capsaicin. Our findings are showing that capsaicin also possesses the same effects of curcumin.

## CONCLUSION

In summary, the study reveals that acute stress is immunostimulatory and chronic stress immunoinhibitory. Curcumin and capsaicin are the two commonly used dietary spices which control the chronic stress-induced immunosuppression and inflammation, and these spices did not show any significant effects in acute stress-induced changes. Our study also reported that there is no significant difference between the effects of these two spices on chronic stress-induced changes.

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