Effect of Feeding of Syzygium Cumini seed Diets and Diets Containing Water Soluble Gummy fibre isolated from Syzygium seeds on Key Enzymes of Carbohydrate and Lipid Metabolism in Normal and Alloxan Treated Diabetic male Albino Rats

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ABSTRACT
Background: Feeding effect of 15% Syzygium cumini seed diet and the diets containing 6, 12&18% water soluble gummy fibre isolated from syzygium cumini seeds by solvent fractionation for 21 days to both normal & diabetic male albino rats were carried out on the activities of the enzymes of carbohydrate metabolism in liver. Significant increase was observed in the activities of the enzymes of glycolytic, HMP shunt and gluconeogenetic pathways. The results obtained of present study in dicate the enhanced activities were mainly due to water soluble gummy fibre.

Keywords: Enzymes of HMP shunt pathway, glycolytic pathway, gluconeogenetic pathway, Syzygium cumini seeds fibre.

INTRODUCTION
Hypoglycamic effect of water soluble gummy fibre isolated from Syzygium cumini seeds have studied earlier¹ which is mainly attributed to its capability to reduce glucose absorption by virtue of its physical characteristic i.e. viscosity.² The information pertaining to the effect of water soluble gummy fibres from plant sources other than Syzygium cumini seeds on catalytic activities of key liver enzymes is scanty.³⁴⁵ There is no report available on the effect of water soluble gummy fibre from Syzygium cumini seeds on catalytic activities of key enzymes.

Alteration in the activities of key enzymes of metabolism measured in vitro can be used as an indicators of changes in flux through metabolic pathway⁶. There for present study was conducted to investigate the effect of water soluble gummy fibre isolated from Syzygium cumini seeds on the activities of carbohydrate and lipid metabolism in Liver.

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Animals and diets
The male albino rats of wistar strain weighing between 150-170g were selected for the present study. They were maintained in the animal facility( room temp 22 °C, proper controlled light, relative humidity, all fresh ventilation 15-20 times /h) . Polystyrene cages equipped with little trays and subjected to the experiment after one week. These animals were divided into five groups each of normal (designed as group I N, II N, III N, IV N, VN ) and diabetic (designated as groups I D, II D, III D, IV D, and group V D ) rats . Rats of diabetic groups were made diabetic by injecting a single intra peritoneal injection of aqueous suspension of alloxan monohydrate in dose of 120mg/kg body weight by the method Lazaro & pallay . Group I from normal and diabetic rats i.e group IN and ID served as control & were fed the control diet. The number of animals in each group are mentioned in the table in parenthesis .Group II, Group III , Group IV & Group V were fed the 20g of 15% Syzygium cumini seeds diets & the diets containing 6, 12, & 18% water soluble gummy fibre isolated from Syzygium cumini seeds. The 1200 mg of gummy fibre present in the diets containing 6% water soluble gummy fibre corresponds to the amount of fibre
present in 3g of Syzygium cumini seeds which happened to be the average consumption of seed present in the 20g of 15% Syzygium cumini seed diets daily.

**Assay of the enzyme activities**

At the end of 21 days, the rats of all the groups were fasted for 18 hours, stunned by blow, decapitated & bled. Their livers were quickly excised & kept in beakers standing on cracked ice, chilled for 5 minutes, blotted on filter paper weighed and used for extraction & assay.

**Chemicals and enzymes**

Glucose–6-phosphate, 6-phosphogluconate, Coenzyme A, NADP, fructose 1,6-bisphosphate, phosphate, potassium chloride, creatine kinase, glucose 1 phosphate, creatine phosphate, malate dehydrogenase, antimycin A, NADH, AMP, fructose diphosphate aldolase, trios phosphate isomerase, glycerol-3-phosphate dehydrogenase (NAD+), ATP citrate (pro-3s)lyase, lactate phosphatase, aldolase, Bovine serum albumin/ ml, 1 mM dithiothreitol, 0.2 mM phenyl methyl sulphonyl fluoride, 2.4 mM fructose-6-phosphate (final pH 7.4). The activity of 6-phosphofructokinase in the medium were stable for upto 6 hours on ice. Extraction medium for 6-phosphofructokinase [e. C. 2. 7. 1. 11] The extraction medium consisted of 250 mM sucrose, 20 mM potassium phosphate, 100 mM ammonium sulphate, 10 mM bovine serum albumin/ ml, 1 mM dithiothreitol, 0.2 mM phenyl methyl sulphonyl fluoride, 2.4 mM fructose-6-phosphate (final pH 7.4). The activity of 6-phosphofructokinase in the medium were stable for upto 6 hours on ice.

Extraction medium for pyruvate kinase [e. C.2.7.1.40.] And fructose-1,6 biphosphatase [e. C. 3. 1. 3. 11] The extraction medium consisted of 20 mM potassium phosphate, 100 mM potassium fluoride, 4 mM EDTA, 250 mM sucrose, 1 mM dithiothreitol (final pH 7.4). The activity of Pyruvate kinase and fructose-1,6 biphosphatase were stable for upto 6 hours on ice.

Since stability of different enzymes require different condition, preparations of separate extraction media were necessary. All liver extracts were centrifuged at 4 c for 6-2 min periods at 12000g in a cold Remi centrifuge. The clear layer between the fat layer and the pellet was used for the assay of enzyme activity and protein determination.

For the assay of lactate dehydrogenase [E.C. 1.1.1.27] the extraction medium consisted of 50 mM triethanol amine, 1 mM EDTA, 0.2 Mm Bovine serum albumin/ ml with pH of 7.6.

The activities of glucose-6-phosphate dehydrogenase, 6-phosphoglucuronate dehydrogenase, malate dehydrogenase (oxaloacetate decarboxylating, NADP+) and ATP - citrate lyase (Pro-3s) were stable in this medium for upto 6 hours on ice.

Extraction medium for 6-phosphofructokinase [e. C. 2. 7. 1. 11] The extraction medium consisted of 250 mM sucrose, 20 mM potassium phosphate, 100 mM ammonium sulphate, 10 mM bovine serum albumin/ml, 1 mM dithiothreitol, 0.2 mM phenyl methyl sulphonyl fluoride, 2.4 mM fructose-6-phosphate (final pH 7.4). The activity of 6-phosphofructokinase in the medium were stable for upto 6 hours on ice. Extraction medium for pyruvate kinase [e. C.2.7.1.40.] And fructose-1,6 biphosphatase [e. C. 3. 1. 3. 11] The extraction medium consisted of 20 mM potassium phosphate, 100 mM potassium fluoride, 4 mM EDTA, 250 mM sucrose, 1 mM dithiothreitol (final pH 7.4). The activity of Pyruvate kinase and fructose-1,6 biphosphatase were stable for upto 6 hours on ice.

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Assay of enzyme activities

Activities of enzymes glucose-6-phosphate dehydrogenase [E.C. 1.1.1.49], 6-phosphogluconate dehydrogenase [E.C.1.1.1.44] and malate dehydrogenase [E.C.1.1.1.40] were assayed by measuring the rate of increase of absorbance at 340 nm due to the reduction of NADP+ according to the method of Newsholme.8

The reaction mixture for glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase contained 50mM tris Hydrochloride, 0.2 mM NADP+, 20mM magnesium chloride, either 2mM glucose-6-phosphate or 6-phosphogluconate (final pH 7.4). Rates of NADP+ reduction measured in the absence of glucose-6-phosphate, 6-phosphogluconate or potassium malate were used to correct for endogenous NADP+ Reduction.

Activity of an enzyme ATP-citrate (pro 3s) lyase was assayed by measuring the rate of decrease of absorbance at 340 nm by coupling the formation of oxaloacetate to the oxidation of NADH via the enzyme malate dehydrogenase according to the method of Inoue et al.9

The reaction mixture for ATP citrate (pro-3s) citrate lyase contained 200mM–dithiothreitol, 10mM magnesium chloride, 0.2 mM NADH, 5mM ATP, 20mM potassium citrate, 10mM creatine phosphate, 100 g creatine kinase [E.C.2.7.3.2], 2.5 g malate dehydrogenase, 5 g antimycin A, 0.2 mM coenzyme A (final pH 8.4). Rates of NADH oxidation measured in the absence of coA were used to correct for endogenous NADH oxidation.

Activity of enzyme fructose-1,6-biphosphatase was assayed by measuring the rate of decrease of absorbance at 340 nm by coupling the formation of fructose 1,6-bisphosphate to the oxidation of NADH via the enzymes glucose phosphate isomerase [E. C. 5. 3. 1. 9] & glucose-6-phosphate dehydrogenase according to the method of Riou et al.10

The reaction mixture for fructose-1,6-biphosphatase contained 100 mM magnesium chloride, 2mM ammonium sulphate, 0.05 mM EDTA, 0.2 mM NADP+, 10 ug glucose phosphate isomerase, 10 ug glucose-6-phosphate dehydrogenase, 10 mM creatine phosphate, 100 ug creatine kinase, 10 ug adenylate kinase[E. C. 2. 7. 4. 3] and 70 nmfructose-1-6-biphosphate (final pH 7.5). Rates of NADP reduction measured in the absence of fructose-1,6-bisphosphate were used to correct for endogenous NADH+ Reduction.
Activities of enzyme lactate dehydrogenase was assayed by measuring the rate of decrease of rate of absorbance at 340 nm due to the oxidation of NADH according to the method of Wroblewski and La due, and Henry et al. The reaction mixture contained 2.7 ml of phosphate buffer in to spectrophotometer cell (1 cm). 0.1 ml of tissue homogenate and 0.1 ml of reduced nicotinamide adenine dinucleotide, allow to stand for 20 minutes and 0.1 ml of sodium pyruvate (2.5 mg/1 ml) was added to the reaction mixture. Decrease in absorbance were measured for 5 minutes at intervals of 15-30 seconds at room temp. Changes in absorbance were measured at 25°C using a Gillford spectrophotometer. The final cuvette volume was 2 ml. Centrifuged liver extract was preincubated at 25°C for 10 minutes in an assay medium omitting substrate. For enzyme assays, the reaction was started by the addition of substrate and for controls the reaction was started with distilled water. Enzyme assays and controls were carried out in duplicate. Protein was estimated by the method of Lowry et al, (15). Enzyme activities are expressed as u moles/min/mg protein and u moles/min/mg protein.

Phosphorylase was assayed by measuring the rate of liberation of inorganic phospho from glucose-1-phosphate in the presence of 5’ AMP according to the method of Sutherland and Wosilait (394). Inorganic phosphate was determined by the method of Fiske and Subarrow(16)

Glucose-6-phosphatase activity was assayed by the method of Swanson (17) by measuring the rate of liberation of inorganic phosphate from glucose-6-phosphate. The inorganic phosphorus liberated was estimated by Fiske and Subarrow method at 660 nm.(365) The reaction mixture contain 0.1 ml of 0.1 M glucose-6-phosphate, 0.3 ml of citric acid, sodium hydroxide buffer (0.1 M, pH6.5) 0.1 ml of 10% (W/v) liver homogenate in 0.25 m sucrose. The contents of the reaction mixture was swirling and all the tubes incubated at 37°C in water bath for 30 minutes. At the end of incubation period, reaction was terminated by the addition of 1 ml of 10% TCA. All the tubes were allowed to stand for 50 minutes and the final volume of reaction mixture was made upto 5 ml by adding 0.5 ml of distilled water and subjected to centrifugation. For this, control tubes were run in similar manner. The supernatant was assayed for inorganic phosphorus by the method of Fiske and Subarrow(16) and the proteins were estimated by the method of Lowry et al.(15)

Enzyme unit is expressed as u moles of inorganic phosphorus liberated /min/mg protein.

RESULT AND DISCUSSION

Results of the activities of the enzyme are given in the (Table 1&2).

Feeding of 15% unextracted (intact) syzygium cumini seed diet and the diets containing 6, 12, 18% water soluble gummy fibre isolated from Syzygium cumini seeds to both normal and diabetic treated rats for 21 days significantly increased the activities of 6-phosphofructokinase, pyruvate kinase and lactate dehydrogenase (enzymes of glycolytic pathway), Fructose-1,6-biphosphatase, glucose-6-phosphatase (enzymes of gluconeogenic pathway), glucose-6-phosphate dehydrogenase, 6-phosphogluconate (enzymes of HMP shunt pathway), ATP citrae (pro-3s) lyase and malate dehydrogenase as compared to their respective controls in our study.

The rats fed the diets containing 12 and 18 % water soluble gummy fibre isolated from syzygium cumini seeds showed more increase in the activities of all the enzymes as compared to the diets containing 6% water soluble gummy fibre isolated from syzygium cumini seeds.

Of the glycolytic enzymes decreased activities for 6-phosphofructokinase (18-19,20 ,21), pyruvate kinase (22-26) and increased activity for lactate dehydrogenase(27-31) have been reported in diabetes. The enhanced activity of lactate dehydrogenase in diabetes could be due to less insulin secretion in diabetes as well as in excessive accumulation of pyruvate in diabetes which in turn reversibly converted to lactate in the presence of enzyme lactate dehydrogenase.

The activities of glucose-6-phophatase (32-42) and fructose-1,6-bisphosphatase (43-45,421) enzymes of gluconeogenetic
pathway have been reported to be increase in diabetes. The increased activity of these hepatic enzymes were also observed in diabetic control rats when compared with there normal control rats. This is confirmed by the earlier reports.

The activities of glucose-6-phosphate dehydrogenase (46-58), 6-phosphogluconate dehydrogenase (59-61) the enzymes of HMP shunt pathway, ATP citrate (pro-3s) lyase (3)& malate dehydrogenase (62-66) have been reported to be decreased in diabetes.

Our study also shows the decreased activities of these hepatic enzymes in diabetic control rats when compared with their normal (untreated rats). Our study also suggest that 15% unextracted (intact) syzygium cumini seed diets and the diets containing 6, 12 & 18% water soluble gummy fibre isolated from syzygium cumini seeds caused a shift from decrease to increase in the activity of these hepatic enzymes in the diabetic rats.

The activities of hepatic glycolytic enzymes have been reported to be elevated in streptozotocin induced diabetic rats fed an African viscous fibre dikanut (Irvingia gabonesis) (67). The activities of key enzymes in the glycolytic pathway i.e. 6-phosphofructokinase and pyruvate kinase were enhanced in the rats fed highly viscous water soluble guar gum (1). It appears that activities of hepatic glycolytic enzymes are elevated to efficiently utilize the low substrates reaching the liver in the rats fed the diets containing 6, 12 &18% viscous water soluble gummy fibre isolated from syzygium cumini seeds caused a shift from decrease to increase in the activity of these hepatic enzymes in the diabetic rats.

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The reaction catalyzed by malate dehydrogenase synthesizes the remaining NADPH required (72), while the reaction catalyzed by ATP citrate (pro-3s) lyase generates Acetyl coA for fatty acid and steroid synthesis (73).

Our study suggest that the demand for NADPH derived from HMP shunt pathway and from the reaction catalyzed by malate dehydrogenase increase in the rats fed the 15% unextracted syzygium cumini seed diets and the diets containing 6, 12 &18% viscous water soluble gummy fibre isolated from syzygium cumini seeds.

One reason for this increased demand for NADPH may be an increased rate of bile acids and cholesterol synthesis required in the rats fed 15% syzygium cumini seed diets and the diets containing 6, 12 &18% viscous water soluble gummy fibre in which the rate of bile acid excretion increased and rate of cholesterol reduced as evident from the data (My Paper 7,8,9).

In other words, the increased rate of bile acid excretion and reduced rate of cholesterol absorption induced the rats to increase the bile acid and cholesterol synthesis .This demand is fullfilled by increasing the activity of glucose -6-phosphate dehydrogenase and malate dehydrogenase to produce NADPH required for the synthesis of cholesterol ,bile acid and fatty acids (74).

An increased rate of bile acid and cholesterol synthesis would require a source of carbon which may be provided by an increased rate of glycolysis observed in treated rats in our study .This source of carbon may also be provided by an increase in the rate of acetyl coA generation in the cytoplasm through the increased activity of ATP citrate (pro-3s) lyase which has been observed in our study.

Feeding of 15 % unextracted ( intact) Syzygium cumini seed diet and the diets containing 6,12 &18 % water soluble gummy fibre isolated from Syzygium cumini seeds to normal and diabetic rats for 21 days ,resulted in significant decrease in the activity of phosphorylase enzymes.

An increase in hepatic phosphorylase (75) activities has been found in the liver of
diabetic rats with respect to normal control. This is confirmed by the results of our study. It appears that the feeding of 15% unextracted (intact) Syzygium cumini seed diet and the diets containing 6, 12 & 18% water soluble gummy fibre isolated from Syzygium cumini seeds caused a shift away from the depletion of glycogen by the diabetic rats to synthesis of glycogen as evident from the higher levels of glycogen in normal and diabetic rats (ref.-chapter 4 or my paper.(76))

Guargum has been reported to alter carbohydrate and lipid metabolism in liver which has been attributed to reduction in the release of gastrointestinal hormone i.e. gastric inhibitory polypeptide (GIP) and glucagon like immunoreactivity (GLI) caused by reduced rate of absorption of glucose and increased rate of bile acid excretion.

Since there are receptors for gut glucagon like reactivity (GLI) on the surface of the hepatocyte membrane (77-83), it has been suggested that these gastrointestinal hormone may have direct effect on hepatic lipogenesis. Therefore, it may be postulated that water soluble gummy fibre from syzygium cumini seeds may operate through similar mechanism and may alter and modify hepatic carbohydrate and lipid metabolism account for changes in the activities of enzymes observed in the present study.

Table: 1 Effect Of Feeding Of Syzygium Cumini Seed Diet And Diets Containing Different Levels Of Water Soluble Fibre Isolated From Syzygium Cumini Seeds On Activities Of Enzymes Of Carbohydrate And Lipids Metabolism In Liver Of Normal And Alloxan Treated And Diabetic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose-6-phosphate Dehydrogenase</th>
<th>6-phosphogluconate dehydrogenase</th>
<th>Malate dehydrogenase</th>
<th>ATP citrate(pro-3s) Lyase</th>
<th>Pyruvate kinase</th>
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<tr>
<td>NORMAL</td>
<td></td>
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<tr>
<td>Group I Control diet fed</td>
<td>3.8 ± 0.29</td>
<td>6.82 ± 0.51</td>
<td>59.39 ± 5.85</td>
<td>9.07 ± 0.69</td>
<td>282.00 ± 20.28</td>
</tr>
<tr>
<td>Group II N(6) 15% syzygium cumini seeds diets containing water soluble gummy fibre fed</td>
<td>4.58* ± 0.34</td>
<td>8.50* ± 0.65</td>
<td>65.58* ± 7.83</td>
<td>11.68* ± 0.88</td>
<td>294.00*±19.50</td>
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<tr>
<td>Group III N (6) diets containing 6% water soluble gummy fiber isolated from syzygium cumini seeds diets fed</td>
<td>5.10* ± 0.39</td>
<td>9.65* ± 0.78</td>
<td>77.04* ± 8.08</td>
<td>13.57* ± 0.92</td>
<td>307.00* ± 22.13</td>
</tr>
<tr>
<td>Group IV N (6) diets containing 12% water soluble gummy fiber isolated from syzygium cumini seeds diet fed</td>
<td>5.93** ± 0.48</td>
<td>10.20** ± 0.88</td>
<td>85.64** ± 8.71</td>
<td>14.50** ± 1.21</td>
<td>316.00** ± 25.00</td>
</tr>
<tr>
<td>Group V N (6) diets containing 18% water soluble gummy fibre isolated from syzygium cumini seeds diet fed</td>
<td>7.02** ± 0.55</td>
<td>11.44** ± 0.91</td>
<td>92.25** ± 9.04</td>
<td>16.42** ± 1.59</td>
<td>331.00** ± 27.00</td>
</tr>
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</table>

The result of the enzymes activities are expressed as means ± SD of 6 rats each. Number of animals used are given in parenthesis. The data were analysed statistically by student’s test. The activities of hepatic enzymes of normal and diabetic treated rats compared with normal and diabetic control rats for statistical analysis.

*p<0.05;  **p<0.01Activities of hepatic enzymes of diabetic controlled rats compared with the activities of normal control rats for statistical analysis. ••†† p<0.01
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<tr>
<td>Group I D (6) Control diet fed</td>
<td>1.98 ± 0.09</td>
<td>4.92 ± 0.39</td>
<td>29.79 ± 0.96</td>
<td>6.84 ± 0.48</td>
<td>160.00 ± 8.99</td>
</tr>
<tr>
<td>Group II D (6) 15% syzygium cumini seeds diets fed</td>
<td>3.66* ± 0.27</td>
<td>6.68* ± 0.51</td>
<td>34.08* ± 2.25</td>
<td>9.00* ± 0.84</td>
<td>201.31* ± 13.11</td>
</tr>
<tr>
<td>Group III D (6) diets containing 6% water soluble gummy fiber isolated from syzygium cumini seeds diets fed</td>
<td>5.30** ± 0.45</td>
<td>9.11** ± 0.66</td>
<td>41.66** ± 3.76</td>
<td>12.05** ± 1.02</td>
<td>250.24** ± 16.63</td>
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<tr>
<td>Group IV D (6) diets containing 2% water soluble gummy fiber isolated from syzygium cumini seeds diets fed</td>
<td>6.89** ± 0.58</td>
<td>11.79** ± 0.79</td>
<td>48.37** ± 4.64</td>
<td>13.11** ± 1.24</td>
<td>290.00** ± 20.93</td>
</tr>
<tr>
<td>Group V D (6) diets containing 18% water soluble gummy fiber isolated from syzygium cumini seeds diets fed</td>
<td>8.26** ± 0.62</td>
<td>13.47** ± 0.91</td>
<td>55.41** ± 5.85</td>
<td>15.83** ± 1.66</td>
<td>328.00** ± 24.71</td>
</tr>
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The result of the enzymes activities are expressed as means ±SD of 6 rats each.
Effect of Feeding of Syzygium Cumini seed Diets and Diets Containing Water Soluble Gummy

Numbers of animals used are given in parenthesis. The data were analysed statistically by student’s t test. The activities of hepatic enzymes of normal and diabetic treated rats compared with normal and diabetic control rats for statistical analysis.

* p<0.05; ** p<0.01

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<th>Groups</th>
<th>6-phosphofructokinase</th>
<th>Fructose-1,6-biphosphatase</th>
<th>Glucose-6-phosphatase</th>
<th>Lactate dehydrogenase</th>
<th>Phosphorylase</th>
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<tr>
<td>Group I D (6) Control diet fed</td>
<td>4.3 ± 0.4</td>
<td>110.34 ± 7.60</td>
<td>142.63 ± 10.26</td>
<td>35.60 ± 3.38</td>
<td>17.43 ± 1.94</td>
</tr>
<tr>
<td>Group II D(6) 15% syzygium cumini seeds diets containing water soluble gummy fibre fed</td>
<td>7.87* ± 0.57</td>
<td>135.57* ± 6.92</td>
<td>149.07* ± 10.69</td>
<td>42.71* ± 7.64</td>
<td>14.00* ± 1.34</td>
</tr>
<tr>
<td>Group III D (6) diets containing 6% water soluble gummy fiber isolated from syzygium cumini seeds diets fed</td>
<td>12.15** ± 0.88</td>
<td>151.30* ± 7.81</td>
<td>154.07** ± 10.69</td>
<td>50.09** ± 7.64</td>
<td>11.00** ± 1.08</td>
</tr>
<tr>
<td>Group IV D (6) diets containing 12% water soluble gummy fiber isolated from syzygium cumini seeds diet fed</td>
<td>11.44** ± 0.91</td>
<td>168.79** ± 8.70</td>
<td>161.37** ± 12.35</td>
<td>56.83** ± 9.25</td>
<td>8.89** ± 0.94</td>
</tr>
<tr>
<td>Group V D (6) diets containing 18% water soluble gummy fibre isolated from syzygium cumini seeds diet fed</td>
<td>13.67** ± 1.17</td>
<td>177.48** ± 9.71</td>
<td>170.75** ± 13.71</td>
<td>61.95** ± 9.25</td>
<td>5.99** ± 0.58</td>
</tr>
</tbody>
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