

Plant derived histidine decarboxylase inhibitor and H₁ antihistamine treatment for atopic dermatitis

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Histidine decarboxylase is a key determinant of the levels of endogenous histamine that play important pathophysiological roles in allergic diseases. Inhibition of histamine synthesis by combination of H₁ receptor antagonist and plant derived histidine decarboxylase inhibitor catechin can be a potential strategy to reduce the side effect of H₁ antagonists. Atopic dermatitis was induced by challenge with 2,4-dinitrofluorobenzene in mice. Evaluation of parameters like clinical scoring, ear weight, vascular permeability, histamine release from mast cell, skin histamine content, histidine decarboxylase enzyme activity, gene expression, histopathology and CNS safety parameters in catechin, cetirizine and combination groups were carried out. All treatment groups showed significant decrease in clinical score, ear weight, vascular permeability, enzyme activity, histamine release from mast cells and skin histamine content. Further, catechin alone and combination group showed beneficial effects in gene expression and histopathology study with reduced side effect. Thus, combination will allow the development of a new strategy using regulation of histamine production and antagonism of H₁ receptor with reduced side effect.

Keywords: Catechin, Cetirizine, Histidine decarboxylase, 2,4-dinitrofluorobenzene, Mast cell, Histamine

The pathological mechanism of atopic dermatitis (AD) is very complex that involves the equal contribution of environmental, genetic and immunological factors¹. It is characterized by rash and severe itching, erythematous skin, lichenification, and intense pruritus of skin. The prevalence of AD is 10–20% in children and 1–3% in adults at the present time; it goes on increasing^{2,3}. The pathological mechanism of AD is very complex that involves the over-activation of immune cells such as mast cells. Histamine is a fundamental mediator released during the immediate allergic response from tissue mast cells and during the late phase response chiefly from recruited basophils. According to Bakrania & Patel, anti-histaminic agents and mast cell stabilisers can be an alternative choice for the treatment of allergic

disorders by blocking H₁ receptors. However, these drugs have a rapid onset of action but have shorter duration as well as having several side effects^{4,5}. Histidine decarboxylase catalyses the production of histamine in mast cell and histidine decarboxylase inhibitors can prevent the synthesis of histidine amino acid to histamine. Therefore, it is supposed to be valuable by reducing the potentially harmful, histamine-related allergic response.

In view of the disadvantages of presently existing synthetic anti-histaminic drugs, it is rational to evaluate the combination of plant derived substance with H₁ blockers that have both high selectivity and good oral efficacy and devoid of side effects can be a major beneficial advancement in the treatment of AD.

Catechin (CAT) is a flavanol found in various medicinal plants like *Acacia catechu*, *Camellia sinensis*. Many studies have reported to have potential efficacy of catechin in various diseases like infectious, neurodegenerative, cancer and metabolic disorders^{6,7}. Catechin also found to have inhibitory action on histidine decarboxylase enzyme in *in vitro* studies^{8,9}. Wide ranges of phytoconstituents were responsible for anti-allergic activity including phenolics, alkaloids and terpenoids. Catechin is a

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Abbreviations: AD, Atopic dermatitis; ANOVA, One way analysis of variance; CAT, catechin; CCSEA, Committee for the Control and Supervision of Experiments on Animals; CET, cetirizine; CNS, Central Nervous System; DNFB, 2, 4-dinitrofluorobenzene; IL, Interlukin; IAEC, Institutional Animal Ethics Committee; OPT, O-pthaldehyde; PBS, phosphate buffer saline; TNF- α , Tumor Necrosis Factor Alpha

flavanol obtained from *Acacia catechu* (Katha) that showed inhibitory action on histamine decarboxylase in *in vivo* and *in vitro* studies and estimated to have low toxicity in human studies¹⁰. Therefore, the aim of the present study was to evaluate the effect of catechin and its combination with H₁ blocker cetirizine (CET) at reduced dose in the treatment of atopic dermatitis.

Materials and Methods

Drugs and chemicals

CAT (Sigma Aldrich chemicals pvt ltd, India), CET (Balaji drugs ltd.), Evans blue dye (Sigma Aldrich chemicals pvt ltd, India) and DNFB (Hi media Pvt. ltd. India) were purchased. Cytokines kits for estimation of IL-12 and TNF- α were purchased from Krishgen Biosystem, Pvt. Ltd., Mumbai. All other reagents and chemicals used in the present experiments were of analytical grade and procured from local suppliers.

Experimental animals

Male 6 week old BALB/c mice (20-25g) and Male Sprague Dawley rats (250-300 g) were procured from registered breeders. Mice and Rats were housed in our departmental animal house under standard 12 h light/dark cycles, pathogen free environment and with free access to standard rodent pellet diet and water *adlibitum*. All the experimental procedures and protocols used in this study were reviewed and approved (IP/PCOL/FAC/13-1/025, IP/PCOL/FAC/14-1/015) by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmadabad, constituted under Committee for the Control and Supervision of Experiments on Animals (CCSEA). CCSEA guidelines were strictly followed during the experiments.

Experimental design

Mice were randomized as per body weight into 5 groups: normal control, disease control, CAT (100 mg/kg, p.o), CET (10 mg/kg, p.o), CAT + CET combination (50 mg/kg & 5 mg/kg, p.o respectively). For induction of AD mice were sensitized by painting 50 μ L of DNFB (0.1%, v/v) in Acetone: Olive oil (4:1) onto the shaved dorsum of each animal for three consecutive days (1, 2 and 3rd day). Four days after sensitization, each mouse was challenged by painting 30 μ L of DNFB (0.2%, v/v) onto the dorsum of both ears every two days (7, 9, 11, 13rd days). The mice were treated with CAT (100 mg/kg, p.o), CET

(10 mg/kg, p.o) and CAT + CET combination (50 mg/kg & 5 mg/kg), p.o respectively every two days (10, 12, 14th day). All animals except normal control mice were sensitized and challenged with DNFB. The normal control animals were treated with acetone: olive oil (4:1). At the end of the treatment period and 24 h later of last DNFB application, clinical scoring was done and animals were anesthetised, blood samples were collected from retro-orbital sinus, centrifuged at 4000 rpm for 15 min at 4°C. The serum samples were stored in -80°C °C until analysis. The serum levels of pro-inflammatory cytokines like TNF- α and IL-12 were determined using commercially available ELISA kits. Determination of ear weight, vascular permeability using Evans blue dye, mast cell histamine content in ears, skin histamine content, histidine decarboxylase (HDC) assay of skin, ear histopathology study, HDC gene expression study was carried out with some modification^{11,12}.

Measurement of clinical scoring

On day 14, The severity of AD in all groups were assessed according to the modified SCORAD (Severity Scoring of Atopic Dermatitis) index described by Leung *et al.*¹³. Clinical signs of dermatitis were evaluated by five categories; erythema, edema, excoriation, erosion, hemorrhage/oozing, and scaling/lichenification. The total score was taken from the grades of each symptom; 0 (No symptoms), 1 (Mild), 2 (Moderate), 3 (Severe). Total dermatitis scores were the sum of these individual scores. Each score was assessed by independent investigator¹⁴.

Determination of mast cell histamine

On the same day, the phosphate buffer saline (PBS) having pH 7.4 was injected by intraperitoneal route to the animals and massaged thoroughly. The PBS was collected from anesthetised animals from peritoneal cavity and was centrifuged at 2000 rpm for 5 min at 4°C. The pellet which formed after centrifugation, was washed twice with PBS and resuspended into 2 mL PBS¹⁵. The cell suspension was pre-incubated at 37°C for 5 min followed by treatment with 0.1 mL compound 48/80 for 10 min. histamine-o-phthaldehyde (OPT) was added to terminate further release of histamine by addition of 0.1 mL of ice-cold phthalaldehyde (OPA) (10 mg/mL) for 4 min at room temperature. Fluorescence was assayed at 450 nm emission and 360 nm excitation wavelength using flurospectrometer.

Determination of vascular permeability

On day 15, 1% Evan's blue dye solution (200 μ L) was injected into the tail vein of mice. After 30 min, mice were sacrificed and right ear was removed. Ear tissue samples were dissolved in 0.7 mL of 1 N potassium hydroxide solution in a stoppered tube at 37°C overnight and 9.3 mL of a mixture of 0.6 N phosphoric acid solution and acetone (5:13) was added. After vigorous shaking and centrifugation at 3000 rpm for 10 min the amount of Evan's blue dye leakage was measured colourimetrically at 620 nm (wavelength for which the absorbance is highest)⁴.

Measurement of ear weight

After determination of vascular permeability, left ears were isolated and ear tissue samples of 6 mm diameter were collected from each animal by using metallic punch. The tissue samples were weighted on analytical balance and results were expressed as increase in ear weight.

Determination of skin histamine content

On day 15, skin was removed and individually homogenized with ice-cold 0.02M phosphate buffer (pH 6.2) containing pyridoxal-5-phosphate (20 mM) and dithiothreitol (200 mM). The homogenates were divided into two. One part was used for estimation of skin histamine content and the other part was used for HDC activity assay. One part of homogenate histamine was then extracted into 5 mL isoamyl alcohol by shaking for 10 min. After centrifugation for 10 min at 4°C at 2000 rpm, the supernatant was washed with 2 mL of alkaline salt saturated chloroform. The washed extract was then shaken with 5 mL heptane and 2.5 mL of 0.1N hydrochloric acid. Histamine was measured fluorometrically as per previously described method⁴.

Determination of skin HDC activity

To another part of homogenate obtained, was incubated with 0.25 mL histidine at 37°C for 4 h. The enzyme reaction was terminated by addition of 0.8 N ice-cold perchloric acid followed by centrifugation for 5 min at 1500 \times g. 1 mL of supernatant was saturated with di-potassium phosphate to yield pH 8.5-8.8. Histamine was then extracted into 5 mL isoamyl alcohol by shaking for 10 min. After centrifugation for 10 min at 4°C at 2000 rpm, the supernatant was washed with 2 mL alkaline NaCl saturated CHCl₃. The extract was mixed with 5 mL heptane and 2.5 mL 0.1N HCl. Histamine was measured fluorometrically as per previously described method¹⁶.

Measurement of TNF- α and IL-12 in serum samples

The levels of pro-inflammatory cytokines such as TNF- α and IL-12 in serum were done using commercially available ELISA kits (Krishgen Biosystem, Mumbai) according to manufacturer instructions.

Histopathological examination of ear tissue

Animals were sacrificed and ear lobes were excised, fixed with 10% formalin and sectioned at 5 μ m thickness. Sections were stained with Hematoxylin and Eosin (H and E) and toluidine stain for changes in thickening of epidermis and dermis, accumulation of inflammatory cells such as mast cells, eosinophils, neutrophils and hyperkeratosis¹⁷.

Determination of mRNA expression of HDC enzyme by PCR

200-300 mg of stomach specimen were taken after 24 h of DNFB application on day 15, homogenized and total RNA was isolated using FastRNA[®] Pro Green Kit (M.P biomedical) following the instructions of user's manual. The RNA integrity was estimated by analysing a portion of RNA sample using gel electrophoresis. 5 μ L of RNA & 2 μ L of bromophenol blue were loaded on 1.2% agarose gel. After electrophoresis, gel was visualized under UV light. The RNA appears as sharp and distinct bands. The RNA concentration was determined by diluting 10 μ L of RNA sample in 990 μ L of diethylpyrocarbonate (DEPC) treated water and absorbance was read using DEPC water as blank at 260 nm. First-strand cDNA was synthesized from 1 μ g of RNA using Revert Aid[™] First Strand cDNA Synthesis Kit (Thermo Scientific) and incubated at 42°C for 60 min followed by 70°C for 10 min. PCR was carried out to check gene expression using primers specific for mouse HDC and β -actin with PCR Master Mix (Thermo Scientific) according to the user manual. PCR products were detected using gel electrophoresis¹⁸.

CNS safety pharmacological evaluation of CAT, CET & CAT + CET combination on locomotor activity, muscle coordination and behavioural changes in mice.

Male BALB/c mice were divided into 4 groups: normal control, CAT (100 mg/kg, p.o), CET (10 mg/kg, p.o), CAT + CET (50 mg/kg & 5 mg/kg, p.o). The treatments of CAT (100 mg/kg, p.o), CET (10 mg/kg, p.o), CAT + CET (50 mg/kg & 5 mg/kg, p.o) were given for 14 days while the normal control group was administered with vehicle carboxy methyl cellulose. 60 min after drug treatment animals were observed for behavioural changes and spontaneous

motor activity. Animals in all groups were also observed for exploratory behaviour like rearing, sniffing, licking and grooming and they were scored accordingly. Another set of animals were used for determination of muscle coordination. Treatment was carried out in different groups as described above and after 2 h individual animal will be kept on rotating rod and its latency to fall down was recorded¹⁹.

Statistical analysis

The results were expressed as mean ± SEM. Comparison of groups was done using one-way analysis of variance (ANOVA) followed by Tukey test. The difference between the groups considered statistically significant when the *P*<0.05 is obtained.

Results

Effect of CAT, CET and CAT + CET combination on clinical scoring and ear weight on DNFB-induced AD

To determine the effects of CAT and CAT + CET combination on the allergic response, the clinical scoring was evaluated. After DNFB induction, the BALB/c mice developed severe erythema, edema, excoriation, erosion, hemorrhage, and scaling on the dorsal skin of disease control group. As illustrated in Table 1, mice treated with CAT, CET and CAT + CET combination has shown significant reduction in clinical scoring induced by DNFB. Furthermore, similar results were observed in ear swelling reaction in mice by CAT, CET and CAT + CET combination treatments.

Effect of CAT, CET and CAT + CET combination on release of histamine from skin mast and peritoneal cells.

The regulatory effect of CAT on histamine release was measured from skin mast and peritoneal cells. The CAT, CET and CAT + CET combination have shown significant inhibition of release of histamine from mast cells of skin as well as peritoneal cavity as compared with disease control group mice (Table 1).

Effect of CAT, CET and CAT + CET combination on skin HDC activity

DNFB challenge to mice showed increased in HDC enzyme activity which showed by increase in the conversion of L-histidine to histamine in disease control group in comparison with normal control group, while the CAT, CET and their combination has showed decreased skin HDC enzyme activity and subsequently decreased conversion of L-histidine to histamine as compared to disease control group mice (Table 1). The results indicate that combination reduces the allergic response induced by DNFB through regulation of histamine synthesis.

Effect of CAT, CET and CAT + CET combination on vascular permeability

DNFB challenge showed significant increase in Evan's blue vascular permeability in disease control group, while the CAT, CET and CAT + CET combination showed significant reduction in vascular permeability as compared to disease control group of animals (Table 1).

Effect of CAT, CET and CAT + CET combination on serum TNF-α and IL-12 levels

Increased levels of TNF-α and IL-12 as a result of DNFB exposure was reversed by CAT, CET and CAT + CET combination groups, although combination has shown better effect as compared to CAT and CET group alone as shown in Fig. 1A & 1B.

Effect of CAT, CET and CAT + CET combination on histopathological examination of ear tissue

To investigate the suppressive effect of treatments on dermis ear tissue, histological analysis of ear tissue was performed. The dermis layer of ear tissue was found to be thicker in the normal control group, whereas the epidermis was thinner in DNFB induced group (Fig. 2A & 2B). However, the dermal thickness greatly decreased in the CAT, CET, CAT + CET treated groups (Fig. 2C- 2E). In order to investigate the effects of treatments on infiltration of mast cells,

Table 1 — Effect of treatments on various parameters in DNFB induced Atopic dermatitis

Parameters	NC	DC	CAT	CET	CAT+CET
Clinical Scoring	1.0±0.0	13.0±0.9 ^{###}	6.8±0.16 ^{***}	8.0±0.3 ^{***}	5.0±0.1 ^{***}
Difference in ear weight (mg)	0.33±0.21	16.3±0.21 ^{###}	11.3±1.30 ^{**}	56.0±0.33 ^{***}	8.0±0.44 ^{***}
Histamine in east cells (ng/mL)	60.6±4.06	142.8±11.9 ^{###}	105.3±78.4 ^{***}	75.5±51.7 ^{***}	83.9±61.4 ^{***}
Histamine in skin (ng/mL)	54.4±3.54	114.1±9.80 ^{###}	87.2±6.44 ^{**}	76.82±6.26 ^{***}	66.51±4.91 ^{***}
Vascular permeability (ng/mL)	216.6±17.2	972.2±61.4 ^{###}	683.3±48.4 ^{**}	377.7±36.9 ^{***}	438.9±28.1 ^{***}
HDC activity (activity/mg protein)	44.8±3.84	105.1±8.53 ^{###}	63.6±4.59 ^{***}	85.5±3.96 ^{**}	76.9±6.75 ^{***}

[Values expressed as Mean ± SEM (n = 6) & analysed by One-way ANOVA followed by Tukey's test. ^{###} *P* < 0.001 as compared to NC group and ^{**} *P* < 0.01 and ^{***} *P* < 0.001 as compared to DC group. NC: Normal control; DC: Disease control; CAT: Catechin; CET: Cetirizine; CAT + CET: Catechin + Cetirizine combination]

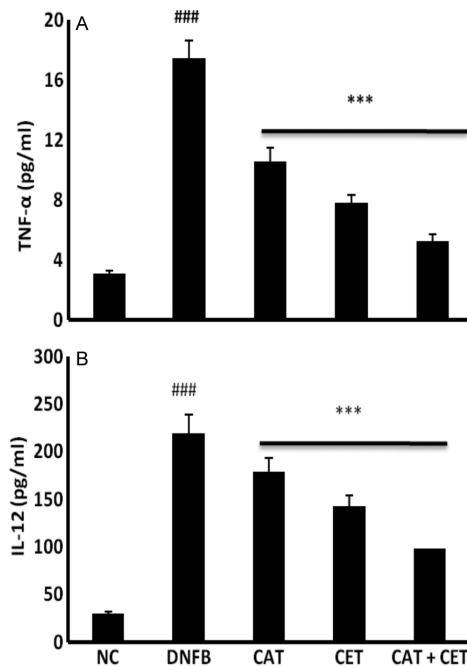


Fig. 1 — Effect of CAT, CET and CAT + CET combination on serum samples in AD (A) TNF- α (B) IL-12. [Values expressed as Mean \pm SEM (n = 6) & analyzed by One-way ANOVA followed by Tukey's test. ### $P < 0.001$ as compared to NC group and * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared to DC group. NC: Normal control; DNFB: Di-Nitro-Fluoro-Benzene; CAT: Catechin; CET: Cetirizine; CAT + CET: Catechin + Cetirizine combination]

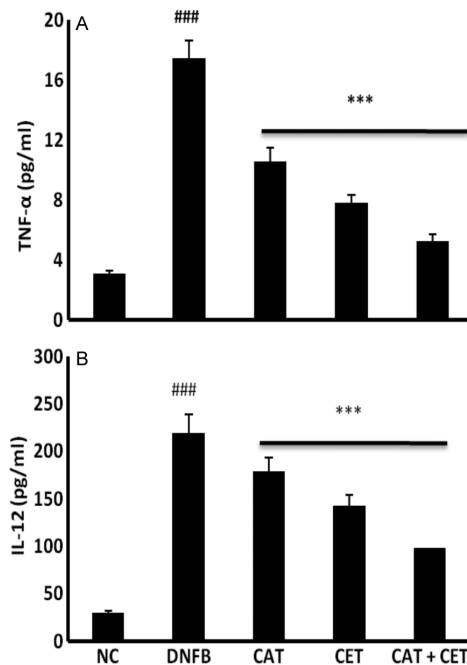


Fig. 2 — Effect of CAT, CET, CAT + CET combination treatment on histopathological examination of ear tissue (H & E stain) in DNFB induced AD in BALB/c mice. [NC: Normal control; DC: Disease control; CAT: Catechin; CET: Cetirizine; CAT + CET: Catechin + Cetirizine combination]

microscopic sections of ear tissue were stained with toluidine blue and slides were observed under a microscope. Size of mast cells were significantly increased in the disease control group compared to the NC group (Fig. 3A & 3B). Size of mast cell found to be reduced in CAT group although it was found to be lower than CAT + CET combination group (Fig. 3C & 3E). These data suggest that CAT and its combination may contribute to the inhibition of mast cell infiltration.

Effect of CAT, CET and CAT + CET combination on HDC mRNA expression

To examine whether CAT could induce modification of atopic response by inhibition of HDC mRNA expression. The expression was measured by real-time PCR study in BALB/c mice. High HDC mRNA expression was found in the disease control group (Fig. 4H), where as a low level was detected in the CAT and CET combination group (Fig. 4K). Whereas, CAT (Fig. 4I) and CET (Fig. 4J) did not show any significant effect on HDC mRNA expression. β -actin expression was observed in all groups of animals (Fig. 4B- 4F).

CNS safety pharmacological evaluation of CAT, CET and CAT + CET combination on locomotor activity, muscle coordination and behavioural changes.

Treatment of mice with CAT didn't show any significant change in the locomotor activity and exploratory behaviour was observed as compared to normal control mice. While, the treatment with CET (10 mg/kg) showed significant decrease in spontaneous activity and exploratory behaviour. CAT + CET combination group has shown no significant effect on these parameters (Table 2). There was no significant change observed in muscle coordination in any treatment groups.

Discussion

The atopic dermatitis (AD) is a "chronic inflammatory skin illness" with remissions and exacerbations itch associated with asthma and allergic rhinitis. It is characterized by erythema, edema, and scaling²⁰. A complex inter-relationship of genetic, environmental, pharmacological and psychological factors leads to the progress and severity of the illness. Different manifestations of immunological disorders are an increment in the liberation of pro-inflammatory mediators by mast cells. Steroid therapy is crucial in the treatment of AD, but it cannot be given for longer duration because of its harmful side

Table 2 — Effect of treatments on Central Nervous system

Parameters	NC	CAT	CET	CAT + CET
Locomotor activity (Number of counts)	51.5 ± 1.14	47.66 ± 1.02	41.33 ± 0.61*	45.66 ± 1.58
Muscle coordination (Falling latency in sec)	33.44 ± 3.9	32.16 ± 4.4	31.72 ± 4.3	30.22 ± 4.7
Exploratory behaviour (score)	15.6 ± 0.87	18.5 ± 0.84	11 ± 1.41*	17.5 ± 1.05

[Values expressed as Mean ± SEM (n = 6) & analysed by One-way ANOVA followed by Tukey's test. *P < 0.05 as compared to NC group. NC: Normal control; CAT: Catechin; CET: Cetirizine; CAT + CET: Catechin + Cetirizine combination]

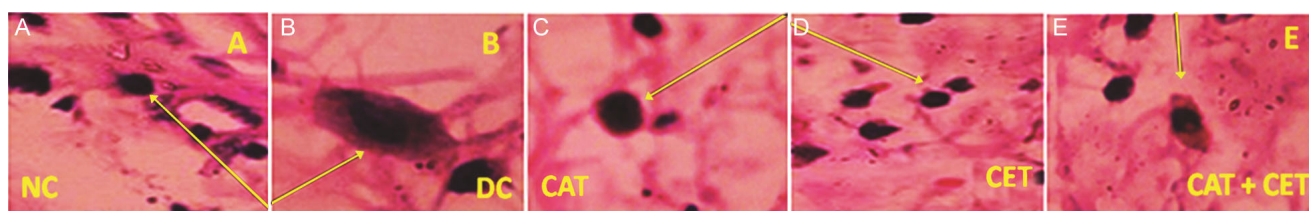


Fig. 3 — Effect of CAT, CET, CAT + CET combination treatment on histopathological examination ear tissue on mast cell size (Toluidine blue) in DNFB induced AD in BALB/c mice. [NC: Normal control; DC: Disease control; CAT: Catechin; CET: Cetirizine; CAT + CET: Catechin + Cetirizine combination]

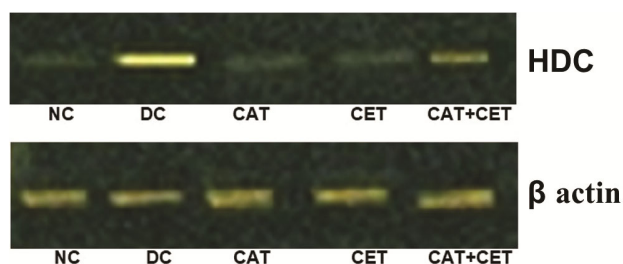


Fig. 4 — Effect of CAT, CET and CAT + CET combination on HDC mRNA expression. [NC group, DC group, CAT group, CET group and CAT + CET combination]

effects. Therefore, several attempts were made to find a new molecule, which is beneficial in the treatment of AD. Special concentration has been focus to medicinal plants with anti-inflammatory activity due to their safety and efficacy. Natural product-based agents with good safety and efficacy lowers the risk of side effects and recommends promising treatment for the prevention of inflammatory conditions. The AD was characterized by a potent skin inflammation associated with an elevated level of IgE against many types of allergens²¹. Previous studies have reported that catechin and its analogues have usefulness as anti-inflammatory, antidiabetic, anticancer, neuroprotective, antibacterial, antirheumatic, and hepato-protective mainly through NF-κB, Nrf-2, TLR4, and MAP Kinase pathways²². On the basis of these studies, we have focused on evaluating the effects of CAT and its combination with CET in mice model of atopic dermatitis. The findings of this study revealed that CAT + CET combination has significantly reduced DNFB-induced atopic dermatitis and ear swelling responses.

Catechin is a flavanol obtained from *Acacia catechu*. CAT is reported to be useful in treatment of ulcers, gastritis, ulcerative colitis and reduces gastric tissue histamine levels^{23,24}. The specific action of CAT is HDC enzyme inhibitor activity has been reported previously²⁵. CET is a well-known anti-allergic drug associated with short life span and CNS side effects whereas; CAT has longer duration of action and no reported side effect.

Allergic reactions are resulted due to release of histamine from mast cells that causes vasodilatation followed by infiltration of inflammatory cells at the site of infection. The clinical scoring was significantly higher in disease control group due to the allergic reaction manifestation by DNFB. Treatment of mice with CAT and CAT + CET combination produces significant decrease in clinical scoring because CAT inhibits histamine synthesis. Thus, there is reduction in the histamine related allergic reaction like vasodilatation, release of infiltration cells, edema, erythema, erosion, hemorrhage and scaling. The challenge of DNFB to ear causes release of histamine, followed by increase in vascular permeability. Due to increase in vascular permeability of mice the adhesion molecules get activated and cause release of infiltration cells at the site of application that resulted into increase in ear weight (ear swelling). This presence of infiltration cells and other fluids in the skin increase the ear weight²⁶. The application of DNFB to BALB/c mice produces increase in ear weight in disease control group animals. Treatment of BALB/c mice with CAT + CET combination produces significant reduction in ear weight due to

inhibition of synthesis of histamine by CAT and an anti-histaminergic activity of CET.

As per the credit of Navi, *et al.* mast cells present in the skin plays important role in the functioning of immune system^{27,28}. The most common well documented immunoglobulin associated with mast cell is IgE, having cell surface high-affinity IgE receptor (FcεRI). After sensitization of mast cell it produce immediate type of hypersensitivity reaction. According to recent developments the mast cells may mediate immune suppression of regulatory T-cells²⁹. These mast cells produce an array of cytokines, chemokines, and growth factors that regulate the enrolment, trafficking, and functioning of other immune cells and cells of skin inflammatory response. The mast cells stimulate cell adhesion molecules on endothelium surface that are essential for interaction of leukocyte with endothelial cells interactions in presence of TNF-α³⁰. Further, secretion of IL-4 and IL-12 activates vascular cell adhesion molecule 1 on endothelial cells which are also involved in inflammatory responses. Mast cells stimulates differentiation of naive T-cells to Th1 and Th2 and enhance T-cell activation³¹. Due to secretion of pro-inflammatory mediators from FcεRI activated mast cells, as well as sensitization to allergens, IgE elevation, and increased mast cells in a majority of AD patients, mast cells are believed to be involved in the pathogenesis of atopic dermatitis. Skin is rich in mast cells and DNFB challenge increases the IgE level of blood. This increase in IgE level of blood causes degranulation of mast cells and increases the skin histamine level³². Skin histamine levels have been found to be increased significantly in disease control group. While, the treatment groups showed reduction in skin histamine levels by virtue of their histamine synthesis inhibitory activity of CAT and anti-histaminic activity of CET.

Vascular permeability assessment was done by use of Evan's blue dye. Histamine has major action on blood vessels and dilates them thereby causes increase in leakage of histamine in blood, which is termed as vascular permeability³³. Disease control group showed significantly increased vascular permeability, while treatment with CAT + CET combination showed significant reduction in vascular permeability by virtue of their HDC enzyme inhibitory activity and antihistaminic activity respectively. The HDC enzyme activity was significantly increased by attack of allergens like DNFB due to release of various pro-

inflammatory mediators in disease control group. Treatment with CAT + CET combination significantly decreased conversion of l-histidine to histamine *via* inhibition of HDC enzyme and anti-histaminergic activity respectively. CAT binds to the catalytic hydrophobic site of HDC causes conformational change in the enzyme and thus inactivates it³⁴.

A number of cytokines including TNF-α, IL-1, IL-6 and IL-12 plays an important role in the development of acute and chronic inflammatory response. These pro-inflammatory cytokines also affect swelling and inflammation in AD-like skin lesions³⁵. TNF-α and IL-12 are produced by activated tissue macrophages and induce acute inflammatory response. The estimation of TNF-α and IL-12 was performed to find inflammatory responses. The increased serum levels of TNF-α and IL-12 were found in DNFB induced mice. While, treatment with CAT + CET combination showed significant reduction both the markers indicated reduction in inflammatory response.

The histopathological studies revealed that DNFB challenge increased the dermal thickening, hyperplasia, presence of inflammatory cells, increase in number and size of mast cells in disease control group. All these changes such as increased vascular permeability causing dermal thickness and infiltration of inflammatory cells are observed due to histamine release from mast cells. When mast cells get degranulate their size seems to be increased or elongated. Here, binding of IgE to mast cells through FcεRI receptor in skin causes an increase in the mast cell size³⁶. Treatment of mice with CAT + CET combination shows reduced dermal thickness and decreased size of mast cells compared to disease control *via* their HDC enzyme inhibitory activity and antihistaminic activity respectively. Increased histamine production leads to increase in HDC mRNA expression in body. HDC mRNA expression studies were carried out by using stomach tissue of mice. PCR amplifies the mRNA expression which is detected using gel electrophoresis^{37,38}. Disease control group showed increased mRNA expression compared to normal control group. Treatment with CAT (50 and 100 mg/kg) showed decreased mRNA expression compared to disease control, as CAT reduces the allergic response by inhibiting HDC enzyme while CET could not reduce HDC mRNA expression. Thus, mRNA expression studies confirm HDC inhibitory action of CAT.

The antihistaminic drug CET produces various CNS side effects like sedation, drowsiness, confusion, dizziness, restlessness *etc.* due to blockade of muscarinic receptor in brain by crossing blood-brain-barrier³⁹. Hence, the effect of CAT, CET and their combination was performed on locomotor activity in BALB/c mice. Moreover, several studies have suggested that CAT is a mood elevator. Thus, combination of CET and CAT can successfully mask the sedative activity of CET by directly reducing the dose of CET and mood-elevating action of CAT. The safety studies proved that combination of CAT + CET reduced the side effects of CET. Treating rats with CET produced a significant decrease in locomotory activity compared to normal control groups. This may be because of sedative effect of CET but combination of CAT + CET produced slight decrease in locomotory activity compared to CET group. This study showed that combination of CAT + CET could be safer compared to CET group alone. Evaluation of the CNS activity was also carried out by rota rod test, which suggested no significant reduction in the muscle coordination or fall of time in treatment groups compared to normal control group. Open field test was used to demonstrate the exploratory behaviour in mice where ambulation with all four paws in different boxes was checked⁴⁰. CET group showed significantly decrease in exploratory behaviour in animals compared to normal control group, while CAT and CET combination and CET group showed no significant increase in exploratory activity compared to normal control group. Lower ambulation and exploration in animals was observed due to sedative activity of CET. Thus, from above study it can be concluded that CAT and CET combination has minimal or reduced CNS side effect as compared to CET treatment alone and can be used in management and treatment of allergic conditions like AD *via* their HDC enzyme inhibitory activity and H₁ receptor antagonist activity.

Conclusion

From the present study it can be concluded that CAT is HDC enzyme inhibitor and inhibits histamine synthesis but not completely reduce histamine levels in body and thus does not interfere with normal physiological role of histamine. The combination of CAT + CET has significant anti-allergic effect on DNFB induced AD in BALB/c mice. The combination of CAT + CET has almost no unwanted effects on brain. Thus, it can be concluded that HDC

enzyme inhibitory activity of CAT and histaminergic receptor antagonist activity of CET their combination is proved to be a better therapeutic regimen in treatment of AD in BALB/c mice. Moreover, it minimizes side effects of CET by reducing the dose of CET when used in combination. These types of combinations will not only reduce the toxicity of currently available drugs but would also enhance their efficacy. Histidine decarboxylase inhibition can be a novel approach towards the development of a safe and effective drug for treatment of atopic dermatitis.

Conflict of interest

The authors declare that there are no conflicts of interest.

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