ANTI DIABETIC ACTIVITY OF AAVARAIVITHAADHI CHOOoranAM IN ANIMAL MODELS

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ABSTRACT
In this modern era many chronic diseases are due to this lifestyle modification. One among the disease is the Diabetes mellitus which is a great threat worldwide. Siddha medicine plays an effective role in treating Diabetes mellitus. Aavarivaithaadhi Chooranam a siddha drug taken from the classical siddha literature used in the treatment of diabetes mellitus. The aim of the present study was to screen the drug for its acute toxicity in animal model and anti-diabetic activity in Streptozotozin induced diabetes in animal model Wistar albino rats. The animals were selected randomly and divided into four groups with six animals in each group. Group 1 taken as normal control, group 2 taken as diabetic control, group 3 taken as standard and group 4 taken as test drug Aavaraivithaadhi Chooranam (200mg/kg). The results were tabulated and it reveals the potency of the drug. The blood glucose level was reduced significantly which was almost equal to that of the standard and the body weight was also maintained. Acute toxicity study showed no toxic effect so it revealed the safety of the drug. Thus I conclude that the Siddha herbal formulation Aavaraivithaadhi chooranam is safe, potent and effective drug against diabetes mellitus and its complications.

Key words: Diabetes Mellitus, Streptozotozin, Anti-Diabetic, Blood glucose level.

INTRODUCTION
Siddha system of medicine is an ancient system of medicine found by Siddhars through their high intellectual, supernatural and spiritual powers. They strongly believed that healthy body is essential to attain the eternal life. In this modern era many chronic diseases are due to this
lifestyle modification. One among the disease is the Diabetes mellitus which is a great threat worldwide.

According to International Diabetes Federation (IDF) Report 2013, 382 million people have diabetes; by 2035 this will rise to 592 million 175 million people with diabetes are undiagnosed. Diabetes caused 5.1 million deaths in 2013; every six seconds a person dies from diabetes. More than 79,000 children developed type 1 diabetes in 2013. More than 21 million live births were affected by diabetes during pregnancy in 2013 [1]. The reasons behind this projected increase in prevalence rate are due to the sudden spurt in lifestyle modification, ethical susceptibility and vast urbanization increase in life expectancy at birth, physical inactivity and obesity and possibly a genetic predisposition [2].

Siddha medicine plays an effective role in treating Diabetes mellitus. Siddhars obtained the products from nature. According to Siddha Materia Medica the medicines are obtained from herbs, metals, minerals and animals products [3]. This paper explains about the study of Cassia auriculata L. (Cesalpinaceae, common name: Tanner’s Cassia) a common plant in Asia. It has been widely used in traditional system of medicine for treating rheumatism, conjunctivitis and diabetes [4]. The drug Aavaraivithaadhi Chooranam has Aavarai seed (Cassia auriculata seeds) as a key ingredient of the drug followed by nine herbal juices in which it is soaked. These juices also possess anti-diabetic action thus enhancing the potency and efficacy of the drug.

Diabetes being a metabolic disorder has to be managed throughout the life. The treatment is mainly based on oral hypo-glycaemic agents and insulin. They are also treated in Indian traditional medicine using anti-diabetic medicinal plants [5]. The oral hypoglycaemic agents taken as long term medication may bring many undesirable side effects. So there is a need for the drug which could be more potent and cause lower side effects to the mankind continues. So the aim of the study was to evaluate the drug “Aavaraivithaadhi Chooranam” for its anti-diabetic activity in animal model.

**MATERIALS AND METHODS**

The siddha herbal preparation Aavaraivithaadhi Chooranam was selected from the Classical Siddha literature [6].

**Ingredients:** Ingredients were listed in the Table 1
Table 1 herbal formulation Aavaraivithaadhi Chooranam

<table>
<thead>
<tr>
<th>S.No</th>
<th>INGREDIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Seeds of Aavarai (tanner’s cassia)</td>
</tr>
<tr>
<td>2</td>
<td>Juice of Atthippattai</td>
</tr>
<tr>
<td>3</td>
<td>Juice of Maruthampattai</td>
</tr>
<tr>
<td>4</td>
<td>Juice of Nellippazham</td>
</tr>
<tr>
<td>5</td>
<td>Juice of Thanneervittan kizhang</td>
</tr>
<tr>
<td>6</td>
<td>Juice of Vazhaik kizhang</td>
</tr>
<tr>
<td>7</td>
<td>Juice of Nerunjiver</td>
</tr>
<tr>
<td>8</td>
<td>Juice of Seendhirkodi</td>
</tr>
<tr>
<td>9</td>
<td>Juice of Sanbagapoo</td>
</tr>
<tr>
<td></td>
<td>Juice of Kattrazhai</td>
</tr>
</tbody>
</table>

Collection, Identification and Authentication of the Drug

Each and every plant materials were freshly collected from in and around Trichy, Tamilnadu. They were identified and authenticated by the Botanist and Gunapadam experts at Government Siddha Medical College, Arumbakkam, Chennai – 106. The specimen sample of all the herbs have been preserved in PG Gunapadam department individually for future reference.

Purification of the drugs

All the ingredients of this herbal formulation were purified according to the proper procedure methods described in Siddha classical literature. [7][8].

Preparation of the Drug

The seeds of Cassia auriculata were soaked and dried each day in the juices of 1 to 9 respectively. Then the seeds were dried in the shade until complete evaporation of the moisture content. It was finely powdered and kept in an air tight container. It was labeled as Aavaraivithaadhi Chooranam (AVC). Then the Chooranam was purified by steam boiling process according to the Siddha classical text. [9].

Animals

The animal model for this activity was the Wistar albino rat of either sex. The weight of each animal ranges from 120-200gm. The animals were purchased from the animal house of king institute of preventive medicine, Guindy, Chennai for the experimental study. The animals were kept in the ventilated room under normal laboratory condition with food and water ad libitum. The animals were acclimatized 2 weeks before they were exposed to the experiment.
The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (approval no: 1545/PO/a11/CPCSEA)

**Acute oral toxicity study**
The study was conducted as per the guidelines of Organization for Economic Cooperation and Development (OECD) \(^{[10]}\). AVC prepared as per the classical Siddha literature was suspended in 2% CMC with uniform mixing and was administered to the groups of Wistar albino rats. It is given in a in a single oral dose by gavage using a feeding needle. After the substance has been administered, food was withheld for a further 3-4 hours. Observations were made and recorded systematically and continuously observed as per the guideline. The toxicological effect was assessed on the basis of mortality. Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out

**Pharmacological activity**

**Screening the drug Aavaraivithaadhi Chooranam against Streptozotocin (STZ) induced Diabetes in Wistar albino Rats**

**Chemicals and drugs**
Streptozotocin used to induce diabetes in animal model and it was bought from Sisco Pharmaceuticals Limited, Mumbai. Glibenclamide was the reference standard for diabetes mellitus was obtained from Zydus Cadila, Ahmedabad. All the drugs and chemicals used in this study were of analytical grade.

**Methodology**

**Induction of Diabetes**
The Diabetes was induced to the animal model Wistar albino rat by injecting Streptozotocin. It was injected as intra peritoneal at the dose of 50mg/kg body weight. Streptozotocin was dissolved in 0.1M sodium citrate buffer (pH - 4.5) and this solution was injected. All the animals were randomly selected before the administration of the Streptozotocin injection and food was withdrawn overnight but water *ad libitum*. After the injection, all rats were access free to food and water. The animals were allowed to drink 5% glucose solution overnight to avoid hypoglycaemic shock. After 48hrs of Streptozotocin injection, the animals having fasting blood glucose level more than 200mg/dl were considered as diabetic rats and used for the experimentation\(^{[11][12]}\)
Experimental Design
Diabetic rats were divided into three groups with six animals in each group. Vehicle
Group I : Normal control received 2 ml Luke warm water which was the vehicle given for 15 days orally
Group II : Diabetic control received the vehicle for 15 days orally.
Group III : Diabetic rats treated with Standard Glibenclamide-5mg/kg orally once a day for 15 days.
Group IV : Diabetic rats treated with test drug Aavaraivithaadhi Chooranam 200 mg/kg body weight once a day orally for 15 days. The dose was established from the result of toxicity studies.

Blood collection
All the experimental rats were fasted overnight and the blood was withdrawn through puncturing retro orbital sinus on the 5th day, 15th day and 20th day of post induction period to determine blood glucose level by GOD-POD kit method. The change in body weight was observed throughout the treatment period in experimental animals.

Statistical Analysis
All the values were expressed as Mean ± S.E.M. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet’s t test. P<0.05 was considered as significant.

RESULTS AND DISCUSSION
Acute oral toxicity in rats – OECD 423
Wistar albino rat was treated with the test drug AVC of single dose of 2000mg/kg in 2%CMC as suspenstion. This study was conducted as per the OECD guidelines. The acute toxicity result shows no mortality rate up to 2000mg/kg. The behavioural changes are normal. Hence the test drug Aavaraivithaadhi Chooranam is a safe herbal drug and can be used for long time administration.

Results of Anti-Diabetic activity
The Anti-Diabetic activity of the test drug AVC was done in the Streptozotozin induced diabetes in animal model Wistar albino rat and the results were noted. The effect of AVC on blood sugar level in Streptozotocin induced diabetic rats from initial, 5th, 15th and 20th day of the treatment were specified in table no 2 and the figure 1 was the representation of the table
2. The table 3 was the effect of AVC on body weight in Streptozotocin induced diabetic rats and the figure 2 was the representation of the Table 3

**Table 2**: Effect of *Aavaraivithaadhi Chooranam* on blood sugar level in Streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Blood glucose level (mg/dl) on post induction days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>70.90±2.50</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>263.54±1.98++</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic rats + Glibenclamide</td>
<td>264.72±1.88</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic rats+ AVC-200mg/kg</td>
<td>271.10±1.54</td>
</tr>
</tbody>
</table>

Values were expressed as Mean±SEM, n=6, Diabetic control was compared with normal control rats - Values are statistically significant at +P<0.05, ++P<0.01, +++P<0.001 Experimental groups(III & IV) were compared with diabetic control rats - Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001

![](https://example.com/image.png)

Fig 1. Showing the effect of AVC on blood glucose level in Wistar albino rats.
NC-Normal Control; DC- Diabetic Control; STD- Standard (Glibenclamide); AVC- Aavaraivithaadhi Chooranam
Table. No 2: Effect of Aavaraivithaadhi chooranam on body weight in Streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Body weight (g) on post induction days</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>Initial: 165.41±2.60 5th day: 167.24±2.63 15th day: 170.10±2.76 20th day: 171.41±2.10</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic Control</td>
<td>Initial: 165.56±2.55 5th day: 162.56±2.07 15th day: 136.82±1.82++ 20th day: 125.60±1.59++</td>
</tr>
<tr>
<td>III</td>
<td>Diabetic rats + Glibenclamide</td>
<td>Initial: 162.51±2.07 5th day: 164.56±2.49 15th day: 169.04±2.67* 20th day: 177.33±2.70**</td>
</tr>
<tr>
<td>IV</td>
<td>Diabetic rats + AVC-200mg/kg</td>
<td>Initial: 167.37±1.92 5th day: 170.83±1.25 15th day: 174.16±2.68* 20th day: 176.50±2.28**</td>
</tr>
</tbody>
</table>

Values were expressed as Mean±SEM, n=6, Diabetic control was compared with normal control rats - Values are statistically significant at +P<0.05, ++P<0.01, +++P<0.001. Experimental groups (III & IV) were compared with diabetic control rats - Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001.

Discussion of Anti-Diabetic activity of Aavaraivithaadhi Chooranam

The Anti-Diabetic Activity of the test drug Aavaraivithaadhi Chooranam has been estimated in the streptozotocin induced diabetes in Wistar albino rat. Administration of the streptozotocin effectively induced diabetes mellitus in the animal model which is known by the increased glucose level.
STZ was considered slightly cytotoxic agent which affects pancreatic beta cells \[^{13}\] , STZ selectively destroys the pancreatic beta cells which secrete insulin. So there is less active cells which lead to diabetes mellitus \[^{14}\] . Thus it is widely used to induce diabetes in animal models. It also interferes with cellular metabolic oxidative mechanisms.

Oral administration of the test drug AVC taken in the dose of 200mg/kg showed significant decrease in the sugar level in 15\(^{th}\) and 20\(^{th}\) day. The drug Glibenclamide is taken as the standard drug which is used for many years in treating diabetes. The test drug shows a significant change and it is almost equal to that of the standard drug.

The possible mechanism by which the test drug AVC brings about a decrease in the blood sugar may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from \(\beta\)-cells of the islets of Langerhans or its release \[^{15}\] . Its effect on the disease may be due to the presence of phytochemical components like flavonoids, alkaloids, glycosides etc., and some biochemical components like zinc, magnesium, etc.,

Body weight of the animal models in this study was also monitored. There is decrease in the body weight of the animal treated with the control. Whereas the animal treated with AVC shows a significant improvement in the body weight in the 15\(^{th}\) and 20\(^{th}\) day. Thus the trial drug not only reduced the sugar level but also maintains the body weight in Diabetes Mellitus.

**CONCLUSION**

AVC is a siddha herbal formulation from the siddha classical literature used in the treatment of diabetes. This drug was screened for its Anti-diabetic activity in Streptozotocin induced diabetes in animal model Wistar albino rats. The result showed significant reduction in the blood sugar level and the body weight was also maintained. These results confirmed the use of AVC in traditional system of medicine to treat diabetes in India. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of AVC and the drug bear the potential for further research.

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