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ANTI-INFLAMMATORY AND ANTI-DIABETIC ACTIVITY OF INDIAN PROPOLIS

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ABSTRACT

Propolis is a natural product with broad spectrum of biological properties collected by *Apis mellifera* bee from buds and exudates of various plant species. The chemical composition of propolis is known to alter with geographical origin leading to variation in pharmacological activities. Propolis from various geographical origins have been evaluated for antioxidant, anti-microbial, anticancer, anti-inflammatory, hepatoprotective activities, however biological properties of Indian propolis have been scarcely studied. The anti-inflammatory and anti-diabetic potential of propolis collected from South India were determined. The in-vitro anti-inflammatory potential of propolis extracts were confirmed by inhibition of albumin denaturation and membrane stabilization assay. The anti-diabetic activity was studied by inhibition of alpha-amylase enzyme and non-enzymatic glycosylation of haemoglobin method. All the studied propolis ethanol extracts possess significant anti-inflammatory and anti-diabetic activity showed dose dependent inhibition of albumin denaturation, haemolysis, alpha-amylase and non-enzymatic glycosylation of haemoglobin. The percentage of inhibition was comparable with that of the standards. The results concluded that the Indian propolis can be a promising therapeutic agent for inflammation and diabetes.

KEY WORDS: *Apis mellifera*, anti-inflammatory, anti–diabetic, albumin denaturation, Alpha-amylase, propolis.

INTRODUCTION

Inflammation is a change in the morphological equilibrium of living tissue towards the cell injury caused by different kinds of agents such as physical trauma, organic and inorganic chemicals and microbial agents. It is represented by oedema and changes in phagocytic activity which contributes to loss of tissue function.[1] Blood is the primary delivery system for inflammatory components. The inflammatory response is characterized by the symptoms like reddening of the localized area, swelling, pain and elevated temperature. The area of inflammation also becomes walled off as a result of the development of fibrinous clots. The deposition of fibrin isolates the inflamed area, cutting off normal circulation. In addition, the histological aspects and specific immune events such as hypersensitive reactions may lead to inflammation.[2] The inflammation injury is also caused by the over production of free radicals from activated neutrophils and macrophages that results in damage of macromolecules and lipid peroxidation of tissue membranes. Although the reactive oxygen species spread the inflammation by stimulating the release of cytokines, the antioxidants and free radical scavenger can extenuate the inflammation and illness caused by their inflammatory reaction.[3] The several currently used steroidal or non steroidal anti-inflammatory drugs used to prevent pain and reduce inflammation may cause undesirable side effects including gastrointestinal irritation, alterations in kidney functions, and cardiovascular effects.[4] The relevance of compounds derived from natural product with anti-inflammatory properties has to be identified, isolated, and developed into an anti-inflammatory drug.

Diabetes mellitus, a metabolic disorder resulting in high level of blood sugar with insufficient or in effective hormone insulin, which regulates the blood glucose within normal limits and stimulates protein, glucose and fat metabolism. Characteristically, diabetes is a long term disease with several serious complications such as cardiovascular, renal, retinal, nerve and micro vascular damages that may occur if it is not controlled optimally.[5] Type I diabetes is characterised by loss of insulin producing beta cells of the Islets of Langerhans in pancreas. The type II diabetes is the most common form in adults where the glucose builds up in blood due to insulin resistance or reduced insulin sensitivity in cells. Both types lead to hyperglycaemia which causes diabetes. Oxidative stress is one of the main mechanisms which results in various complications of diabetes.[6] It is estimated that currently about 170 million of the population suffers from this disease throughout the world and the number will increase to over 366 million by 2030 and that large increases will occur in developing countries.[7, 8]

Propolis is a natural resinous mixture produced by honeybees by the substances collected from parts of

plants, buds and exudates that exhibits many biological activities. It provides beneficial effect on human health especially in folk medicine to treat many diseases. Several pharmacological significance was reported about the presence of bioactive compounds in propolis and their derivatives may exhibit novel properties which may be beneficial in the pharmacological view.[9,10,11] The composition of the propolis, its physico-chemical properties, biological activities and therapeutic uses depend on the vegetation where the hives are placed and the climatic conditions.[12] Based on the antioxidant activity of Indian propolis[13], the present study was conducted to evaluate the in vitro anti-inflammatory and anti-diabetic potential of ethanol extract of propolis collected from different regions of South India.

MATERIALS AND METHODS

The propolis samples were collected from 4 Southern states of India like Tamil Nadu, Karnataka, Kerala and Andhra Pradesh by scrapping the frames of *Apis mellifera* bee hives. The raw propolis samples were stored in cool place for further investigations.

Preparation of ethanol extract of propolis

The ethanol extract of propolis was prepared by crushing 10gm of propolis into small pieces and extracted with 100ml of 70% ethanol and left overnight followed by intermittent shaking at room temperature. The suspension obtained was filtered and the extraction was repeated twice. The extracts were combined and evaporated to dryness under pressure by using rotor evaporator and the dried extract was weighed to determine the yield of soluble components.[14] The resulting extract was kept in refrigerator at 4^{o} C to assess the anti-inflammatory and anti-diabetic potential through various assays.

ANTI-INFLAMMATORY ACTIVITY

Inhibition of albumin denaturation assay

The anti-inflammatory activity of ethanol extract of propolis was studied by using inhibition of albumin denaturation technique [15]. The reaction mixture consists of 1ml of propolis extract and 1% aqueous solution of bovine albumin fraction, the sample extracts were incubated at 37ºC for 20min and then heated to 70º C for 20min, after cooling the samples the turbidity was measured at 660nm using UV Visible Spectrophotometer.

Membrane stabilization

The blood was collected from healthy human and washed with 0.4% saline and centrifuged simultaneously for 10min at 3000 rpm. The suspension of RBC was made with 10% saline phosphate buffer.[15] The reaction mixture consisted of 1ml propolis extract of different concentrations (100 - 500 µg/ml) and 1ml of 10% RBCs suspension. Instead of test sample only saline was added to the control test tube and aspirin was used as a standard drug and all the centrifuge tubes containing reaction mixture were incubated in water bath at 50ºC for 30min. At the end of the incubation the tubes were cooled, centrifuged at 2500rpm for 5min and the absorbance of the supernatants were measured at 560nm.[16]

The experiments were performed in triplicates for all the test samples. The percentage inhibition of protein denaturation and inhibition of haemolysis was calculated as: Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control.

ANTI-DIABETIC ACTIVITY

Inhibition of alpha-amylase enzyme

In this method, the enzyme solution was prepared by dissolving alpha -amylase in 20mM phosphate buffer (pH 6.9) at the concentration of 0.5mg/ml. One ml of propolis extract of various concentrations (100, 200, 300, 400, 500µg/ml) and 1ml of enzyme solution was mixed together and incubated at 25°C for 10min. After incubation, 1ml of starch (0.5%) solution was added to the mixture and further incubated at 25°C for 10min. The reaction was then stopped by adding 2ml of dinitrosalicylic acid and heat the reaction mixture in a boiling water bath for 5min. After cooling, the absorbance was measured at 565nm.[17] The inhibition percentage was calculated and acarbose was used as standard drug.

Non-enzymatic glycosylation of haemoglobin assay

The anti-diabetic activity of propolis extracts were investigated by estimating degree of non-enzymatic haemoglobin glycosylation. Glucose (2%), haemoglobin (0.06%) and Sodium azide (0.02%) solutions were prepared in 0.01M phosphate buffer with pH 7.4. One ml of the above solution was mixed with 1ml of various concentrations of ethanol extract of propolis. The mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of haemoglobin was measured at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug for assay.[18] All the tests were performed in triplicate and the percentage inhibition of alpha-amylase enzyme and inhibition of non-enzymatic glycosylation of haemoglobin was calculated as

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Statistical Analysis

All the analysis was performed in triplicate for each sample and at each concentration and the data has been statistically analyzed by using SPSS and Mega state software

RESULTS AND DISCUSSION

Albumin denaturation is a process where protein loses their biological function by the disruption and destruction of both secondary and tertiary structures, results in inflammation.[18] Inflammation is response to injury characterized by heat, redness, pain, swelling and disturbed physiological function. In the present study, ethanol extracts of propolis collected from different parts of South India were investigated for their potential to

inhibition of albumin denaturation. The different concentrations (100 -500µg/ml) of propolis extracts were tested for the inhibition of albumin denaturation activity (Table 1 and Fig. 1). According to the result, propolis sample collected from Tamil Nadu at 500µg/ml concentration had the highest albumin denaturation inhibition of 64.20±4.85% followed by Kerala (61.45±3.38%), Karnataka (55.35±1.65%) and Andhra Pradesh (52.98±3.75%). The standard anti-inflammatory drug aspirin showed the maximum inhibition of 65.30±2.86% at the concentration of 500µg/ml. All the tested extracts possess significant activity comparable with that of the standard and showed dose dependent inhibition of albumin denaturation.

Table1. Albumin denaturation assay

	Percentage of inhibition of albumin denaturation					
	Standard and propolis extract					
Sl. No	**Concentrations (µg/ml)**	**Standard (Aspirin)**	**Karnataka**	**Tamil Nadu**	**Andhra Pradesh**	**Kerala**
1	100	28.60±2.89	19.26±0.45	30.00±2.20	15.15±2.58	22.90±2.56
2	200	44.50±3.62	30.58±0.56	38.45±2.69	22.45±1.90	28.80±2.20
3	300	47.38±3.54	41.87±0.97	47.45±4.20	36.90±2.90	32.12±3.45
4	400	56.30±3.76	49.75±2.98	60.35±3.80	44.85±3.50	48.46±1.98
5	500	65.30±2.86	55.35±1.65	64.20±4.85	52.98±3.75	61.45±3.38

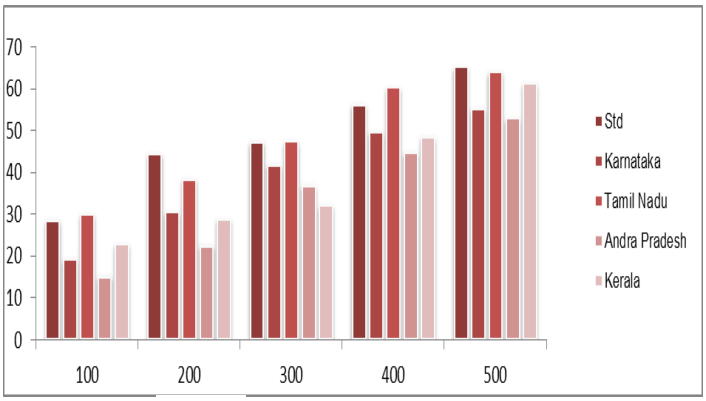

Fig.1. Albumin denaturation assay

The vitality of red blood cells depends on the integrity of their membrane. The erythrocyte membrane is similar to lysosomal membrane and the stabilisation of lysosomal membrane or inhibiting the release of lysosome enzyme is important in limiting inflammation process.[19] Therefore, it is expected that the compounds with membrane stabilizing property should offer significant protection of cell membrane against injurious substances. The studied propolis extracts showed significant anti-inflammatory activity by HRBC membrane stabilization assay in a concentration depended manner and the results are tabulated in Table 2 and Fig. 2. The ethanol extract of propolis from Tamil Nadu, Kerala and Karnataka at the concentration of 500µg/ml showed a maximum of 68.90± 2.75%, 65.47 ±5.60% and 65.36± 2.85% protection of HRBC membrane respectively followed by Andhra Pradesh with 52.45±3.45%. The recorded results are compared with standard aspirin which showed 72.70±4.05% of inhibition of lyses.

Table 2. Membrane stabilisation assay

	Percentage of inhibition haemolysis					
	Standard and propolis extract					
Sl. No	**Concentrations (µg/ml)**	**Standard (Aspirin)**	**Karnataka**	**Tamil Nadu**	**Andhra Pradesh**	**Kerala**
1	100	8.00±2.04	19.38±2.59	26.38±0.90	4.80±1.09	16.47±1.85
2	200	23.35±2.82	28.45±1.45	39.48±2.75	21.65±1.78	32.90±1.39
3	300	42.50±3.50	52.15±1.08	52.90±2.54	38.47±3.86	55.25±2.35
4	400	66.48±4.45	56.90±3.75	60.68±4.58	45.80±3.90	62.90±3.78
5	500	72.70±4.05	65.36±2.85	68.90±2.75	52.45±3.45	65.47±5.60

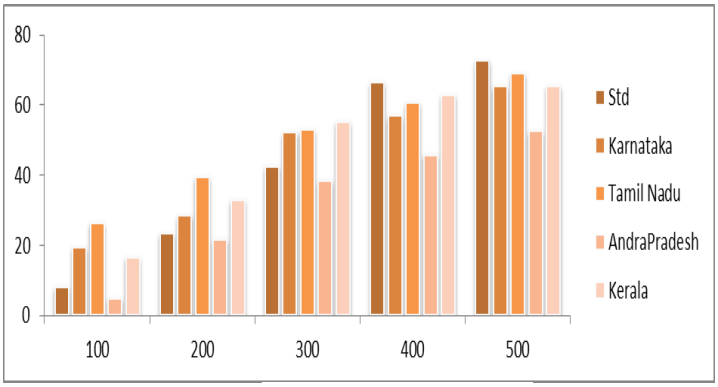

Fig. 2. Membrane stabilisation

The digestive enzyme alpha-amylase is responsible for hydrolyzing polysaccharides such as glycogen and starch, which breaks down into glucose prior to absorption. The inhibition of alpha-amylase would delay the degradation of polysaccharides which would decrease the absorption of glucose as a result there is a reduction of post prandial blood sugar level.[20, 21, 22] The alpha amylase inhibitory studies of propolis extracts revealed that the percentage of inhibition was concentration dependent. The samples from Kerala (74.67±2.57%) and Tamil Nadu (72.85±3.89%) showed the highest percentage inhibition of alpha amylase at 500µg/ml, whereas Andhra Pradesh and Karnataka sample showed 62.95± 0.69 % and 57.45±3.11% inhibition at 500µg/ml. All the tested propolis samples are compared for inhibitory activity with standard drug (Table 3 and Fig. 3).

Table 3. Alpha- amylase inhibition assay

	Percentage of inhibition of alpha- amylase					
	Standard and propolis extract					
Sl. No	**Concentrations (µg/ml)**	**Standard (Acarbose)**	**Karnataka**	**Tamil Nadu**	**Andhra Pradesh**	**Kerala**
1	100	53.04±0.56	19.28±4.25	48.50±1.78	24.78±0.98	32.78±3.88
2	200	62.27±2.47	30.47±2.22	54.76±1.39	38.90±0.47	45.02±2.45
3	300	67.94±1.44	48.89±2.87	65.30±1.48	49.48±0.85	53.73±2.11
4	400	73.45±1.85	52.98±3.46	69.35±2.34	55.80±0.57	61.75±1.75
5	500	78.62±2.56	57.45±3.11	72.85±3.89	62.95±0.69	74.67±2.57

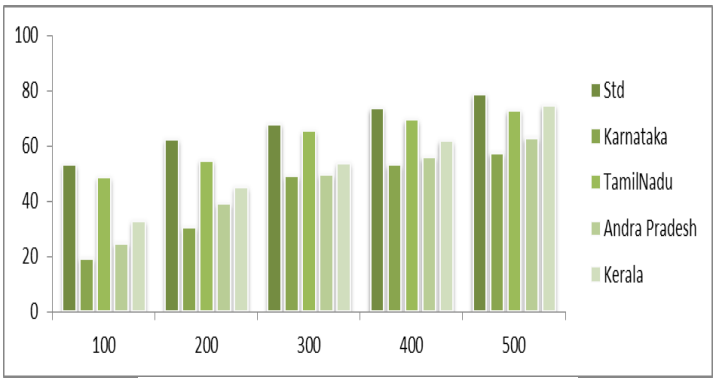

Fig. 3. Alpha- amylase inhibition assay

When blood glucose level is high, glucose molecules get bound to the haemoglobin in red blood cells, which is called as glycosylated haemoglobin. Such glucose haemoglobin complex is quite stable and results in the formation of reactive oxygen species.[23] The concentration of glycosylated haemoglobin is an indication of glucose in blood. In the present study, all the tested the propolis sample exhibited significant inhibitory activity with inhibition of glycosylation as 68.90±1.48%, 67.30±1.46%, 65.89±1.85% and 64.38 ±2.58% from Kerala, Tamil Nadu, Andhra Pradesh and Karnataka at the concentration of 500µg/ml (Table 4 and Fig. 4). All the samples exhibited a comparable inhibition of glycosylation with the standard drug indicating the propolis extracts decreases the formation of glycosylation. As the concentration of propolis extract increases, the formation of glucose-haemoglobin complex decreases and also the concentration of free haemoglobin in red blood cells increases.

Table 4. Non-enzymatic glycosylation of haemoglobin assay

	Percentage of inhibition of glycosylation					
	Standard and propolis extract					
Sl. No	**Concentrations (µg/ml)**	**Standard ((Acarbose)**	**Karnataka**	**Tamil Nadu**	**Andhra Pradesh**	**Kerala**
1	100	32.75±3.55	17.37±1.49	37.94±0.40	28.48±1.45	34.45±0.49
2	200	44.67±3.30	22.89±1.34	49.90±0.86	37.56±2.35	39.89±0.45
3	300	52.48±3.69	37.90±3.48	56.34±0.58	50.56±2.78	51.56±0.78
4	400	66.90±2.65	58.75±2.40	62.56±1.95	57.45±1.90	60.40±0.95
5	500	70.68±2.60	64.38±2.58	67.30±1.46	65.89±1.85	68.90±1.48

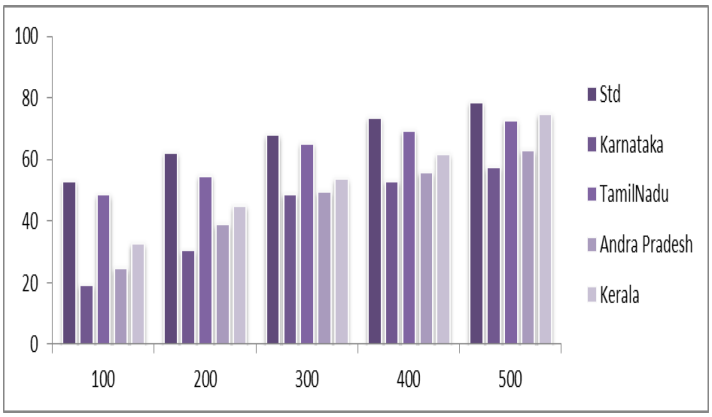

Fig. 4: Non-enzymatic glycosylation of haemoglobin assay

CONCLUSION

The bee propolis provides a wide range of natural compounds which has broad spectrum of biological properties to treat and cure many diseases. The present study indicates that in- vitro anti-inflammatory and anti-diabetic potential of the propolis extracts provide a promising result for the utilisation as a drug to treat inflammation and diabetes. Further, the isolation of the active principles responsible for these beneficial properties would help in the finding of novel compounds as anti-inflammatory and anti-diabetic drugs from this natural product.

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