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Evaluation of mast cell stabilizing activity of *Camellia sinensis* and *Ocimum basilicum* and correlation with their antioxidant property

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Abstract

Mastocytes are vital for human immunity. Mast cell degranulation leads to several immune/allergic reactions. A major factor responsible for mast cell disruption is oxidative stress. Hence antioxidants may play a significant role in mast cell stabilization. Antioxidant plants such as *Camellia sinensis* (Theaceae) and *Ocimum basilicum* (Lamiaceae) are used in allergic conditions in traditional medicine. However, a little is known about their mast cell stabilizing potential. The present study evaluated the mast cell stabilizing activity of two polyphenol rich plants viz. *C. sinensis* and *O. basilicum* and correlated this with their antioxidant capacity. Acetone and hydroalcoholic extracts of the two plants were prepared and standardized with respect to total phenolic and flavonoid content. Their antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay. Mast cell stabilization was assessed by the ability to inhibit histamine release in an *in vitro* model that employs compound 48/80 (a potent mast cell activator) to induce rat peritoneal mast cell degranulation. Acetone extract of *C. sinensis* showed significant free radical scavenging activity (IC₅₀ value 8 µg/ml) and the potent inhibition (>80%) of histamine release at a concentration of 100 µg/ml. Significant positive linear correlation (correlation coefficients <1) for all extracts suggests that antioxidant activity contributes to mast cell stabilization. Management of oxidative stress may aid in preventing histamine release possibly by reducing mast cell degranulation. The strongly antioxidant acetone extract of *C. sinensis* may be a potential mast cell stabilizer.

Keywords: *Camellia sinensis*, free radical scavengers, mast cell stabilizers, polyphenol-rich plants

Introduction

Mast cells or mastocytes are vital for the immune surveillance of the human body [1]. These large connective tissue cells originate in the hematopoietic progenitor cells and are present in various tissues such as the skin, mucosa of the lungs, and digestive tract, as well as in the mouth, conjunctiva and nose [2]. When activated by antigen, mast cells initiate subcellular signal transduction pathways that expel several mediators including tryptase, hydrogen peroxide, chymase, cytokines and histamine, resulting in progression of allergic responses [3]. Moreover, mast cells release certain prostaglandins and leukotrienes that contribute to the immediate inflammatory response [4]. Mast cell stabilizers are thus important for prevention of different types of allergic and inflammatory reactions. A vast number of physiological stimuli including oxidative stress trigger granular decomposition of mast cells [3]. Hence antioxidants may play a significant role in mast cell stabilization.

Conventional pharmacotherapy has limited effectiveness and numerous side effects, including nervousness, fatigue, headache, drowsiness, and bleeding [5]. Hence, there is a need for safe and effective mast cell stabilizers. Plants are a rich source for drug discovery. Plants rich in polyphenols are strong antioxidants, have anti-inflammatory activity and inhibit histamine release [6, 7]. Thus plants containing phenolic compounds may be explored as potential mast cell stabilizers. In the present investigation two plants containing polyphenols have been selected to evaluate their mast cell stabilizing potential. *Camellia sinensis* (Linn.) O. Kuntze (syn. *C. thea* Link) (Theaceae), commonly known as tea, is the one of the most popular beverage worldwide [8]. It is consumed as ethno-botanical remedy in Traditional Chinese Medicine and Ayurveda. It has many therapeutic effects including lowering body temperature, lowers blood sugar levels, boosting immune system, enhancing heart function, suppressing aging [9]. Systematic scientific studies have attributed several biological activities of tea such as anticancer, anti-inflammatory and anti-arthritis to the potent antioxidant phytoconstituents, specifically catechin polyphenols [8]. *Ocimum basilicum* Linn. (Lamiaceae), commonly known

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as Sweet basil, is an ornamental and medicinal herb [10] and is a rich source of polyphenols that possess significant antioxidant activities [11]. Basil has anti-inflammatory, neuroprotective, and adaptogen properties [12-14]. Both plants are potent antioxidants and are traditionally used as immunomodulators and in allergic conditions [15, 16].

In spite of the traditional indications, high polyphenol content and marked free radical scavenging capacity, the mast cell stabilization potential of *C. sinensis* and *O. basilicum* has not been investigated. The present study examined this activity and attempted to correlate with their antioxidant property.

Material and methods

Plant material: The Kashmiri tea or Kahwah (processed leaves of *Camellia sinensis*) was purchased from Jammu in August 2013 and plant identity was confirmed by Dr. Sunita Garg, Chief Scientist, Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communications and Information Resources (NISCAIR), New Delhi (Ref.No.NISCAIR/RHMD/Consult/2013/2298/78). The leaves of *Ocimum basilicum*, were collected from a cultivated local source in September 2013 and were identified and authenticated by Dr. Gurpaul Singh Dhingra, Professor, Department of Botany, Punjabi University, Patiala.

Chemicals: Analytical grade acetone, methanol and petroleum ether (Loba Chemie Pvt. Ltd., Mumbai) were used for extraction of plant material. Compound 48/80, histamine hydrochloride and O-phthaldehyde were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium cromoglycate (Cromal Forte, Cipla Ltd.) was used as standard mast cell stabilizer. Compound 48/80 is a polymer formed by the condensation of N-methyl-p-methoxyphenethylamine with formaldehyde. This produces mast cell degranulation leading to release of histamine

Preparation of extracts: The collected plant materials were air dried, coarsely powdered, defatted with petroleum ether and subsequently extracted sequentially by maceration with acetone and a mixture of methanol and water (70:30) in shaking incubator (100 rpm; temperature 25 ± 2 °C; 24 h). The obtained extracts were concentrated on rotary evaporator and percentage yield of dried extracts was calculated in terms of the dry weight of the plant material. The prepared extracts were subjected to qualitative phytochemical tests [17].

Standardization of extracts: The extracts were standardized with respect to total phenol content (TPC) and total flavonoid content (TFC). TPC was determined by Folin Ciocalteu procedure [18]. The amount of total phenolic components was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions and expressed as percentage w/w. TFC was determined according to the method of Jay *et al* [19]. The flavonoid content was determined as quercetin equivalent from the calibration curve of quercetin standard solutions and expressed as percentage w/w. All measurements were done in triplicate.

In vitro antioxidant activity: The antioxidant activity of various plant extract was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [20, 21]. A methanol solution of DPPH (0.1 mM) was prepared immediately before the assay and used as experimental control. About 2 ml of various concentrations of each sample prepared in 50% methanol

were added to 2 ml of DPPH solution. The reaction mixtures were shaken vigorously and allowed to stand at room temperature in dark for 30 min. The absorbance of the resulting mixture was measured by a UV-visible spectrophotometer at 517 nm. Ascorbic acid was used as positive control. The DPPH radical scavenging effect was measured as follows:

% scavenging effect = $(1 - \text{Absorption of test solution} / \text{absorption of control solution}) \times 100$

The IC₅₀ values for different extracts were determined graphically from the graph with percentage inhibition plotted on y-axis and concentration on x-axis.

Evaluation of in vitro mast cell stabilization activity: Mast cell stabilization activity was examined by *in vitro* rat peritoneal mast cell (RPMC) degranulation model [22]. In this method Wistar rats were administered with 50 ml of normal saline in peritoneal cavity under strong anesthesia; massaged gently for few minutes to stimulate the release of mast cells, then peritoneum was cut open to collect the fluid which was centrifuged at 2000 rpm for 15 min, supernatant was discarded and cells were re-suspended in normal saline. Obtained mast cell suspension (1 ml) was incubated with different concentrations (1, 10 and 100 µg/ml) of plant extracts (1 ml) at room temperature. After 20 min, one ml of compound 48/80 (10 µg/ml) was added and mixture was allowed to incubate at room temperature for further 20 min to induce histamine release. Then 0.4 ml of 1N NaOH and 0.1 ml of o-phthaldehyde (1% w/v) was added and again incubated for 5 min followed by addition of 0.3 ml of 3N HCl. Fluorescence of resulted mixture was noted using spectrofluorometer at excitation wavelength of 350 nm and emission wavelength of 450 nm. Sodium cromoglycate (10 µg/ml) was used as standard drug. The amount of histamine released was calculated from corresponding fluorescence using the standard plot of histamine hydrochloride. The percent inhibition of histamine release was calculated as -:

$$\% \text{ Inhibition of histamine release} = [(a-b/a)] \times 100$$

Where, a = histamine release without extract; b = histamine release with extract

Statistical Analysis: Data is expressed as mean \pm SD. All measurements were taken in triplicate. The data were analyzed by one way analysis of variance ($p < 0.05$) followed by *post hoc* Tukey's test. The IC₅₀ values (µg/ml) for DPPH scavenging assay were calculated by using linear regression analysis. Pearson's coefficient (r) of correlation of different extracts to determine the relationship between antioxidant activity and mast cell stabilization potential was calculated using CORREL statistical function in MS Excel 2007 software.

Results

Phytochemical studies and standardization of extracts: The yields of various prepared extracts are reported as percentage w/w (on dry weight basis) in Table 1. The preliminary phytochemical screening of prepared extracts revealed the presence of alkaloids, flavonoids and sterols in acetone extracts of both plants, whereas hydroalcoholic extracts showed the presence of alkaloids, flavonoids, phenols, carbohydrates and saponins. Further, the extracts were standardized by quantitative determination of TPC and

TFC (Table 1). The acetone extract of *C. sinensis* gave maximum phenol content ($26.16 \pm 0.017\%$ w/w) while acetone extract of *O. basilicum* had lowest phenol content ($3.81 \pm 0.014\%$ w/w).

In-vitro Antioxidant activity: In the present study, the antioxidant activity of extracts was evaluated by determining their DPPH radical scavenging abilities and results were presented as IC_{50} ($\mu\text{g/ml}$). The standard, ascorbic acid, had IC_{50} value $4 \mu\text{g/ml}$. All test extracts exhibited significant *in-vitro* scavenging of DPPH radicals (Table 2). Acetone and hydro-methanol extracts of *C. sinensis* had IC_{50} values of 8.0 and $13.5 \mu\text{g/ml}$, respectively. The IC_{50} values of acetone and hydro-methanol extracts of *O. basilicum* were 34.7 and $29.0 \mu\text{g/ml}$ respectively. The order of free radical scavenging potential of extracts under study was as follows: acetone extract of *C. sinensis* > hydromethanol extract of *C. sinensis* > hydro-methanol extract of *O. basilicum* > acetone extract of *O. basilicum*.

In vitro mast cell stabilization activity: The mast cell stabilizing activity was evaluated by studying the inhibition of histamine release using spectrophurometric method.

Significant higher amount of histamine was released from RPMC upon stimulation with compound 48/80 which was concentration dependently inhibited by test extracts (Fig. 1). It is noteworthy here that the degree of inhibition caused by pretreatment with acetone extract ($100 \mu\text{g/ml}$) of *C. sinensis* against compound 48/80 induced mast cell degranulation was significantly different ($p < 0.05$) from the control and comparable with standard drug (Sodium cromoglycate $10 \mu\text{g/ml}$). The hydro-methanol extract of *O. basilicum* at the highest concentration used ($100 \mu\text{g/ml}$) causes only 50.76% inhibition of histamine release. The acetone extract of *O. basilicum* was least effective among all extracts with maximum percent inhibition (35.35%) at the highest concentration employed.

Correlation between antioxidant activity and % histamine release inhibition: The relationship between antioxidant and histamine release inhibition activities for acetone and hydro-methanol extracts of *C. sinensis* and *O. basilicum* are shown in Table 3. As the values of Pearson's coefficient of correlation (r) was close to +1, it indicated the positive linear correlation between two studied activities.

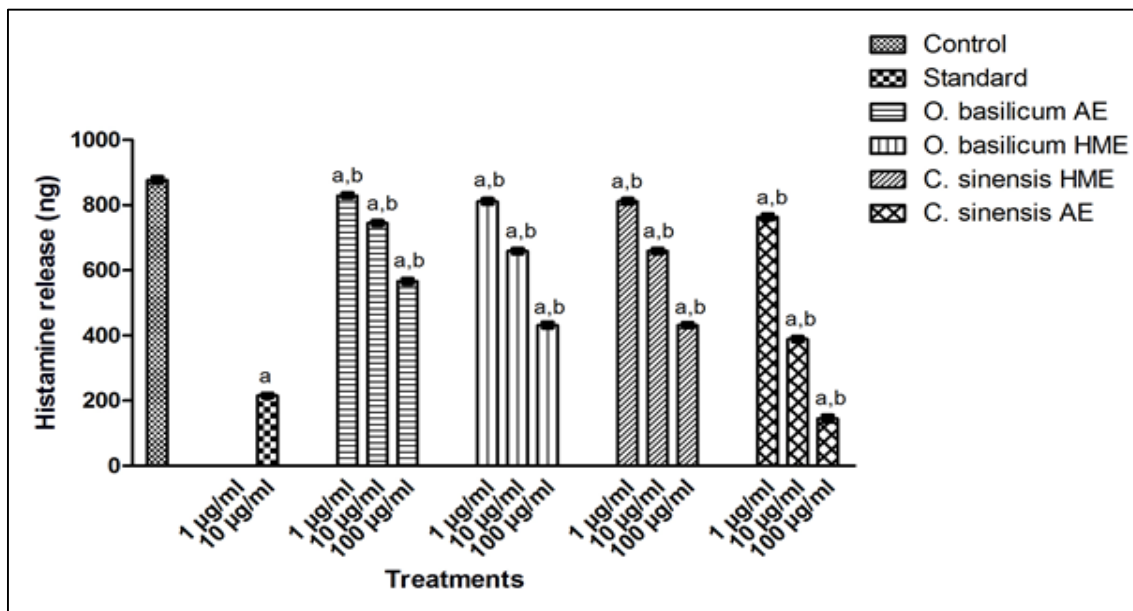


Fig 1: Effect of different plant extracts on histamine released from RPMC

Data is expressed as mean \pm SD and analyzed by one way ANOVA followed by *post hoc* Tukey's multiple range test, $a = p < 0.05$ vs. Standard (Sodium Cromoglycate). AE- Acetone extract; HME- Hydro-methanol (30:70) extract.

Table 1: Percentage yield, total phenol content and total flavonoid content of test extracts

Plant	% yield (w/w; dry weight basis)		Total phenol content (%w/w)		Total flavonoid content (%w/w)	
	AE	HME	AE	HME	AE	HME
<i>Camellia sinensis</i>	3.10	8.95	26.16 ± 0.01	23.31 ± 0.05	1.63 ± 0.06	1.94 ± 0.16
<i>Ocimum basilicum</i>	2.75	8.10	3.81 ± 0.01	7.90 ± 0.004	2.04 ± 0.13	1.70 ± 0.04

Data are expressed as Mean \pm S.D.; n =3; AE- Acetone extract; HME- Hydromethanol extract

Table 2: In-vitro DPPH scavenging activity of prepared extracts

Plant	DPPH scavenging activity ($IC_{50} \mu\text{g/ml}$)	
	Acetone extract	Hydro-methanol extract
<i>Camellia sinensis</i>	8.0	13.5
<i>Ocimum basilicum</i>	34.7	29.0
Ascorbic acid	4.0	

Table 3: Correlation coefficients for different plant extracts

Plants Extract	<i>C. sinensis</i>		<i>O. basilicum</i>	
	Acetone extract	Hydro alcoholic extract	Acetone extract	Hydro alcoholic extract
Correlation coefficient(r)	0.9474	0.9517	0.9839	0.97822

Discussion

The present study evaluated the mast cell stabilization activity and its relation with antioxidant potential of test extracts prepared from traditionally used immunomodulatory plant drugs i.e. *Camellia sinensis* and *Ocimum basilicum*. Mast cells are present virtually in all organs and are well known participants in allergic diseases [23]. Their activation and subsequently degranulation can be elicited by not only the aggregation of cell surface-specific receptor for IgE, FcεRI, but also by the number of positively charged substances like compound 48/80 [24]. Compound 48/80 increases intracellular calcium level and generates ROS endogenously which results in mast cell disruption to produce proinflammatory mediators [1, 25]. Degranulated mast cells release number of mediators including histamine, a potent vasoactive mediator, which may precipitate hypersensitive reactions [26]. Compound 48/80 was used to induce mast cell degranulation and histamine release from RPMC which was measured spectrophotometrically as histamine o-phthalaldehyde complex. Quantitative decrease in histamine release from RPMC was taken as an index of mast cell stabilization.

The extracts of *C. sinensis* and *O. basilicum* showed the attenuation of compound 48/80 induced mast cell degranulation in dose dependent fashion. The acetone extract of *C. sinensis* showed most significant mast cell stabilizing activity. Further, to explore the mechanism by which test extracts exerted mast cell stabilizing properties, the antioxidant activity of the was also determined. It was found that acetone extract of *C. sinensis* was potent inhibitor of DPPH free radical while basil was least active.

Phytochemical screening of acetone extract *C. sinensis* and *O. basilicum* showed the presence of alkaloids, phenols, flavonoids and sterols. Plants containing polyphenols have demonstrated marked mast cell stabilizing activity, for example *Woodfordia fruticosa* extract containing gallic acid and flavonoids reduced histamine release after challenge with compound 48/80 [27]. Silibinin, a polyphenol obtained from *Silybum marianum*, inhibited histamine release from RPMCs induced by compound 48/80 [28]. Flavonoids such as luteolin, quercetin, kaempferol, myricetin etc possess antihistaminic and antiallergic effects [29]. Tea catechins have antioxidant and cytotoxic property and also exert anti-asthmatic, anti-allergic, immunomodulatory and anti-inflammatory effects [9, 30]. Recently mast cell stabilizing potential of crude aqueous extract of green tea was reported [31]. However it did not correlate the activity with the antioxidant effects.

The results in the present study revealed that the standardized acetone extract was highly active as mast cell stabilizer at very low concentrations (1, 10 and 100 µg/ml). Also, a strong positive linear correlation between antioxidant and mast cell stabilization potential was observed for all the test extracts suggesting that antioxidant activity is likely to contribute in mast cell stabilization. A positive linear correlation has already been established between antioxidant potential and phenolic content of tea and basil [32, 33]. This provides evidence that high content of phenolic compounds and antioxidant activity prevent histamine release and may be closely associated with mast cell stabilization.

It may be concluded that *C. sinensis* and *O. basilicum* possess

mast cell stabilization property mediated through their antioxidant capacity. The strongly antioxidant acetone extract of *C. sinensis* may be a developed as mast cell stabilizer.

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