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Dietary effect of water soluble gummy fibre and water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds on blood glucose, glucose tolerance and some intestinal enzymes in normal and diabetic male albino rats

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Abstract

In the present study, the effect of feeding of diets containing 12% and 18% water soluble gummy fibre and 5% and 10% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds were carried out in normal and alloxan treated diabetic rats for 21 days. The results obtained from present study indicates that incorporation of 12% and 18% water soluble gummy fibre significantly reduces blood glucose, notably improved glucose tolerance and decreased activity of intestinal enzymes *i.e.* amylase and invertase while 5% and 10 % water insoluble neutral detergent fibre (NDF) did not exhibit any hypoglycaemic effect in both normal and treated diabetic rats.

Keywords:- *Syzygium cumini seed fibre, Neutral detergent fibre (NDF), Blood glucose, Glucose tolerance, Intestinal enzymes*

Introduction

Hypoglycaemic effect of *Syzygium cumini* seeds are mainly due to water soluble gummy fibre has been studied earlier (Pandey and Khan, 2002). In earlier study, feeding of diet containing 6% water soluble gummy fibre isolated from *Syzygium cumini* seeds by solvent fractionation method for 21days significantly reduces the blood glucose levels, notably improve the oral glucose tolerance (Pandey and Khan, 2002) and decreases the activity of intestinal enzymes *i.e.* amylase and invertase in both normal and diabetic treated rats whereas feeding of diets containing 2.25 % water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds did not show any hypoglycaemic effect (Pandey and Khan, 2002). Keeping in view the hypoglycaemic effect of 6% water soluble gummy fibre, the present study was conducted to investigate whether the increase in the amount of fibre *i.e.* 12% and 18% water soluble gummy fibre and 5% and 10% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds had same or any enhancing hypoglycaemic effect in both normal and alloxan treated diabetic rats for 21 days.

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Materials and Method

Chemicals

Casein (Vitamin free), soluble starch, glucose oxidase, peroxidase, O-dianisidine were purchased from Sigma Chemicals, St. Louis, Co, USA. Benzoic acid, Sodium hydroxide, Zinc Sulphate, Sodium dihydrogen phosphate and D-glucose were procured from Qualigen's fine chemicals, Co. Bombay. Rest of the chemicals used were of analytical grade.

Animal and diet

The male albino rats of Wistar strain weighing between 150-170 gm were divided into five groups each of normal (designated as Group I N, Group II N, Group III N, Group IV N, Group V N and diabetic

(designated as Group I D, Group II D, Group III D Group IV D and Group V D) rats. The animals were made diabetic by the method of Lazaro and Pallay (Pandey and Khan, 2002). Group I from normal (*i.e.*, Group I N) and diabetic rats (*i.e.*, Group I D) served as control and were fed the control diets for 21 days whereas remaining groups were fed the experimental diets containing different amounts of water soluble gummy fibres and water insoluble detergent fibre (NDF) isolated from *Syzygium cumini* seeds as mentioned below:

Groups Diets

Group II N and Group II D diets containing 12% water soluble gummy fibre. Group III N and Group III D diets containing 18% water soluble gummy fibre. Group IV N and Group IV D diets containing 5% water insoluble neutral detergent fibre. Group V N and Group V D diets containing 10% water insoluble neutral detergent fibre. The numbers of animals in each group are mentioned in the table in parenthesis. The 20.00 gm of all the diets were fed daily for 21 days. The composition of control and experimental diets are given in (Table 1). All the animals were allowed free access to deionized distilled water. Body weights were examined at weekly intervals before feeding till the termination of the experiment. At the end of 21 days, the rats of all the groups were fasted for 18 hours. After fasting, half of the animals from each group were sacrificed by decapitation, small intestine were removed and used for the determination of activity of intestinal enzymes, amylase and invertase (Williams and James, 1979). After fasting for 18 hours followed by feeding of 2400 mg and 3600 mg which corresponds to 12% and 18% of water soluble gummy fibre and 1000 mg and 2000 mg which corresponds to water insoluble neutral detergent fibre (NDF) to the animals of respective groups, blood samples were collected from the tail vein of the remaining animals and blood glucose was estimated by glucose oxidase method by (Pandey and Khan, 2002). In order to determine the effect of feeding of diets containing 12% and 18% water soluble gummy fibre and 5% and 10% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seeds on oral glucose tolerance of the rats, the same pattern of grouping of animals, feeding of diets to the respective group and fasting was followed as described for above parameters.

The glucose tolerance was performed by administrating glucose orally (10 mg/Kg body

Table 1: Composition of controled and experimental diet

Ingradients	Control diet	Experimental diets containing water soluble gummy fibre		Experimental diet containing water insoluble neutral detergent fibre	
		12%	18%	5%	10%
Casein	20.00	20.00	20.00	20.00	20.00
Water soluble gummy fibre isolated from *Syzygium cumini* seeds	-	12.00	18.00	-	-
Water insoluble neutral detergent fibre (NDF)isolated from *Syzygium cumini* seeds	-	-	-	5.00	10.00
Hydrogenated oil	10.00	10.00	10.00	10.00	10.00
Starch	65.00	53.00	47.00	60.00	55.00
Hawk's Oser salt mixute*	4.00	4.00	4.00	4.00	4.00
Vitamin mixture*	1.00	1.00	1.00	1.00	1.00

Each diet contains (%w/w) salt mixture *4 g ; Vitamin mixture 1g.

Table 2: Effect of feeding of diets containing water soluble gummy fibre and water insoluble neutral detergent fiber isolated from *Syzygium cumini* seeds for 21 days on blood glucose and glucose tolerance in normal and alloxan treated diabetic male albino rats

Group	Blood glucose (mg/100 ml)	Glucose tolerance test				
		0 hr	1/2 hr	1 hr	11/2 hr	2 hr
Normal						
Group I N (6) Control diet fed	97.56±8.09	96.11±7.79	138.00±9.61	163.00±10.00	177.00±8.99	95.25±7.01
Group II N (6) diets containing 12% water soluble gummy fibre fed	72.43**±5.98	71.00**±6.00	92.00**±7.43	94.00**±7.67	84.00**±6.59	65.00**±6.03
Group II N (6) diets containing 18% water soluble gummy fibre fed	67.25***±5.75	72.06***±5.95	85.13***±6.60	91.00***±6.86	80.00***±6.00	63.00***±5.81
Group IV N (6) diets containing 5% waer insoluble neutral detergentfibre fed	62.33^{NS}±5.53	62.13^{NS}±5.49	82.00^{NS}±6.64	84.00^{NS}±6.86	70.00^{NS}±4.84	60.36^{NS}±4.07
Group V N (6) diets containing 10% water insoluble neutral detergent fibre fed	96.22^{NS}±7.94	91.05^{NS}±7.06	132.00^{NS}±8.60	158.00^{NS}±9.14	112.31^{NS}±7.02	89.00^{NS}±6.91
Diabetic						
Group I D (6) Control diet fed	285.10±14.63	281.03±15.07	357.00±18.25	442.19±21.20	316.16±16.40	309.00± 16.11
Group II D (6) diets containing 12% water soluble gummy fiber fed	132.00**±8.04	130.00**±7.33	243.00**±9.80	256.00**±14.00	165.00**±9.92	139.00**±9.90
Group III D (6) diets contiaining 18% water soluble gummy fiber fed	125.00***±7.00	126.00***±6.92	230.00***±9.00	240.00 ***±12.52	163.00***±9.65	132.00**±7.61
Group IV D (6) diets contiaining 5% water insoluble neutral detergent fiber fed	280.25**±15.16	277.00^{NS}±12.76	356.00^{NS}±17.49	438.00^{NS}±19.91	313.00^{NS}±16.00	305.00^{NS}±15.89
Group V D (6) diets containing 10% water insoluble neutral detergent fibre fed	280.00^{NS}±15.01	277.05^{NS}±12.80	355.00^{NS}±17.00	438.00^{NS}±19.90	308.00^{NS}±15.95	298.00^{NS}±15.04

The results presented in table are means ± SD of 6 rats each .Number of animals used are given in parenthesis .The data were analyzed statistically by student t-test. The blood glucose levels of normal and diabetic treated rats at 0, 1/2, 1, 1 ½, 2 hrs. compared with corresponding glucose levels at 0, 1/2, 1, 1 ½ ,and 2 hrs of normal and diabetic control rats after glucose loading for statistical analysis. p< 0.01 . ***p< 0.001. NS Not Significant**

weight) to the animals of all groups and blood samples were collected for glucose estimation at 0, ½, 1, 1 ½ and 2 hours after the administration of glucose by glucose oxidase method (Pandey and Khan, 2002). The experiments were conducted in accordance with the internationally accepted principles for laboratory animals.

Results and Discussion

In previous study, feeding of diets containing 6% water soluble gummy fibre isolated from *Syzygium cumini* seeds significantly reduced the blood glucose, improved the glucose tolerance (Pandey and Khan, 2002) and significantly decreased the activity of intestinal enzymes *i.e.* amylase and invertase in both diabetic and normal rats when compared with their respective controls while feeding of diets containing 2.25% water insoluble neutral detergent fibre (NDF) isolated from *Syzygium cumini* seed diets did not show any hypoglycaemic effect (Pandey and Khan, 2002). Feeding of diets containing 12% and 18% water soluble gummy fibre isolated from *Syzygium cumini* seed resulted in more decrease in blood glucose level and more improvement in glucose tolerance than the feeding of diet containing 6% water soluble gummy fibre isolated from *Syzygium cumini* seeds is observed by the result of our study (Table 2). A significant decrease in the activity of intestinal enzymes *i.e.* amylase and invertase was observed in the normal and diabetic rats fed the diets containing 12% and 18% water soluble gummy fibre

Table 3: Effect of feeding of diet containing water soluble gummy fibre and water insoluble neutral detergent fibre isolated from *Syzygium cumini* for 21 days on intestinal enzymes in normal and alloxan treated diabetic male albino rats

Group	α-Amylase (U/l)	Total invertale (U/mg protein)
Normal		
Group 1 N (6) control diet fed	5.449±0.486	0.135±0.051
Group II N (6) diets containing 12% water soluble gummy fibre fed	3.505**±0.367	0.086**±0.032
Group III N (6) diets containing 18% water soluble gummy fibre fed	2.338***±0.247	0.056***±0.021
Group IV N (6) diets containing 5% water insoluble neutral detergent fibre fed	4.885^{NS}±0.430	0.140^{NS}±0.056
Group V N (6) diets containg 10% water insoluble neutral detergent fibre fed	3.982^{NS}±0.383	0.148^{NS}±0.056
Diabetic		
Group I D (d) control diet fed	5.943±0.507	0.149±0.020
Group II D (6) diets containing 12% water soluble gummy fibre fed	3.602**±0.377	0.079**±0.010
Group III D (6) diets containing 18% water soluble gummy fibre fed	2.891***±0.305	0.039***±0.005
Group III D (6) diet containing 5% water insoluble neutral detergent fibre fed	4.874^{NS}±0.429	0.144^{NS}±0.019
Group V D (6) diets containing 10% water insoluble neutral detergent fibre fed	4.117^{NS}±0.395	0.150^{NS}±0.020

The results presented in table are means ± SD of 6 rats each. Numbers of animals used are given in parenthesis. The data were analyzed statistically, by student's 't' test. The activity of intestinal enzymes, α- amylase and total invertase of normal and diabetic treated rats compared with the corresponding activities of intestinal enzymes, α-amylase and total invertase of normal and diabetic control rats for statistical analysis. **P<0.01,***P<0.001

isolated from *Syzygium cumini* seeds where as 5% and 10% water insoluble neutral detergent fibre (NDF) did not exhibit any hypoglycaemic effect shown in (Table 3).

The amount of water soluble gummy fibre is very high *i.e.* (40 g %) in *Syzygium cumini* seeds and viscosity of water soluble gummy fibre *i.e.* (14 centipoises) is very nearer to that of guargum *i.e.* (16 to 20 centipoises) which has been shown to be gummy fibre (Jenkins *et al.*, 1977). Our observation confirmed by guar gum studies. The guar gum has been reported to reduce serum levels of glucose of both in normal and diabetic animals and human beings (Anderson and Chen, 1979 and Anderson and Clark, 1986). It has been demonstrated that viscous guar gum and water soluble gummy fibre isolated from *Syzygium cumini* seed caused more impairment in glucose diffusion than the less viscous gum Arabica.

Our observation that increase in the amount of water soluble gummy fibre caused enhancing hypoglycaemic effect is mainly due to factors like impaired gastric emptying or decreased intestinal transit time which may be dscribed to physical property of the water soluble gummy fibre *i.e.* viscosity. It was confirmed by in invitro dialysis bag experiment that increase in the amount of guargum and water soluble gummy fibre from *Syzygium cumini* seeds caused more impairment of glucose diffusion due to increased viscosity. In invitro dialysis bag experiment, impairment in glucose, diffusion in presence of water soluble fibre isolated from *Syzygium cumini* seeds has been correlated to its viscosity (Morgan, 1979). Viscosity has a considerable on absorption and transit time in that guar gummy fibre, and the most viscous substance was more effective in decreasing post prandial glucose and insulin concentration. This was confirmed by the fact that the destruction of the viscous character of guar by hydrolysis prevented these actions. The two fold actions of viscous agents may depends on both delayed gastric emptying and delayed absorption of glucose from the small intestinal lumen (Nuttall, 1993). The increase in the sugar at 2 hrs and its correlation with viscosity may be another factor of slower absorption. In other words, the enhanced flattening of oral glucose tolerance curve observed in the rats fed diets containing 12% and 18% in both normal and diabetic treated rats in our study may be considered an another factor of slower glucose absorption caused by viscosity of fibre. Other factors such as a changed hormonal background and the gut endocrine system play an important role in reducing blood glucose concentration. GIP and GLI, *i.e.* gastric inhibitory polypeptide and gut glucagon like immuno reactivity, these two gastric hormones markedly stimulate the release of insulin in the presence of glucose, were not carried out in our study but their corelationship with gummy fibre in the animals fed gummy fibre has been established as also reported by Ribes *et al.*, 1984 and Vohouny and Washington, 1981.They are released from the gut in response to glucose and may be regulated by the rate of absorption of glucose (Williams and James, 1979).

Reduced level of blood glucose observed in our study in treated rats may partly be attributed to the decreased activity of intestinal enzymes i.e. amylase and invertase, which are responsible for digestion of carbohydrates and are trapped in the gummy gel which reduces their accessibility for the substrates resulting in the slowing down of the absorption of end product *i.e.* glucose.

In conclusion, the present study clearly indicates the enhancing hypoglycaemic effect of the water soluble gummy fibre isolated from *Syzygium Cumini* seeds and not due to water insoluble neutral detergent gummy fibre [NDF] or other constituents isolated from *Syzygium cumini* seeds.

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