Hypoglycaemic effect of defatted seeds and water soluble fibre from the seeds of \textit{Syzygium cumini} (Linn.) Skeels in alloxan diabetic rats

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The effect of feeding orally along with diet of different fractions obtained from the seeds of \textit{S. cumini} was tried on fasting blood glucose and glucose tolerance in normal and alloxan diabetic rats. The quantitative determination showed that \textit{S. cumini} seeds contained 40\% of water-soluble gummy fibre and 15\% of water-insoluble neutral detergent fibre (NDF). This study demonstrated that feeding for 21 days of the diets containing 15\% powdered unextracted (intact) seeds containing water-soluble gummy fibre, 15\% powdered defatted seeds from which lipid and saponins were removed only and 6\% water-soluble gummy fibre isolated from \textit{S. cumini} seeds significantly lowered blood glucose levels and improved oral glucose tolerance whereas feeding of the diets containing 15\% powdered degummed \textit{S. cumini} seeds from which water-soluble gummy fibre was removed but which contained neutral detergent fibre (NDF) and 2.25\% water-insoluble neutral detergent fibre (NDF) isolated from \textit{S. cumini} seeds neither lowered blood glucose levels nor improved oral glucose tolerance in both normal and diabetic rats. These observations indicate that the hypoglycaemic effect of \textit{S. cumini} seeds was due to water-soluble gummy fibre and also that water-insoluble neutral detergent fibre (NDF) and other constituents of the seeds had no significant hypoglycaemic effects.

The seeds, fruits and bark of \textit{Syzygium cumini} Skeels. or \textit{Syzygium jambolana} Lam. or \textit{Eugenia jambolana} Lam. (Myrtaceae) commonly known as Jamun in Hindi, Jambudo in Gujarati, Kala Jam in Bengali, Jambu in Marathi, Neredu in Telugu and Black plum in English have been used in Ayurveda and Unani traditional system of medicine as antidiabetic drugs\textsuperscript{1-5}.

The powdered seeds, the solvent and aqueous extract of seeds, bark, leaves and fruits have been reported to lower blood glucose level in diabetic patients and in experimentally induced diabetic animals\textsuperscript{6,9}. Shukla et al.\textsuperscript{5} isolated few highly active hypoglycaemic compounds from the seeds and the pulp of \textit{E. jambolana} and applied for patent. The reports on confirmed hypoglycaemic effect of certain types of plant fibres\textsuperscript{10-12} and the reports demonstrating the presence of fair amount of fibres in \textit{S. cumini} seeds\textsuperscript{13,15} tempted us to speculate that fibres in \textit{S. cumini} seeds might be responsible for their attributed hypoglycaemic effect reported earlier. Therefore the objective of this study was to investigate the hypoglycaemic effects of \textit{S. cumini} seeds and also to determine whether the hypoglycaemic effect was due to the presence of water soluble gummy fibre and/or water insoluble neutral detergent fibre (NDF) or some other constituents in \textit{S. cumini} seeds. For this purpose the \textit{S. cumini} seeds were subjected to a solvent fractionation to remove lipid, saponins, water soluble gummy fibre and water insoluble neutral detergent fibre (NDF). The hypoglycaemic effects of diets containing defatted, degummed, unextracted (intact) \textit{S. cumini} seeds, 6\% water-soluble gummy fibre and 2.25\% water-insoluble neutral detergent fibre (NDF) isolated from the seeds were investigated in normal and diabetic rats.

Materials and Methods

\textbf{Chemicals—}Casein (vitamin-free) was the product of E. Merck, Co. Germany. Glucose oxidase, peroxidase, o-dianisidine were purchased from Sigma Chemicals Co. St. Louis U.S.A.

Rest of the chemicals used were of analytical grade from companies in Bombay.

\textbf{Solvent fractionation of \textit{Syzygium cumini} seeds}

(A) \textbf{Collection of seeds sample}

Dried seeds of \textit{S. cumini} Skeels. (Myrtaceae) collected from the local market were washed thoroughly with distilled water three to four times (10 min each time). The water washing which did not show any hypoglycaemic activity was discarded. The seeds were dried at room temperature, powdered in an electrical grinder and the powdered form of unextracted

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(intact) *S. cumini* seeds were used throughout the investigations. Sample of seeds powder was always stored at 5°C.

(B) Extraction of seeds

(1) Preparation of defatted seed sample

In order to remove lipid and saponins, each batch of 100 g of air dried powder of *S. cumini* seeds contained in thimble was extracted with diethyl ether for 16 hours followed by extraction with absolute alcohol for 24 hr in soxhlet extractor. The defatted seed powder was then air dried for seven days on ordinary filter paper. The defatted material (97.3 g) thus obtained was free from lipid and saponins (2.4 g fat and 0.3 g saponins).

(2) Isolation of gum and preparation of degummed seed sample

The defatted seeds powder devoid of lipid and saponin obtained in the first step (97.3 g) was then stirred with 5% glacial acetic acid at room temperature for 30 min followed by centrifugation for 30 min at 20,000g and the supernatant recovered. The supernatants obtained from repeated extraction for four times and centrifugation were pooled. Ethanol was added to the pooled supernatants with stirring till 50% concentration was reached. This precipitated the gum from the pooled supernatants. The precipitate was then centrifuged and the supernatant was decanted. The precipitate thus obtained was washed with ethanol and acetone and dried at 40°C and weighed. The yield of the gum was 40 g percent on the basis of dry seed weight.

The residue (57.3 g) left after the treatment with 5% acetic acid in above step was washed 3 to 4 times with distilled water followed by alcohol and acetone. The degummed seeds material (57.3g) thus obtained was spread on a filter paper and dried at room temperature for 24 hr.

The neutral detergent fibres were estimated by the technique of Goering and Vansoest. Seeds powder defatted as mentioned above was finally extracted with cold neutral detergent solution(sodium lauryl sulphate USP, disodium ethylene diamine tetraacetate dihydrate crystal, sodium borate decahydrate, disodium hydrogen phosphate, anhydrous 2-ethoxy ethanol) as described by Goering and Vansoest in reflux apparatus with filter manifold unit.

The *S. cumini* seeds were analysed for moisture, ash, fat, protein and starch content and were found to contain (g/100g): moisture-5.9, ash-21.7, lipid-2.4, protein-8.5, starch-6.5, neutral detergent fibre (NDF)-15.0, gum-40.0. 15g of NDF contained: cellulose-6.9, hemicellulose-5.9, lignin-1.0, cutin-0.76 and silica-0.44.

The male albino rats of Wistar strain weighing between 150 and 170 g were divided into six groups each of normal (designated as Group I N, Group II N, Group III N, Group IV N, Group V N, Group VI N) and diabetic (designated as Group I D, Group II D, Group III D, Group IV D, Group V D and Group VI D) rats. The number of animals in each group was six. 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 diet. Group II, Group III, Group IV, Group V and Group VI of both normal and diabetic rats were fed the 20 g of experimental diets daily containing 15% (3g) unextracted (intact), 15% (3g) extracted (defatted), and 15% (3g) degummed *S. cumini* seeds, 6% water soluble gummy fibre and 2.25% of water insoluble neutral detergent fibre (NDF) respectively for 21 days.

The experimental diet contained (g/100g): casein-20.0, unextracted (intact) or extracted (defatted) or degummed *S. cumini* seeds-15.0 or water soluble gummy fibre-6.0 or water insoluble neutral detergent fibre-2.25, hydrogenated oil-10.0, Hawk's Oser salt mixture-4.0 and vitamin mixture-1.0. The weights of all the experimental diets were made up to 100g with starch. The control diet had the same composition without the supplementation of *S. cumini* seeds or its fractions and the starch content was 65 g. Vitamin mixture contained (1g/100g): thiamine HCl-10 mg, riboflavin-20 mg, pyridoxine-10 mg, calcium pantothenate-40 mg, folic acid-12.5 mg, choline chloride-200 mg, biotin-10 mg, menadione-5 mg, L-tocopherol-50 mg, cyanocobalamin-10 mg, vitamin A-2000 IU, vitamin D-200 IU.

All the animals were allowed free access to deionized distilled water. Body weights were examined before feeding and at weekly intervals till the termination of the experiment. At the end of experiment the animals were fasted for 18 hr. Blood samples were collected from the tail vein of the rats at 4 hr after feeding of 3 g of each of 15% unextracted (intact), extracted (defatted) and *S. cumini* seeds, 1.2 g of water soluble gummy fibre and 0.45 g of water insoluble neutral detergent fibre (NDF) to the animals of respective groups and blood glucose was estimated by glucose oxidase method. The amount of the powdered intact seeds, defatted and degummed seeds or
water soluble fibre and NDF were chosen based on the daily consumption of 20g of diet.

The glucose tolerance test was performed by administrating glucose orally (10 g/kg body weight) to the animals of all the groups and blood samples were collected for glucose estimation at 0, 1/2, 1, 1 1/2 and 2 hr after the administration of glucose by glucose oxidase method

Results and Discussion

Feeding of diets containing 15% unextracted (intact), 15% defatted \textit{S. cumini} seeds and 6% water soluble gummy fibre for 21 days significantly lowered (26-28%) the blood glucose level (Table 1) and significantly improved oral glucose tolerance (Table 2) whereas feeding of the diets containing 15% degummed seeds and 2.25% of water insoluble neutral detergent fibre (NDF) neither lowered blood glucose level (Table 1) nor improved oral glucose tolerance (Table 2) in both normal and diabetic rats when compared with their respective controls.

After glucose loading to normal and diabetic control rats, the blood glucose levels were increased to maximum value at 1 hr and then returned to almost initial values at 2 hr in the case of normal rats but remained high with untreated diabetic rats. Feeding of diets containing 15% unextracted (intact), 15% ex-

The glucose tolerance test was performed by administrating glucose orally (10 g/kg body weight) to the animals of all the groups and blood samples were collected for glucose estimation at 0, 1/2, 1, 1 1/2 and 2 hr after the administration of glucose by glucose oxidase method

\textit{Induction of diabetes by alloxan monohydrate}

Rats were made diabetic by injecting a single rapid injection (ip) of alloxan monohydrate in a dose of 120 mg/kg body weight (2% w/v in water) by the method of Lazaro and Pallay\textsuperscript{20}. After 72 hr of alloxan injection stable hyperglycaemia was confirmed by estimating the glucose level in urine of rats by Benetic's qualitative test.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|}
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Groups & Fasting blood glucose (mg/100ml) & %Change \\
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&&
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Normal & & \\
Group I N & 91.2 ± 7.00 & \\
Control diet & 91.2 ± 7.00 & \\
Group II N & 67.0*** ± 5.62 & 26.5 \\
15% unextracted (intact) seed diet & 67.2*** ± 5.20 & 26.3 \\
Group III N & 90.5 NS ± 6.88 & 0.8 \\
15% defatted seed diet & 65.2*** ± 5.90 & 28.5 \\
Group IV N & 91.0 NS ± 7.40 & 0.2 \\
15% degummed seed diet & 282.1 ± 14.64 & 0.2 \\
Group V N & 282.5 ± 14.00 & 0.14 \\
6% gummy fibre diet & 113.7*** ± 5.87 & 59.7 \\
Group VI N & 113.4*** ± 5.91 & 59.8 \\
2.25% NDF diet & 113.1*** ± 4.95 & 59.9 \\
& & \\
Diabetic & & \\
Group I D & 282.1 ± 16.05 & \\
Control diet & 282.1 ± 16.05 & \\
Group II D & 113.7*** ± 5.87 & 59.7 \\
15% unextracted (intact) seed diet & 113.4*** ± 5.91 & 59.8 \\
Group III D & 282.5 ± 14.00 & 0.14 \\
15% defatted seed diet & 113.1*** ± 4.95 & 59.9 \\
Group IV D & 281.5 NS ± 14.64 & 0.2 \\
15% degummed seed diet & 282.5 ± 14.00 & 0.14 \\
Group V D & 113.7*** ± 5.87 & 59.7 \\
6% gummy fibre diet & 113.4*** ± 5.91 & 59.8 \\
Group VI D & 281.5 NS ± 14.64 & 0.2 \\
2.25% NDF diet & 282.1 ± 16.05 & \\
\hline
\end{tabular}
\caption{Effect of feeding diet containing \textit{S. cumini} intact, defatted and degummed seeds, water soluble gummy fibre and water insoluble neutral detergent fibre on blood glucose in normal and diabetic rats for 21 days
[Values are means ±SD of 6 rats]}
\end{table}

\*\*\* \(p<0.001\), NS : Not significant. Blood glucose levels of treated rats compared with the control rats.
tracted (defatted) and 6% water soluble gummy fibre isolated from *S. cumini* seeds to both normal and diabetic rats for 21 days resulted in a considerable improvement in oral glucose tolerance.

The observation that the diets containing 15% unextracted (intact) seed, 15% extracted (defatted) *S. cumini* seed from which only fat and saponins were removed but contained water soluble gummy fibre and the diet containing 6% water soluble gummy fibre isolated from *S. cumini* seeds exhibited the pronounced hypoglycaemic effect whereas the 15% degummed seed diet devoid of water soluble gummy fibre and the diet containing 2.25% water insoluble neutral detergent fibre (NDF) isolated from *S. cumini* seeds did not show hypoglycaemic effect both in normal and diabetic rats point towards the fact that the hypoglycaemic effect was due to the water soluble gummy fibre and not due to the water insoluble neutral detergent fibre (NDF) and other constituents of the seeds.

Our observation pertaining to the effect of neutral detergent fibre (NDF) and water soluble gummy fibre in *S. cumini* seeds are confirmed by earlier reports that neutral detergent (NDF) such as cellulose, hemicellulose and lignin found in dietary sources had little impact on plasma glucose and insulin\(^{21-22}\) while water soluble fibres present in plant materials have been shown to reduce blood glucose in animals and human beings\(^{23}\).

The effect of lipid and saponin on lowering the blood glucose levels both in normal and diabetic rats is also ruled out because extracted (defatted) seeds from which lipid and saponins were removed had the similar hypoglycaemic effect as the 15% unextracted Table 2—Effect of feeding diet containing *S. cumini* intact, defatted and degummed seeds, water soluble gummy fibre and water insoluble neutral detergent fibre on oral glucose tolerance in normal and diabetic rats for 21 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/100ml)</th>
<th>Normal</th>
<th>0 hr</th>
<th>1/2 hr.</th>
<th>1 hr</th>
<th>1 1/2 hr</th>
<th>2 hr</th>
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<td>Normal</td>
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<td>Control diet</td>
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<td>15% unextracted (intact) seed diet</td>
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<td>2.25% NDF diet</td>
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<td>15% unextracted (intact) seed diet</td>
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The data were analysed statistically by student's 't' test. For statistical analysis the values of treated rats were compared with those of normal and diabetic control.

**P<0.01; N.S.: Not significant.**
In conclusion it may be stated that our observations are suggestive of the fact that the hypoglycaemic effect of *S. cumini* seed diets was due to the water soluble gummy fibre while water insoluble neutral detergent fibres (NDF) and other constituents of the seeds had no significant hypoglycaemic effect.

References