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Research Article

IN VITRO ANTI-INFLAMMATORY AND ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *CARDANTHERA DIFFORMIS* DRUCE

Somnath De [1], Dulal Chandra Das [2], Tanusri Mandal [3]*
[1]Department of Biotechnology, Panskura Banamali Collage, West Bengal, India
[2]Department of Botany, Raja Narendranal Khan Womens' College, West Bengal, India
[3]Department of Biotechnology, Vidyasagar University, West Bengal, India
*Corresponding Author Email: tanusrimandaloist@gmail.com

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ABSTRACT

In rural and backward areas of West Bengal in India, several plants were commonly used as herbal medicine for the treatment of many diseases without studying any photochemical and biological information in detail. The current study was to investigate the anti-inflammatory and antidiabetic study of methanolic extract of *Cardanthera difformis*. Methanolic extract of *Candanthera difformis* was screened for anti-inflammatory activity by denaturation of egg albumin. The methanolic extract of *Candanthera difformis* was subjected to in vitro inhibition of protein denaturation in various concentrations i.e. 100, 200, 400, 800, 1000 µg/ml. Methanolic extract exhibited a concentration dependent inhibition of protein (albumin) denaturation. The anti-diabetic behaviour of methanol extracts of whole plant of *Cardanthera difformis* using the standard optical density assay were investigated against the two very essential enzyme α-amylase and α-glucosidase. The intestinal digestive enzymes play a vital role in the carbohydrate digestion. Pancreatic alpha-amylase and glucosidase inhibitors offer an effective strategy to lower the levels of post prandial hyperglycaemia via control of starch breakdown. The goal of the present study is to improve in vitro evidence of potential inhibition of alpha-amylase and alpha-glucosidase enzymes by using the methanolic extract of *Cardanthera difformis*. The present study suggests that the crude extract of *Cardanthera difformis* effectively act as in vitro anti-inflammatory and in vitro anti-diabetic activity.

Key words: Anti-inflammatory, denaturation of egg albumin , anti-diabetic, α-amylase and α-glucosidase inhibition, *Cardanthera difformis*.

INTRODUCTION

Inflammation is a complicated and not fully understood communication between cellular and humoral elements[1,2] . It is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organism, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemicals mediators from injured tissue and migrating cells[3]. The production of auto antigens in certain arthritic diseases may be due to in vivo denaturation of proteins [4]. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding5. So, by controlling the production of auto antigen and inhibiting denaturation of protein and membrane lysis in rheumatic disease leads to anti-arthritic or anti-inflammatory activity. Hence, inhibition of protein denaturation and membrane lysis were taken as a measure of the in vitro anti-inflammatory activity. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs(NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers[6,7].

Diabetes mellitus(DM) is a chronic disease characterized by a deficiency in insulin production and disturbances the metabolism process[8]. Now a days it is like a life style major health problem, in worldwide; gradually increases in epidemic rate[9]. Diabetes mellitus is considered to be a dangerous issue in many countries and traditional medicinal plants are used to control the problem[10]. Herbal medicines should have anti-diabetic potential activity to delaying the glucose absorption[11]. The intestinal digestive enzymes alpha-glucosidase and alpha-amylase plays a vital role in the carbohydrate digestion. One anti-diabetic therapeutic approach reduces the postprandial glucose level in blood by the inhibition of alpha-glcosidase and alpha-amylase enzymes. Inhibition of alpha-amylase and alpha-glucosidase enzymes can be an important strategy in management of postprandial blood glucose level in type 2 diabetes patient.[12] Some inhibitors currently in clinical use are acarbose, miglitol, and voglibose are known to inhibit a wide range of glycosidases such as α-glucosidase and α-amylase. Because of their non specificity in targeting different glycosidases, these hypoglycaemic agents have their limitations and are known to produce serious side effects. The main side effects of these inhibitors are gastrointestinal viz., bloating, abdominal discomfort, diarrhoea and flatulence[13]. Therefore, the search for more safer, specific, and effective hypoglycaemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering great potential for discovery of new anti-diabetic drugs[14,15,16]. The plant *Cardanthera difformis* which is chosen is a weed. It is a tropical aquarium plant under the family *Acanthacae* and common known as water wisteria, used as environmental ornaments, found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan, and Nepal[17]. This plant should have antibacterial, antifungal, antioxidant and anthelmentic activity[18,19,20]. But no report was found regarding

anti-inflammatory and anti-diabetic activity of *Cardanthera differmis* till the date. In the present investigation attempts have been made to find out the anti-inflammatory and anti-diabetic properties of *Candanthera differmis* by protein denaturation and α-amylase and α-glucosidase inhibition of methanolic extract respectively.

MATERIALS AND METHODS

Plant material

Cardanthera differmis Druce has been selected for experiment tools. It is collected in the month of march , 2015 form Paschim Medinipur district (Latitude- 22˚25'00'' to 22˚57'00'' north, Longitude- 87˚11' east, Altitude- 23 meters from mean sea level), West Bengal , India and it is available in any season of year.

Plant material extraction

The taxonomic identities of this plant are determined by the expertise of the department of botany of Vidyasagar University. The leaves were washed thoroughly using tap water and dried under shed for 11 days, then finely grinded to a powder. Then the powdered material was extracted with methanol using soxhlet apparatus. About 10 grams of powder was loaded in soxhlet extraction unit and exhaustively extracted using 100ml of solvents such as methanol at 60^0C for 12 hours. Thereafter, it was filtered with the help of Whatman No.1 filter paper[21]. The extracts were concentrated by rotary evaporator and used for testing anti-inflammatory and anti-diabetic activity.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

The following procedure was followed by for evaluating the percentage of inhibition of protein denaturation[22].

Control solution (50ml)
2 ml of egg albumin, 14 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.

Standard drug (50ml)
2 ml of egg albumin, 28 ml of phosphate buffer (pH 6.4) and 10ml various concentration of standard drug (Diclofenac sodium) concentration of 100, 200, 400, 800 and 1000µg/ml.

Test solution (50ml)
2ml of egg albumin, 28 ml of phosphate buffer (pH 6.4) and 10ml various concentration of methanol extract *Candanthera differmis* concentration of 100, 200, 400, 800 and 1000µg/ml.
All of the above solutions were adjusted to pH using a small amount of 1N HCl. The samples were incubated at 37˚ C for 15 minutes and heated at 70 ˚ C for 5 minutes. After cooling, the absorbance of turbidity was measured at 660 nm in UV-vis spectrophotometer the above solutions percentage inhibition of protein denaturation was calculated using the following formula [23].

Percentage inhibition = [Vt/Vc -1] X 100
Where, Vt= Absorbance of test sample ,
Vc = Absorbance of control

In vitro anti-diabetic activity

α-amylase inhibition assay

The α-amylase inhibitory activity was determined according to the method[24]. Briefly, the total assay mixture containing 200 µl of 0.02M sodium phosphate buffer, 20 µl of enzyme, and the plant extracts in the concentration range 10-100µg/ml were incubated for 10 min at room temperature followed by addition of 200 µl of 1% starch in all the test tubes. The reaction was terminated with addition of 400 µl of 3,5 dintrosalycylic acid (DNSA) color reagent, placed in boiling water bath for 5 minutes, cooled at room temperature and diluted with 15 ml of distilled water and the absorbance measured at 540nm. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts prepared with different solvent prepared with DMSO. The results were expressed as % inhibition calculated using the formula:

Inhibition activity (%) = Abs (control) -Abs (extract)/ Abs(control)×100

The IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined b y performing the assay as above with varying concentrations of the plant extracts ranging 20 to 100µg. The IC_{50} values were determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

α-glucosidase inhibition assay

The yeast α-glucosidase was dissolved in 100mM phosphate buffer, pH 6.8 was used as enzyme source; 10mM paranitrophenyl-α-D glucopyranoside was used as substrate. *Cardanthera differmis* extract powder was weighed and mixed with dimethylsulfoxide to get a concentration of 20-100µg/ml. The different concentration of plant extract was mixed with 320µl of 100mM phosphate buffer (pH 6.8) and 50 µl of 10mM PNPG in the buffer and then it was incubated at 30°C for 5 minutes. After the incubation, 20µl of the buffer containing 0.5 mg/ml of the enzyme was added and further incubated at 30°C for five minutes. Finally, 3.0 ml of 50mM sodium hydroxide was added to the mixture and the absorbance (A)was measured at 410nm on a spectrophotometer. The enzyme without plant extract was used as a control[25].

% Inhibition = A_{410} control –A410 test/ A410 control×100

The IC_{50} values (inhibitor concentration at which 50% inhibition of the enzyme activity occurs) of the plant extracts were determined by performing the assay as above with varying concentrations of the plant extracts ranging 20 to 100µg. The IC_{50} values were determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

RESULTS

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

In in-vitro anti- inflammatory activity by egg albumin denaturation method at concentration of 100, 200,400,800 and 1000 µg/ml showed 172.50, 175.00, 195.00, 223.75 and 225.00% inhibition of egg albumin denaturation (Table 1) whereas, standard Diclofenac sodium at 100, 200, 400, 800 and 1000 µg/ml which showed 180.00, 197.50, 211.25, 233.13 and 234.37% inhibition of egg albumin denaturation (Table 2) and their comparison between sample and standard (Table 3). From this experimental results showed significant inhibition of denaturation of egg albumin in concentration dependent manner.

Table 1: **In vitro anti-inflammatory activity of methanolic extract of *Cardanthera difformis* on protein denaturation (Fresh egg albumin)**

Treatment	Concentration (µg/ml)	Percentage of inhibition (%)
Methanolic extract of *Cardanthera difformis*	100	172.50
	200	175.00
	400	195.00
	800	223.75
	1000	225.00

Table 2: **In vitro anti-inflammatory activity of Diclofenac sodium on protein denaturation (Fresh egg albumin)**

Treatment	Concentration (µg/ml)	Percentage of inhibition (%)
Diclofenac sodium	100	180.00
	200	197.50
	400	211.25
	800	233.13
	1000	234.37

Table 3: **In vitro anti-inflammatory activity of Diclofenac sodium & methanol extract of *Cardanthera difformis* on sodium on protein denaturation (Fresh egg albumin)**

Sl.No.	Concentration (µg/ml)	% inhibition Diclofenac sodium	% inhibition *Cardanthera difformis*
1	100	180.00	172.50
2	200	197.50	175.00
3	400	211.25	195.00
4	800	233.13	223.75
5	1000	234.37	225.00

In vitro antidiabetic activity

In vitro α-amylase inhibition assay

Table 4: **In vitro antidiabetic activity of alpha- amylase inhibition method of standard(Acarbose) methanol extract of *Cardanthera difformis***

Sample	Concentration(µg/ml)	% of inhibition	IC_{50}(µg/ml)
Methanol extract	20	23.62	74.88
	40	33.89	
	60	39.66	
	80	55.63	
	100	61.31	
ACARBOSE (Standard)	20	33.75	46.46
	40	49.22	
	60	59.20	
	80	67.22	
	100	73.97	

In vitro α-glucosidase inhibition assay

Table 5: In vitro anti-diabetic activity of alpha-glucosidase inhibition method of standard and methanol extract of *Cardanthera difformis*

Sample	Concentration(µg/ml)	% of inhibition	IC_{50} (µg/ml)
Methanol extract	20	30.78	52.17
	40	39.67	
	60	56.08	
	80	64.01	
	100	85.71	
ACARBOSE (Standard)	20	40.73	38.36
	40	49.34	
	60	63.48	
	80	72.56	
	100	91.58	

DISCUSSION

Protein denaturation is a process in which protein lose their tertiary structure and secondary structure by application of external stress or compound such as strong acid or base concentration inorganic salt, an organic solvent or heat most biological protein lose their biological function when denaturation. Denaturation of protein is a well-documented cause of inflammation. As a part of the investigation on the mechanism of the anti- inflammatory and anti-arthritic activity, ability of plant extract to inhibit protein denaturation was studied. Several anti-inflammatory drugs have showed dose dependent ability to inhibit thermally induced protein denaturation[26]. Denaturation of protein is a well document cause of inflammation in condition like Rheumatoid arthritis.

Diabetes mellitus (DM) is a common endocrine system disease that causes metabolic disorders and which leads to multiple organ damage syndrome. Clinical admiral diabetes is divided into two types, with more than 90% of patients having Type II diabetes[27]. The number of diabetes cases was 171 million in 2000 and is expected to rise to 366 million in 2030 .Inhibition of

α-glucosidase and α-amylase, enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients[28]. Intestinal α – glucosidase is a glucosidase acting as a key enzyme for carbohydrate digestion, located at the epithelium of the small intestine. α-glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in Type II DM[29]. Several natural α-glucosidase and α-amylase inhibitors including acarbose, voglibose and miglitol are clinically used as a treatment, but their prices are high and clinical side effects occur [30,31]. Natural products are still the most available source of α-glucosidase inhibitors. Therefore, screening of alpha-amylase and glucosidase inhibitors in medicinal plants has received much attention. Therefore, in the present study we investigated α-amylase and α-glucosidase inhibitory activity by using the methanolic extract of *Cardanthera difformis*.

In vitro anti-diabetic studies demonstrated that *Cardanthera difformis* extract h as both α-glucosidase and α-amylase inhibitory activity. The percentage of inhibition at 100, 80, 60, 40 and 20 µg/ml concentrations of plant extract showed a concentration-dependent reduction in percentage inhibition. Acarbose like drugs, that inhibit α-glucosidase and amylase present in the epithelium of the small intestine, have been demonstrated to decrease post-prandial hyperglycaemia[32] and improve impaired glucose metabolism without promoting insulin secretion in NIDMM patients[33]. These medications are most useful for people who have just been diagnosed with type 2 diabetes and who have blood glucose levels only slightly above the level considered serious for diabetes. They also are useful for people taking sulfonylurea medication or metformin, who need an additional medication to keep their blood glucose levels within a safe range. Therefore, the retardation and delay of carbohydrate absorption with a plant-based α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus and borderline patients[34,35]. The results of this study indicate that the administration of *Cardanthera difformis* an probably manage the postprandial blood glucose levels and confirm the usage of these plants.

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