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## RESEARCH ARTICLE

### ANTIDIABETIC ACTIVITY OF SINGLE BLEND NUTRACEUTICAL INCORPORATED COOKIES ON ALLOXAN INDUCED DIABETIC RATS

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#### ABSTRACT

The present study on antidiabetic activity of single blend nutraceutical incorporated cookies on alloxan induced diabetic rats was undertaken to develop low-glycemic index cookies using functional ingredient like flaxseed and as well as to assess its functional efficacy in terms of hypoglycaemic, hypocholesterolemic effect. The cookies were developed at different level of incorporation of flaxseed flour (20%, 30% and 40%) and were subjected to acceptability trials. The cookies incorporated with 30% flaxseed flour was highly acceptable in terms of all the sensory attributes. The GI and anti diabetic efficacy of the cookies was estimated through animal experimentation. The GI of the developed cookies was 30 indicating that the cookies were low glycemic. Impact of supplementation of the cookies to alloxan induced diabetic rats revealed that there was a significant decrease ( $p<0.001$ ) in blood glucose level in the experimental group fed with flax seed cookies as compared to diabetic control group after 15 days of intervention. The overall decrease in mean blood glucose level in experimental animals fed with flaxseed cookies was 93 mg/dl at the end of 15 days intervention period. Alloxan mediated body weight reduction was also reversed due to flaxseed cookies supplementation as well as the total protein level increased from 5.5g/dl in diabetic rats to 6.45 g/dl in experimental group fed with flaxseed cookies suggesting the regeneration process. The total cholesterol level reduced significantly from 201mg/dl in diabetic rats to 104mg/dl in experimental group fed with flaxseed cookies and similarly SGPT and SGOT reduced from 102u/l and 110u/l in diabetic rats to 51u/l and 59u/l respectively in experimental group fed with flaxseed cookies at the end of the intervention period.

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## INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. India is presently home to 62 million diabetic people and by 2030, India's diabetes numbers are expected to cross the 100 million (Wild *et al.*, 2000). As the rate of diabetic population is increasing alarmingly, one of the best approach to prevent diabetes is modification of food by increasing the availability of more therapeutic and functional foods with anti-diabetic activity. Functional foods like flaxseed has been part of the human diet for thousands of years, and more recently it has been used as a

source of nutraceutical. Its benefits to health are generally attributed to high concentrations of linolenic acids (omega-3) as well as significant quantities of dietary fibre like lignin. Flaxseed contains approximately 28 per cent fibre, of which one third is soluble and has proved to reduce cholesterol as well as regulate blood sugar. The remaining two thirds of insoluble dietary fibre can increase faecal mass, reducing transit time in the lumen, preventing constipation (Hussain *et al.*, 2006). Hence the present study on antidiabetic activity of single blend nutraceutical incorporated cookies on alloxan induced diabetic rats was undertaken with the following objectives:

1. To develop low-glycemic index cookies using functional ingredient like flaxseed.
2. To assess the functional efficacy of flaxseed cookies in terms of hypoglycaemic and hypocholesterolemic effect.

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**Table 1. Sensory scores of developed flaxseed cookies**

Formulations	Quality attributes					
	Colour	Appearance	Taste	Flavour	Crispness	Overall acceptability
FC1 (20%)	7.50	7.10	8.00	8.30	7.90	8.00
FC2 (30%)	7.73	7.66	8.50	8.63	7.93	8.16
FC3 (40%)	7.23	7.30	7.20	7.43	7.23	7.36

**Table 2. Glycemic Index of developed Flaxseed cookies**

Groups	iAUC mg.min/100ml	Glycemic index
Reference group( glucose)	3757	
Experimental group(30% flaxseed cookies )	1132	30

**Table 3. Effect of supplementation of developed Flaxseed cookies on blood glucose of alloxan induced diabetic rats**

Animal groups	Blood glucose after induction of diabetes(mg/dl)	Effect of supplementation on blood glucose after induction of diabetes(mg/dl)		
		0 day	5 days	10 days
Diabetic control	375 ±7.93	380 ±4.25	378 ±4.90	382± 3.70
Experimental Group	352 ±8.37	300 ±6.69	280 ±7.33	259 ±6.69

**Table 4. Effect of supplementation of developed Flaxseed cookies on SGPT, SGOT, Total protein and Cholesterol level of alloxan induced diabetic rats**

Animal Groups	SGPT	SGOT	Total protein	Cholesterol
Diabetic control	102u/l	110u/l	5.50g/dl	201mg/dl
Experimental Group	51u/l	59u/l	6.45g/dl	104mg/dl

## MATERIALS AND METHODS

**Formulation of cookies:** Three cookies namely FC1, FC2 and FC3 were developed from flour mix which was prepared by substituting wheat flour with flaxseed flour at 20%, 30% and 40% level of incorporation respectively. The cookies were prepared following the guidelines outlined by AACC (Anon., 1990) and were subjected to sensory evaluation using 9-point hedonic scale

**Induction of Diabetes:** Diabetes was induced in experimental animals by a single intraperitoneal administration of alloxan (140 mg/kg) with 4% saline solution (Florían *et al.*, 2013). Before administering alloxan, baseline glycemia was tested. After 48 hours blood samples by puncture of the tail vein was taken and blood glucose level was checked and the rats showed levels above 300 mg/dL were considered diabetic.

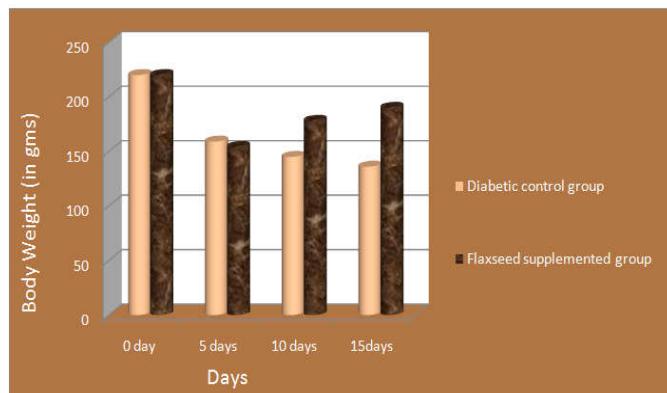
**Determination of glycemia and body weight:** Blood samples were taken from tail tipping of the animals at 0, 5<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> day. Blood glucose was determined by using glucose tester Device Accu check Roche and body weight of alloxan induced rats were also monitored at regular interval of 0, 5<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> days.

**Biochemical analysis:** Blood was collected from retro orbital plexus of rat eyes under mild ether anaesthesia at the end of 15 days of intervention, during the course of study for biochemical analyses, blood sera was separated by centrifugation at 3000 rpm for 10 minutes and stored at 4 - 8° C until use. SGPT, SGOT, total protein and cholesterol was analyzed using commercially available kits.

**Statistical analysis:** Mean and standard deviation was done for tabulation

## RESULTS AND DISCUSSION

**1. Sensory evaluation of developed flaxseed cookies:** Acceptability trials were conducted by a semi-trained panel consisting of 15 numbers of judges. Scoring was done on nine point hedonic scale. For Sensory evaluation of flaxseed cookies three formulations were developed namely FC1, FC2 and



**Fig 1. Effect of supplementation of developed Flaxseed cookies on body weight of alloxan induced diabetic rats**

**Glucose tolerance test:** Glycemic Index (GI) for developed cookies was determined through animal experiment by calculation of Incremental Area Under two hours of blood glucose response or Curve (IAUC) for the cookies and compared with the IAUC for glucose solution standard according to the method of Wolever *et al.* (1991)

FC3.Table1 revealed that out of the three different level of incorporation of flaxseed cookies, FC2 (30% incorporated cookies) had highest mean scores in terms of flavour, colour, taste appearance and overall acceptability. Therefore based on the acceptability trials30% incorporated cookies was selected for further animal experimentation.

### **Estimation of glycemic index**

The Glycemic Index (GI) is a scale that ranks carbohydrate-rich foods by how much they raise blood glucose levels compared to a standard food. The standard food is glucose or white bread. The foods with high GI are those which are rapidly digested and absorbed as a result increase blood sugar levels. Low GI foods by virtue of their slow digestion and absorption produce gradual rise in blood sugar and insulin level. The GI is a way of ranking carbohydrate-containing foods (from 0-100) based on whether they raise blood sugar levels highly, moderately or at lower rates. Carbohydrate containing foods that are digested quickly will result in a high blood glucose level and have a high glycaemic index (high GI foods). Foods with a GI 70 and above are classified as high GI foods. Carbohydrate foods that are digested more slowly raise blood glucose levels more slowly, and so have a lower glycaemic index (low GI foods). Foods with a GI 55 and below are low GI foods (Foster-Powell, 2002). Table 2 revealed that the GI of Flaxseed cookies was 30, this low GI of Flaxseed cookies can be attributed to high soluble fibre in flaxseed flour. The soluble dietary fiber component has been reported to decrease the activity of digestive enzymes, thus resulting in incomplete hydrolysis of carbohydrates and decreases the rate of starch degradation and digestion which is related to the regulation of postprandial glucose levels. (Brennan and Samyue, 2004; Wursch and Pi-Sunyer, 1997).

### **Effect of supplementation of developed Flaxseed cookies on blood glucose level**

The diabetic induced animals were characterized with symptoms of IDDM i.e. persistent hyperglycaemia, excessive fasting, polydypsia and severe loss of body weight. These results are in agreement with previous studies by Robert (2001) and Oliveira *et al.* (2013). After development of diabetes the animals are grouped and supplemented with developed cookies. After 15 days of supplementation of flaxseed cookies marked decrease in blood glucose level was observed as compared to diabetic control. The effect of oral supplementation of Flaxseed cookies on blood glucose level of alloxan induced diabetic rats are presented in Table 3. A significant decrease in blood glucose level was observed between different days of intervention from initial level of  $352 \pm 8.37$  mg/dl to  $259 \pm 6.69$  mg/dl at the end of 15 days. Various studies reported that lignin in flaxseed decrease ROS generation which downregulates TNF-a and IL-6 (inflammatory genes) production which decrease inflammation and blood glucose level. The active ingredient of flaxseed [lignin, secoisolaricresinol diglucoside (SDG)] has significant antioxidant effects by inhibiting DNA scissions and lipid peroxidation and decreasing ROS and thereby reducing ROS mediated disorder like diabetes and inflammation (Lee *et al.*, 2009)

### **Effect of supplementation of developed Flaxseed cookies body weight of alloxan induced diabetic rats**

In the present study, Alloxan mediated body weight loss was also reduced by flaxseed cookies supplementation, the increase in body weight in diabetic untreated rats was less than that in the flaxseed cookies fed animals. (Fig. 1) In control group initial body weight was 220 gms which reduced to 136 gms at the end of 15 days of intervention whereas in experimental group fed with flaxseed cookies the initial body weight was 220gms which reduced to 190 gms at the end of 15 days.

### **Estimation of biochemical parameters**

From Table 4 it was revealed that the total protein level increased from 5.5g/dl in diabetic rats to 6.45 g/dl in experimental group fed with flaxseed cookies suggesting the regeneration process. The total cholesterol level reduced significantly from 201mg/dl in diabetic rats to 104mg/dl in experimental group fed with flaxseed cookies. In the present study, the levels of SGPT and SGOT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan SGPT and SGOT were elevated in diabetic control rats to 102u/l and 110u/l which were brought back to normal levels after flaxseed cookies supplementation to 51u/l and 59u/l at the end of the intervention period, SGPT and SGOT levels act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver.

### **Conclusion**

It is evident from the present study that cookies can be made with substitution of wheat flour with functional ingredient like flaxseed flour without affecting the sensory attributes in terms of colour, taste, flavour, crispiness and over all acceptability. The developed flaxseed cookies had low glycemic index with enhanced hypoglycemic and hypocholesterolemic effect providing potential health benefits from degenerative diseases like diabetes mellitus. The potential utilization of functional ingredients like flaxseed in developing new novel value added products with enhanced therapeutic properties will not only improve the overall health of the population but also help in the prevention of the onset of degenerative disease as well as in the management of metabolic disorder like diabetes and obesity

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