INVITRO ANTIDIABETIC ACTIVITY OF PENTACYCLIC TRITRPENOIDS AND FATTY ACID ESTERS FROM BAUHINIA PURPUREA

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Abstract-The objective of present work was to evaluate the invitro antidiabetic activity of Pentacyclic tritrpenoids and fatty acid esters from stem bark of Bauhinia purpurea. The samples were studied for their effect on inhibition of glycosylation of haemoglobin, glucose transport across yeast cells and α- Amylase inhibition. Inhibition of glycosylation of haemoglobin and α- Amylase inhibition was in a dose dependent manner and glucose transport differs with the sample and glucose concentration. From the results of the study, it is inferred that, B. purpurea stem bark possesses antidiabetic activity. However, these effects need to be confirmed using in vivo models and clinical trials for its effective utilization as therapeutic agents.

Keywords- Bauhinia purpurea, antidiabetic, Acarbose, Metronidazole

I. INTRODUCTION

Bauhinia is a well-known and established genus belonging to the family Fabaceae comprising of trees and shrubs that grow in warm climate. Bauhinia purpurea is rare in southern most districts in India, 5-7 m tall and found in deciduous forests which are often planted in gardens along roadside for its large purple flowers. The leaves are 10-20 cm long and broad, rounded, alternate and bilobed at the base and apex. The flowers are conspicuous, pink, and fragrant, with five petals. The fruit is a pod 30 cm long, containing 12 to 16 seeds and have long seeds as pea. Flowers and fruits appear in the month of December. The plant is commonly known as Purple Orchid tree or Mandaram[1].

B. purpurea is native to South China (which includes Hong Kong) and South-eastern Asia and it is found throughout India, ascending to an altitude of 1300 m in Himalaya[2].

The different species of Bauhinia viz., B. reticulata, B. rufescens and B. variegata have been traditionally used to treat roundworm infections, conjunctivitis, anthrax, ulcers, dysentery, blood-poisoning, leprosy, lung and skin diseases in Africa; while in India, extracts of the bark of B. variegata are used for treatment of cancer[3]. Hartwell mentions the traditional use of B. purpurea for inflammatory tumors and B. tomentosa and B. variegata for cancer in India[4]. The bark is used as fiber in dyeing and tannin extraction and its decoction is used in diarrhea. The decoction of root is used for expelling gases, flatulence and gripping pain from the stomach and bowels. The decoction of flowers work as a maturant for boils and abscesses[5]. Preliminary screening of the methanolic extract of the plant showed the presence of carbohydrates, glycosides, saponins, sterols and triterpenoids[6]. A novel flavone glycoside has also been isolated from the seeds of Bauhinia purpurea L[7].

B. purpurea extracts exhibit anti-oxidant activity[8]. An allied species B. racemosa was reported to possess anti-inflammatory, analgesic and anti-pyretic activities [9]. Compounds isolated from this plant, bauhinastatins have been reported to possess cytotoxic activity. The present work was under taken to explore the in-vitro antidiabetic potential of Pentacyclic tritrpenoids and fatty acid esters from Bauhinia purpurea (Fabaceae).

II. MATERIALS AND METHODS

Plant material
The stem bark of Bauhinia purpurea L. were collected from local area of Manipal, Karnataka, India during August 2011 and were authenticated by Dr. Chandrashekar KS, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal.

Preparation of petroleum ether extract [10, 11]
The stem bark of Bauhinia purpurea L. were dried in hot air oven at 50-60º C for 5-6 days and then ground to a fine powder in a grinder. The powdered plant material (2.5 kg) was subjected to maceration using petroleum ether for 4 days, then filtered with muslin cloth and evaporated to dryness. The extract was kept in desiccator.

Isolation of bioactive compounds from the Petroleum ether extract
The petroleum ether extract (10g) was dissolved in chloroform (20 ml) and adsorbed onto silica gel (20 g). After evaporation of the solvent, it was loaded onto silica gel column (200 g) prepared in petroleum
Concentration was added to above mixture. Mixture 12.5-100 µg/ml solutions were prepared. 1 ml of each concentration was added to 1 mL of yeast suspension, vortex and further incubated for 1 hr. Then 0.1 ml Iodine-iodide chloride in 100 ml distilled water) was added in the mixture. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

% increase in glucose uptake = \( \frac{\text{Abssample} - \text{Abscontrol}}{\text{Abscontrol}} \times 100 \)

Where, Abssample is the absorbance of the test sample, and Abscontrol is the absorbance of the control.

**Characterization by 1HNMR, IR and GCMS**

The characterization by 1HNMR, IR and GCMS led to the identification of Hopanol (A), 5, 11-Dimethyl hentriacontane (B), Ursdienol (C), Octadecedienoic acid methyl ester (D) in the stem bark of B. purpurea.

**Statistical Analysis**

All determinations were carried out in triplicates. Statistical Analysis- All determinations were carried out in triplicates and data were analyzed by ANOVA followed by Tukey’s multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at p≤0.05. Graphs were plotted using Origin 8.1 software.

**Inhibition of alpha-Amylase (%)**

\[
\text{Inhibition of alpha-Amylase (%) = } \frac{\text{Abssample} - \text{Abscontrol}}{\text{Abssample}} \times 100
\]

Where, Abssample is the absorbance of the test sample, and Abscontrol is the absorbance of the control.

**Alpha amylase inhibition**

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 inhibitory activity was based on the starch iodine method that was originally developed by Fuwa 1954[17] and later employed by others for determination of amylase activity in plant extracts[18] with some modifications. In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), 1 ml of drug solution (Acarbose std drug/ Pentacyclic triterpenoids and fatty acid esters) of four different concentration such as 12.5, 25, 50 and 100 µg/ml, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer (820.3 mg Sodium acetate and 18.7mg sodium chloride in 100ml distilled water). The above mixture was incubated for 1 hr. Then 0.1 ml Iodine-iodide indicator (635mg Iodine and 1gm potassium iodide in 250ml distilled water) was added in the mixture. Absorbance was taken at 565 nm in UV-Visible spectroscopy.

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\text{Inhibition of alpha-Amylase } = \frac{\text{Abssample} - \text{Abscontrol}}{\text{Abssample}} \times 100
\]
were carried out in triplicates and data were analyzed by ANOVA followed by Tukey’s multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at \( p \leq 0.05 \). Graphs were plotted using Origin 8.1 software.

III. RESULTS

In-vitro Non-enzymatic glycosylation of haemoglobin method The Pentacyclic triterpenoids of Bauhinia purpurea L. exhibited significant antidiabetic activity. The percentage inhibition of glycosylation is dose dependent, as dose increases, inhibition increases. Because as the concentration of drug increases formation of glucose-haemoglobin complex decreases and free haemoglobin increases, which show the inhibition of glycosylated haemoglobin. The activity of isolated compounds from the stem bark of Bauhinia purpurea L. was found to be better than standard drug acarbose.

Glucose uptake in Yeast cells

The rate of glucose transport across cell membrane in yeast cells system is presented in Fig. 2, 3 and 4. 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 3 glucose concentrations. The Pentacyclic triterpenoids exhibited significantly higher activity than at all concentrations. However the highest uptake of glucose was seen in 20mM Glucose concentration.

α- Amylase inhibition

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharides 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. As the result shows petroleum ether and aqueous extract of B. purpurea shows significant activity as compared to acarbose standard drug, and 12.5, 25, 50 and 100 µg/ml concentration of petroleum ether extract shows greater activity than Acarbose.

IV. DISCUSSION

In-vitro Non-enzymatic glycosylation of haemoglobin method is not important to detect diabetes. It is more important to judge the control of diabetes. The haemoglobin present in the red blood corpuscles has a tendency to get bound to glucose and form an abduct Alc. The greater the blood-glucose concentration, the greater is the amount of glucose-bound (called glycosylated) haemoglobin. Such glucose haemoglobin linkage is quite stable and lasts for 60 to 120 days (the life-span of red blood corpuscles). 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). Amount of Glycated haemoglobin should not be more than 12%. Our study demonstrates promising activity of Pentacyclic triterpenoids in preventing the binding of glucose to surface proteins of erythrocytes mostly due to antioxidant property of pentacyclic triterpenoids.

In the Glucose uptake in Yeast cells method the mechanism of glucose transport across the yeast cell membrane has been receiving attention as in vitro screening method for hypoglycaemic effect of various compounds/ medicinal plants. Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells (Saccharomyces cerevisia) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose.

In α- Amylase inhibition method α- Amylase hydrolyses α- bond of large α linked polysaccharides such as glycogen and starch to yield glucose and maltose. This assay is based on the formation of starch – iodine complex due to inhibition of α-amylase. The pentacyclic triterpenoids from the stem bark showed a good result in α-amylase inhibition assay, suggesting that B. purpurea might be effective in braking down starch to minimized glucose availability. The results infer that the pentacyclic triterpenoids may have significant effect in maintaining postprandial glucose concentration.

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