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Anti-Inflammatory and Antidiabetic Potential of Ethanolic Fruit Extract of *Physalis Peruviana* L. – an *In Vitro* Study

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ABSTRACT
Physalis peruvianaL. is the commercially most important herbal plant among the over 100 species of the genus Physalis (family Solanaceae). In this study was carried out for preliminary assessment of anti-inflammatory and antidiabetic potential of P. peruviana L fruit. In vitro anti-inflammatory activity was evaluated by HRBC membrane method and albumin denaturation assay. The assays of Non-enzymatic glycosylation of hemoglobin, Glucose uptake in yeast cell and α-Amylase inhibitory were used for evaluation of anti diabetic potential. In inflammatory activity, the 500 µg/ml fruit extract showed maximum protection of HRBC in hypotonic solution and albumin denaturation process and they are higher than that of the standard drugs. For antidiabetic activity, the inhibition rate in all concentrations of fruit extract exhibited higher inhibition of glycosylation, glucose uptake in yeast cells assay and α amylase as compared with the standard drug. The results of the work indicate that the fruit extract has possessed considerable in vitro anti inflammatory and anti-diabetic potential and can be applied as alternative in the treatment of various inflammatory diseases and diabetes complications.
Keywords: *Physalis peruviana, anti inflammatory, anti diabetic, in vitro.*

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INTRODUCTION

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [1]. However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis etc., [2]. It is believed that current drugs available such as opoids and non –steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorders, because of their side effects and potency [3]. As a result, a search for other alternatives seems necessary and beneficial.

Diabetes mellitus is a chronic disease caused by inheritance or acquired deficiency in insulin secretion and by decreased responsiveness of the organs to secreted insulin. Such deficiency caused the increased blood glucose level, which in turn can damage many of the body's systems, including blood vessels and nervous system [4]. Food habits and genetic factors are main responsible for diabetes. According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3%[5]. The treatment of diabetes without any side effect is still a challenge to the medical community. Thus searching for a new class of compounds is essential to overcome diabetic problems. Medicinal plants can be used for the treatment of diabetes since they are devoid of side effects. WHO recommends the use of herbal medicines as a cure for diabetes [6].

Physalis peruviana L. (Family: *Solanaceae*) is an herbaceous, semi-shrub plant, annual in the temperate zones and perennial in the tropics and sub-tropics and widely used in folk medicine for treating diseases such as malaria, asthma, hepatitis, dermatitis, cancer, diuretic, rheumatism, antispasmodic, diuretic, antiseptic, sedative, analgesic, cataract-cleaning, antidiabetic, and anti-parasitic properties [7]. Various therapeutic applications have been attributed to the Cape gooseberry, including anti-asthmatic, anti-septic, strengthener of optic nerve, remedy for throat infections, anti-ulcer potential, antiparasitic, antiamoebic, as well as albumin from kidneys, controlling cholesterol level [8]. It was first reported in the Andean region, mostly Colombia, Peru, Ecuador and presently cultivated in several regions throughout

the world [9,10]. In the view of above all, the present study was made to evaluate the *in vitro* anti-inflammatory and antidiabetic activity of ethanolic fruit extracts of *Physalis peruviana* L.

MATERIALS AND METHODS

The fruits of *Physalis peruviana* L. were collected from medicinal plant garden, Sri Akilandeswari Women's College, Wandiwash, Tamil Nadu, India**.** The fruits were subjected to shade drying for 10-15 days and the dried samples were crushed into fine powder using an electronic blender. About 40 gm of fruit powder was extracted with 200 ml of ethanol and the extraction was done by hot percolation method using Soxhlet apparatus. It was concentrated to dryness under controlled temperature 40-50°C and preserved in refrigerator till further use.

In-vitro anti-inflammatory activity

HRBC membrane stabilization method[11]

The human red blood cell (HRBC) membrane stabilization method was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with iso-saline and a 10% suspension was made. Drug was prepared by 4 g of fruit was macerated with 10ml of hyposaline (0.36% NaCl) and extracts is centrifuged at 3000 rpm. Various concentrations of fruit extracts were prepared (100, 200, 300, 400 and 500µg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added. Test solution was incubated at 37^0C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. 100 – 500 µg/ ml of Hydrocortisone (50 mg) were used as reference standard and a control was prepared omitting the extracts.

Inhibition of albumin denaturation[12]

The reaction mixture was consisting of fruit extracts (100 – 500 µg/ ml) and 1% aqueous solution of bovine albumin fraction, pH (7.2) of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 51°C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug of varied concentration 100 – 500 µg/ ml. The experiment was performed in triplicate. % of protein denaturation was calculated as follows:

$$\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

Invitro Antidiabetic activity

Non-enzymatic glycosylation of haemoglobin method[13]

The estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. The fruit extract was weighed and dissolved in DMSO to obtain stock solution and then 250 to 1250 ppm solutions were prepared. 1.0 ml of 250, 500, 750, 1000 and 1250 was added individually to above mixture. Mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. 100 – 500 µg/ ml of Metformin (50 mg) were used as a standard drug for assay.

$$\% \text{ of inhibition} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

α- Amylase Inhibition method[14]

1.0 ml of substrate-potato starch (1% w/v) and 1.0 ml of 5 different concentrations of ethanolic fruit extract such as 250, 500, 750, 1000 and 1250 ppm was mixed individually with 1.0 ml of α- amylase enzyme (1% w/v) and 2.0 ml of acetate buffer (0.1 M, 7.2pH) were added. Instead of test sample, 1.0 ml of drug solution (Glinil – 5 mg) was used for positive control. The mixture was incubated for 1hr.then 0.1 ml iodine-iodide indicator (635.0 mg iodine and 1.0 gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565nm in UV-Visible spectroscopy.

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Glucose uptake in Yeast cells method [15]

The commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of fruit extracts (250, 500, 750, 1000 and1250 ppm) were added to 1ml of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. 1.0 ml of drug solution Glinase – 5 mg was taken as standard drug.

Statistical analysis

The results of the study were expressed as mean ± SEM. ANOVA was used to analyze and compare the data, followed by Dunnet's test for multiple comparisons. The value of probability less than 5 % ($p < 0.05$) was considered statistically significant.

RESULT

***In vitro* anti-inflammatory activity**

HRBC membrane stabilization method

In the present study, *in vitro*anti-inflammatory activity by HRBC membrane stabilization method of fruit extract *P. peruviana* at different concentrations (250 - 1250 ppm) was shown in Fig 1. The fruit extract *P. peruviana* of at the dose of 500 µg/ml concentration showed high significant activity (***P***<0.05) at 72 h, where it caused 85.10 ± 0.02% inhibition, as compared to that of 50 mg/kg of hydrocortisone which exhibited up to 86.48 ± 0.11% inhibition.

Inhibition of albumin denaturation

Stabilization of fresh egg albumin against heat-induced protein denaturation also indicated pronounced in vitro anti-inflammatory activity (Fig 2). The fruit extract at the concentration of 500µg/mL has exhibited a significantly higher (***p*** < 0.05) level of inhibition (78.15±0.128 %) compared to other concentrations studied, whereas 100µg/mL of fruit extract showed the lowest inhibition level (11.43 ±0.11%).

***In vitro* antidiabetic activity**

Non-enzymatic glycosylation of haemoglobin method

In the present study *in vitro* anti diabetic activity by Non enzymatic glycosylation of haemoglobin of ethanol extracts of *P. peruviana* was represented in Fig 3. The concentration of 1250 µg/ml has registered a significantly maximum (***p*** < 0.05) level of inhibition (80.28 ± 0.23%) compared to other concentrations studied, whereas 250 µg/mL of fruit extract showed the lowest inhibition level (17.27 ±0.09%). The standard drug of Metformin inhibition was performed the maximum rate of inhibition 85.81±0.18% in 1250µg.

α- Amylase Inhibition method

Results presented in Fig. 4 showed that α-amylase inhibition potential of the fruit extract had a significantly (p≤ 0.05) higher amount of inhibition (71.54±0.81%) at the concentration of 1250 µg/mL than the other concentrations tested, whereas the standard Glinil has registered the maximum rate of inhibition 77.08±0.23% in 1250µg/ml.

Glucose uptake in Yeast cells method

Fig.5 depicts the level of glucose uptake in yeast cells of ethanolic fruit extract of *P. peruviana*. The result suggests that the fruit extract of *P. peruviana* exhibited significantly maximum level glucose uptake was recorded more than 71.54±0.18% in 1250 ppm and minimum level of glucose uptake 25.18± 0.26% in 250 ppm. In standard drug of Glinase the rate of glucose uptake, the maximum and minimum level of glucose uptake were 76.10± 0.23% in 1200 ppm and 25.75± 0.46% in 250 ppm respectively.

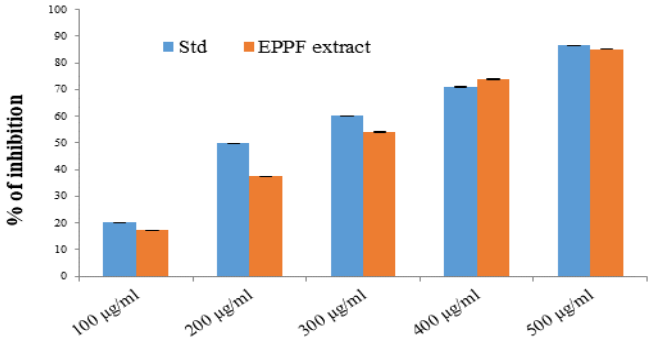

Fig 1. HRBC membrane stabilization

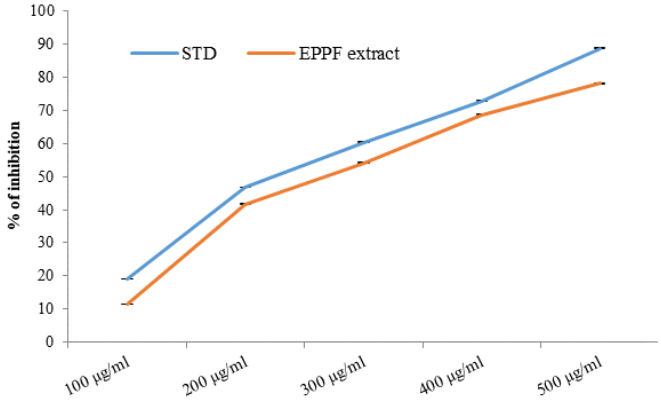

Fig 2. Inhibition of albumin denaturation

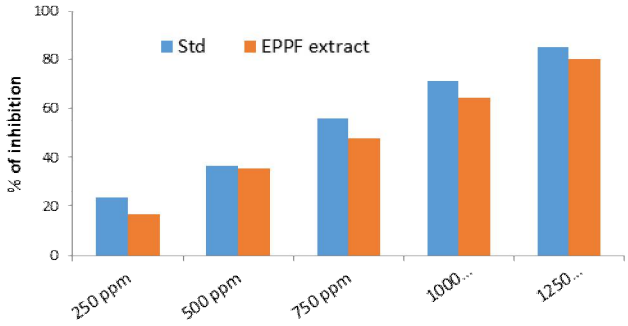

Fig 3. Non-enzymatic glycosylation of haemoglobin

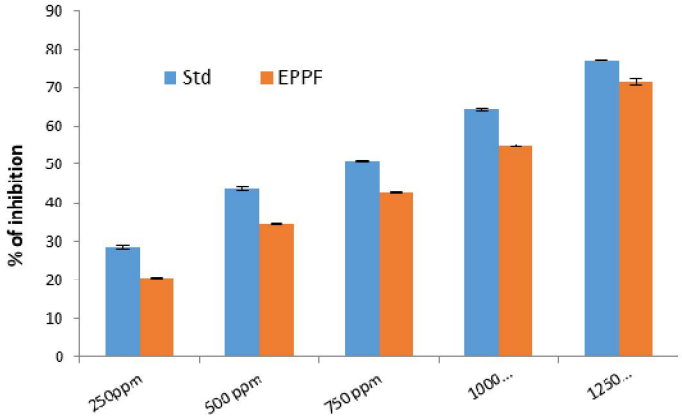

Fig 4. **α- Amylase Inhibition**

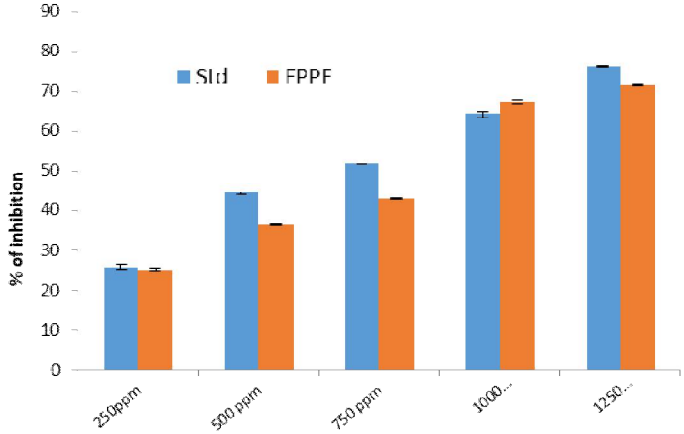

Fig 5. Glucose uptake in Yeast cells

DISCUSSION

Stabilization of liposomal membrane is important in limiting the inflammatory response by inhibiting the release of liposomal constituents of activated neutrophil such as bactericidal enzymes and protease, which cause further tissue inflammation and damage upon extracellular release[16]. In the present study, The different concentrations of ethanolic fruit extract of *P. peruviana* were studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method. Among all the extracts, the fruit extract of *T. chebula* at a concentration of 500 µg/ml showed maximum protection of HRBC in hypotonic solution. All the results were compared with standard hydrocortisone which showed above 70% to 86% protection. Denaturation of proteins is responsible for the cause of inflammation. Neutrophils are known to be a rich source of proteinases which carry in their lysosomal granules, and it was previously reported that leukocytes proteinase plays an important role in the development of tissue damage during the inflammatory response[17]. So, prevention of protein denaturation may help in preventing inflammatory conditions [18, 19]. In the present investigation, the ethanolic fruit extracts of *P.peruviana* showed maximum percentage of albumin denaturation at 500 µg/ml concentration (78%) which lower than that of the standard (88%). In this result is supported by previous *in vitro* antiflammatory works of Solanaceae plants done viz., *S. xanthocarpum* [20, 21] and*S. torvum* [22].

High glucose levels in body leads to its binding to hemoglobin which may result in the production of reactive oxygen species. The amount of glycosylated haemoglobin is a sure guide to the concentration of glucose in the blood (i.e., the degree of control over the disease achieved). and the amount of glycated haemoglobin should not be more than 12%.End products of glycosylation can be inhibited by plant extracts[23].In the present study, ethanolic fruit extracts of *P. peruviana* at the concentration 1250 ppm was exhibited higher inhibition of glycosylation (80%) and the same concentration of standard drug has registered 85% of glycosylation inhibition. Likely, Acharya *et al.,*[13] reported that the *invitro* antidiabetic activity of *Bauhinia purpurea* stem barks. Thus the amount of glycosylated haemoglobin is a sure guide to the concentration of glucose in the blood (i.e., the degree of control over the disease achieved). The amount of glycosylated hemoglobin should not be more than 12%.

In the present study *in vitro* anti diabetic activity by glucose uptake in yeast cells of ethanol extracts of *P. peruviana* at different concentrations. The result suggests that the fruit extract of *P. peruviana* exhibited significantly maximum level glucose uptake was recorded more than 70% in 1200 ppm. The mechanism of glucose transport across the yeast cell membrane has been receiving attention as an ***in vitro*** method for hypoglycemic effect of various compounds and medicinal plants [24].Our finding reveals the rate of glucose transport across cell membrane in yeast cells system is presented. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in all the glucose concentrations.

The α-amylase inhibition was assayed by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide in mono and disaccharide. In the present investigation of alpha amylase inhibition method are carried out ethanolic fruit extracts of *P. peruviana* in the concentration of 250, 500, 750, 1000 and 1250µl/ml. The inhibition rate in all the concentrations of fruit extracts exhibited significantly, the maximum level of activity (71%) was registered in 1250µg/ml and standard drug of Glinil exhibited the rate of glucose inhibition maximum level 77%. Similarly, Conforti *et al.,*[25] reported that the seed extracts of *Amaranthus caudatus* reported 50% α-amylase inhibition at a concentration of 25 µg/ml.

CONCLUSION

From the above study it was concluded that the fruit extracts of *P. peruviana* has significant anti-inflammatory activity (membrane stabilization property, albumin denaturation) and anti-diabetic activity (non - enzymatic glycosylation hemoglobin, glucose uptake in yeast cells and alpha amylase inhibition methods). However further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and helpful in projecting medicinal plant of *P. peruviana* as a therapeutic target in anti-inflammatory and anti-diabetic research.

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