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VIEWS

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Anti Anaphylactic and Mast Cell Stabilizing Activities of Fluoro Benzothiazoles

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ABSTRACT

Background: The aim of the study is to investigate Synthetic fluoro substituted benzothiazole derivatives (FBTDs) for anti anaphylactic and mast cell stabilizing activities in Compound 48/80 induced allergic reactions in mice. Allergic disorders are in rise every year and stated as an endemic disease of the 21st century. various allergens which are existing in the co system of our daily life and also originating from immune system are responsible for asthma, eczema, hay fever, anaphylaxis, autoimmune diseases.The acute oral toxicity studies was determined as per OECD guideline 423 and minimum effective dose was also determined for pharmacological screening. **Materials and methods**:The anti allergic activity of FBTDs was studied against 48/80 induced allergy(mast cell stabiling and anaphylacting reactions). **Results and discussion**: P-7, OX-9, shown equal to standard P-13 ,P-4 and P-2 show significant as degranulated mast cells considered. whereas in anaphyalactic model OX-9 and P-13 were also shown 66.66% of protection which are equal to standard. P-4 & P-6 was shown 50% protection which are near to standard. Whereas P-7 shown 33.33% and P-1,P-5, & P-9 were exhibit 16.66% protection.

Key words: compound 48/80, Fluoro substituted benzothiazole derivatives, anaphylactic reaction, mast cells.

INTRODUCTION

Allergic disorders are in rise every year and stated as an endemic disease of the 21st century. various allergens may be provoked from existing ecosystem and our own immune system and held responsible for asthma, eczema, hay fever, anaphylaxis, autoimmune diseases.[1] The interaction between genetic and environmental factors is generally accepted to cause individuals to be sensitized with environmental allergens and to suffer from allergic diseases. However, it is believed that recent changes in the environment have contributed to the increase more significantly than genetic factors, since it seems unlikely that genes would change over one or two generations. Thus, it is a central issue to reveal what environmental factors cause such high prevalence and to find strategies to prevent their development.[2] The allergic process has an important inflammatory component in which mast cell activation and degranulation are the first phenomena observed.3 This led us to initiate us towards the allergic research which kick started through the synthesis of FBTS's with rationale of designing anti allergic drugs.

Mast cells are constituents of virtually all organs and tissues and are important mediators of inflammatory responses such as allergy and anaphylaxis in which histamine remains the best characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity upon release. Mast cell degranulation can also be evoked by the compound 48/80, which is a mast cell degranulator and has been used as a direct and convenient reagent to study the mechanism of anaphylaxis.[4]

Anaphylaxis is an acute hypersensitivity reaction with multi-organ-system involvement that rapidly progresses to, a severe life-threatening reaction. Mast cells are the primary effector cells involved in an allergic or immediate hypersensitivity response. Activation of mast cells occurs in response to a challenge by a specific antigen against which the surface immunoglobulin E (IgE) is directed, or by other IgE-directed ligands. Activated mast cells can produce histamine and a wide variety of inflammatory mediators .which result into various acute and chronic allergic responses. Mast cell degranulation can also be elicited by the synthetic compound 48/80. Compared with the natural process, compound 48/80 induces histamine release from mast cells and used as a direct and convenient reagent to investigate the mechanisms of allergy and anaphylaxis.So, the murine mast cell is a good experimental model for the study of compound 48/80-induced histamine release.

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MATERIALS AND METHODS

Procurement of synthetic derivatives for Pharmacological screening

The novel synthesized substituted Benzothiazole derivatives (FBTDs) were synthesized by Patel Snehal kumar Vishnubhai. under the guidance of Dr. E. Jayachandran, Professor and head, PG Department of Pharmaceutical Chemistry, S.C.S. College of Pharmacy, Harapanahalli. The same SSBDs were procured for screening anticonvulsant activities in validated experimental animal model.

2.1.1 Animals:

Wister albino rats (weighing 150-200 g) and albino mice (weighing 20-25 g) of either sex were used in this study. They were procured from Sri Venkateshwara Enterprises, Bengaluru. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C under 12 hrs dark / light cycle. They were fed with standard rat feed (Gold Mohur Lipton India Ltd.) and water *ad libitum* was provided. The husk in the cages was renewed thrice a week to ensure hygienity and maximum comfort for animals.

Ethical clearance for usage of the animals was obtained from the Institutional animal ethical committee **(Certificate reference no: *SCSCP/*626/A 2012-13 dated: 09-01-2013)** prior to the beginning of the project work.

Acute toxicity Studies:

The toxicity of FBTDs were determined by using female albino mice (20-25 g), maintained under standard husbandry conditions. The animals were fasted for 3-4 hours prior to the experiment. Animals were administered with single dose of Fluoro substituted Benzothiazole derivatives observed up to 48 hours study period for its mortality (short term toxicity). Based on the short-term toxicity profile, the next dose were determined as per OECD guidelines No 423[5]

Determination of minimum effective dose:

The minimum effective dose of FBTDs was determinated at dose of 30, 100, 150 and 250 mg/kg body weight of an animal to carry out screening of anticonvulsant and ant allergic activities.

Antianaphylactic activity induced by compound 48/80 in mice:

Albino mice of either sex with a body weight 22-25g were divided into 13 groups of 6 animals in each. Group 1 was served as control and receives normal saline. Group 2 was served as positive control and receives 8 mg/kg i.p injection of compound 48/80 for induction of anaphylactic reaction. Group 3 was served as standard and treated with sodium chromoglycate (10mg/kg; ip), Group 4-13 were administered orally with compound 1–10 respectively 1 hour prior to compound 48/80. Mortality was monitored for 1 hour after induction of anaphylactic shock in both control and treated groups[6,7].

$$\text{Mortality (\%)} = \frac{\text{Number of dead mice}}{\text{Total number of experimental mice}} \times 100$$

Mast cell stabilizing activity induced by compound 48/80 in rats:

Albino rats of either sex with a body weight 250-275g were divided into 13groups of 6 animals in each. Group 1 was served as negative control (normal). Group 2 was served as positive control and receives 8 mg/kg i.p injection of compound 48/80. Group 3 was served as standard which treated with sodium chromoglycate (10 mg/kg;ip) and Group 4-13 were administered orally with compound 1–10 respectively to rats daily 5 days prior to collection of mast cells. The animals were anesthetized by diethyl ether and injected normal saline (8-10 ml) into the peritoneal cavity of albino rats. After gentle massage, the peritoneal fluid were collected and transferred into test tubes containing RPMI-1640 reagent (pH 7.2-7.4). Mast cells were washed three times by centrifugation at a low speed (400-500 rpm) discarding the supernatant and taking the pellets of mast cells into the medium. Mast cells from control group and treated groups were incubated with compound 48/80 (1 µg/ ml) at 37^0 c for 10 min. After incubation, mast cells were stained with toluidine blue (0.1 %) and percentage protection against degranulation were counted under high-power microscope (45X) [8-10].

Statistical Analysis

Results were expressed as mean + SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test by using graph pad instant software. p value less than 0.05 was considered to be statistically significant. *p<0.05, *p<0.01 and **p<0.01, when compared with control and toxicant group as applicable.

RESULTS

Table No:1

Mean data of effect of synthesized substituted Benzothiazole derivatives on compound 48/80 induced anaphylactic reaction in mice

Sl. No.	Treatment	Dose	Percentage of Mortality	Percentage of Protection
1	Negative Control	----	0	100
2	Positive Control	10mg/kg, (i.p.)	100	0
3	Standard	10 mg/kg (i.p.)	33.33	66.66
4	P1	100mg/kg (p.o.)	83.33	16.66
5	P2	100mg/kg (p.o.)	100	0
6	P3	100mg/kg (p.o.)	100	0
7	P4	100mg/kg (p.o.)	50	50
8	P5	100mg/kg (p.o.)	83.33	16.66
9	P6	100mg/kg (p.o.)	50	50
10	P7	100mg/kg (p.o.)	66.66	33.33
11	P9	100mg/kg (p.o.)	83.33	16.66
12	P13	100mg/kg (p.o.)	33.33	66.66
13	OX9	100mg/kg (p.o)	33.33	66.66

The data are presented as mean±SEM, n=6. Statistical analysis were performed using One-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Levels of significance: **P*<0.05, ***P*<0.01, *P*<***0.001 compared to control group.

Table No:2

Mean data of effect of synthesized substituted Benzothiazole derivatives on mast cell degranulation induced by Compound 48/80

Sl. No.	Treatment	Dose	Granulated mast cell	Degranulated mast cell	(%) Percentage of protection
1	Negative Control	**----**	85.17 ± 1.701	15.83 ± 1.078	85.17
2	Positive Control Compound 48/48	10mg/kg	16.50 ± 1.333	84.17 ± 1.078	16.50
3	Standard	10 mg/kg (i.p.)	69.67 ± 2.499	21.00 ±0.930***	69.67
4	P1	100mg/kg (p.o.)	28.00 ± 1.483	75.83 ± 1.721NS	28.00
5	P2	100mg/kg (p.o.)	40.83 ± 1.014	68.50 ± 3.871*	40.83
6	P3	100mg/kg (p.o.)	37.67 ± 4.137	70.17±4.003NS	37.67
7	P4	100mg/kg (p.o.)	26.50 ± 1.703	62.17 ± 5.896**	26.50
8	P5	100mg/kg (p.o.)	23.83 ± 1.838	76.17 ± 1.833NS	23.83
9	P6	100mg/kg (p.o.)	40.50 ± 1.565	78.33 ± 1.563NS	40.50
10	P7	100mg/kg (p.o.)	50.50 ± 2.717	49.17 ±4.771***	50.50
11	P9	100mg/kg (p.o.)	37.17±1.327	72.50 ± 3.274NS	37.17
12	P13	100mg/kg (p.o.)	54.83 ± 6.529	66.67 ± 5.129*	54.83
13	OX9	100mg/kg(p.o)	63.67 ± 1.909	43.33 ±6.791***	63.67

The data are presented as mean±SEM, n=6. Statistical analysis were performed using One-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Levels of significance: **P*<0.05, ***P*<0.01, *P*<***0.001 compared to control group.

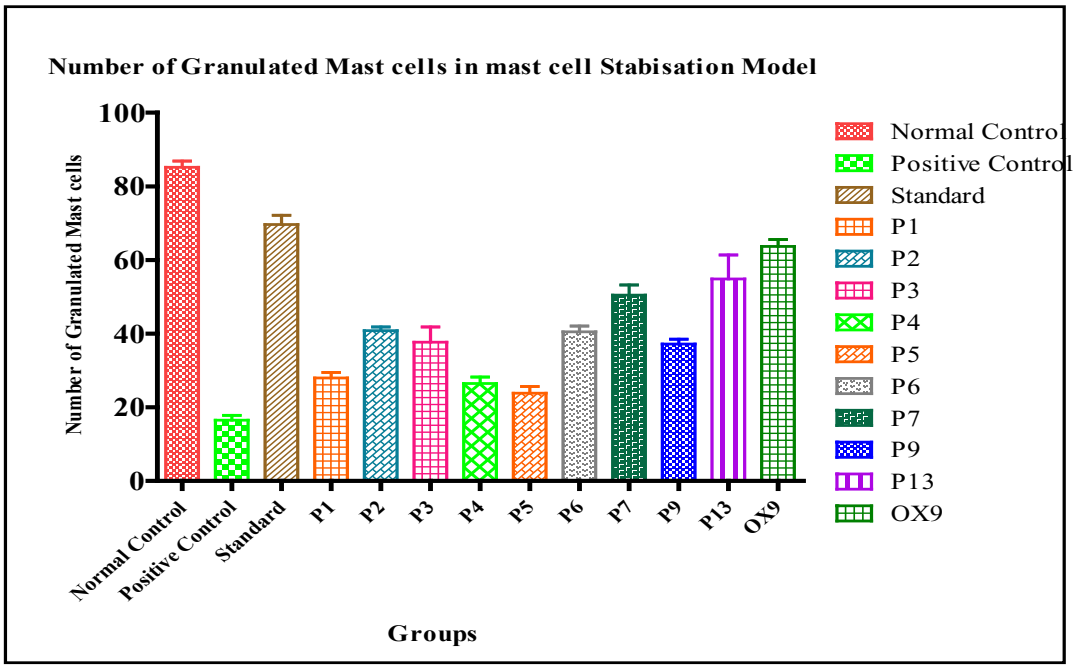

Fig. 01 : Number of granulated mast cell in mast cell stabilization model

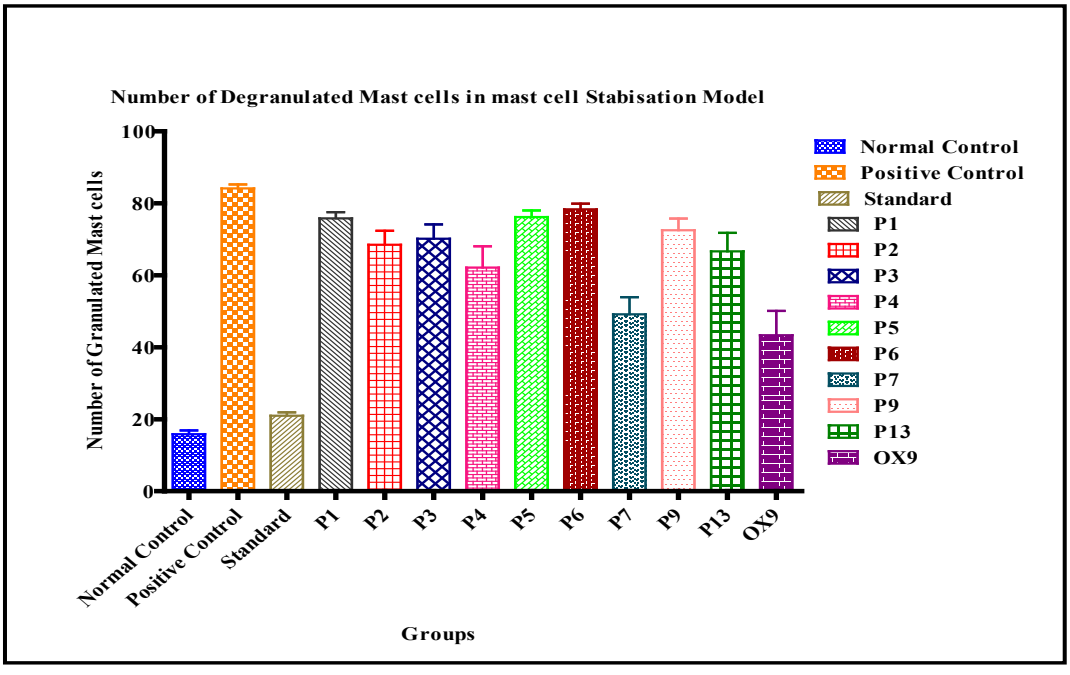

Fig. 02 : Number of De granulated mast cell in mast cell stabilization model

DISCUSSION

Compound 48/80, which is a mixed polymer of phenethylamine cross-linked by formaldehyde, induces almost a 90% release of histamine from mast cells. Hence, compound 48/80 has been used as a direct and convenient reagent to study the mechanism of allergy and anaphylaxis**.** Mast cells play a pivotal role for the development of allergic asthma, but so far therapeutic approaches to directly target mast cells have not been very successful.[11]

Compound 48/80 initiates the generation of superoxide anion by Akinase inactivation through decreasing the intracellular cAMP concentration in mast cells**.** generated superoxide anion results in the inositol 1, 4, 5- triphosphate or GTP induced calcium release from endoplasmic reticulum which increases intracellular calcium content, which leads to histamine release from mast cells which is known as degranulation of mast cells.

Present data demonstrate that FBTDs suppresses both compound 48/80-induced Systemic anaphylaxis and mast cell degranulation. Several reports have shown that compound 48/80 increases the permeability of the lipid bilayer membrane of mast cells by causing perturbation of the membrane . These findings indicate that the increase in cell membrane permeability may be an essential trigger for the release of mediators from mast cells.[12]

Anaphylaxis is a severe and systemic allergic reaction caused by systemic release of histamine and other pharmacological mediators. The common cause of anaphylaxis was Ige mediated hypersensitivity reaction. One of the newer method of anaphylaxis treatment involves use of immunotherapeutic agent by decreasing production of IgE2.[13]

Study was conducted at a dose of 100mg/kg body weight of an animal. It was observed that, sodium cromoglycate as a standard shown extremely significant (***P<0.001) as degranulated mast cells considered. P-7, OX-9, shown equal to standard P-13 ,P-4 and P-2 show significant as degranulated mast cells considered were clearly mentioned in table no 1 and figure no.1

As anaphylaxis concerned , sodium cromoglycate as a standard shown 66.66% of protection. OX-9 and P-13 were also shown 66.66% of protection which are equal to standard. P-4 & P-6 was shown 50% protection which are near to standard. Whereas P-7 shown 33.33% and P-1,P-5, & P-9 were exhibit 16.66% protection were in table no. 2 and figure no.2.

CONCLUSION

The Present investigation also revealed that the FBTDs found to have a remarkable anti-allergic activity. **P-4, P-7,** and **OX-9.** were shown significant effect in compound 48/80 induced anti-anaphylactic and mast cell stabilizing activities

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Invitro and *Invivo* Antidiabetic Activity of Hydroalcoholic Leaves of *Soyamida Febrifuga* in Albino Rats

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ABSTRACT

Objective: The present study aimed to evaluate antihyperglycemic effects of 70% ethanolic extract of *Soyamida febrifuga* leaves (EESFL) in alloxan-induced diabetic rats. **Methodology:** The extract was prepared, and hyperglycaemia was induced in wistar rats by injecting alloxan monohydrate (120 mg/kg b.w). After 72 hours of alloxan monohydrate injection, the hyperglycaemic rats (glucose level > 250 mg/dl) were separated. The diabetic rats were administered orally with extract at three different doses (250, 333.33 & 500mg/kg b.w/day) for 10 days. The various biochemical markers like fasting blood glucose, serum cholesterol, serum urea, serum creatinine and total serum protein, triglyceride, HDL, LDL, VLDL, the hepatic glycogen content were estimated. Inhibition of hydrolyzing enzymes plays an important role in the management of diabetes. Hence the extract was further screened for alpha-amylase enzyme inhibitory activity. **Results:** Test extracts demonstrated *in-vitro* and *in-vivo* antioxidant activities. Significant decrease in the elevated level of fasting blood glucose, serum cholesterol, total serum protein, triglyceride, HDL, LDL, VLDL and also there is a decrease in the pancreas weight and significant increase in the hepatic glycogen content was estimated in alloxan induced diabetic model. Significant decrease in the GSH, super oxide dismutase, catalase and significant increase in the lipid peroxidation was estimated. **Conclusion:** The extract exhibited significant *invivo* and *invitro* antidiabetic and α-amylase enzyme inhibition. The antidiabetic and α-amylase enzyme inhibition of the plant may be due to the polyphenolic compounds like flavonoids, tannins and total phenols that are present in the plant and for evaluation in future clinical studies.

Keywords: *soyamida febrifuga*, antidiabetic, alloxan, *Invito* α-amylase enzyme.

Introduction

Diabetes is the world's largest endocrine disease with deranged carbohydrate, fat and protein metabolism. As per a WHO report, approximately 150 million people have diabetes mellitus worldwide, and this number may double by the year 2025[1]. Diabetes is a growing challenge in India with estimated 8.7% diabetic population in the age group of 20 and 70 years [2]. India currently represents 49 percent of the world's diabetes burden, with an estimated 72 million cases in 2017, a figure expected to almost double to 134 million by 2025[3]. Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycaemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic beta cells (negative nitrogen balance), glycosuria, and sometimes ketonemia[4]. It is caused by heredity, increasing age, poor diet, imperfect digestion, obesity, sedentary lifestyle, stress, drug-induced, infection in the pancreas, hypertension, high serum lipid and lipoproteins, less glucose utilization and other factors. DM is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and anginopathy and several others. Type 1 and Type 2 are forms of diabetes differ in their pathogenesis, but have hyperglycemias as a common hall mark [5]. Patients suffering from type I are therefore totally dependent on the exogenous source of insulin while, patients suffering from Type II diabetes can be treated with dietary changes, exercise and medication. Type II diabetes is the more common form of diabetes constituting 90% of the diabetic population [6]. Even though different classes of antidiabetic drugs are available to control type 2- diabetes, still it is a challenging task to bring a better molecule which is devoid of undesirable adverse effects than existing drugs. Currently available synthetic drugs like sulfonyl ureas, biguanides, α-glucosidase and α-amylase inhibitors, etc. besides being expensive, produces serious adverse effects. Since ancient time, in India, traditional medicines have been effectively used to treat diabetes. *Soymida febrifuga* is a tall tree belonging to family meliaceae; commonly known as Indian redwood, bastrol cedar. Pharmacologically

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the plant is of great importance in the ethanomedicinal use. It contains some important constituents like in bark lupeol, sitosterol, methyl angolensate, leaves contains quercetin, rutin and fruits abundantly contain tetraterpenoids. The ethno botanical used in the treatment of diarrhea, dysentery and fever, as a bitter tonic in general debility, treatment of rheumatic swelling, in gargles, vaginal infection etc [7].

Materials and Methods

Collection, Preparation of Extract and Preliminary phytochemical screening

Soyamida febrifuga leaves were identified and collected in the surrounding of Harapanahalli, and authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S College of Pharmacy, Harapanahalli. A voucher Specimen No. SCSCOP.Ph.Col Herb.001/2011-12. Bulk collection of leaves was carried out, and it was cleaned, shade-dried, and powdered. The leaves were dried, and extracted with Soxhlet extractor using petroleum ether, chloroform, 70% ethanol and distilled water at room temperature. The extracts were concentrated under reduced pressure using rotary evaporator and stored in airtight container in refrigerator below 10°C. Preliminary phytochemical investigation was carried out on all extracted solvents to evaluated for the presence of carbohydrates, proteins, flavonoids, tannins, and alkaloid [8-9].

Experimental animals

The Wistar strains of albino rats of either sex, weighing between 100 and 150g were obtained for the present study, from Venkateshwara Enterprises, Bangalore (CPCSEA Reg. no: 157/1999/CPCSEA). The animals were housed in larger spacious cages and they were fed with commercial pelleted rat chow marketed by Hindustan Lever Ltd., Bangalore, India, under the trade name Gold Mohur Rat Feed and had free access to water ad labium. The animals were well acclimatized to standard environmental conditions of temperature (22 ±5°C) and humidity (55±5%) and 12h light dark cycles throughout the experimental period. The animals used in the present study were approved by the Institutional Animal Ethical Committee (SCSCP/583/3/2011-12)

Acute toxicity test

The acute toxicity of EESFL was evaluated using female albino mice. The animals were fasted overnight prior to the experiment, the fixed dose method was adopted as per OECD Guideline No. 423 (Annexure-2d) of CPCSEA. A Group of three mice was taken for each test dose. Briefly, rats were administered different concentrations of extract and were examined for any signs of behavioural changes and / or mortality for the following 21 days. 70%EESFL: 250 mg/kg ($1/10^{th}$ of LD50 cut off value), 333 mg/kg ($1/7.5^{th}$ of LD50 cut off value), 500 mg/kg ($1/5^{th}$ of LD50 cut off value) [10].

Quantitative determination

a) Total Phenolic Content (TPC) [11]

The total phenolic content of 70% ethanolic extract was determined. The aliquot of the extract was taken in a 10 ml glass tube and the volume was made up to 3 ml with distilled water. Then 0.5 ml Folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue colour was developed in each tube and the intensity of the colour was directly proportional to the phenolic content. The blue colouration in the tube is due to the formation of molybdenum blue as a result of complex redox reaction between phenols and phosphomolibdic acid in Folin ciocalteau reagent in alkaline medium. The test solutions were warmed for 1minute, cooled and absorbance was measured at 650 nm. The calibration curve was prepared using catechol as standard. The phenolic content of the plant was expressed as a mg. equivalent of phenol per gm. of extract.

b) Total Flavonoid content (TFC) [12]

The aliquots of each extract were pipetted out in series of test tubes and volume was made up to 0.5ml with distilled water; sodium nitrate (5%: 0.3ml) was added to each tube & incubated for 5 min, at room temperature; aluminium chloride solution (10%; 0.06ml) was added and incubated for 5 min at room temperature; Sodium hydroxide (1M; 0.25ml) was added and total volume was made to 1ml with distilled water. Absorbance was measured at 510nm against a reagent blank using Shimadzu model 1700 double beam spectrophotometer and concentration of flavonoids in the test sample was determined and expressed as mg equivalent of quercetin per gram of sample

c) Total tannin content (TTC) [13]

Prior the quantitative estimation of the tannin content in the samples, the presence of tannins was identified using the classic $FeCl_3$ and Gelatine tests. The quantitative tannin content in samples was estimated by the method of Price and Butler with some modifications. In short, of a dry plant sample was transferred to 100 ml flask; 50 ml water was added and boiled for 30 min. After filtration with cotton filter, the solution was further transferred to a 500 ml flask and water was added ad 500 ml mark. 0.5 ml aliquots were finally transferred to vials, 1 ml K_3Fe (CN) 6 and 1 ml $FeCl_3$ were added and water was added upto 10 ml volume. After five min time period, the solutions were measured spectrophotometrically at 720 nm. The actual tannin concentrations were calculated on the basis of the optical absorbance values obtained for the standard solutions in range 5 -25 µg /10 ml.

Animal Studies

Induction of Hyperglycemia in Albino Rats [14, 15, 16]

Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan monohydrate (s.d. fine-chem. Ltd., Mumbai, India) in sterile saline. The albino rats were then kept for the next 24 hour on 10% glucose solution bottles,

in their cages, to prevent hypoglycemia. After 72 hour of alloxan injection, the hyperglycemic rats (glucose level > 250 mg/dl) were separated and divided into different groups comprising of 6 rats each.

Group I- Received normal saline (i.p) + vehicle (p.o).

Group II- Received alloxan monohydrate 120 mg/kg (i.p) + normal saline (p.o).

Group III- Alloxan + Glibenclamide (10 mg/kg p.o.) served as standard.

Group IV- Alloxan monohydrate + 70% EESFL (250 mg/kg, p.o.).

Group V- Alloxan monohydrate + 70% EESFL (333mg/kg, p.o.).

Group VI- Alloxan monohydrate + 70% EESFL (500 mg/kg, p.o.).

The treatment (p.o.) was started from the same day except normal control and diabetic control groups for a period of 10 days. During this period, animals in all groups had free access to standard diet and water. Body weight and blood glucose levels were estimated one hour after the drug administration and the day 5th, 10th, 15th day of the treatment.

On the 15th day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for biochemical estimations. The pancreas from all the animals was removed immediately and kept in 10% formalin solution for histopathological examination.

Results

Acute toxicity and Preliminary phytochemical screening

A preliminary toxicity study was designed to demonstrate the appropriate safe dose range that could be used for subsequent experiments rather than to provide complete toxicity data on the extract. It is observed from the preliminary phytochemical screening that glycoside, flavonoid, tannin, alkaloid, saponins, protein and carbohydrate found in 70% ethanolic extracts. It was qualitatively observed that 70% ethanolic extract contain higher concentration of polyphenol, flavonoid, alkaloid, protein and tannin components and hence selected this extract for further study.

Quantitative estimation of Total Phenolic, Flavonoids and Tannins

The total phenolic content was 1.15 mg/g expressed as equivalent to catechol. Similarly, flavonoid content was found to be 11.44 mg/g expressed as equivalent to quercetin, and total tannin content was to be 42 mg/g expressed as equivalent to tannic acid as shown in table1 and figure no 1,2&3 respectively.

Table - 1 :

Quantitative determination of secondary metabolites

Sl. No.	Name of the secondary metabolites	Absorbance at	Ethanolic extract (mg/g)
1	Total Phenol	650nm	1.15 mg/g Catechol
2	Flavonoid	510nm	11.44 mg/g Quercetin
3	Tannin	700nm	42 mg/g Tannic acid

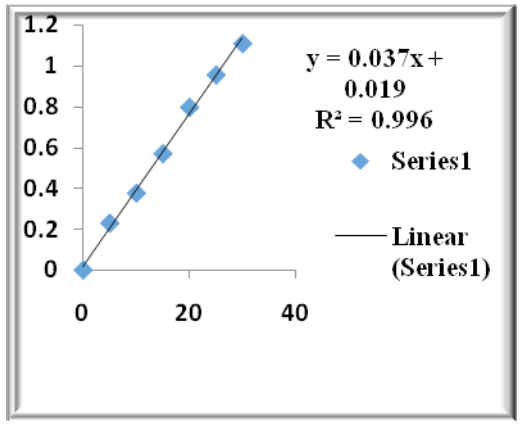

Fig. 1 : Calibration Curve of Catechol

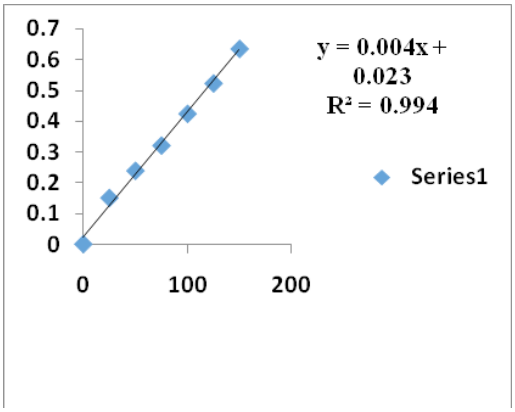

Fig. 2 : Calibration Curve of Quercetin

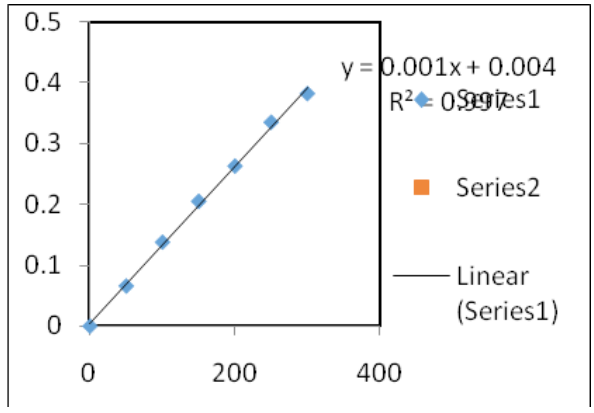

Fig. 3 : Calibration Curve of Tannic acid

***In vitro* α-amylase inhibition study** [17-18]

α-Amylase is a protein enzyme that hydrolysis alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose. It is the major form of amylase found in humans and other mammals. The inhibition of this enzyme can delay the carbohydrate digestion and reduce the rate of glucose absorption. Consequently, postprandial rise in blood glucose is decreased. Hence, they have long been though to improve glucose tolerance in diabetic patients. On the other hand, natural polyphenols and phenolic compounds have been reported to inhibit the activity of carbohydrate hydrolyzing enzymes and acts as natural antioxidants. The maximum inhibition of 70% EESFL was 84.8% at a concentration 6mg/ml. The percentage inhibition ranged from 84.8 – 78.09%. Aqueous extract of *Soyamidafebrifuga* leaves produced a maximum inhibition of 87.3% at a concentration 6mg/ml. At the lowest concentration 1mg/ml, there was about 82.9% inhibition. Aqueous extract of *Soyamidafebrifuga* leaves showed a higher inhibitory potential than 70% EESFL.

Table - 2
In vitro α-amylase inhibition activity

Sl. No.	Concentration	70%Ethanolic Extract		Aqueous Extract	
		Mean ± SEM	% inhibition	Mean ± SEM	% inhibition
1	Control	0.9280 ± 0.000894	__	0.9280 ± 0.000894	__
2	1mg/ml	0.2033 ± 0.02402	78.09%	0.1582 ± 0.01879	82.9%
3	2mg/ml	0.1923 ± 0.03470	79.27%	0.1177 ± 0.01705	84.2%
4	3mg/ml	0.1898 ± 0.03938	79.54%	0.1447 ± 0.01823	84.4%
5	4mg/ml	0.1685 ± 0.02373	81.8%	0.1393 ± 0.01585	84.9%
6	5mg/ml	0.1625 ± 0.01874	82.4%	0.1290 ± 0.01485	86.0%
7	6mg/ml	0.1403 ± 0.01642	84.8%	0.1458 ± 0.01983	87.3%

***In vivo* Alloxan induced Antidiabetic activity**

The diabetic control group have shown a change in body weight from a mean ± SEM value of 181.7±5.426g to 157.5±4.787g in initial and final (15 days) days respectively. The Glibenclamide and the extract treated groups have protective action from loosing body weight. A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats. 70% EESFL exhibited a significant anti-hyperglycaemic activity on 5th, 10th and 15th day post treatment in alloxan induced diabetic rats. The mean fasting serum glucose (± SEM) in the diabetic control group of rats was found to be 181.7±5.426, 179.5±6.850, 160.0±6.325and 157.5±4.787on 0, 5th, 10th and 15th day respectively, which was found to be significantly ($p \leq 0.05$) higher when compared with the normal rats.. This reduction in FBG compared to Positive control group indicates the anti-hyperglycaemic activity of reference standard glibenclamide and 70% EESFL. The biochemical parameters like serum triglyceride, serum HDL, serum LDL, serum VLDL are normal in negative control group during treatment period. Same parameters are significantly increased in positive control group ($p \leq 0.05$) compared to negative control group. The serum protein and hepatic glycogen concentration gets reduced in positive control group compared to negative control group. It was further confirmed by pancreatic biopsy observations.

Table-3 :

Effect of 70% EESFL on different biochemical parameters

Groups	Dose (mg/kg)	Body weight	Blood glucose	Total Cholesterol	Triglycerides	HDL	LDL	VLDL	Total protein	Hepatic Glycogen content	Pancreas weight
Control	Vehicle	235.0± 4.83	157.5± 4.78	65.02± 5.39	0.198± 0.017	5.189± 0.10	59.86± 5.47	0.038± 0.003***	10.69± 0.33	0.073± 0.004	0.92± 0.02
Diabetic control	Alloxan (120mg/kg)	157.5± 4.78	235.0± 4.83	82.81± 4.52	0.715± 0.09	6.458± 0.45	69.35± 5.97	0.134± 0.02	12.00± 0.60	0.023± 0.0005	0.51± 0.01
Standard	Alloxan + Glibenclamide (10mg/kg)	208.3± 11.7 ***	208.3± 11.7 ***	46.36± 8.53***	0.369± 0.04***	4.393± 0.49**	46.31± 2.58**	0.068± 0.007**	4.59± 0.18***	0.066± 0.009***	0.79± 0.06**
Lower Dose	Alloxan+ 70%EESFL (250mg/kg)	203.3± 6.14 ***	203.3± 6.14 ***	47.11± 5.00***	0.353± 0.03***	3.581± 0.39***	46.98± 3.45**	0.070± 0.006**	7.34± 0.34***	0.058± 0.006**	0.76± 0.09**
Median Dose	Alloxan + 70%EESFL (334mg/kg)	199.2± 7.79 **	199.2± 7.79 **	51.35± 3.91**	0.298± 0.05***	4.547± 0.23**	46.55± 5.40**	0.045± 0.01***	7.47± 0.43***	0.058± 0.004**	0.73± 0.03*
Higher Dose	Alloxan+ 70%EESFL (500mg/kg)	204.2± 7.68 ***	204.2± 7.68 ***	46.10± 2.60***	0.233± 0.06***	3.931± 0.52***	42.96± 2.40**	0.064± 0.01***	4.21± 0.21***	0.077± 0.006***	0.76± 0.008**

Values are Mean ± S.E.M; n=6* P<0.05, **P < 0.01 and ***P < 0.001 vs Positive control

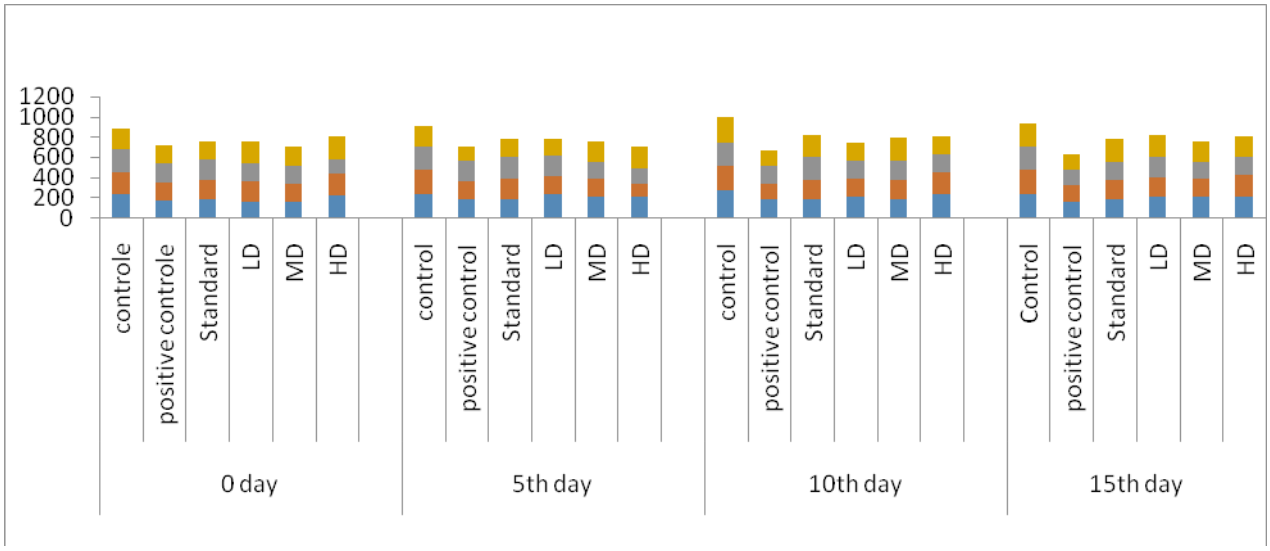

Fig. 2A : Effect of 70% EESFL on body weight in alloxan induced diabetic rats

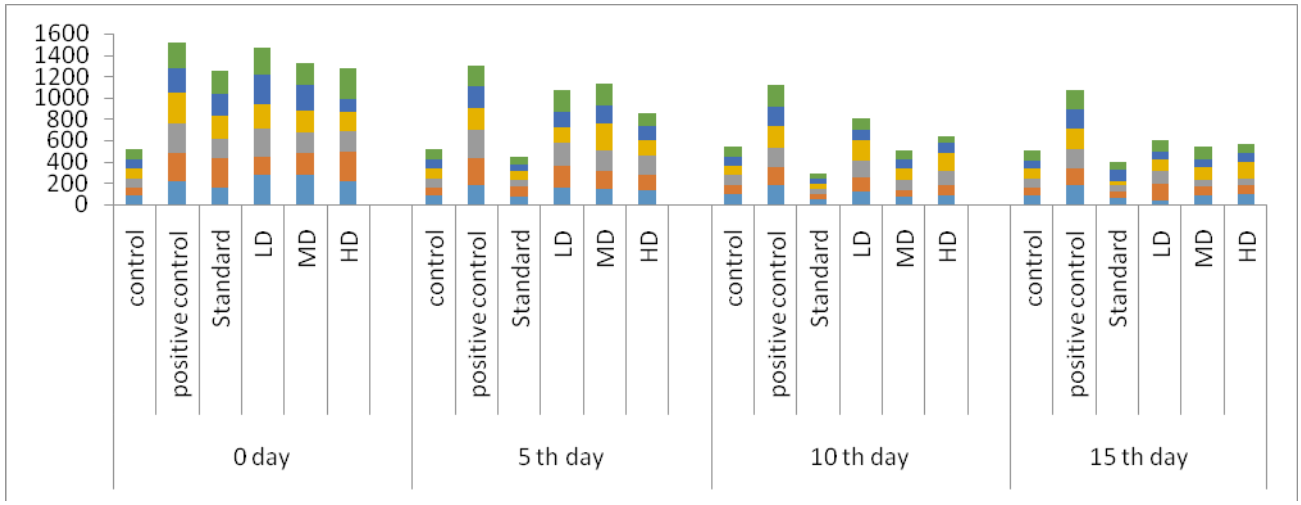

Fig. 2B: Effect of 70% EESFL on blood glucose in alloxan induced diabetic rats

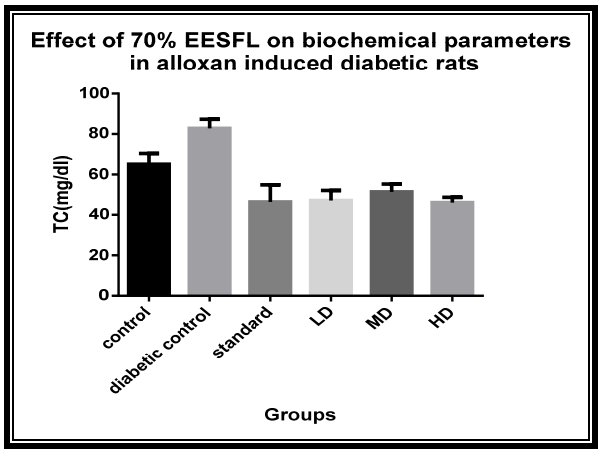

Figure 3A

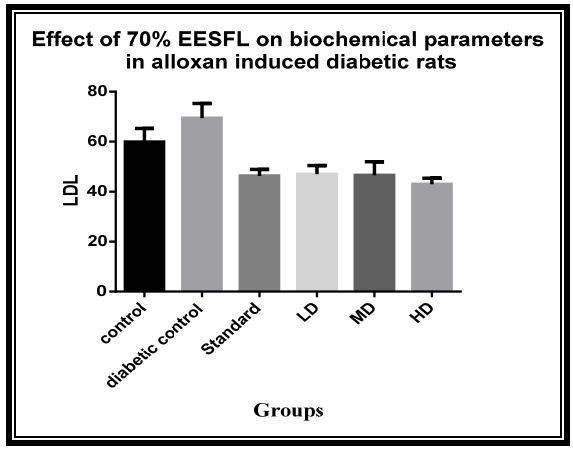

Figure 3D

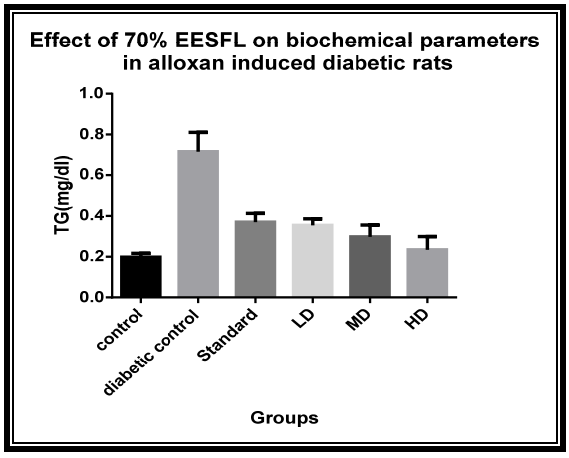

Figure 3B

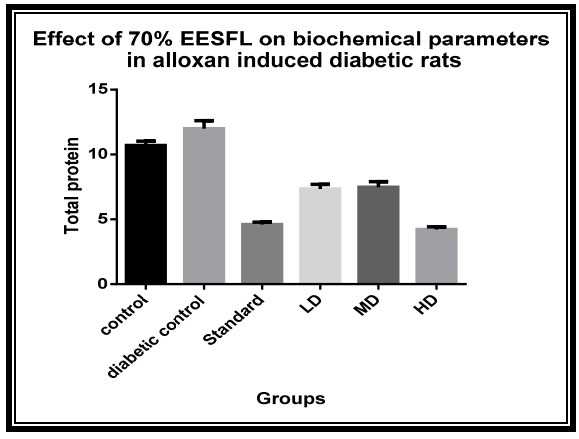

Figure 3E

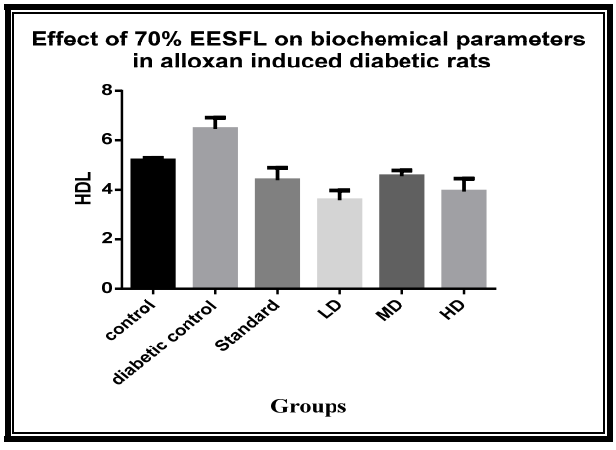

Figure 3C

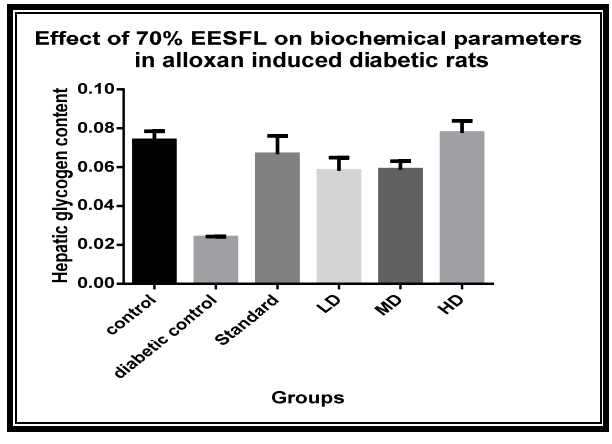

Figure 3F

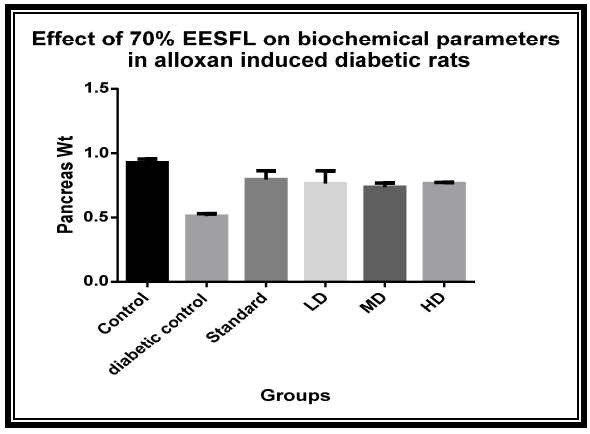

Figure 3G

Figure 3 Shows the Bar graphs of different Biochemical Parameters

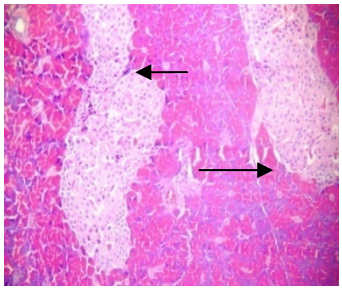

Figure 4A Normal Group

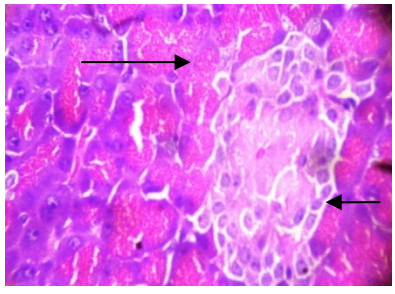

Figure 4B Positive Group

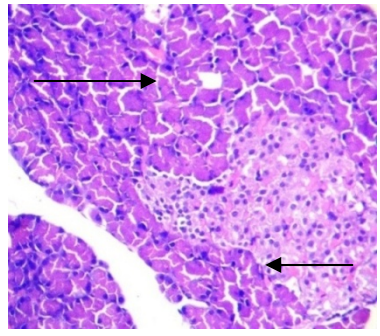

Figure 4C Standard Group

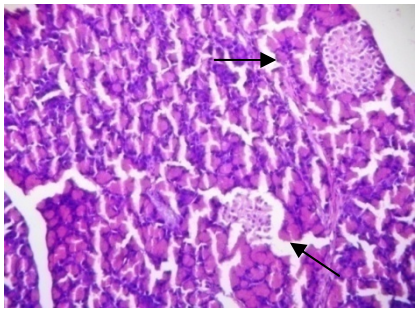

Figure 4D Lower dose

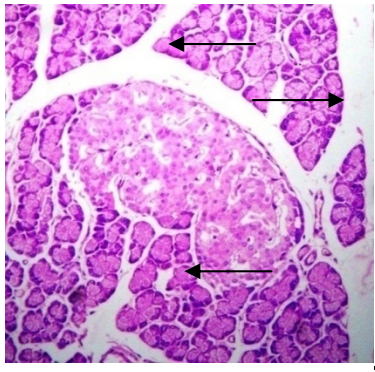

Figure 4E Median dose

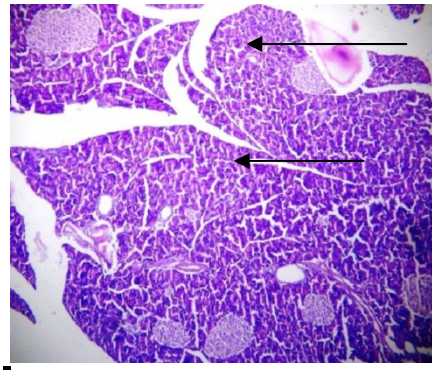

Figure 4F Higher dose

Figure 4 Biopsy report alloxan induced diabetic rats

Discussion

Diabetes mellitus (DM) is possibly the world's fastest growing metabolic disorder. In this study, the pancreatic β cells were destroyed using alloxan (chemical diabetogen), a toxic glucose analogue that accumulates in pancreatic beta cells via GLUT 2 glucose transporters. In the presence of thiols, especially glutathione (GSH), alloxan generates reactive oxygen species (ROS) in cyclic redox reactions. The reduction product of alloxan is dialuric acid. Auto-oxidation of dialuric acid generates ROS, which are responsible for the death of the β cells. Alloxan also inhibits glucose-induced insulin secretion through its ability to inhibit the β cell glucose sensor, glucokinase. Inappropriate activation of NFκB by ROS might start a cascade of events that result in an inflammatory and autoimmune response in pancreas, so the inhibition of NFκB activation by antioxidants could improve the severity of type 1diabetes [19-20].

Flavonoids are known to be bioactive antidiabetic Principles [21]. Flavonoids constitute the largest and the most important group of polyphenolic compounds in plants. They are widely distributed food products of plant origin such as fruit, vegetables, wine, tea and cocoa.

It is now widely accepted that dietary poly phenolics have beneficial effect in protecting the body against chronic diseases, such as cancer, cardiovascular diseases, and diabetes mellitus. Several works have demonstrated that flavonoids may reduce hyperglycaemia and exert protective effect against nonenzymatic glycosylation of proteins in animals [22]. In most diets, carbohydrates are the greatest source of calories. The carbohydrates are ranges from monosaccharide to polysaccharides are breakdown by two major enzymes like 'alpha-amylase' and 'glucosidase' were being absorbed by the body. Alpha-amylase inhibitor indicates that it reduces the rate of absorption of carbohydrates, thereby reducing the GI (Glycemic Index) of foods [23]. Natural α-amylase and α-glucosidase inhibitors from plant sources offer an attractive therapeutic approach to the treatment of post-prandial hyperglycemia by decreasing glucose release from starch and delaying carbohydrate absorption by inhibiting the activity of the carbohydrate hydrolyzing enzymes in the small intestine and may have potential for use in the treatment of diabetes mellitus and obesity. Inhibition of α-amylase, maltase and sucrase by a polyphenolic extract of green tea has been reported[24]. Our present research suggests that the presence of polyphenolic compounds of *Soyamida febrifuga* leaves may have a potentially important role in managing diabetes via the inhibition of α-amylase enzyme activity.

Conclusion

From the results and discussion, it may be concluded that, the plant possesses a significant quantity of phenol, flavonoids, tannin, very good antioxidant properties and also its possess alpha amylase enzyme inhibition as good antidiabetic. Conclusively, the 70% ethanolic extract posses antidiabetic activity against alloxan induced diabetic rats due presence alpha amylase inhibition. However, further research is required to elucidate the specific mode of action.

Acknowledgments

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Anti-Ulcer Activity of Hydroalcoholic Extract of Trichosanthes Cucumerina Fruits in Experimental Rats

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ABSTRACT

The present study was designed to investigate the effect of *Trichosanthes cucumerina* Plant against experimental gastric ulcer. The 50% of hydroalcoholic extract of the fruits of *Trichosanthes Cucumerina* was studied in pylorus ligation & Indomethacin induced Gastric ulcer model. Ranitidine (30mg/kg.) was used as a reference standard for the purpose of comparison.

Fruit extract was tested for acute toxicity studies in albono rats and found to be safe up to the dose of 2000mg/ Kg body weight by orally. Fruit extract in dose of 100,200 and 400mg/kg produce dose dependent inhibition of gastric lesion in indomethacin induce gastric ulcer model. In 4H Pylorus- ligated animals, Fruit extract showed significant reduction in number and severity of ulcers. Also, there was a significant reduction observed in gastric volume, free Acidity, total acidity, when compared with control group. Significant increase in PH by fruit extract was observed. Fruit extract also showed significant decrease in ulcer index in Indomethacin treated rats.

KEY WORDS: antiulcer activity, *Trichosanthes cucumerina,*pylorus ligation,indomethacin.

Introduction

Trichosanthes cucumerina is a highly reputed plant in the Ayurvedic system of medicine which is commonly known as 'Ran Patol', 'Ran Parval', or 'Vahd Karela'.The whole plant is used in liver disease and alimentary canal disorders. It has also been reported to exhibit hepatoprotectic, antibacterial and antifungal activities[1]. *Trichosanthes cucumerina* contains cucurbitacin, which is reported to be responsible for its cytotoxic activity. Fruits of *Trichosanthes cucumerina* is used in ulcer disorders[2]. The *Trichosanthes cucumerna* fruit has been claimed to have antiulcer activity but no detailed scientific investigations have been carried out to define the antiulcer activities. Thus the present investigation sets out to study the antiulcer activity of *Trichosanthes cucumerna* unripe fruit extract. The effect produced by *Trichosanthes cucumerna* were compared with that of ranitidine, a standard drug.

Materials and Methods:

Plant collection:

The unripe fruits of *Trichosanthes cucumerna* required for the study were collected from in and around Mangalore, Dakshina Kannada (dist), Karnataka; in the month of august. The plant material was authenticated by Dr.Noeline J.Pinto, Head of the Dept. of Botany, St. Agnes College, Mangalore.

Extraction:

The unripe fruits of *Trichosanthes curcumina* were cleaned and cut into small pieces were shade dried for about 7 days. The shade dried fruits were then ground to coarse powder using a mechanical grinder. The coarse powder was subjected to cold maceration extraction using 50% hydroalcoholic as a solvent. Cold maceration was done in two parts of the plant powder (100g) for all the solvents, each for 7 days. After the extraction, the macerated powder was filtered using a muslin cloth. The marc obtained was air dried. The filtrate obtained was subjected to steam distillation, to concentrate the extract and the solvent was recollected and was used for further extraction process. A dark green residue was obtained on further concentrating and evaporating the extract on a water bath. The dried extract thus obtained was kept in the desiccator and was used for further phytochemical and pharmacological investigations.

Preliminary Phytochemical screening (Qualitative Analysis)

The ethanol extract of was subjected to preliminary qualitative phytochemical screening, to determine and characterize the different chemical groups present in it. Chemical tests were carried out as per the standard methods [3].

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Animals

Wistar albino rats of both were obtained from Institutional animal house and were maintained under standard laboratory conditions with commercial pellet diet and water ad libitum. Acute toxicity study was performed for the extracts to ascertain safe dose by the acute oral toxicity by OECD guidelines 425[4].

4H Pyloric ligation model [5]

Trichosanthes curcumina (100,200 and 400 mg/kg) was administered for a period of 7 days. On day 7, after the last dose of *Trichosanthes curcumina*, the rats were kept for 24 hours fasting and care was taken to avoid coprophagy. Under light ether anesthesia, the abdomen was opened and pylorus was ligated without causing any damage to its blood vessels. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures. The animals were deprived of water during the postoperative period. Four hours after ligation, stomachs were dissected out and contents were collected into clean tubes. Volume, pH, free acid and total acid content of gastric juice were determined. The contents were centrifuged, filtered and subjected to titration for estimation of free and total acidity. 1ml of centrifuged and filtered gastric secretion was titrated against 0.1N Sodium hydroxide using Topfers reagent as indicator for determination of free acidity and 1% phenolphthalein as indicator for combined acidity. The sum of the two titrations was total acidity. The stomach was opened along the greater curvature and examined for ulcers. The ulcer index was evaluated using the method described earlier [6].

Indomethacin induced Ulcer model

Thirty rats were randomized into five groups of six rats each. Group 1 (normal control) animals received only distilled water. Rats in group 2 treated with standard drug ranitidine. Groups 3,4 and 5 animals were administered respectively with 100,200 % 400 mg/kg b.w. 50% hydroalcholic fruit extract of *Trichosanthes curcumina* for 21 days prior to indomethacin administration. These were orally administered once daily using oral intubator with *ad libitum* provision of food and water throughout the experimental period. Indomethacin was suspended in 1% CMC in water and administered orally in a dose of 200mg/kg to 36 hrs fasted rats. The animals were sacrificed after 6 hrs. After Indomethacin administration, the stomachs were removed and opened along the greater curvature to determine ulcer index of glandular mucosa.

Results

Table - 1

Effect of *Trichosanthes Cucumeria* Friut extract on Pylorus ligation Method

Treatment(mg/kgbw)	Volume of gastric juice(9ml/100gm)	Free acidity	Toatal acidity	pH
Control	2.95±0.038	72.32±3.22	130.8±4.87	1.56±0.16
Standard(Ranitidine)	2.1±0.1479	65.88±1.74	115.9±2.88	2.3±0.10*
100 HAETC	1.5±0.097*	57.98±1.68*	103.12±1.50*	2.62+0.09*
200 HAETC	1.2±0.059*	49.18±1.41*	94.18±1.56*	2.98±0.195*
400 HAETC	0.62±0.08*	39.78±1.31*	83.02±1.91*	3.2±0.148*

All the values are expressed as mean ± SEM (n=6)* P<0.001, Significant compared to control

Table - 2

Effect of *Trichosanthes Cucumeria* Friut extract on Indomethacin-Induced Gastric Ulcer Model

Treatment (mg/kgbw)	Mean Ulcer±SEM	% Protection
Control	43.33±2.11	-
Standard (Ranitidine)	25.0±2.24*	57.69
100 HAETC	23.33±3.34*	53.85
200 HAETC	30.0±3.99*	69.23
400 HAETC	33.33±2.37*	76.92

All the values are expressed as mean ± SEM(n=6) * P<0.001, Significant compared to control

Discussion:

The experiment used in the present study was designed to validate the use of *Trichosanthes Cucumerina* against gastro duodenal ulcers. The 50% Ethanolic extract of *Trichosanthes Cucumerina* (Fruit Extract) was tested in pylorus-ligation & Indomethacin induced gastro duodenal ulcer model.

In the present study, Fruit extract at the dose of 100,200 & 400mg/kg (p.o.) showed significant anti erosion effects as indicated by significant decrease in ulcer index when compared with the control group in Indomethacin-induced gastric ulceration. In the Indomethacin model was also suppressed by ranitidine pretreatment. It has been reported that ranitidine protect the gastric mucosa against variety of agents such as aspirin, indomethacin and ethanol. Reduction in ulcer index by fruit extract (400mg/kg) was found to be more (76.92%) than that observed by more 30mg/kg of Ranitidine (57.69%).

It has been established that *Trichosanthes cucumerina* possesses anti-inflammatory activity and hepatoprotective activity 40 that may offer gastro protection in this model. Further, the antiulcer activity produced by this drug against indomethacin-induced ulceration also confirms its cytoprotection property.

Gastric hyper secretion plays an important role in reducing experimental ulcer by pylorus- ligation. Pylorus ligation has been reported to reduce mucosal blood flow and enhance acid secretion. In the pylorus- ligation model it has been proposed that the digestive effect of accumulated gastric juice and interference with gastric blood circulation are responsible for the induction of ulcers. This procedure also induces a substantial release of histamine, an event that seen to be associated with an increase in free histamine level in the gastric juice. Increased synthesis of nucleic acids and increased metabolism of carbohydrate & therapy exhaustation of carbohydrate andother compensatory mechanism could also be responsible for ulceration due to pylorus- ligation[8].

In the present study, Fruit extract at the dose level of 400mg/kg showed significant reduction in ulcer index & Total acidity. Fruit extract showed increase in pH and this rise in pH by fruit extract was found to be stastically significant.

Ranitidine is known to reduce gastric acid secretion through the blockade of histamine H2 receptor in both man and experimental animals. In the present study,Ranitidine showed significant decrease in ulcer- index & total acidity and significant rise in pH indicating its antigastric effect. The mechanism of their antiulcer activity can be related to the acid neutralizing property, reduction in acid –pepsin secretion & increase in mucin activity.Indomethacin remains one of the most effective treatments for the inflammation and pain associated with joint disease. However, the major limitation to use of indomethacin and the more recently developed NSAIDs continue to be their detrimental effects on the mucosa of the gastrointestinal tract, particularly the stomach. This injury commonly referred to as 'NSAID gastropathy', represent more frequent adverse reaction to medication. Oral administration of Indomethacin causes marked reduction of histologically observed mucosubstance present both within and on the surface of mucus cells in the gastric mucosa of experimental animal[9].

Indomethacin has been reported to cause mucosal damage by affecting PG synthesis,enhancing acid secretion, increasing back diffusion of H^+ ions, decreasing mucin secretion and breaking of mucosal barrier etc. It has long been known to cause gastric ulceration in animals and man, which is attributed to the mucosal prostaglandindeficiency caused by the inhibition of key enzyme cyclo-oxygenase. By inhibiting prostaglandin synthesis it decrease the gastric mucosal blood flow, the mucus content and bicarbonate secretion.

In the present study; Fruit extract (400mg/kg, orally) showed significant decrease in ulcer index in indomethacin-induced ulcer. Fruit extract showed 76.92% protection of stomach mucosa. This indicates that *Trichosanthes cucumerina* has antiulcer activity against indomethacin–induced ulceration. The mechanism of this antiulcer activity could be correlated with that of pylorus ligated model. In this model, *Trichosanthes Cucumerina* might be preventing back diffusion of H+ and thus strengthening gastric mucosal barrier, which is evident from the rise in gastric pH, inhibition of acid secretion and mucoprotective effect.

Thus, the extract of *Trichosanthes cucumerina* plant showed significant antiulcer and cytoprotection effect on various experimentally induced gastric and duodenal ulcer models. These results confirm folk information regarding the use of the plant in ulcer disorders. As gastric ulcer is a multifactorial entity, it is possible that *Trichosanthes Cucumerina* extract exerts antiulcer activity through the involvement of more than one mechanism. Further studies are necessary to elucidate its extract mechanism of action. From the finding of the present study, the mechanisms of antiulcer effect of *Trichosanthes Cucumerina* are increasing the mucosal defensive factor or affording cytoprotection, inhibitory effect on acid and pepsin secretion and decreasing gastric acidity.

Summary:

The present study was designed to investigate the effect of *Trichosanthes Cucumerina* Plant against experimental gastric ulcer. The 50% of Alcoholic extract of the fruits of *Trichosanthes Cucumerina* was studied in pylorus ligation & Indomethacin induced Gastric ulcer model. Ranitidine (30mg/kg.) was used as a reference standard for the purpose of comparison.

Fruit extracted tested in Rats up to the dose of 2000 mg/

Kg., orally did not produce any sign of toxicity. Fruit extract in dose of 100,200 and 400mg/kg produce dose dependent inhibition of gastric lesion in indomethacin induced gastric ulcer model. In 4H Pylorus- ligated animals, fruit extract showed significant reduction in number and severity of ulcers. Also, there was a significant reduction observed in gastric volume, free Acidity, total acidity, when compared with control group. Significant increase in pH by fruit extract was observed. Fruit extract also showed significant decrease in ulcer index in Indomethacin treated rats.

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Utilisation Of *Kalakand* in The Preparation of Chocolate Like Product

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ABSTRACT

Kalakand is a milk based sweet product prepared by continuous heat desiccation of milk along with sugar. In this project work, the *kalakand* was utilised as base material for chocolate like product preparation. The finalized proportions of ingredients on *kalakand* basis were: cream 25%, sugar 25%, skim milk powder (SMP) 20%, cocoa powder 7%, vanilla essence 0.6% and vegetable oil 25%. SMP was used for obtaining milky flavour and vegetable oil for getting desirable body in the final product. The optimized process involved mixing of *kalakand* with SMP and cream, warming, addition of sugar and cocoa powder, heating, addition of refined vegetable oil, heating to semi-solid consistency, cooling, addition of vanilla essence, mixing and and setting at 30oC for 16 – 18 hrs. The set block was cut into pieces and evaluated for quality analysis. The *kalakand* based chocolate thus produced contained on an average 24.6% fat, 17.7% protein and 26.1% carbohydrates including sucrose.

Key words: *Kalakand*, milk chocolate, cream, milk powder, sugar.

Introduction

Chocolates are made in different varieties, namely, *white chocolate, milk chocolate, dark chocolate, sweet dark chocolate, semi sweet chocolate, bittersweet chocolate* and *unsweetened chocolate*; the variation among these depends on the extent of cocoa solids and sugar present in the product, the last variety containing almost 100% cocoa solids. Of these varieties, the one with milky flavour is called the Milk chocolate; it is the mainstay of chocolate industry ever since its invention in 1876 by Daniel Peters of Switzerland. As per FSSAI [1], milk chocolate means the chocolate containing minimum of 10.5% (db) milk solids with a minimum of 2.5% cocoa solids.

Cocoa butter is used as base material for chocolate preparation. Cocoa butter provides smooth body and texture to chocolate and imparts typical melting property to it for which it is famous and most relished. Other ingredients are cocoa powder, chocolate liquor, sugar and milk solids. Other minor ingredients depending on the type of chocolate are dextrose, maltodextrins, whey proteins, emulsifiers and a variety of flavour compounds. Milk powders, dairy cream powders, sweetened condensed milk and milk ingredients like lactose, whey proteins are extensively used in chocolate manufacture [2,3,4]. These provide nutritional and technological benefits like higher proteins, carbohydrates, accelerating conching process etc. [5,6]. In case of milk chocolate, constituents like milk powder and whey powders are important for providing functional properties.

Kalakand is a heat desiccated dairy product prepared by heat-concentration of milk to about 2.5 times, addition of sugar and further desiccation to a semi-solid consistency [7]. Since *kalakand* contains significant amounts of sucrose (25-30%) and fat (15-18%) [8], it *kalakand* could be utilized in the preparation of chocolate like product. In chocolates, fat forms dominant phase in which other ingredients are dispersed; this determines final body and texture of chocolate which is solid as in chocolate bars and liquid as in case of liquid-chocolate products. Chocolate of smooth consistency and semi-solid consistency are popular fillings in several bakery products and desserts. The smooth consistency of chocolates can be obtained by conching of dominant fat medium which melts fast in the mouth. If milk protein is used as continuous medium or matrix, then it is possible to obtain chocolate with slow melting characteristic. Children and youth relish such chocolates with slower melting characteristic. As per our social observation, children and youth are discouraged from eating the chocolates because of dental caries problem, however, chocolate prepared with enhanced protein content can be a solution to such problem. *Kalakand* has milk proteins as well as sugar both of which are suitable as ingredients for chocolate. Hence, in this project, *kalakand* was tried as a base material for chocolate like product manu-

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facture which could be an attractive proposition for small scale entrepreneurs.

Materials and Methods

Materials

Commercially available Cadbury's plain cocoa powder was used to prepare chocolate like product from *kalakand*. Fresh cream was made available from experimental dairy of the Institute. Skim milk powder (medium heat) required for product standardization was procured from the local market. Good quality *kalakand* was collected from Experimental Dairy of NDRI, Bengaluru. Other ingredients, namely, crystalline refined sugar, vanilla essence (Bush Boake Allen), shelled groundnuts and refined vegetable sunflower oil were purchased from local market.

Methods

Preparation of Chocolate like Product from *Kalakand*

The *kalakand* used in the study contained 20-25% moisture, 25-30% sucrose, 15-18% fat, 12-15% proteins, 12-15% of lactose and 2.0 – 2.2% of minerals. *Kalakand* was taken in shallow pan *karahi* and warmed over LPG flame. Then weighed quantities of SMP and cream were added and mixed. At this stage, sugar and cocoa powder were added and heated. The mixture was mixed and scraped thoroughly during heating. Care was taken to avoid burning and charring of the contents. When the contents attained uniform body, the required quantity of vegetable oil was added. The contents were heated and uniformly mixed into a semi-solid consistency. The container was taken off from the flame and cooled. When the chocolate mass cooled to 45-50oC, vanilla essence was added and mixed thoroughly. The molten *kalakand*-chocolate mass was then spread in a tray into a uniformly thick slab and allowed to cool to about 30oC. The tray was then kept at 30oC for 16-18 h for setting purpose. After setting the *kalakand*-chocolate slab was cut into required size pieces for evaluation purposes.

Optimisation of Various Parameters

The effect of the following additives on the sensory quality of chocolate was studied and optimized:

Cream: 15, 20 and 25% (w/w), Sugar: 15, 20 and 25% (w/w), SMP: 15, 20 and 25% (w/w); Cocoa powder: 3, 5 and 7% (w/w); Vanilla essence: 0.2, 0.4 and 0.6% (v/w); refined vegetable oil: 15, 20 and 25% (w/w) all on *kalakand* basis.

When the effect of one additive was to be studied, its content was varied but all other additives were kept constant at one level.

Evaluation of *Kalakand*-chocolate

The organoleptic quality of *kalakand*-chocolate was evaluated by a panel of judges, who were comprised of Institute's staff and PG students who are well exposed to the quality of milk choclate, on a 9.0 point Hedonic scale wherein a score of 1.0 represented dislike extremely and a score of 9.0 represented like extremely [9]. The chocolate samples for evaluation were taken out from the tray and coded appropriately before serving them to the judges for sensory evaluation. Sensory evaluation of the samples was carried out in the sensory evaluation room under appropriate lighting. The following parameters were studied: colour and appearance, flavour, body and texture and overall acceptance. The judges were asked to comment on the after taste, any lingering taste of sweetness, unnaturality etc. The judges were also requested to record the specific observations on the quality of the product other than above parameters.

Analyses

Moisture percentage of *kalakand*-chocolate was determined according to BIS [10] and fat content was analysed by Soxhlet extraction method [11]. For ash analysis, procedure described in Manual of Dairy Chemistry [12] was followed. The per cent protein in chocolate was determined by standard macro Kjeldahl method described in AOAC [13].

Statistical Analysis

The sensory evaluation data were tabulated and analyzed by one way ANOVA by MS Excel software. Significance of treatments was determined by Critical Difference at 5% level as per Snedecor and Cochran [14] and Sundararaj *et al.* [15].

Results and Discussion

Effect of Addition of Cream on the Quality of *Kalakand*-chocolate

Cow milk cream (50% fat) at three levels (15, 20 and 25% on the basis of *kalaknd*) was used in the chocolate formulation in order to improve consistency and flavor of the final product. The effect of addition of cream on the quality of the product was evaluated by sensory evaluation of the 'chocolate' on 9- point Hedonic scale (Table-1). There was no influence of incorporation of cream on the colour and appearance of the chocolate ($P>0.05$), but body and texture and flavour of the product significantly improved by using cream ($P<0.05$). This is indicated by the scores presented in Table-1. The use of cream provided smoothness and plasticity to the product. Since *kalakand* has slight granular texture, it is essential to convert it to smooth texture. This was done by addition of cream as well as employing thorough mixing. Milk fat has a plastic character [16], hence improved the texture of the chocolate. It also imparted pleasant taste to the chocolate because the milk fat is known for its relishing and desirable taste [17]. Milk fat is also known to contribute pleasing flavor to many products [18]. Though cocoa flavor is the major contributor of flavor in chocolate, the milk fat, in combination with cocoa powder, further enhanced the chocolate flavor. This is the reason why many consumers relish milk chocolate more than dark chocolate. Thus in the

study, cream at 25% level of addition scored highest and was recommended.

Effect of Addition of Sugar on the Quality of *Kalakand*-chocolate

Kalakand used in the study already contained 25-30% sugar . Since this level was not enough for the making of chocolate like product, extra sugar was used and in order to optimize the sugar addition, three levels were tried viz. 15, 20 and 25%. The use of extra sugar influenced the sensory characteristics of the *kalakand*-chocolate. Use of the sugar enhanced the textural quality as can be found by enhanced body and textural scores (Table-2). Optimium flavour and thereby overall quality of the chocolate was found at 25% sugar level. Though there was no significant difference between 15 and 20% sugar levels, use of 25% sugar significantly enhanced the overall acceptance of the chocolate. Normally in white products, colour difference because of sugar content could be expected because of sugar caramelisation during heat treatment. But in chocolate, the colour difference because of sugar level was masked by the colour of cocoa powder. Hence there was no statistically significant effect of sugar level on the colour and appearance of the chocolate. By addition of extra sugar the body and texture of the chocolate improved because it is the sugar along with fat that contributes to the body and texture of chocolate. Therefore, in the present study use of 25% sugar was optimum for getting a good body and texture in the chocolate like product. As sugar had significant effect on body and texutre and flavor of the chocolate, use of 25% sugar has been recommended.

Effect of Addition of Skim Milk Powder (SMP) on the Qua lity of Chocolate

Milk powder is commonly used in milk chocolate. It provides typical milky flavor as well as consistency to the chocolate. In the present project, though milk sweet is used as base material, SMP was additionally added in order to derive the benefits of using SMP. Three levels of SMP were used viz. 15, 20 and 25% on *kalakand* basis (Table-3). The addition of SMP slightly whitened the colour of the chocolate, but the judges felt that the acceptance of appearance remained the same meaning that they did not mind little whitening of the chocolate by addition of SMP. However, its use might have sorbed moisture and enhanced the consistency of the chocolate. Proteins in SMP and lactose are known to possess good water sorbing properties [19]. As discussed earlier, milk solids contribute to the flavour of chocolate because of presence of casein and lactose. Use of SMP enhanced the body and texture, flavour and overall quality of the chocolate. Hence, in the present study improvement in flavor was observed by using SMP at 20% level. Overall, an SMP level of 20% yielded a good quality chocolate from *kalakand*.

Effect of Addition of Cocoa Powder on the Quality of *Kalakand*-chocolate

Cocoa powder was used at three levels 3, 5 and 7% in order to arrive at an optimum level for *kalakand* based chocolate. The effect of these levels on the sensory quality of the chocolate was studied. The colour and appearance scores as influenced by the cocoa powder levels are presented in Table - 4. Addition of cocoa powder imparted dark brown colour to the product which was statistically significant ($P<0.05$). Though cocoa powder did not contribute to body and texture, it enhanced flavour aspect because of pleasant cocoa taste. The cocoa powder level of 7% yielded chocolate like product of highest flavor acceptance. Addition of cocoa powder not only imparts flavor but also colour to the product, because during production of cocoa powder the beans undergo fermentation resulting in brown coloured compounds [20]. These have the property of imparting colour to foods to which they are added. Hence, higher the cocoa powder greater the colour intensity. In the present study, cocoa powder at 7% yielded optimum intensity of colour. The body and texture scores were not statistically significantly different which showed that cocoa powder had no effect on the body and texture of the chocolate. This was expected because chocolate was added at 3-7 % which is not large enough to cause any body and textural change. Moreover cocoa solids are not known for any water binding characteristic. Cocoa powder has a pleasing cocoa taste which get enhanced by the presence of sugar and milk solids. In *kalakand* based product, sufficient amounts of milk solids and sugar are present. These along with cocoa produced a good quality chocolate like product. As a result, 7% cocoa level yielded a good quality chocolate better than the 3 and 5% levels. Hence, 7% cocoa powder addition was recommended.

Effect of Addition of Vanilla on the Quality of *Kalakand*-chocolate

Vanilla forms a good background flavor for the chocolate taste. It means that its use enhances the chocolate flavor. Therefore vanilla was tried in the preset study to enhance the flavour acceptance of the chocolate. Vanilla flavor was added at three levels namely 0.2, 0.4 and 0.6 %. There was no effect of vanilla essence level on the colour and appearance and body and texture scores as shown in Table-5. The flavour acceptability of the chocolate improved by using different vanilla essence levels. However, there was no difference between 0.2 and 0.4% levels, but 0.6& level significantly enhanced the flavour of the chocolate ($P<0.05$). For the same reason, overall acceptance also increased with enhanced vanilla levels.

Vanilla essence was added in small quantities, so it had no influence on the colour and appearance and body and texture of chocolate. However, flavour attribute improved by using vanilla essence because it enhanced synergistically the cocoa flavour. In several formulations, vanilla flavor is used in combination with chocolate flavour.

Hence, overall acceptance also improved by using the vanilla flavour.

Effect of Addition of Vegetable Oil on the Quality of *Kalakand*-chocolate

The chocolate like product to be prepared in the project was envisaged to have a soft and chewy texture and not crispy body. Hence, it was thought that use of vegetable oil would impart more softness and smoothness to the chocolate. In order to study the effect of vegetable oil, three levels were used viz. 15, 20 and 25%. Addition of vegetable oil did not in any way influence the colour and appearance acceptance score. However, the body and texture attribute was significantly influenced by addition of the oil as indicated by the scores which were 7.3, 7.4 and 7.8 respectively for 15, 20 and 25% oil levels (Table-6). There was not much difference between 15 and 20% levels but there was a significant difference between 25% oil level and other levels. This indicates that addition of the oil helped in enhancing the body and texture attribute of the chocolate like product, but there was no effect on flavour attribute. Use of 25% oil effected a significant improvement in the overall acceptance of the *kalakand* based chocolate. During milk chocolate preparation, the ingredients are thoroughly mixed and distributed in the dominant phase i.e. fat. In case of *kalakand* based chocolate, the dominant phases are fat and protein. So the moisture, minerals and flavour lay uniformly distributed in the matrix. So the addition of oil did not much influence the colour and appearance of the chocolate. However, the proteins and fat might have got mixed thoroughly during the stirring process to obtain a smooth consistency of the chocolate. That is the reason why when vegetable oil was added, the smoothness of the chocolate increased. As refined vegetable oil was used in the study, it did not have much influence on the flavour component of the chocolate. Moreover, chocolate flavour was more dominating than the oil flavor. Hence, there was no significant effect of oil on the flavour of the chocolate. However, because of considerable effect on the body and textural attribute, the addition of vegetable oil improved the overall acceptance of the *kalakand* based chocolate.

Optimised Process of Preparation of *Kalakand*-chocolate

Based on the studies conducted on optimization of various parameters, a method of preparation of *kalakand*-chocolate is recommended. It is shown in Fig.1.

Physico-chemical Characteristics of *Kalakand*-chocolate

The appearance of *kalakand* based chocolate is shown in Fig.2. The composition of the product is given in Table-7. It may be seen that the 'chocolate' is rich in protein content.

Conclusion

A process has been standardized for the preparation of *kalakand* based chocolate which is rich in fat, protein and carbohydrate contents. Since the study showed that *kalakand* could be utilized for manufacture of chocolate, other dairy products rich in proteins and fat contents may also be utilized to manufacture newer varieties of chocolate.

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Kalakand (100g)

⇩

Mixing into uniform consistency and warming

⇩

Addition of SMP (20g) & Cream (25g)

⇩

Warming and mixing thoroughly

⇩

Addition of sugar (25g) & cocoa powder (7g)

⇩

Heating and scraping thoroughly

⇩

Addition of refined vegetable oil (25g)

⇩

Heating to a semi-solid consistency and

⇩

Cooling to about 45-50oC

⇩

Addition of vanilla flavor (0.6ml)

⇩

Setting in a tray at 30oC for 16-18h

⇩

Cutting and packing

⇩

Kalakand-chocolate

⇩

(Chocolate like product from *kalakand*)

Fig. 1: Flow diagram of manufacture of *kalakand*-chocolate

Fig.2: *Kalakand* based milk chocolates

Table-1

Effect of addition of cream on sensory scores of *kalakand* based chocolate like product

Sensory attribute	Level of addition of cream		
	15%	20%	25%
Colour and appearance	7.8[a]	7.3[a]	7.5[a]
Body and texture	7.1[a]	7.3[a]	7.6[b]
Flavour	7.1[a]	7.3[a]	7.7[b]
Overall acceptance	7.3[a]	7.3[a]	7.7[b]

Note: Mean values with similar superscripts in a row are not significantly different (P>0.05)

Table-2

Effect of addition of sugar on sensory scores of *kalakand* based chocolate like product

Sensory attribute	Level of addition of sugar		
	15%	20%	25%
Colour and appearance	7.3[a]	7.5[a]	7.6[a]
Body and texture	7.5[a]	7.7[ab]	7.9[b]
Flavour	7.2[a]	7.5[b]	8.0[c]
Overall acceptance	7.3[a]	7.5[a]	8.0[b]

Note: Mean values with similar superscripts in a row are not significantly different (P>0.05)

Table-3

Effect of addition of SMP on sensory scores of *kalakand* based chocolate like product

Sensory attribute	Level of addition of SMP		
	15%	20%	25%
Colour and appearance	7.3[a]	7.4[a]	7.4[a]
Body and texture	7.2[a]	7.3[a]	7.6[b]
Flavour	7.2[a]	7.5[b]	7.6[b]
Overall acceptance	7.2[a]	7.5[b]	7.6[b]

Note: Mean values with similar superscripts in a row are not significantly different (P>0.05)

Table-4

Effect of addition of cocoa powder on sensory scores of *kalakand* based chocolate like product

Sensory attribute	Level of addition of cocoa powder		
	3%	5%	7%
Colour and appearance	7.3[a]	7.7[a]	7.9[b]
Body and texture	7.3[a]	7.4[a]	7.6[a]
Flavour	7.3[a]	7.6[b]	7.9[c]
Overall acceptance	7.3[a]	7.5[a]	7.8[b]

Note: Mean values with similar superscripts in a row are not significantly different (P>0.05)

Table-5

Effect of addition of vanilla essence on sensory scores of *kalakand* based chocolate like product

Sensory attribute	Level of addition of vanilla essence		
	0.2%	0.4%	0.6%
Colour and appearance	7.8[a]	7.8[a]	7.8[a]
Body and texture	7.5[a]	7.5[a]	7.8[a]
Flavour	7.3[a]	7.6[b]	7.9[c]
Overall acceptance	7.4[a]	7.5[a]	7.8[b]

Note: Mean values with similar superscripts in a row are not significantly different (P>0.05)

Table-6

Effect of addition of vegetable oil on sensory scores of *kalakand* based chocolate like product

Sensory attribute	Level of addition of vegetable oil		
	15%	20%	25%
Colour and appearance	7.3[a]	7.5[a]	7.6[a]
Body and texture	7.3[a]	7.4[a]	7.8[b]
Flavour	7.5[a]	7.5[a]	7.5[a]
Overall acceptance	7.3[a]	7.5[ab]	7.8[b]

Note: Mean values with similar superscripts in a row are not significantly different (P>0.05)

Table- 7

Physico-chemical characteristics of *kalakand* based chocolate like product

S.No.	Constituent	Percentage
1	Moisture	28.9
2	Fat	24.8
3	Protein	17.7
4	Ash	2.5
5	Carbohydrates	26.1

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