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Protective effect of catechin on humoral and cell mediated immunity in rat model

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ABSTRACT

The present study was focused on examining the effect of catechin on the cellular and humoral immunity in rat model. Immunomodulatory effect of catechin was determined by delayed-type hypersensitivity (DTH) response, carbon clearance assay, leucocyte mobilization test and cyclophosphamide-induced myelosuppression and hemagglutinating antibody (HA) titer assay. Catechin in experimental dose (25, 50 and 100 mg/kg, p.o.) elevated a significant increase in antibody titer in the hemagglutination test with increased levels of immunoglobulin. There was an enhancement in the delayed-type hypersensitivity reaction produced by sheep red blood cells. There was also restoration in the functioning of leucocytes in cyclophosphamide-treated rats with an increased clearance of carbon particles. The results of the present study signify that catechin possesses sufficient potential for modulating immune activity by cellular and humoral mechanisms.

1. Introduction

Immunity is the ability of the body to defend itself against various types of chemical and biological challenges [1]. The immune system can distinguish between 'own' cellular machinery and extraneous bodies. Once these 'extraneous bodies' are identified, a group of immune cells activate and 'institute' the immune response, which is, collectively predisposed due to innate and adaptive immune response [2]. The event of cellular infiltration, redness, inflammation, swelling, and allergy are seen during such consequences. In such event, use of 'immunomodulators' is advocated which promotes and strengthens the immune system [3]. Immunomodulators from natural sources have the potential for development of therapeutic leads [4,5]. Many herbs [6–9] and polyherbal formulations [10,11] have been evaluated for their potential immunomodulatory effects.

Flavonoids are one of the important classes of phytochemicals abundantly found in all plants. Tea (*Camellia sinensis*) is one of the second most devoured beverage worldwide [12]. Chinese traditional medicine documents the use of tea to cure many ailments possibly due to the presence of one of the constituents 'catechin' [13]. Catechin is a 'flavonol,' and the term 'catechin' originated from catechu, which is obtained from the boiled extract of *Mimosa catechu* [14].Catechin is richly found in green tea [15], black tea [16], coconuts [17] onion and grape seeds [18].Catechin has demonstrated various 'pharmacological effects' on the biological system [19] and has shown significant analgesic [20], anti-inflammatory [21] and antarthritic [22] effect. However, no study about the protective effect of catechin on the cellular and humoral system has been performed. Therefore, present work aimed to determine immunomodulatory effects of catechin in the rat model.

2. Experimental

2.1. Animals

Laboratory-bred Wistar rats (180–200 g) of either sex were housed in polypropylene cages, maintained under standardized condition (12 h light/dark cycles, 28 ± 2°) were used in the study. Animals were provided with standard pellet food and had free access to drinking water. All the animal study protocols were duly approved by Institutional Animal Ethics Committee.

2.2. Chemicals

Catechin was purchased from Central Drug House, Mumbai, India. All the other chemicals used in the study were of analytical grade.

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2.3. Carbon ink

Carbon ink suspension Camel, India was injected in a dose of 10 μl/g.

2.4. Antigen

Sheep red blood cells (SRBCs), collected in Alsevier's solution, washed with large volumes of sterile normal saline thrice and adjusted to a concentration of 5×10^9 cells/ml was used for immunization and challenge.

2.5. Selection of dose

As per studies performed by Hamaishi et al. [23], the catechin in the dose of 25, 50, and 100 mg/kg per oral was used in the study.

2.6. Animal group and dosing

Animals were divided into five groups with six animals in each.
Group I Distilled water 2 ml/kg
Group II Cyclophosphamide (30 mg/kg)
Group III Catechin (25 mg/kg)
Group IV Catechin (50 mg/kg)
Group V Catechin (100 mg/kg)

2.7. Immunomodulatory study protocols

2.7.1. Hypersensitivity reaction (delayed type)

On day 0, all the animal groups were immunized by subcutaneous administration of 1 ml of SRBC cell suspension into the right hind footpad. On day 15, all groups were challenged by subcutaneously injecting 0.5 mL of SRBC cell suspension into the left hind footpad [24], and the thickness of the left hind footpad was calculated after 24 and 48 h of a challenge using vernier calipers. The difference in the thickness of the right hind paw and the left hind paw was used as a measure of DTH reaction and was expressed as a mean percent increment in thickness/edema.

DTH reaction
= (Left foot pad challenged with antigen-Right footpad (control))
/(Left foot pad challenged with antigen) × 100

2.7.2. Carbon clearance test

The phagocytic activity of the reticuloendothelial system (RES) was assessed by carbon clearance method. Briefly, 1 ml of Indian ink was administered intravenously to all five groups of adult Wister rats on the 8th day of daily administration of catechin. Blood samples were collected at 0 and 15 min intervals and transferred directly into the centrifuge tube, allowed to coagulate at room temperature, and centrifuged at 2000 rpm for 10 min. Then, 50 μL of clear supernatant (serum) was collected and transferred to the different volumetric flask. The volume was made up to 25 mL using distilled water; absorbance was measured at 650 nm using a spectrophotometer [25,26].

The phagocytic index K was calculated using the following equation:

$$K = (\text{Loge } OD_1 - \text{Loge } OD_2)/15$$

where OD_1 and OD_2 are the optical densities at 0 and 15 min respectively.

2.7.3. Cyclophosphamide-induced neutropenia

The method described by Manjrekar et al. [27] was adopted. The animals of groups II–V were injected with cyclophosphamide (100 mg/kg, i.p.) on the 11th, 12th, and 13th day, 1 h after the administration of the respective drug treatments. Blood samples were collected from retro-orbital plexus on the 14th day of the experiment. Determination of total white blood cells was carried out.

2.7.4. Neutrophil adhesion test

The rats were treated orally with standard and test compounds as mentioned above for the period of 14 days. On day 14, blood samples were collected from the retro-orbital plexus into heparinized vials and analyzed for total leukocyte count (TLC) differential leukocyte count (DLC). After the initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 10 min at 37 °C. The incubated blood samples were again analyzed for DLC. The percentage of neutrophils in the treated and untreated blood was determined, and the difference was taken as an index of neutrophil adhesion [28,29].

The product of total leukocyte count and % neutrophil known as the neutrophil index was determined for each of the respective groups [30]. The % neutrophil adhesion for each of the test groups was determined as follows:

%Neutrophil adhesion
= (Difference in neutrophil count in untreated and fiber treated blood)
/(Neutrophil count of untreated blood) × 100

2.7.5. Indirect hemagglutination test

Rats were pretreated with the drugs for 14 days, and each rat was immunized with 0.5×10^9 sheep red blood cells (SRBCs) intraperitoneally. The day of immunization was referred to as day 0. The drug treatment was continued for 14 more days, and blood samples were collected from each rat at the end of the drug treatment, and the titer value was determined by titrating serum dilutions with SRBC (0.025×10^9 cells) in microtiter plates. The plates were incubated at room temperature for 2 h and examined visually for agglutination. The minimum volume of serum showing haemagglutination was expressed as haemagglutination (HA) titer [31].

2.7.6. Serum immunoglobulin

The five groups of rats were treated with standard/test drug orally for 21 days. Six hours after the last dose, blood samples were collected, and the serum was separated by centrifugation, the collected serum was used for estimation of immunoglobulin levels. Briefly, for each serum sample to be analyzed, a control tube containing 6 ml of distilled water and a test tube containing 6 ml of zinc sulfate solution was prepared. To each, 0.1 ml of serum was added from a pipette. They were inverted to enable complete mixing of the reagents and left to stand for 1 h at room temperature in plugged tubes. The first tube served as blank. The turbidity developed was measured spectrophotometrically at 580 nm. The turbidity obtained (sample-blank) was compared with that obtained with standard barium sulfate ($BaSO_4$) solution. The standard $BaSO_4$ solution was prepared by adding 3 ml of barium chloride solution (1.15% w/v) to 97 ml of 0.2 N sulphuric acid. The turbidity obtained with this solution was expressed as 20 zinc sulfate turbidity (ZST) units [32].

2.8. Statistical analysis

All the values were expressed as mean ± SEM. Statistics was applied using Graph Pad Prism version 5.0 for Windows, Graph Pad software, San Diego, California, USA. One-way ANOVA followed by Dunnet's comparison test was used to determine the statistical significance between various groups. Differences were considered to be statistically significant when $^*p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$.