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EXPERIMENTAL RESEARCH

In Vitro Assay Of Alpha Amylase Inhibitory Activity Of Indian Medicinal Herb *Acalypha Indica*

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Introduction

Diabetes is defined as the state in which the homeostasis of the carbohydrate and lipid metabolism is improperly regulated by insulin. It results in the elevation of postprandial blood glucose levels and leads to hyperglycemia, which turns into a syndrome called diabetes mellitus. It has been estimated that 143 million people of the worldwide population are suffering from diabetes and the number is expected to increase to 333 million by the year 2030 [1]. The exact cause for diabetes in India is unknown, where genetics and lifestyle factors are being named as the causes. It is being aimed to provide cheap, effective, easily available and socio-culturally nutraceuticals to the rural Indian population suffering from diabetes [2]. As a result of modernization and globalization, rural people prefer to buy two wheelers as a symbol of status. Sedentary lifestyles, genetic susceptibility, environmental and lifestyle changes from industrialization and migration to the urban environment could be responsible for the incidence of diabetes mellitus [3]. A majority of the people in India suffer from malnutrition, where they consume more amounts of carbohydrates than fats and proteins. A person on a low fat, high carbohydrate diet utilizes the fatty acid synthesis pathway. It results in the generation of malonyl-CoA and inhibits the pathway to fatty-acid oxidation,

which could lead to diabetes [4]. The plant *Acalypha indica* is commonly known as Indian Acalypha and it belongs to the family Euphorbiaceae. The common names of *Acalypha indica* are Indian acalypha (English), Brennkraut (German), Alcalifa (Brazil) and Ricinela (Spanish). It is a common annual herb, found mostly in the backyards of houses and waste places throughout the plains of India. The plant is traditionally used as an expectorant against asthma and pneumonia, and also as an emetic, emmenagogue and anthelmintic [5]. *Acalypha indica* contains acalyphine which is used in the treatment of sore gums [6]. The plant is reported to have a post-coital antifertility effect [5], anti-venom properties [7], wound healing effects [8], antioxidant activities [9], anti-inflammatory effects [10], acaricidal effects [11], diuretic effects [12] and anti bacterial activities [13]. Till now, the investigation of anti-diabetic activity has not been done on *Acalypha indica*. In the present study, the ethanol, chloroform and hexane extracts of *Acalypha indica* were evaluated for inhibition of the α -amylase activity by using the *in vitro* method.

Materials and Methods

Drug and Chemicals: Starch azure, porcine pancreatic amylase and Tris-HCl were purchased from Sigma Aldrich, India. Hexane, chloroform, ethanol, dimethyl sulfoxide and acetic acid were purchased from Merck, India. The whole plant of *Acalypha indica* was collected from *Arakkonam*, Tamilnadu state, India, in the months from July to September 2007. The identification of the plant was confirmed by Dr. Senthilkumar M, Plant Biotechnologist, University of Madras, Chennai. The herbarium of the plant was deposited in

PITAM against voucher no. PITAM/CH/00025/2007. The whole plant was dried at an ambient temperature for 20-30 days. After complete drying, the plant was ground into fine powder using a domestic electric grinder (Product: (GX 21), Bajaj appliances, Mumbai, India). The dried powdered whole plant of *Acalypha indica* (50g) was extracted using a soxhlet apparatus with hexane, chloroform and ethanol sequentially, to get different solvent extracts. Each solvent extract was evaporated using a rotary evaporator (Buchi R-210), under reduced pressure. The percentage yield of hexane, Chloroform and ethanol extracts were 1%, 3% and 3.5%, respectively. Each dried extract was dissolved in dimethyl sulfoxide at different concentrations and was subjected to an Alpha amylase inhibitory assay.

PORCINE PANCREATIC AMYLASE (PPA) INHIBITORY ASSAY

2 mg of starch azure was suspended in each of the tubes containing 0.2ml of 0.5 M Tris-HCl buffer (pH 6.9) and 0.01 M CaCl₂. The tubes containing the substrate solution were boiled for 5 min and were then incubated at 37°C for 5 min. 0.2 ml of *Acalypha indica* extract (hexane, chloroform and ethanol), was taken in each tube containing different concentrations (10, 20, 40, 60, 80 and 100 µg/ml) of dimethyl sulfoxide. Porcine pancreatic amylase (PPA) was dissolved in Tris-HCl buffer to form a concentration of 2 units/ml and 0.1 ml of this enzyme solution were added to each of the above mentioned tubes. The reaction was carried out at 37°C for 10 min and was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer (Perkin Elmer Lambda 25 UV-VIS spectrophotometer). The α-amylase inhibitory activity was calculated as follows:

$$= \frac{[(Ac^+) - (Ac^-)] - [(As - Ab)]}{[(Ac^+) - (Ac^-)]} \times 100$$

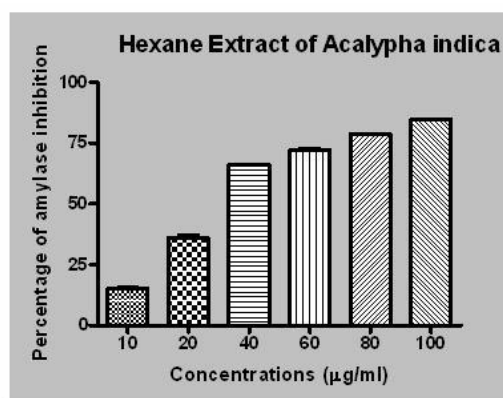
Where

Ac⁺, Ac⁻, As and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only

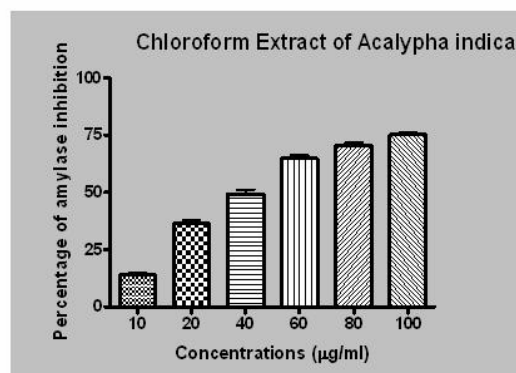
solvent without enzyme activity), a test sample (with enzyme) and a blank (a test sample without enzyme), respectively [14].

Results

Different extracts of (hexane, chloroform and ethanol) of *Acalypha indica* were prepared using a soxhlet apparatus. These extracts were tested for their α-amylase inhibitory activity against porcine pancreatic amylase. The ethanol extract of *Acalypha indica* failed to inhibit the α-amylase, whereas the chloroform and hexane extracts showed 75.32 % and 84.51% [Table/Fig 1],[Table/Fig 2] dose dependent amylase inhibitory activity against porcine pancreatic amylase *in vitro* [Table/Fig 3], respectively. The graphs were made by Graph Pad Prism 4 Demo software.



(Table/Fig 1)



(Table/Fig 2)

(Table/Fig 3) α -amylase inhibitory activity of different extracts of *Acalypha indica*

Concentration of <i>Acalypha indica</i> extracts(μ g/ml)	% Inhibition of α -amylase Hexane Extract	% Inhibition of α -amylase Chloroform Extract	% Inhibition of α amylase Ethanol Extract
10	14.95	13.90	ND
20	35.95	36.48	ND
40	65.87	49.33	ND
60	72.43	64.82	ND
80	78.73	70.60	ND
100	84.51	75.32	ND

All determinations were done in triplicate
ND defines- no α -amylase inhibitory activity

Discussions

In the previous studies, the petroleum ether and ethanol extracts of *Acalypha indica* at 600mg/kg body weight of female albino rats, showed oestrogenic activity and it was effective in causing anti-implantation activity [5]. The ethanol leaf extracts at the dose level of 500mg/kg, inhibited the Viper russelli venom induced lethality, haemorrhage, necrotizing and mast cell degranulation in rats and at the dose level of 750 mg/kg, inhibited the cardiotoxic and neurotoxic effects in isolated frog tissue [7]. The ethanol extract of *Acalypha indica* (ten percent weight/volume) prepared with saline, showed wound healing activity with low tensile strength [8]. The aqueous ethanolic leaf extracts of *Acalypha indica* showed 89-93% anti-oxidant activity in the Diphenylpicryl Hydrazyl (DPPH) method at a test concentration of 50 μ g/ml [9]. The fresh juice of the leaves of *Acalypha indica* showed anti-inflammatory activity in fasted albino rats by the inhibition of paw volume and oedema [10]. The paste *Acalypha indica* leaves showed acaricidal activity, both by *in vitro* and *in vivo* methods. The *in vitro* method showed maximum inhibition, with the suppression of lesions after 48 hours. The lethal effect on live mite count and the suppression of lesions in naturally infested broiler rabbits was observed after 4 hours of treatment by the *in vivo* method [11]. The methanol extracts of *Acalypha indica*, at the dose level of 400mg/kg in albino mice, showed diuretic activity after five hours of ingestion [12]. The hexane, chloroform, ethyl acetate and methanol extracts of *Acalypha indica* showed antibacterial activity against gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus* and *Streptococcus faecalis*), with the inhibitory concentration between 0.156-2.5mg/ml [13].

In this present study, the chloroform and hexane extracts of *Acalypha indica* at different concentrations, showed dose dependent α -amylase inhibition against porcine pancreatic amylase by the *in vitro* method, whereas the ethanol extract had no amylase inhibition. Further, it was suggested that *Acalypha indica* may have a beneficial effect in the management of diabetes.

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