Anti-hyperglycaemic study of *Eladi Churna* in streptozotocin (STZ) induced diabetic rats

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Diabetes mellitus is the seventh leading cause of death worldwide, with a frequent occurrence of Type II diabetes as compared to Type I. The aim of this present study was to evaluate the anti-hyperglycaemic effect of a classical herbomineral preparation *Eladi Churna* in STZ induced Type II diabetic rats. Thirty adult charles foster albino rats were allocated in to five groups with six animals in each group: Group I (Control Group), Group II (Diabetic control group without any medication), group III (Standard drug Glibenclamide at 1mg/kg of body weight), group IV (*Eladi Churna* at 300 mg/kg of body weight), group V (*Eladi Churna* at 600 mg/kg of body weight). Diabetes was induced by intraperitoneal injection of STZ at dose level of 35mg/kg. The whole study was conducted for 28 days. The animals were examined for blood glucose levels on 0, 7, 14, 21, 28 days respectively and other biochemical parameters such as serum cholesterol, HDL, LDL, Triglycerides, SGOT, SGPT were evaluated on day 0 and 28, respectively. The results showed that a gradual reduction in blood glucose level was assessed on administration of *Eladi Churna* at two different dose levels. But it was more significant at dose level of 600 mg/kg as p<0.05 with a mean±SD value of 107.34±2.67 on day 28 as compared to mean±SD value of 494.40±43.99 on day 3 (after induction of diabetes mellitus). And the results were almost analogous to potent antidiabetic drug glibenclamide with a mean±SD value of 95.44±7.44 on day 28. And the drug was also responsible for maintenance of alterations in other biochemical parameters, associated with diabetes mellitus, thus depicting its potent anti hyperglycaemic and hypolipidaemic actions.

**Keywords:** Anti-hyperglycaemic, Diabetes mellitus, *Eladi Churna*, Streptozotocin.

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Diabetes mellitus is a metabolic syndrome characterized by hyperglycaemia due to disturbances in carbohydrates, proteins and fats metabolism resulting from defect in insulin secretion, insulin action or both. Diabetes mellitus is not new to 21st century rather it was widely known problem since 1000 BC. Globally an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. WHO projects that diabetes will be the 7th leading cause of death in 2030. China, India and USA are amongst the top three countries with a high number of diabetic populations. The diabetes epidemic relates particularly to Type II diabetes mellitus and is taking place both in developed and developing nations and meticulously in India. The diabetic burden in India mainly relates to adoption of western lifestyle and dietary habits. Health experts are alarmed because the onset of Type II diabetes mellitus tends to affect peoples in the west in 40 and 50 yrs of age group, but the disease strikes Indians even in young age. Type II diabetes mellitus currently affects about 26 million peoples in the U.S. and more than 382 million peoples worldwide. As many as 79 million adults in the U.S. have “pre-diabetes” and are at high risk of developing Type II diabetes mellitus. Diabetes Mellitus is not a disease rather it is a syndrome leads to disturbance in normal physiological conditions of body and incessant malfunctioning related with glucose consumption. The treatment of diabetes mellitus don’t rely only upon the reduction of blood glucose levels but also to cope up with the complications associated

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with the increased blood glucose levels. Though a wide range of antidiabetic drugs are available in contemporary science but even then people desire to pivot on herbal drugs as they carry very little or no side effects if administered in a prescribed way, i.e., right dose to be taken on right time with right adjuvant as rightly advised by the physician. Ayurvedic herbal and herbomineral formulations, a combination of multiple ingredients with multidimensional approach carry the competence to target the multiple etiology in diabetes mellitus like insulin management in β cells of pancreas, utilization of blood glucose by peripheral tissues, inhibit gluconeogenesis from liver cells, etc. with the help of therapeutic properties of individual ingredients as well as synergistic effect of other ingredients used in the formulation.

Eladi Churna, one of the classical herbomineral formulations has been mentioned as Prameha Rogahara by Acharya Vallabhacharya in the treatise Vaidya Chintamani (15th Century). Eladi Churna contains four ingredients in equal proportion (Ela-Elettaria cardamomum L., Pippali-Piper longum L., Pashanbheda-Bergenia lingulata Wall., Shilajit-Asphaltatum punjbinum). Prameha or Madhumeha (a type of Prameha) can be correlated with Diabetes mellitus on account of prodromal symptoms, clinical symptoms and complications associated with the both. The anti-hyperglycaemic potential of individual ingredients of Eladi Churna has been validated through many scientific research. The purpose of this research was to experimentally assess the anti-hyperglycaemic effect of Eladi Churna in Streptozotocin-induced diabetic rats and to compare it with glibenclamide as a reference standard. Clinically used glibenclamide (a sulphonylurea drug) is known to lower the serum glucose by stimulating β-cells to release insulin.

Materials and Methods

Chemicals

Streptozotocin was sponsored by Department of Rasa-Shastra and Bhaishajya Kalpana, Faculty of Ayurveda, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, wherein the chemical was purchased from Himedia Laboratories Pvt. Ltd. Dindori, Nasik, India. And glibenclamide, used as standard anti-diabetic agent, was purchased from Emcure SANOFI, trade name Daonil in India.

Procurement of drugs and preparation of Churna

The herbal ingredients of Eladi Churna, i.e., Ela, Pippali and Pashanbheda, were procured from Gola Deenanath (Raw drug market), Varanasi. All the collected crude drugs were pharmacognostically identified and confirmed in the Department of Dravyaguna, Faculty of Ayurveda, IMS, BHU. The collected herbal drugs were cleaned and dried in the sunlight. The dried plant materials were then grounded into fine powder form by using mechanical pulverizer in Ayurvedic Pharmacy, Banaras Hindu University, Varanasi, India. Mineral ingredient Shilajit was procured from Dabur India Limited, Kaushambi, Sahibabad, Ghaziabad (U.P.) and its physico-chemical assessment was done in the Department of Rasa-Shastra and Bhaishajya Kalpana, Faculty of Ayurveda, IMS, BHU. After authentication, Shodhana of Shilajit was done and kept for dryness. After complete drying, it was grounded in to fine powder form by end runner. Afterwards all the powdered ingredients i.e. herbal and mineral drugs were mixed properly to prepare Eladi Churna.

Experimental Animals

Thirty adult charles foster albino rats with an average weight of 180±20 gm and with either sex were selected for this study. The animals were obtained from the Central Animal House, IMS, BHU, Varanasi. The animals were housed in suitable cages and acclimatized for about one week. All the rats were freely allowed to eat pellet chow (obtained from Amrut Laboratory Animal Feed, Pranav Agro Industries Limited, Sangali) and water ad libitum during the study period. The study was conducted after getting approval from Institutional Animal Ethical Committee (No. Dean/2016/CAEC/47) of BHU and the principles of laboratory animal care as per NIH guidelines were followed continuously during the whole study.

Experimental design

The study was conducted in the animal house of the Department of Pharmacology, IMS, BHU. Thirty animals were erratically allocated in to five groups with six animals in each group namely group I (Normal Control), group II (Diabetic Control without any medicines), group III (Standard drug Glibenclamide 1mg/kg of body weight), group IV (Eladi Churna 300 mg/kg of body weight), group V (Eladi Churna 600 mg/kg of body weight). Considering adult dose of Churna Kalpana with reference to various Churnas mentioned in AFI, 3-6 gm of human adult dose of Eladi Churna was...
converted in to animal dose based on the body surface area ratio using the table of Paget and Barnes 1969 (Human adult dose × Body surface area ratio convertible factor i.e. 0.018) And human dose of glibenclamide is given as 10 mg OD and the suitable dose for rats was calculated by referring to table of Paget and Barnes.

The rats were kept on fasting overnight prior to STZ administration day. STZ solution was induced in rats of group II, group III, group IV and group V at a dose of 35 mg/kg through insulin syringes as it has been depicted through various researches that STZ at a lower dose is known to resemble Type II diabetic model and does not kill pancreatic β cells. The STZ solution was freshly prepared by dissolving it in 50 mg of sodium citrate buffer (pH 4.5) to prepare a final concentration of 1 mg/mL, just prior to its intraperitoneal administration in rats for the induction of Diabetes Mellitus. Blood glucose levels of STZ induced rats were estimated after 72 h with one touch verio flex glucometer. On affirmation of hyperglycaemia after 72 h, rats of group III were treated with standard antidiabetic drug Glibenclamide at a dose of 1mg/kg of body weight and rats of group IV and V were administered with Eladi Churna at a dose of 300 mg/kg and 600 mg/kg of body weight, respectively. While the rats of group II were not given any treatment. 5 mg tablet of Glibenclamide (Daonil) was finely powdered and dissolve in distilled water. The strength of the Glibenclamide solution was made up to 1mg/mL/day per oral. Similarly the stock solution for the trial drug Eladi Churna was prepared freshly by adding adequate quantity of distilled water. Fresh stock solution was prepared for all the three drug samples i.e. Glibenclamide, Eladi Churna at 300 mg/kg and Eladi Churna at 600 mg/kg every day prior to its administration. The medicaments were administered orally with the help of intragastric tube. And then the blood glucose levels were recorded on day 7, 14, 21 and 28 days, respectively or the study was conducted for 28 consecutive days. At the end of the experiment, the animals were sacrificed under anesthesia with diethyl ether. Apart from blood glucose levels, the animals were also assessed for biochemical parameters such as serum cholestrol, serum high density lipoproteins (HDL), serum low density lipoproteins (LDL), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase activity (SGPT) on day 0 (initial day) and day 28 (final day).

Statistical analysis

Results were expressed as mean±standard deviation of mean by using statistical software SPSS version 16.0. All statistical comparisons between the groups were made by means of One Way ANOVA (Analysis of Variance) with post hoc multiple comparison test. Within the group comparison was done by paired ‘t’ test. The p value < 0.05 was regarded as statistically significant and < 0.01, < 0.001 were taken as statistically highly significant.

Results

Modulatory effects of medications on concentration of glucose are depicted in Table 1 which revealed that a significant difference in the levels of blood glucose were present in intragroups on day 0 and on day 28 which was confirmed by applying t-test. Blood glucose concentration was significantly increased in group II as compared to control group. And in groups treated with glibenclamide and Eladi Churna at two different doses showed a significant decrement in blood glucose levels from the day 3 onwards. The results were highly significant in group IV and V as p < 0.001. Decrement in mean values of blood glucose of group V was almost comparable to group III. Similarly, in intergroup comparison, by applying one way Anova, it was found that a highly significant difference (p < 0.01) in blood glucose levels of different groups were present from day 3 onwards while it was non-significant (p > 0.05) on day 0. To compare the blood glucose levels of group II with that of group III, group IV and group V, post-hoc test was applied and it was seen that a highly significant difference in glucose levels were present from day 7 onwards or it can be said that there was a decrement in blood glucose levels on applying medicines, i.e., Glibenclamide and Eladi Churna at two different dose levels. And Eladi Churna at dose level 600 mg/kg showed results almost similar to that of glibenclamide treated group.

The results of serum cholestrol, serum high density lipoproteins (HDL), serum low density lipoproteins (LDL), serum triglycerides, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase activity (SGPT) are compiled in Table 2. The data depict that in the rats of group II, there was an increment in the mean values of above mentioned biochemical parameters while in control group it remained almost constant on day 0 and day 28. In treatment groups, i.e., group III, group IV and
group V, on day 0, the observed mean values were comparable to that of control group and on day 28, there was not much divergence in the values of biochemical parameters or it was nearly analogous to control group showed that even after administration of STZ, if there was an augmentation in biochemical parameters, it got normalized after administration of medications. The utmost significant decrease was observed in group V concerning total cholestrol (mean±sd=131.00±2.985) as p value is <0.05. Though both group IV and V were found to be responsible for maintenance of biochemical parameters in a requisite range but rats of group V were screening results approximately comparable to rats of group III, i.e., treated with standard antidiabetic drug glibenclamide. No significant results were obtained on the levels of SGOT and SGPT in all the treatment groups.

### Discussion

Diabetes mellitus is a complex and heterogeneous disorder presently affecting more than 100 million people worldwide and causing serious socio-economic problems. Animal models of diabetes are constructive and indispensable research tools to perceive the molecular basis, pathogenesis of complications, and the utility of therapeutic agents in diabetes. The most prominent diabetogenic chemical in diabetes research is streptozotocin (STZ). Administration of STZ at a dose of 35 mg/kg replicates the natural pathogenesis of Type II Diabetes mellitus which resembles to human disease with presence of insulin resistance. Following STZ injection, an acute triphasic response has been observed in rats: (a) early hyperglycemia at 2–4 h which may be due to mobilization of liver glycogen (b) hypoglycemia observed 6–10 h after injection due
to increased serum insulin levels and (c) permanent hyperglycemia from 24 h onwards, characterized by polyuria, glycosuria, hyperglycemia\textsuperscript{12,13}.

In this present research work, it was found that \textit{Eladi Churna} at two different dose levels showed the significant anti-hyperglycaemic potential in STZ induced diabetic rats. But the utmost significant reduction was observed with \textit{Eladi Churna} at 600 mg/kg body weight dose level and the results were almost comparable to that of standard drug glibenclamide treated group. The therapeutic effectiveness of \textit{Eladi Churna} can be alleged on the account of its individual ingredients. All the individual ingredients of \textit{Eladi Churna} (\textit{Ela}, \textit{Pippali}, \textit{Pashanbheda} and \textit{Shilajit}) possess significant anti-hyperglycaemic activities that have already been validated through various scientific researches. Fine powder suspension of \textit{Ela} (\textit{Elettaria cardamomum} L.) or queen of spices was studied on albino rats having hyperglycaemia due to induction of dexamethasone and its therapeutic efficacy was compared with pioglitazone (45 mg/kg). Cardamom showed comparable efficacy to pioglitazone in preventing dexamethasone induced hyperglycaemia\textsuperscript{14}. And also ethyl acetate extract of four different varieties of cardamom obtained from Mysore, Malabar, Vazhukka and Guatemala showed wide antioxidant potential and found to be effective against oxidative stress caused by hyperglycaemic condition in diabetics\textsuperscript{15}. Similarly research work done on oral administration of ethanolic extract of plant \textit{Pippali} (\textit{Piper longum} L.) showed that it restored the normal blood glucose levels in diabetic rats within 45 days, which indicates that the extract stimulated the activities of the liver to maintain the normal homeostasis of blood glucose during diabetes\textsuperscript{16}. And aqueous extract of the plant at a dose of 300 mg/kg of body weight, \textit{EC 600- Eladi Churna} with dose of 600 mg/kg of body weight).

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group I (NC)</th>
<th>Group II (DC)</th>
<th>Group III (SDG)</th>
<th>Group IV (EC 300)</th>
<th>Group V (EC 600)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholestrol mg/dl (Day0)</td>
<td>141.90±4.822</td>
<td>139.36±9.09</td>
<td>139.68±1.801</td>
<td>138.88±2.119</td>
<td>139.92±5.76</td>
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<td>Total Cholestrol mg/dl (Day28)</td>
<td>141.72±3.662</td>
<td>216.76±4.966 ***</td>
<td>134.06±5.119 #</td>
<td>120.66±7.810 *</td>
<td>131.00±2.985 **</td>
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<tr>
<td>HDL mg/dl (Day0)</td>
<td>39.92±8.075</td>
<td>39.62±8.075</td>
<td>38.32±1.409</td>
<td>38.58±5.21</td>
<td>38.82±1.110</td>
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<tr>
<td>HDL mg/dl (Day28)</td>
<td>40.38±1.293</td>
<td>34.42±1.543</td>
<td>37.04±7.23</td>
<td>38.88±6.90</td>
<td>36.94±1.006 *</td>
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<td>LDL mg/dl (Day0)</td>
<td>59.64±8.84</td>
<td>59.26±9.04</td>
<td>58.84±5.36</td>
<td>58.22±6.26</td>
<td>59.44±5.17</td>
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<td>LDL mg/dl (Day28)</td>
<td>59.12±9.14</td>
<td>70.26±1.348 ***</td>
<td>57.10±1.210 #</td>
<td>57.42±1.75</td>
<td>56.32±3.610</td>
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<tr>
<td>Triglycerides mg/dl (Day0)</td>
<td>101.96±2.51</td>
<td>102.12±4.43</td>
<td>102.20±1.224</td>
<td>103.26±1.219</td>
<td>101.98±4.65</td>
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<tr>
<td>Triglycerides mg/dl (Day28)</td>
<td>101.98±1.247</td>
<td>140.34±3.700 ***</td>
<td>101.16±2.352 #</td>
<td>120.20±5.711 *</td>
<td>101.84±1.346 **</td>
</tr>
<tr>
<td>SGOT IU/L (Day0)</td>
<td>30.72±8.16</td>
<td>31.88±2.068</td>
<td>31.46±1.043</td>
<td>30.48±1.311</td>
<td>32.16±9.04</td>
</tr>
<tr>
<td>SGOT IU/L (Day28)</td>
<td>33.66±1.86</td>
<td>39.80±6.20</td>
<td>31.32±1.439</td>
<td>34.12±1.084</td>
<td>31.64±9.71</td>
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<tr>
<td>SGPT IU/L (Day0)</td>
<td>27.88±4.91</td>
<td>28.22±8.78</td>
<td>27.58±3.56</td>
<td>28.32±1.071</td>
<td>27.86±5.77</td>
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<tr>
<td>SGPT IU/L (Day28)</td>
<td>28.56±5.89</td>
<td>33.92±7.29</td>
<td>28.68±5.93</td>
<td>29.98±3.56</td>
<td>29.94±5.17</td>
</tr>
</tbody>
</table>

(HDL - High Density Lipoproteins, LDL - Low Density Lipoproteins, SGOT- Serum Glutamate Oxaloacetate transaminase, SGPT- Serum Glutamate pyruvate transaminase, * p<0.05, ** p<0.01, ***p<0.001 (* in intra group comparison), #p<0.05, ## p<0.01, ### p<0.001 (# intergroup comparison of treatment groups with diabetic control group), NC- Normal Control, DC- Diabetic Control (No medication), SDG- Standard Drug Glibenclamide with dose of 1mg/kg of body weight, EC 300- Eladi Churna with dose of 300mg/kg of body weight, EC 600- Eladi Churna with dose of 600 mg/kg of body weight).
due to the stimulation of cells of pancreatic islets or mediated through stimulation of insulin release resembling the oral hypoglycemic sulphonylureas. And flavanoids present in root of plant also exhibits alpha-glucosidase inhibitory activity and acting as antidiabetic agents. The mineral Shilajit present in the herbomineral formulation is a complex natural mixture of organic (60-80%) and inorganic compounds (20-40%) along with presence of trace elements. Shilajit at a dose of 100mg/kg leads to significant reduction in blood glucose level and also shows improvement in lipid profile of alloxan induced diabetic rats. Aqueous extract of Shilajit exhibited DPPH radical-scavenging activity with IC50 value of 11.9 μg/mL and found to be helpful in diabetes associated oxidative stress and also inhibits lipid peroxidation.

As it is splendidly recognized that plant material are a complex mixture of different phytoconstituents which are principally accountable for a plant to exhibit its therapeutic potential. The individual plant drug mainly consists of flavanoids, alkaloids, tannins, terpenes, polyphenols, essential oil, etc. and mineral Shilajit chiefly depicts the presence of 14–20% humidity; 18–20% minerals; 13–17% proteins (with marked amylase activity); 4–4.5% lipids; 3.3–6.5% steroids; 18–20% nitrogen-free compounds; 1.5–2% carbohydrates; and 0.05–0.08% alkaloids, amino acids and other compounds. These constituents are known to possess glucose lowering affect by various mechanisms of actions.

Flavanoids regulate the glucose levels by inhibiting starch digestion, delaying the gastric emptying rate and reducing active transport of glucose across intestinal brush border membrane. Also inhibition of intestine sodium–glucose cotransporter-1 (Na-Glut-1) along with inhibition of α-amylase and α-glucosidase activity makes them a potential candidate in the management of hyperglycemia. Tannins have been observed to enhance the glucose uptake through mediators of the insulin-signaling pathways, such as PI 3K (Phosphoinositide 3-Kinase) and p38 MAPK (Mitogen-Activated Protein Kinase) activation and GLUT-4 translocation. Tannins are known to induce β cell regeneration and a direct action on adipose cells that enhances insulin activity. The mechanism of action of alkaloids include inhibition of aldose reductase, inducing glycolysis, preventing insulin resistance through increasing insulin receptor expression. Terpenes act by inhibiting the activity of α-amylase. α-amylases are mainly responsible to breakdown complex carbohydrates molecule in to glucose molecule and inhibition of these enzymes leads to decrement in production of glucose: hence helpful in lowering blood glucose concentration in diabetes mellitus. D-limonene, a monocyclic monoterpen was administered orally at doses of 50, 100 and 200 mg/kg body weight and glibenclamide at a dose of 600 g/kg body weight daily for 45 days in STZ induced diabetic rats. At the dose of 100 mg/kg the levels of blood glucose and HbA1c got decreased. As Eladi Churna consists of four ingredients, it can be said that due to synergistic action of a number of phytoconstituents with their specified mechanism of actions in the final preparation, i.e., Eladi Churna makes it a more persuasive antidiabetic agent in comparison to individual drug material. The results of drug have also been found to be analogous to that of glibenclamide. Glibenclamide is a second generation potent sulfonylurea oral antidiabetic drug. It performs its action by closure of ATP sensitive K+ channels (prevents K+ efflux) causing depolarization of membrane. This further opens the voltage dependent Ca channels, leads to Ca2+ influx. This Ca brings about release of insulin that is stored in granules of beta cells and also increases the sensitivity of peripheral tissues to insulin by increasing the number of insulin receptors. As Eladi Churna at 600 mg/kg showed the effects similar to that of glibenclamide. So it can also be assumed that Eladi Churna may possess the action similar to that of glibenclamide and reduces blood glucose levels by release of insulin from the pancreatic β cells and increasing the sensitivity of peripheral tissues to insulin and it also support the fact that STZ used at lower dose level induces Type II Diabetes mellitus by decreasing sensitivity of peripheral tissue to insulin instead of destructing β cells. And also showed positive effects on other biochemical parameters associated with diabetic condition such as alteration in lipid profile and liver functions because the individual drug material has also been documented for their hepatoprotective and hypolipidaemic activities.

Conclusion
From the above mentioned results, it can be concluded that oral administration of Eladi Churna in STZ induced diabetic rats showed a gradual remarkable reduction in blood glucose levels and also helpful in normalizing the alterations present in other biochemical parameters.
parameters such as serum cholesterol, HDL, LDL and triglycerides. The modulatory effect of the *Eladi Churna* was partial, but rather it was significant in a dose dependent manner, i.e., more significant at a dose level of 600 mg/kg and was nearly analogous to potent antidiabetic drug glibenclamide.

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**References**