“AAVARAI KUDINEER”- A POTENT POLYHERBAL SIDDHA FORMULATION FOR MANAGEMENT OF DIABETES MELLITUS

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ABSTRACT

Diabetes mellitus is caused due to deficiency in production of insulin. It is a global problem and number of those affected is increasing day by day. Oral hypoglycemic agents use is restricted by their pharmacokinetic properties, secondary failure rates, and accompanying side effects and the world health organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated. Management of Diabetes mellitus in the Traditional, Indian, Siddha system of medicine is time tested. Many Siddha anti-diabetic formulations are reputed and popular. Many of the Siddha anti-diabetic formulations have been scientifically studied. Literature survey revealed that Siddha “Kudineer” anti-diabetic formulations are scientifically under explored. Siddha “Kudineer” anti-diabetic formulations are tailor made, effective, tried and trusted. Many of them are official also. They are economical. They can be prepared by the himself. This review work is a small step towards, scientifically studying the Siddha “Aavarai Kudineer” anti-diabetic formulation, so as to standardize and improve the formulation for the benefit of mankind.

Keywords: Diabetes mellitus, Siddha system of medicine, Aavarai Kudineer.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. Type I diabetes (insulin dependent) is caused due to insulin insufficiency because of lack of functional β-cells. Patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication [1]. 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. More than 80% of diabetes deaths occur in low- and middle-income countries. WHO projects those diabetes deaths will double between 2005 and 2030.

Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of type 2 diabetes. Over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves. Diabetes increases the risk of heart disease and stroke. 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Combined with reduced blood flow, neuropathy in the foot increases the chance of foot ulcers and eventual limb amputation. Diabetic retinopathy is an important cause of blindness and occurs as a result of long-term accumulated damage to the small blood vessels in the retina. After 15 years of diabetes, approximately 2% of people become blind and about 10% develop severe visual impairment. Diabetes is among the leading causes of kidney failure. 10-20% of people with diabetes die of kidney failure [2].

Wide arrays of plant derived active principles representing numerous phytochemicals have demonstrated consistent hypoglycemic activity and their possible use in the treatment of diabetes mellitus2. In the traditional system of Indian medicinal plant formulation and several cases, combined extracts of plants are used as drug of choice rather than individual. Many of these

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have shown promising effect [3].

Diabetes Mellitus is a condition which can be compared with Neerizhivu in Siddha. The other names described in the texts are the Madhumegham and Nippunneer. The signs and symptoms explained is increased urination both in frequency and quantity, there will be flies surrounding the urine voided place, weight loss, dryness of the skin, etc.

In Siddha, the management of a disease not only depends on the medicine but the modification of food, habits, and lifestyle also. There are several medicines said in the literatures and practiced successfully by Siddha practitioners. The regulations in food, daily habits etc. are the specialty of most of these medicines. Some commonly used medicines are
1. Madhumegha choornam
2. Seenthil choornam
3. Naval choornam
4. Seenthil Kudineer
5. Aavarai kudineer
6. Abraga Pappam
7. Vanga parpam etc.,

In addition to the prepared medicines there are several herbal combinations said in the texts for the management of this disease. All these medicines are to be used with the prescription of a Siddha medical practitioner and with proper regimen. These medicines include several plants with anti-diabetic property like Jambolinor Naval (Syzygium cuminii)
Sarkarai Kolli (Gynmema Sylvestre)
Kadalazhinjil or Eganayakam (Salacia reticulate)
Seenthil or Amrithu (Tinospora cordifolia)
Vilwam (Aigil marmalose) etc [4],

Siddha systems of medicines are more effective to control the type-2 diabetes. Yoga, traditional food and siddha medicines to control and prevent diabetes. In which kudineer is one of most important polyherbal formulations equally referred to khashayas in Ayurveda are more useful to prevent the diabetes and their associated complications. Besides, these formulations are only time tested, not scientifically proven and the ingredients are not well established in their scientific terms. Keeping the above information in view, it was thought worthy to study selected antidiabetic Siddha Kudineer polyherbal formulations to create scientific evidence.

SELECTION OF KUDINEER FORMULATION

Among the several anti-diabetic Siddha kudineer formulations in theraiyar’s kudineer in the ancient Siddha literature, the Aavarai kudineer have been selected for this current research. Since it is very popular, reputed and the ingredients have been proved to be useful in the management of diabetes mellitus. It contains the following ingredients [5].

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aavarai ilai (Cassia auriculata, Leguminosae)</td>
<td>1 part</td>
</tr>
<tr>
<td>Kondrai ilai (Cassia fistula, Caesalpiniaceae)</td>
<td>1 part</td>
</tr>
<tr>
<td>Naval kottai (Syzygium cumini Fam:Myrtaceae)</td>
<td>1 part</td>
</tr>
<tr>
<td>Kadal azhingil (Salacia reticulata, Celastraceae)</td>
<td>1 part</td>
</tr>
<tr>
<td>Korai kizhangu (Cyperus rotundus, Cyperaceae)</td>
<td>1 part</td>
</tr>
<tr>
<td>Koshtam (Costus spicicous, Costaceae)</td>
<td>1 part</td>
</tr>
<tr>
<td>Maratham pattai (Terminalia arjuna, Combretaceae)</td>
<td>1 part</td>
</tr>
</tbody>
</table>

1) Cassia auriculata Linn. [6-7]

Biological Source & Family: Cassia auriculata (Caesalpinaceae)

Habitat: Wild in dry regions of Madhya Pradesh, Tamil Nadu and Rajasthan. Cultivated in other parts of India.

Botanical description: Avaram (Cassia auriculata Linn), family Caesalpiniaceae, is also known as Avaram tree. The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5 cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5 cm long 1-1.3 cm broad, slightly overlapping, oval oblong, obtuse, at both ends, mucronate, glabrous or minutely downy, dull green, paler beneath, stipules very large, reniform-rotund, produced at base on side of next petiole into a filliform point and persistent. Its flowers are irregular, bisexual, bright yellow and large (nearly 5 cm across), the pedicels glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, crowded in axils of upper leaves so as to form a large terminal inflorescence (leaves except stipules are suppressed at the upper nodes). The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than the inner ones. The petals also number 5, are free, imbricate and crisped along the margin, bright yellow veined with orange. The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules.

The fruit is a short legume, 7.5–11 cm long, 1.5 cm broad, oblong, obtuse, tipped with long style base, flat, thin, papery, undulately crimpled, pilose, pale brown. 12-20 seeds per fruit are carried each in its separate cavity.

Chemical constituents: Pod husk contains nonacosane and nonacosen-6-one, chrysophanol, eomdin and rubadin.
Medicinal uses: Roots are used in skin diseases and asthma. The flowers are used in diabetes, urinary disorders and nocturnal emissions. The bark is used as astringent and the leaves and flowers were used as anti-diabetic.

2) *Cassia fistula* Linn. [8-11]

**Biological Source & Family:** *Cassia fistula* (Caesalpinaceae)

*Cassia fistula* Linn. (Caesalpinaceae)

**Habitat:** Cultivated as an ornamental throughout India.

**Botanical description:** A large, branched shrub or small tree. Leaves wholly glabrous with yellowish or greenish – grey bark. Leaves paripinnate, 20-40 cm. long, leaflets 4-8 pairs, 5-16 x 4-7 cm. distinctly stalked, ovate or ovate-oblong, acute or acuminate, entire, coriaceous. Flowers bright-yellow, 30-55 cm. long, in axillary, pendulous lax racemes. Bracts minute, caducous. Calyx 5-partite, glabrous, caduceus. Stamens- 10, all with anthers. Pods 30-60 cm. long, cylindrical, pendulous, indehiscent, smooth, hard, dark brown or black, transversely divided or ribbed into numerous 1-seeded cells. Seeds embedded in soft, sweet, albuminous pulp.

**Chemical constituents:** The plant contains Rhein, aloe-emodin, kaempferol and emodin.

**Medicinal uses:**

Flowers and pods- purgative, febrifugal, astringent and antibilious. Seed powder used in amoebiasis. Leaves contain Laxative property.

3) *Syzygium cumini* (Linn.) Skeels. [12-15]

**Biological Source & Family:** *Syzygium cumini* (Myrtaceae)

*Syzgium cumini (Linn.) Skeels.* (Myrtaceae)

**Habitat:** Cultivated throughout India up to 1,800 m.

**Botanical description:** A moderate-sized, branched tree with dull-white or grey bark. Leaves oblong-lanceolate, falcately acuminate, petiolate, entire. Flowers whitish-green in short panicles with stouter branched. Berry globose, Ovoid-oblong, smaller in size.

**Chemical constituents:** Jambolan is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin.

**Medicinal uses:**

Fruit are stomachic, carminative and diuretic. The bark and seed: anti-diarrhoeal. Seed: hypoglycaemic. Leaf: antibacterial, anti-dysenteric.

4) *Salacia chinensis* Linn. [16-17]

**Biological Source & Family:** *Salacia chinensis* (Hippocrateaceae/Celastraceae)

*Salacia chinensis* Linn. (Celastraceae)

**Habitat:** A large, climbing shrub or small tree occurring throughout India, including the Andaman Islands. The plant is common in sacred groves and along hedges.

**Botanical description:** It is a straggling shrub with deep yellow coloured root, leaves up to 7.5 X 3 cm, oblong or ovate, crenate – serrate, obtusely-acuminate at apex, coriaceous, glabrous, flowers 6 mm across, honey – scented, fascicled on axillary tubercles, pedicels ca 12 mm long. Calyx has puberulous outside. Petals are yellowish. Stamens reflexed when the flower is open over the conical disk.

**Chemical constituents:** The root bark contains proanthocyanidins, consisting of monomeric leucopelargonidin, its monomer, dimer and tetramer; triterpenoids (friedelin and its derivatives), mangiferine, phlobatannin and glucosidal tannins. The stem yielded gutta, dulcitol and proanthocyanidin consisting of dimer of leucopelargonidin.
Medicinal uses: Roots- used in diabetes. Also used for amenorrhea, dysmenorrhea and venereal diseases.

5) *Costus speciosus* (Koenig) Sm. [18-20]

**Biological Source & Family:** *Costus speciosus* (Coataceae)

**Habitat:** Assam, North Bengal, Khasi and Jaintia Hills, sub Himalayan tracts of Uttar Pradesh and Himachal Pradesh and Western Ghats.

**Botanical description:** An erect, perennial, 2-4 ft. high herb with tuberous, horizontal, rhizomatous, root stock. Leaves 15-30 x 5-8.5 cm., sub sessile or very shortly petioled, oblong-ovate to oblanceolate, base rounded, acute or acuminate, sometimes caudate apex, dark-green, glabrous above and appressed pubescent beneath, white ciliate margined, sheath coriaceous, ligule none. Flowers showy, white with red bracts, many arranged in 4-10 cm. long, terminal, dense flowered spikes. Bracts bright-red, showy, oblong-lanceolate, 2.5-3.5 x 1-1.5 cm., bracteoles red. Calyx long, deltoid-ovate, cuspidate. Corolla white with yellowish centre, tube usually equal to the calyx, lobes 4.5 x 1.5-2 cm. obovate-oblanceolate, apiculate. Stamens filament with a tuft of hairs at their base, staminodes none. Style long, stigmas with a semi-lunar ciliate mouth. Capsule 3-gonous, bright red. Seeds black.

**Chemical constituents:** The rhizomes contain saponins-dioscin, gracillin, diosgenin, beta-sitosterol and beta-D-glucoside.

**Medicinal uses:** Astringent, purgative, depurative, anti-inflammatory (used in gout, rheumatism; bronchitis, asthma, catarrhal fevers, dysuria), anthelmintic, antiermin, maggoticide, antifungal. The alkaloids show papaverine-like smooth-muscle-relaxant activity, cardiotoxic activity like that of digitalis and antispasmodic.

6) *Cyperus rotundus* Linn.[21-22]

**Biological Source & Family:** *Cyperus rotundus* (Cyperaceae)

**Habitat:** Throughout India.

**Botanical description:** An erect, perennial with woody, subterranean, stoloniferous rhizome often clothed with fibrous remains of leaf-sheaths. Stem 15-60 cm. high, triquongous. Leaves basal, usually shorter than the stem, narrowly linear, finely acuminate. Inflorescence an umbel of condensed spikes. Bracts- 3, foliar, unequal, exceeding the umbel, longest one up to 15 cm. long. Spikelets pale- yellow and brown with reddish tinge, 4-1- on a spike. Glumes 3-4 mm. long, decurrent on the rachilla, ovate or boat shaped. Stamens-3, anthers apiculate. Style 0.4 cm. long Nuts ovoid-ellipsoid with 3- flat or concave sides, glabrous.

**Chemical constituents:** The plant contains essential oils, flavonoids, terpenoids, mono- and sesquiterpenes. Such as Cyproteone, cypera-2, 4-diene, a-copaene, cyperene, aselinene, rotundene, valencene, ylanga-2, 4-diene, g-gurjunene, trans-calamenene and d-cadinene.

**Medicinal uses:** Carminative, astringent, anti-inflammatory, antirheumatic, hepatoprotective, diuretic, antispetic, analgesic, hypotensive and nervine tonic.

7) *Terminalia arjuna* (Roxb.) W. & A. [23-25]

**Biological Source & Family:** *Terminalia arjuna* (Combretaceae)

**Habitat:** Throughout the greater part of India, also grown as an avenue tree.
**Botanical description:** A medium-sized or large handsome tree with grey bark. Leaves 8-15 x 4-7 cm, sub-opposite, oblong or elliptic, obtuse, acute, with 1 or 2 glands at the base of lamina or on the petiole, narrowed towards the base, slightly crenulate. Flowers light-yellow, in terminal or axillary, pendulous, often panicked spikes. Bracts very small. Young ovary pubescent. Fruit ovoid or obovoid, oblong, wood with 5-7 hard, coriaceous, narrow wins.

**Chemical constituents:** It contains Beta-sitosterol, ellagic acid and arjunic acid.

**Medicinal uses:** Bark is used as a cardio protective and cardio tonic in angina and poor coronary circulation; as a diuretic in cirrhosis of liver and for symptomatic relief in hypertension. It is used externally in skin diseases, herpes and leuoderma.

**CONCLUSION**

In the traditional medicinal practice number of anti-diabetic herbal drug(s) and their formulations are widely used by the physicians. They are prescribed from time to time to the patients according to patient’s need. Therefore many of the formulations are not well established in their scientific parameters. One such formulation is Siddha kudineer formulation “Aavarai Kudineer” which are highly prescribed but not standardized by scientific methods. Development of scientific evidence for these formulations may pave a pathway to use and or export of this potential drug globally.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors do not have any conflict of interest for this review work on Aavarai kudineer.


PROMISING INVITRO ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF “AAVARAI KUDINEER” - A POTENT POLYHERBAL SIDDHA FORMULATION

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³Professor and Head, Department of Pathology, Sri Ramachandra Medical College & Research Institute, Sri Ramachandra University, Porur, Chennai-600 116, Tamil Nadu, India.

ABSTRACT

Diabetes mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction. It is a global problem and number of those affected is increasing day by day. Currently used oral hypoglycemic agents use is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects. Many Siddha antidiabetic formulations are reputed, popular but scientifically under-explored. One such formulation is Aavarai kudineer which is tailor made, effective, tried and trusted. α-glucosidase and α-amylase inhibitors from plant sources offer an attractive strategy for the control of post prandial hyperglycemia in type –II diabetes mellitus. Thus this research work is a small step towards, scientifically studying the Siddha “Aavarai kudineer” antidiabetic formulation, by invitro α-glucosidase and α-amylase inhibitory methods so as to standardize and improve the formulation for the benefit of mankind. From this study, the formulation has been proved to be a significant antidiabetic and antioxidant through invitro studies.

Key words: Diabetes mellitus, Siddha system of medicine, Aavarai kudineer, Invitro antidiabetic, Antioxidant activity.

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder due to defects in carbohydrate metabolism. 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. More than 80% of diabetes deaths occur in low- and middle-income countries, WHO projects those diabetes deaths will double between 2005 and 2030. Type I diabetes (insulin dependent) is caused due to insulin insufficiency because of lack of functional β-cells. Patients suffering from this are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medication (Frier BM and Fisher M, 2006).

Healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use can prevent or delay the onset of type II diabetes. Over time, diabetes can damage the heart, blood vessels, eyes, kidneys, and nerves. Diabetes increases the risk of heart disease and stroke. 50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Combined with reduced blood flow, neuropathy in the foot increases the chance of foot ulcers and eventual limb amputation. Diabetic retinopathy is an important cause of blindness and occurs as a result of long-term accumulated damage to the small blood vessels.

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in the retina. After 15 years of diabetes, approximately 2% of people become blind and about 10% develop severe visual impairment. Diabetes is among the leading causes of kidney failure. 10-20% of people with diabetes die of kidney failure (Davis SN and Granner DK, 2001). Wide arrays of plant derived active principles representing numerous phytochemicals have demonstrated consistent hypoglycemic activity and their possible use in the treatment of diabetes mellitus. In the traditional system of Indian medicinal plant formulation and several cases, combined extracts of plants are used as drug of choice rather than individual. Many of these have shown promising effect (Mukherjee PK et al., 2006). There are several medicines said in the literatures and practiced successfully by Siddha practitioners. The regulations in food, daily habits etc. are the speciality of most of these medicines. In addition to the prepared medicines there are several herbal combinations said in the texts for the management of this disease. All these medicines are to be used with the prescription of a Siddha medical practitioner and with proper regimen. In which kudineer is one of most important polyherbal formulations equally referred to khashayas in Ayurveda are more useful to prevent the diabetes and their associated complications. Besides, these formulations are only time tested, not scientifically proven and the ingredients are not well established in their scientific terms (Anonymous, 2011). Keeping the above information in view, Aavarai kudineer formulation (AKF) is selected to study the antidiabetic and antioxidant activity so as to create scientific evidence (Anonymous 1979).

MATERIALS AND METHODS

a) In-vitro α-Glucosidase Inhibitory Activity

Yeast α-glucosidase was dissolved at a concentration of 0.1 U/ml in 100 mM phosphate buffer, pH 7.0, containing bovine serum albumin 200 mg/ml and sodium azide 200 mg/ml which was used as enzyme source; p-nitrophenyl-α-D-glucopyranoside were used as substrate. Weighed Aavarai Kudineer Formulation (AKF) was made into a concentration of 1 mg/ml and serial dilutions of 500, 250, 125, 62.5, 31.25μg/ml were made with equal volumes of DMSO and distilled water. 10μl of AKF dilutions were made into a microplate reader (Hamdan II and Afifi FU, 2004). Percent α-glucosidase inhibition was calculated as follows:

\[
\% \text{Inhibition} = \left(\frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}}\right) \times 100
\]

The inhibitory concentration of the AKF required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by graphical method. Experiments were performed in triplicate. Acarbose were dissolved in distilled water and serial dilutions were done and it was used as positive control.

b) In-vitro α-amylase Inhibitory Activity

A total of 500 μl of test samples and standard drug (31.25-500μg/ml) were added to 500 μl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 μl of a 1% starch solution in 0.20 M phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm (Thalapaneni NR et al 2008). Control represent 100% enzyme activity and were conducted in similar way by replacing AKF with vehicle.

\% \text{Inhibition} = 100 - \% \text{Reaction}

For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The concentration of AKF required to inhibit the activity of the enzyme by 50% (IC_{50}) was calculated by graphical method. Experiments were performed in triplicate (Heidari R et al 2005).

c) DPPH radical scavenging activity

In the DPPH test, the antioxidants reduce the purple coloured DPPH radical (2, 2-diphenyl-1-picrylhydrazyl hydrate) to a yellow-coloured 2, 2-diphenyl-1-picrylhydrazine and the extent of the reaction depended on the hydrogen donating ability of the antioxidants. The changes in colour (from deep-violet to light-yellow) was measured at 517 nm wavelength. As DPPH is sensitive to light, it is exposed to minimum possible light (James O and Jacob BO 2010).

![DPPH radical scavenging activity](image-url)

Procedure
1 ml each of different concentrations (500, 250, 125, 62.5, 31.25 µg/ml in DMSO and distilled water) of AKF and standard (Ascorbic acid) were added to 2.5 ml of a 0.3 mM DPPH-methanol solution. The mixture should be shaken vigorously and allowed to stand at room temperature in the dark for 30 mins. The absorbance was measured at 517 nm. The % DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical Scavenging activity} = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100$$

Where, \(A=\text{Absorbance}\).

Lower absorbance of the reaction mixture indicates higher radical scavenging activity. All the tests were performed in triplicate and the graph were plotted with the mean values. The concentration of sample required to scavenge 50% of DPPH (IC\(_{50}\)) was determined by graphical method (Badami S et al 2003).

d) Nitric oxide scavenging method

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess illosvoy reaction. In the present investigation, Griess illosvoy reagent is modified by using naphthyl ethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). Scavengers of nitric oxide compete with oxygen leading to reduce the production of nitric oxide (Ardestani A and Yazdanparast R, 2007).

Procedure

The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and AKF and standard solution (0.5 ml) were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite were pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylenediamine-di-hydrochloride (0.1%) was added, mixed and allowed to stand for 30 min. A pink colored chromophore was formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions (Jie Yin et al 2008). The amount of nitric oxide radical inhibited by the extract was calculated using the following equation:

$$\% \text{ of Scavenging activity} = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100$$

Where \(A=\text{Absorbance}\).

RESULTS

Table 1. \(\alpha\)-Glucosidase Inhibitory Activity of aqueous extract of AKF

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (µg/ml)</th>
<th>Log conc.</th>
<th>Standard (Acarbose)</th>
<th>AKF extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% of Inhibition (Mean ± SEM)</td>
<td>IC(_{50}) (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>31.25</td>
<td>1.4948</td>
<td>31.56 ± 0.460</td>
<td>25.06 ± 0.045</td>
</tr>
<tr>
<td>2</td>
<td>62.5</td>
<td>1.7958</td>
<td>53.20 ± 0.400</td>
<td>49.41 ± 0.005</td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>2.0969</td>
<td>62.03 ± 0.015</td>
<td>58.72 ± 0.030</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>2.3979</td>
<td>73.07 ± 0.050</td>
<td>74.72 ± 0.015</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>2.6989</td>
<td>81.87 ± 0.050</td>
<td>64.0 ± 0.23</td>
</tr>
</tbody>
</table>

Data were expressed as Mean ± SEM, (n=3)

Table 2. \(\alpha\)-Amylase Inhibitory Activity of aqueous extract of AKF

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (µg/ml)</th>
<th>Log conc.</th>
<th>Standard(Acarbose)</th>
<th>AKF extract</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% of Inhibition (Mean ± SEM)</td>
<td>IC(_{50}) (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>31.25</td>
<td>1.49485</td>
<td>43.75 ± 0.225</td>
<td>33.81 ± 0.225</td>
</tr>
<tr>
<td>2</td>
<td>62.5</td>
<td>1.79588</td>
<td>59.22 ± 0.370</td>
<td>49.35 ± 0.015</td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>2.09691</td>
<td>69.20 ± 0.33</td>
<td>58.72 ± 0.030</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>2.39794</td>
<td>77.22 ± 0.133</td>
<td>69.02 ± 0.452</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>2.69897</td>
<td>93.97 ± 0.356</td>
<td>82.92 ± 0.371</td>
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</tbody>
</table>

Data were expressed as Mean ± SEM, (n=3)
Table 3. DPPH scavenging activity of aqueous extract of AKF

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (µg/ml)</th>
<th>Log conc.</th>
<th>Standard (Ascorbic acid)</th>
<th>AKF extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Scavenging (Mean ± SEM)</td>
<td>IC₅₀ (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>31.25</td>
<td>1.49485</td>
<td>39.06±0.370</td>
<td>47.37±0.22</td>
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<tr>
<td>2</td>
<td>62.5</td>
<td>1.79588</td>
<td>58.74±0.130</td>
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<tr>
<td>3</td>
<td>125</td>
<td>2.09691</td>
<td>64.35±0.375</td>
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</tr>
<tr>
<td>4</td>
<td>250</td>
<td>2.39794</td>
<td>72.03±0.050</td>
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<tr>
<td>5</td>
<td>500</td>
<td>2.69897</td>
<td>89.87±0.210</td>
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</tbody>
</table>

Data were expressed as Mean ± SEM, (n=3)

Table 4. Nitric oxide scavenging activity of aqueous extract of AKF

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Concentration (µg/ml)</th>
<th>Log conc.</th>
<th>Standard (Ascorbic acid)</th>
<th>AKF extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Scavenging (Mean ± SEM)</td>
<td>IC₅₀ (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>31.25</td>
<td>1.49485</td>
<td>41.83 ± 0.19</td>
<td>47.87±0.15</td>
</tr>
<tr>
<td>2</td>
<td>62.5</td>
<td>1.79588</td>
<td>57.07 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>125</td>
<td>2.09691</td>
<td>69.98 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>2.39794</td>
<td>73.54 ± 0.01</td>
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</tr>
<tr>
<td>5</td>
<td>500</td>
<td>2.69897</td>
<td>82.21 ± 0.21</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as Mean ± SEM, (n=3)

Table 5. Comparison of IC₅₀ value of In-vitro antidiabetic activity:

<table>
<thead>
<tr>
<th>Methods used</th>
<th>IC₅₀ value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard (Acarbose)</td>
</tr>
<tr>
<td>α-glucosidase inhibitory method</td>
<td>41.82±0.28</td>
</tr>
<tr>
<td>α-amylase inhibitory method</td>
<td>56.92±0.20</td>
</tr>
</tbody>
</table>

Table 6. Comparison of IC₅₀ value of In-vitro antioxidant activity:

<table>
<thead>
<tr>
<th>Methods used</th>
<th>IC₅₀ value (µg/ml)</th>
<th>Standard (Ascorbic acid)</th>
<th>Test drug (AKF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH</td>
<td>47.87±0.15</td>
<td>47.37±0.22</td>
<td>70.05±0.28</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td></td>
<td></td>
<td>61.30±0.12</td>
</tr>
</tbody>
</table>

Figure 1. Comparison α-Glucosidase Inhibitory activity of aqueous extract of AKF with standard Acarbose

Figure 2. Comparison α–Amylase inhibition activity of aqueous extract of AKF with standard Acarbose
DISCUSSION

**In-vitro antidiabetic screening of AKF by α-glucosidase & α-amylase inhibitory methods**

Lack of insulin affects the metabolism of carbohydrates, proteins, fat and causes significance disturbance in metabolic function (Neelesh M et al., 2010). Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes and is more strongly associated with the risk for cardiovascular diseases than are fasting blood glucose. α-glucosidase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation (Puls W et al., 1997). α-glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption. Acarbose are competitive inhibitor of α-glucosidases and reduces absorption of starch and disaccharides (Mukherjee PK et al., 2006).

The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faeces in the colon. This finding reveals that aqueous extract of AKF effectively inhibits α-glucosidase and α-amylase enzyme by in vitro method when compared with standard (Acarbose) at different concentrations in serial dilution 500, 250, 125, 62.5, 31.25µg/ml showed significant (P<0.05) antidiabetic activity (Table 5).

**In-vitro antioxidant activity by DPPH and Nitric oxide free radical scavenging methods**

DPPH is usually used as a substrate to evaluate antioxidant activity. DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. This method is based on the reduction of purple colored methanol solution of DPPH in the presence of hydrogen donating antioxidants, by the formation of yellow colored non radical form of DPPH. Lower the absorbance higher the free radical scavenging activity. Aqueous extract of AKF were able to reduce purple colored DPPH to yellow colored picryl hydrazine at different concentrations in serial dilution 500, 250, 125, 62.5, 31.25µg/ml showed significant (P<0.05) antioxidant activity.

Nitric oxide is implicated in diseases such as cancer and inflammation. It also mediates smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated cytotoxicity. Sodium nitroprusside spontaneously generates nitric oxide at physiological pH, in aqueous solutions. The nitric oxide, generated is converted into nitric and nitrous acids on contact with dissolved oxygen and water. The liberated nitrous acid was estimated using a modified Griess-Iloslov method. Nitrous acid reacts with Griess reagent, to form a purple azo dye. In presence of antioxidants, the amount of nitrous acid will decrease and the degree of decrease in the formation of purple azo dye will reflect the extent of scavenging. Aqueous extract of AKF when compared with standard (Acarbose) at different concentrations in serial dilution 500, 250, 125, 62.5, 31.25µg/ml showed significant (P<0.05) antioxidant activity (Table 6).
CONCLUSION

The Siddha system of medicine is a traditional Indian system of medicine. Among the various Siddha formulations useful in the treatment of diabetes mellitus, “Aavarai Kudineer” (AKF) is a reputed formulation. AKF is, “make your own medicine” type, formulation. It is freshly prepared and consumed. Lot of variation has been observed in the preparation of AKF; hence to avoid the variation and to have reproducible results a small step has been taken through the raw materials and finished product of this formulation have been standardized. This study will be helpful in maintaining the quality of AKF. Further the formulation has been proved to be an effective antidiabetic through in-vitro studies. Also the selected formulation was found to possess excellent antioxidant properties. These studies will help the formulation attain the quality standards at par with their modern medicine counterparts.

ACKNOWLEDGEMENT

The authors are sincerely thanked the Dean, Mother Theresa PG & RI of Health Sciences, Puducherry for his kind support and constant engagement for carrying out this research work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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James O, Jacob BO. Evaluation of bio-safety and antioxidant activity of the fruit and leaf of Saba florida (Benth.) from Ibaji forest. International Journal of Medicine and Medical Sciences. 2010; 2(3): 100-05.
Jie Yin, Seong- II Heo and Myeong-Hyeonwang. Antioxidant and antidiabetic activities of extract from Cirsium japonicum roots. Nutritional Research and Practice 2008; 2(4); 247-51.
PHYTOCHEMICAL STANDARDIZATION OF AAVARAI KUDINEER FORMULATION (AKF) - AN OFFICIAL SIDDHA POLY HERBAL FOR DIABETES MELLITUS

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2Professor & Head, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, (A Govt. of Puducherry Institution), Puducherry-605 006.
3Professor and Head, Department of Pathology, Sri Ramachandra Medical College & Research Institute, Sri Ramachandra University, Porur, Chennai-600 116, Tamil Nadu.

ABSTRACT

The Siddha system of medicine is a traditional Indian system of medicine. Among the various Siddha formulations useful in the treatment of diabetes mellitus, “Aavarai Kudineer Formulation” (AKF) is a reputed formulation. This formulation is scientifically under explored in the field of Phytochemistry. Hence an attempt was made to scientifically standardize the AKF under Phytochemical parameters. Phytochemical evaluation such as preliminary phytochemical screening, fluorescence analysis, TLC and HPTLC studies were performed for the first time in this formulation. The information obtained from preliminary phytochemical screening and fluorescence analysis for seven individual drugs as well as AKF would be useful in finding out the genuineness of the drug. TLC studies of individual drug was compared with AKF in the newly developed solvent system: Toluene: Ethyl acetate: Methanol: Water (9.5: 5: 0.7: 0.9) and number of spots are observed under UV254nm and UV365nm. HPTLC Qualitative analysis of AKF shows different peaks scanned under UV254nm and UV365nm. The results of these investigations could, therefore, serves as a basis for proper identification, authentication and standardization of the AKF.

KEYWORDS: Aavarai Kudineer, Siddha system of medicine, Diabetes mellitus, Phytochemical evaluation.
INTRODUCTION
Siddha medicine means medicine that is perfect. Siddha medicine is claimed to revitalize and rejuvenate dysfunctional organs that cause the disease and to maintain the ratio of vata, pitta and kapham. Diabetes mellitus is a condition which can be compared with Neerizhivu in Siddha. The other names described in the texts are the Madhumegham and Inippuneer. The signs and symptoms explained is increased urination both in frequency and quantity, there will be flies surrounding the urine voided place, weight loss, dryness of the skin, etc.\(^1\)\(^-\)\(^2\) Siddha kudineer antidiabetic formulations are tailor made, effective, tried and trusted. Many of them are official also. They are economical and they can be prepared by himself.\(^3\)

Among the several anti-diabetic siddha kudineer formulations in, “Theraiyar kudineer” an ancient siddha literature, the “Aavarai kudineer” is selected for this current research. Since it is very popular, reputed and the ingredients have been proved to be useful in the management of diabetes mellitus. It contains Leaves of *Cassia auriculata* (Leguminosae), *Cassia fistula* (Caesalpinaceae), Seeds of *Syzygium cumini* (Myrtaceae), Roots of *Salacia chinensis* (Celastraceae), Rhizomes of *Cyperus rotundus* (Cyperaceae), *Costus specious* (Coataceae) and Barks of *Terminalia arjuna* (Combretaceae) in equal parts.\(^4\)\(^-\)\(^5\) This research work is a small step towards, scientifically studying the Siddha antidiabetic kudineer formulation, so as to standardize and improve the formulation for the benefit of mankind.

MATERIALS AND METHODS
PRELIMINARY PHYTOCHEMICAL SCREENING
The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The following tests such as detection of Alkaloids, Carbohydrates, Glycosides, Saponins, Proteins and Amino acids, Phytosterols, Fixed Oils and Fats, Phenolic compounds and Tannins, Gum and Mucilage and Volatile Oil have been performed on extracts to detect various phytoconstituents present in them.\(^6\)\(^-\)\(^10\)

FLUORESCENCE ANALYSIS
A molecule can be excited from its ground electronic state to an excited electronic state by absorbing energy in the form of visible or ultraviolet light. Many molecules are capable of emitting this energy as radiation, thus returning to the ground state. The emitted radiation is called fluorescence. For fluorescence analysis the plant powder was treated with different solvents (1N aqueous sodium hydroxide and 1N alcoholic sodium hydroxide, acids like 1N
hydrochloric acid, 50% sulphuric acid and nitric acid) separately and then extracts were subjected to fluorescence analysis in visible/day light and UV light (254nm & 365nm).[11-12]

THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS
Chromatography represents a group of methods for separating molecular mixtures that depend on the differential affinities of the solute between two immiscible phases. In TLC, the separation is carried on a glass plate, which is coated with a thin uniform layer of finely divided inert adsorbent silica gel-G. The special advantages of TLC compared to PC (Paper chromatography) include versatility (a number of different adsorbents besides cellulose may be used), greater speed (due to more compact nature of the adsorbent when spread on a plate) and sensitivity (separations on less than µg amounts of materials can be achieved). One more advantage over PC is that glass plates may be spread with conc. H2SO4, a useful detection reagent for steroids and lipids. The glass plates have carefully cleaned with acetone to remove grease. Then the slurry of silica gel (or other adsorbent) in water has been vigorously shaken for a set time interval before spreading. Finally, plates after spreading have been air dried and then activated by heating in an oven at 100-110°C for 30 minutes. The solution of the sample in a volatile solvent was applied by using a capillary tube or a micropipette to a spot keeping 1.2 cm from the bottom of the TLC plate. The position of the sample spot was indicated by making a ‘origin line’ on the plate with the lead pencil. When the spot has dried, the plate is placed vertically in a suitable tank (which is paper-lined so that the atmosphere inside is saturated with the solvent phase) with its lower edge immersed in selected mobile phases. The solvent rises by capillary action, resolving the sample mixture into discrete spots. The different solvent system used to detect various phytoconstituents are Toluene: Ethyl acetate: Diethylamine (70:20:10) for Alkaloids, Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26) for Flavonoids. Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8) for Saponins, Diethyl ether: Toluene (1:1; Saturated with 10 % acetic acid) for Coumarins, Toluene: Ethyl acetate (93:7) for Essential oils and Ethyl acetate: Methanol: Water (100:13.5:10) for Glycosides.

The developed chromatogram was first inspected under UV light. After preliminary inspection in UV light, each chromatogram was analyzed for the presence of drug constituents by spraying with an appropriate group reagent. The Rf value is the distance of a compound moves in chromatography relative to the solvent front. It is calculated by using the formula [13-15]
HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC is the most simple separation technique today available to the analyst. It can be considered a time machine that can speed our work and allows us to do many things at a time usually not possible with other analytical techniques. For scanning use of UV / Visible / Fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and the scanner is an advanced type of densitometer. The samples were applied at a concentration of 2-10µl and standard at about 5µl were applied using CAMAG Linomat V Sample applied on aluminium sheets precoated with silicagel 60F-254 (Merck) of 0.2mm layer thickness and 5x20 cm was used as a stationary phase in different track. The plate was developed in the mobile phase to a distance of 120mm for developing the chromatogram. The developments were carried out in CAMAG twin trough glass chamber. The band width as 6mm was maintained and the band on the plate at a distance of 6mm was applied. The different tracks were scanned using CAMAG densitometer scanner 3VI.13 equipped with CATS V 4.04 software at a wavelength of 254 and 366 nm using deuterium lamp and record the finger print profile.[16-19]

RESULTS AND DISCUSSION

Table 1: Preliminary phytochemical screening of AKF

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemical groups</th>
<th>C. au</th>
<th>C. fi</th>
<th>S. cu</th>
<th>S. ch</th>
<th>C. sp</th>
<th>C. ro</th>
<th>T. ar</th>
<th>AKF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>+</td>
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<td>2</td>
<td>Glycosides</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
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<td>Carbohydrates</td>
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<td>Phenols</td>
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<td>+</td>
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<tr>
<td>9</td>
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<td>+</td>
<td>+</td>
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<tr>
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<td>Fixed oils and fats</td>
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<td>-</td>
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<tr>
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<tr>
<td>12</td>
<td>Volatile oil</td>
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<td>-</td>
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<tr>
<td>13</td>
<td>Mucilage and gums</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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Table 2: Fluorescent analysis of AKF

<table>
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<tr>
<th>S.NO</th>
<th>Experiments</th>
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<th>U.V light (365 nm)</th>
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<td>Drug powder as such</td>
<td>Pale green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>2</td>
<td>Powder + 1N NaOH</td>
<td>Dark brown</td>
<td>Pale yellow</td>
<td>Dark green</td>
</tr>
<tr>
<td>3</td>
<td>Powder + 1N HCl</td>
<td>Brownish yellow</td>
<td>Brown</td>
<td>Green</td>
</tr>
<tr>
<td>4</td>
<td>Powder + 50% H$_2$SO$_4$</td>
<td>Blackish brown</td>
<td>Fluorescent green</td>
<td>Dark green</td>
</tr>
<tr>
<td>5</td>
<td>Drug powder + Nitric acid</td>
<td>Light brown</td>
<td>Pale yellow</td>
<td>Fluorescent green</td>
</tr>
</tbody>
</table>

TLC IDENTIFICATION OF AKF

<table>
<thead>
<tr>
<th>Plant Name</th>
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</thead>
<tbody>
<tr>
<td>C. auriculata</td>
<td>0.87</td>
</tr>
<tr>
<td>C. fistula</td>
<td>0.9, 0.67</td>
</tr>
<tr>
<td>S. cumini</td>
<td>0.32</td>
</tr>
<tr>
<td>S. chinensis</td>
<td>0.80</td>
</tr>
<tr>
<td>C. speciosus</td>
<td>0.79</td>
</tr>
<tr>
<td>C. rotundus</td>
<td>0.47</td>
</tr>
<tr>
<td>T. arjuna</td>
<td>0.79, 0.84</td>
</tr>
</tbody>
</table>

HPTLC CHROMATOGRAM OF AKF
The information obtained from preliminary phytochemical screening and fluorescence analysis for seven individual drugs as well as AKF would be useful in finding out the genuineness of the drug. TLC studies of individual drug was compared with AKF in the newly developed solvent system: Toluene: Ethyl acetate: Methanol: Water (9.5: 5: 0.7: 0.9) and number of spot are observed under UV254nm and UV365nm. HPTLC Qualitative analysis of AKF shows different peaks scanned under UV254nm and UV365nm. The results of these investigations could, therefore, serve as a basis for proper identification, authentication and standardization of the AKF.

CONCLUSION
AKF is, “make your own medicine” type, formulation. It is freshly prepared and consumed. The physician advises the patient to prepare himself the formulation and consume it daily for a period, in multiplication of 48 days (mandalam). Lot of variation have been observed in the preparation of AKF, hence to avoid the variation and to have reproducible results a small step has been taken through this research work.

The raw materials and finished product of this formulation have been standardized. This study will be helpful in maintaining the quality of AKF. Further the formulation has been proved to be an effective anti-diabetic through in-vitro studies in earlier. Also the selected formulation was found to possess excellent antioxidant properties. These studies will help the formulation attain the quality standards at par with their modern medicine counterparts.

REFERENCES


POTENTIAL INVIVO ANTIDIABETIC ACTIVITY OF “AAVARI KUDINEER FORMULATION” (AKF) IN NORMAL AND STREPTOZOTOCIN INDUCED TYPE-II DIABETIC RATS

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2Professor & Head, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, (A Govt. of Puducherry Institution), Puducherry-605006, India.
3Professor and Head, Department of Pathology, Sri Ramachandra University, Porur, Chennai-600116, Tamil Nadu, India.

ABSTRACT

The present study demonstrates that “Aavarai Kudineer formulation” (AKF) exhibits promising antidiabetic activity and help to maintain good glycemic and metabolic control. The herbal formulation, AKF elicit hypo-glycaemic and antidiabetic effects in both normal and Streptozotocin (STZ) induced type- II hyperglycemic rats. The herbal formulation under acute toxicity studies shows, it is non toxic upto 2000mg/kg/BW. It is possible that the herbal formulation may act through both, pancreatic and extra-pancreatic mechanism(s). This Aavarai Kudineer formulation elicited a significant antidiabetic effect in Streptozotocin induced diabetic rats as reflected by its ability to inhibit lipid peroxidation and to elevate the enzymatic antioxidants in pancreatic tissue. This extract showed improvement in parameters like body weight, food consumption, organ weight and biochemical parameters and might be of great valuable in diabetic treatment. The histopathological studies during 28 days long term treatment have shown to ameliorate the Streptozotocin induced histological damage of Islets of Langerhans. Moreover the inhibitory effects on biochemical and histological parameters induced by herbal formulation at a dose of 500mg/kg were almost comparable to that of standard drug, Glibenclamide (5mg/kg).

KEY WORDS: Aavarai Kudineer formulation, Antidiabetic, Streptozotocin, Biochemical parameters, Histopathological studies.

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INTRODUCTION

Diabetes is a metabolic disorder in which a person has high blood sugar level because either the pancreas does not produce enough insulin or cells do not respond to it. Type 1 or Insulin Dependent Diabetes Mellitus known as Juvenile diabetes Mellitus. 5-10% cases are diagnosed as Type 1. It is an autoimmune disorder when the immune system goes against its own self causing destruction of the pancreatic cells that produce insulin. Type 2 or Non Insulin Dependent Diabetes Mellitus is the most common type. Slow onset with symptoms like increased thirst, increased urination and weight loss. Gestational Diabetes is seen during pregnancy. About 3-5% ladies have chances to develop Gestational diabetes. 1 In Siddha system of medicine, many single and polyherbal formulations and higher medicines like parpam, chendooram and chunnam have been practiced to cure or control diabetes mellitus from time immemorial. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural products with anti-diabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. Recent overwhelming attention to plant products and alternative medicine has encouraged plant chemists, pharmacologists, biochemists and molecular biologists to combine their efforts in a search for natural agents that can limit diabetes mellitus and its complications. Siddha polyherbal formulations like Seenthalil Kudineer, Seenthith Choornam, Vilva kudineer, Aavarai kudineer, Madumhegaa choornam, Nyavalkottai Choornam, Silasuthu parpam, Abraka chendooram, Triphala Choornam etc are used in treatment of Diabetes. 2 For the current research, Aavarai Kudineer formulation-AKF is selected based on survey among the Siddha physicians in and around Puducherry. It consists of equal parts of leaves of Cassia auriculata & Cassia fistula, seeds of Syzygium cumini, roots of Salacia chinensis, rhizomes of Costus speciosus & Cyprus rotundus and bark of Terminalia arjuna. The literature review revealed that there is no scientific work has been carried out on this formulation. Hence it was thought worth to carryout invitro anti-diabetic studies on this traditional anti-diabetic herbal formulation.

MATERIALS AND METHODS

Experimental animals

Albino Wistar rats (180-220g) of either sex bred in the animal house were used in this study. The animals were fed on a standard pellet diet (Hindustan Unilever Ltd, Mumbai-400 099) and had free access to ozonised filter water ad libitum. The animals were maintained in their respective groups under controlled conditions of temperature and humidity. All the studies were conducted in accordance with CPCSEA guidelines and the experiments were carried out as per the approval of institutional ethics committee (IAEC-MTPG&RIHS/6040/JAN-16).3-4

Dose and drug solution

Traditionally 1 to 2g of the AKF is used in diabetes. Further for this study, in-house prepared AKF was extracted with distilled water, concentrated under vacuum, dried and dissolved in 1% CMC solution to have a desired dose of 125, 250 and 500 mg/kg BW in 1ml solution. Glibenclamide was obtained as a gift sample from USV Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade and procured locally.

Acute toxicity studies

The study was carried out according to the OECD guidelines 423. Female Wistar rats of weight 180-220g were taken for the study and kept for overnight fasting. Next day, body weight was taken and AKF was administered orally at a dose of 5, 50, 300 and 2000mg/kg in distilled water. Then the animals were observed for mortality and morbidity at 0, ½, 1, 2, 4, 6, 8, 12 and 24 hr. Feed was given to the animals after 4 hr of the dosing and the body weight was checked prior and at 6 hr after dosing. The animals were observed twice daily for 14 days and body weight was taken. The same experiment was repeated again on 3 rats as there was no observable clinical toxicity for the animals on the acute toxicity study.5

Antidiabetic screening of aavarai kudineer formulation (akf) in normal and streptozotocin induced diabetic rats.

Antidiabetic activity of Avarai Kudineer Formulation (AKF) in normal rats

Normal fasted rats: Normal albino rats (180-220 mg) were first used for the screening of the herbal formulation AKF for hypoglycemic activity. Overnight fasted normal rats were randomly divided into 5 groups, of 6 rats each. The group I served as control, which received vehicle i.e. 1%CMC (1ml/kg, orally). Group II, III and IV were treated orally with test AKF 125, 250 and 500 mg/kg, respectively. Group V received standard drug Glibenclamide 5 mg/kg orally.6 Blood samples were collected from tail vein prior and 1, 2 and 4 hour after treatment using CONTOUR™TS blood glucose meter with same test strips. Fasting blood glucose was estimated by glucose oxidase and peroxidise (GOD/POD kit) method. Intensity of the red quinoneimine was measured at 540 nm in auto analyzer.7,8

Oral Glucose Tolerance Test

Overnight fasted rats were divided into 5 groups of six animals each as mentioned as above except the diabetic control and received the respective treatments. After 30 minutes of drug administration the rats of all the groups were orally administered with 2g/kg of glucose. Blood samples were collected from tail vein just prior to drug administration and at 30, 60, 120 and 240 minutes after glucose loading. Blood glucose levels were measured immediately using CONTOUR™TS blood glucose meter with same test strips.9

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Antidiabetic activity of Aavarai Kudineer Formulation (AKF) in Streptozotocin (STZ) induced diabetic rats

Adult albino Wistar rats (180-220 g) of either sex were made diabetic with an intra-peritoneal injection of 65 mg/kg body weight of Streptozotocin (Sigma Aldrich chemicals, USA) dissolved in 0.1 M cold citrate buffer, pH 4.5, immediately before use. Streptozotocin injected animals exhibited massive glucosuria and hyperglycemia within 2-4 days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, on 4th day after the injection with STZ. Adult albino Wistar rats with blood glucose levels more than 200 mg/dl were considered to be diabetic and were used in this experiment.

Single dose, short term study

After induction of diabetes, the rats were divided into 6 groups of six animals each and screened for anti-hyperglycemic activity of the various concentration of AKF in overnight fasted diabetic rats. The AKF at the dose of 125, 250 and 500 mg/kg body weight were administered orally after suspending in 1% CMC solution. The blood samples were collected from tail vein and the blood glucose levels were determined using CONTOUR™TS blood glucose meter with same test strips.10-11 The blood samples were collected from tail vein and the blood glucose levels were determined using CONTOUR™TS blood glucose meter with same test strips.

Multiple doses, long term study

In multiple dose studies the AKF at the dose of 125, 250 and 500 mg/kg bodyweight once daily was given for 28 days and blood glucose levels were monitored only at seven days intervals. Blood sample were collected from tail veins of the animals. Blood glucose levels were determined using CONTOUR™TS blood glucose meter with same test strip at intervals of seven days. After 4 weeks of drug treatment, animals were sacrificed, blood withdrawn for biochemical detection. The pancreas was removed and a portion of pancreatic tissue was homogenized and the extract was used for the estimation of activity of enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), lipid peroxidase (LPO), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP) by colorimetric method. The body weights of all the animals of all the groups were recorded before starting the treatment and at end of the treatment period12-15.

Haemoglobin and glycosylated Hb (HbA1C)

Haemoglobin was estimated by the method of Drabkin’s method. Intensity of the color formed by oxidized haemoglobin with potassium ferricyanide was measured at 530 nm in UV-Visible spectrophotometer (Shimadzu, Japan). Glycosylated Hb (HbA1C) was estimated by following the method of Sudhakar Nayak and Pattabiramam, 1982. Briefly, saline washed red cells were treated with water/CCl4 for lysis and incubated at 37°C for 15 minutes and oxalate or HCl solution was then added and mixed. The filtrate was heated in a boiling water bath for 4 hrs, cooled with ice-cold water, treated with 40% TCA and again centrifuged at 10000g for 10 minutes. The supernatant obtained was then heated with 80% phenol and sulphuric acid and the colour developed using thiobarbituric acid was read at 480nm after 30 minutes.16-17

Histopathological study of pancreas

Pancreas were isolated and preserved in 10% formalin. All parafformaldehyde fixed tissues were embedded in paraffin, sections 6µm thick cut with a cryostat microtome and then stained with haematoxylin and eosin. Histopathological observation of the tissues was carried out under a light microscope. Photomicrograph was taken to substantiate the findings under low power (10X). The alteration and changes in the histology of pancreas were shown in vide plate and the results with photomicrograph were given in the result section.18

Statistical analysis

The data obtained was analyzed using prism software and the results were expressed as mean ± SEM, n=6. Statistical significance was determined by using one way analysis of variance (ANOVA) followed by dunnett’s multiple comparison tests. The AKF and Glibenclamide treated groups were compared with the corresponding normal or diabetic control. P<0.01 and p<0.05 were considered to be significant.

RESULTS AND DISCUSSION

Acute toxicity studies

In the acute toxicity study, Aavarai kudineer formulation up to the dose level of 2000 mg/kg of body weight did not exhibit any lethality or toxic symptoms. Further dosing to estimate the LD50 of the formulation was not performed. According to Organization for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity, an LD50 dose of 2000 mg/kg and above is categorized as unclassified and hence the drug is safe. As 2000 mg/kg of body weight was well tolerated by the animals without any behavioral changes further studies were carried out with in the 500 mg/kg of body weight and the drug was found to be safe.

Antidiabetic activity in normal fasted rats

The onset of hypoglycemic activity of Aavarai kudineer formulation at 125 and 250 mg/kg was evident between 1-2 hrs, the peak was found to be at 4hs. The rats receiving 500 mg/kg of Aavarai kudineer formulation showed the onset of effect at 1 hr with peak effect at 4hr. The hypoglycemic effect of Aavarai kudineer formulation at 500 mg/kg was found to be nearly comparable to that of Glibenclamide 5mg/kg (Table 1).

Oral glucose tolerance test

Results of OGGT are presented in Table 2. An over dose of glucose (2g/kg BW) was fed to normal rats to evaluate the efficacy of various concentration of Aavarai kudineer formulation on anti-hyperglycemic properties. Results from this study showed that 250 mg and 500 mg/kg were highly effective in bringing down the BGL from 170.3 to 127 mg/dl and 109 to 85.10 mg/dl at the end of 240 minutes which was on par with the Glibenclamide that reduce BGL from 108 to 75.80 mg/dl. These results are in relationship with the earlier experiments suggesting that all concentrations are anti-hyperglycemic.
Antidiabetic activity in STZ induced diabetic rats (single dose, short term study)

Acute effect of various concentration of Aavarai kudineer formulation in overnight fasted diabetic rats presented in table 2. Blood glucose level (BGL) of rats of group-I was compared with BGL of group-II, diabetic control rats to confirm that the drug STZ has induced diabetes in experimental animals (p<0.01) at all interval of sampling. It was noticed that all the concentration of Aavarai kudineer formulation resulted in reduction of BGL significantly except 125mg/kg BW. 250mg and 500mg were significantly (p<0.01) effective in reducing initial BGL of 352.3 to 210 mg/dl and 360 to 170 mg/dl respectively which was on par with Glibenclamide that reduce BGL from 370 to 158 mg/dl at the end of 240 minutes (Table 3).

Antidiabetic activity in STZ induced diabetic rats (Multiple doses, long term studies)

The changes in BGL and body weight are reported in the Table 4 and 5. Changes in serum lipid profile are reported Table 6. There was significant (p<0.01) decrease in body weight in all diabetic rats within 28 days ranging from 13.78 to 27.78% (Table 5). Significant (p<0.01) decrease in BGL was observed in rats treated with 500mg/kg BW which was on par with Glibenclamide in reducing the BGL from 240 to 72.2 mg/dl and 258 to 64.5 mg/dl respectively. The 125mg and 250mg/kg BW also lowered BGL significantly (p<0.05) compared to diabetic control by bringing down from 241.2 to 162.1 and 260.2 to 102.2mg/dl respectively (Table 4). The triglycerides level of the animal treated with all the concentration have come down significantly compared to normal control group and Glibenclamide treated group which is a desired effects. Further the concentration of TC and TG decreased in 500mg/kg but in 250 and 125mg/kg it was less (Table 7). This results suggested that 250 and 500mg/kg of Aavarai kudineer formulation are better than 125mg/kg BW and equivalent to standard drug Glibenclamide 5mg/kg BW. The anti-hyperglycemic activity of a drug is the ability of drug to lower very high blood sugar levels to acceptable lower levels. In literature very less work has been reported for this Aavarai kudineer formulation.19 In this study the report that resulted from three different independent experiments suggest that all the three concentration were anti-hyperglycemic. 250 and 500mg/kg BW were superior to 125mg/kg BW in bringing down the BGL from very high level to acceptable level within 240 minutes and the same was verified for it reproducibility of results in long duration multiple dose studies. It was confirmed that 250mg and 500mg/kg BW were on par with standard drug Glibenclamide, 5mg/kg BW in maintaining pancreatic enzyme profiles (Table 6). Further the Aavarai kudineer formulation in 250mg and 500mg/kg caused reduction in triglycerides (TG) and showed significant decreased in total cholesterol (TC) and raised insulin levels (Table 7). The Aavarai kudineer formulation in above mentioned effects was comparable with Glibenclamide 5mg/kg. Glycosylated haemoglobin (HbA1C) level in STZ induced diabetic rat was decreased significantly after treatment with Aavarai kudineer formulation for 28 days (Table 7). In addition to decreased blood glucose level, the formulation has also effect on overall metabolic variables as evidenced by its property to lower lipid profile. Since type 2 diabetes mellitus is a metabolic disorder characterized by hyperglycemia, hyperlipidemia and insulin resistance drug therapy aiming at overall amelioration of the disorder is more desirable than a drug which decreases blood glucose alone. The results of our study shows that AKF not only normalizes blood glucose but also the hyperglycemia. It also showed in improvement in body weight and insulin levels and lipid profile of the animals. Thus the current study my pave a pathway to develop a novel formulation from a traditional dosage forms to combat diabetes mellitus and its associated complications.
Table 3
Effect of Aavarai kudineer formulation on blood glucose level in Streptozotocin-induced diabetic rats (single dose, short term study)

<table>
<thead>
<tr>
<th>Group &amp; Treatment (dose mg/kg, po)</th>
<th>Blood Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 minutes</td>
</tr>
<tr>
<td>Normal control</td>
<td>80.8±2.40</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>355.8±1.96</td>
</tr>
<tr>
<td>Aavarai kudineer formulation (125)</td>
<td>369.5±2.07</td>
</tr>
<tr>
<td>Aavarai kudineer formulation (250)</td>
<td>352.3±1.00</td>
</tr>
<tr>
<td>Aavarai kudineer formulation (500)</td>
<td>360.0±1.46</td>
</tr>
<tr>
<td>Glibenclamide (5)</td>
<td>370.0±2.20</td>
</tr>
</tbody>
</table>

Results were represented as mean ±SEM of n=6 rats in each group. One way ANOVA followed by Dunnett’s multiple comparison test was applied for comparing with the control group and treatment groups. P value: <0.01; compared to normal group, *diabetic group.

Table 4
Effect of Aavarai kudineer formulation on blood glucose level in Streptozotocin-induced diabetic rats (multiple doses, long term study of 28 days)

<table>
<thead>
<tr>
<th>Group &amp; treatment (dose, mg/kg, p.o)</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>76.1±1.02</td>
<td>81.0±2.10</td>
<td>80.5±2.80</td>
<td>82.8±2.40</td>
<td>78.9±4.49</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>352.5±1.20</td>
<td>266.2±2.50</td>
<td>289.7±3.80</td>
<td>309.8±1.60</td>
<td>371.6±2.30</td>
</tr>
<tr>
<td>Aavarai kudineer formulation (125)</td>
<td>241.2±2.26</td>
<td>200.5±1.40</td>
<td>190.2±2.30</td>
<td>182.0±3.02</td>
<td>162.0±6.22</td>
</tr>
<tr>
<td>Aavarai kudineer formulation (250)</td>
<td>260.2±0.10</td>
<td>240.2±2.40</td>
<td>228.0±0.48</td>
<td>174.0±2.70</td>
<td>102.2±1.50</td>
</tr>
<tr>
<td>Aavarai kudineer formulation (500)</td>
<td>240.2±2.21</td>
<td>222.2±2.12</td>
<td>110.0±2.12</td>
<td>804.2±1.10</td>
<td>072.2±1.10</td>
</tr>
<tr>
<td>Glibenclamide (5)</td>
<td>258.5±2.30</td>
<td>100.2±2.12</td>
<td>090.6±1.40</td>
<td>082.5±0.80</td>
<td>64.5±2.12</td>
</tr>
</tbody>
</table>

Results are mean ±SEM of 6 rats in each group. One way ANOVA followed by Dunnett’s multiple comparisons test was applied for comparing with the control group and treatment groups. P values: <0.01** and <0.05* as compared to normal group, *diabetic group.

Table 5

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>Initial Body weight (g)</th>
<th>Final Body weight (g)</th>
<th>%Increased/decreased of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>182.2±2.70</td>
<td>210.0±2.82</td>
<td>+27.81%</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic control</td>
<td>186.8±0.20</td>
<td>160.1±2.90</td>
<td>-29.48%</td>
</tr>
<tr>
<td>3</td>
<td>Aavarai kudineer formulation (125)</td>
<td>182.6±2.34</td>
<td>161.8±8.92</td>
<td>-20.78%</td>
</tr>
<tr>
<td>4</td>
<td>Aavarai kudineer formulation (250)</td>
<td>184.22±1.22</td>
<td>168.44±2.10</td>
<td>-15.78%</td>
</tr>
<tr>
<td>5</td>
<td>Aavarai kudineer formulation (500)</td>
<td>188.12±8.44</td>
<td>174.10±4.22</td>
<td>-14.02%</td>
</tr>
<tr>
<td>6</td>
<td>Glibenclamide (5mg/kg)</td>
<td>190.0±1.02</td>
<td>176.22±8.52</td>
<td>-13.78%</td>
</tr>
</tbody>
</table>

Values are mean ± SD from 6 animals in each group. Where (+) indicates % increase of body weight, (-) indicates % decrease of body weight.

Table 6
Effect of Aavarai kudineer formulation on biochemical parameters in Streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>Group &amp; treatment (dose mg/kg, po)</th>
<th>SGOT (I/U/L)</th>
<th>SGPT (I/U/L)</th>
<th>Alkaline phosphatase (I/U/L)</th>
<th>% Lipid Peroxidation</th>
<th>CAT (U/mg)</th>
<th>GPx (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>62.12±1.10</td>
<td>51.08±0.30</td>
<td>137.90±1.20</td>
<td>67.22±4.02</td>
<td>3.26±2.14</td>
<td>2.67±0.82</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>142.70±1.25</td>
<td>104.10±0.44</td>
<td>260.20±1.12</td>
<td>112.0±0.50</td>
<td>1.00±1.66</td>
<td>1.66±1.48</td>
</tr>
<tr>
<td>AKF 125</td>
<td>115.8±1.06</td>
<td>85.16±0.64</td>
<td>194.30±1.76</td>
<td>80.56±0.24</td>
<td>2.20±0.17</td>
<td>2.10±1.09</td>
</tr>
<tr>
<td>AKF 250</td>
<td>95.23±5.02</td>
<td>74.66±1.58</td>
<td>164.20±0.42</td>
<td>74.36±0.50</td>
<td>2.46±0.69</td>
<td>2.26±0.04</td>
</tr>
<tr>
<td>AKF 500</td>
<td>80.36±2.11</td>
<td>65.34±0.24</td>
<td>149.20±0.49</td>
<td>65.32±0.85</td>
<td>2.96±1.3</td>
<td>2.68±1.17</td>
</tr>
<tr>
<td>Glibenclamide (5mg/kg)</td>
<td>73.33±0.18</td>
<td>58.30±2.45</td>
<td>145.30±0.14</td>
<td>60.93±0.13</td>
<td>3.10±3.32</td>
<td>2.50±0.15</td>
</tr>
</tbody>
</table>

Results are mean ±SEM of 6 rats in each group. One way ANOVA followed by Dunnett’s multiple comparisons test was applied for comparing with the control group and treatment groups. P value: <0.01; compared to normal group, *diabetic group.

Table 7
Effect of Aavarai kudineer formulation on Total Cholesterol (TC), Triglycerides (TG), Insulin (I), Hemoglobin (Hb) and Glycosylated hemoglobin (HbA,C) levels in diabetic rats.

<table>
<thead>
<tr>
<th>Group Treatment (dose, mg/kg, po)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>I (µU/ml)</th>
<th>Hb (%)</th>
<th>HbA,C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>101.5±1.40</td>
<td>67.6±1.12</td>
<td>33.6±1.60</td>
<td>13.2±0.84</td>
<td>4.63±1.20</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>307.3±0.46</td>
<td>179.6±2.02</td>
<td>25.9±1.30</td>
<td>9.7±0.81</td>
<td>7.14±0.07</td>
</tr>
<tr>
<td>AKF (125)</td>
<td>282.0±2.42</td>
<td>147.0±6.86</td>
<td>24.0±1.10</td>
<td>8.0±2.81</td>
<td>6.08±0.06</td>
</tr>
<tr>
<td>AKF (250)</td>
<td>174.1±0.98</td>
<td>75.0±2.10</td>
<td>33.5±0.64</td>
<td>10.3±1.18</td>
<td>5.70±0.98</td>
</tr>
<tr>
<td>AKF (500)</td>
<td>169.0±1.82</td>
<td>69.0±4.01</td>
<td>32.1±2.42</td>
<td>13.0±2.60</td>
<td>5.29±4.10</td>
</tr>
<tr>
<td>Glibenclamide (5)</td>
<td>166.1±2.18</td>
<td>71.0±2.14</td>
<td>35.3±1.20</td>
<td>12.3±0.98</td>
<td>5.27±1.96</td>
</tr>
</tbody>
</table>

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HISTOPATHOLOGICAL STUDIES

Pancreas section of rat of normal group (Fig.1) revealing endocrine Islets of Langerhans and exocrine pancreatic acini of serous epithelial cells. No fibrosis or inflammation was found. Pancreas section of rat of diabetic control group (Fig.2 and 2A) Islets showing disrupted beta cells and reduction in beta cell mass. Degenerative changes and necrosis in islets of langerhans and acini were observed in this group. Pancreas section of rat treated with Aavarai kudineer formulation 125mg/kg (Fig.3) showed that minimal necrosis and mild to moderate atrophy of islets and fibrotic changes were found. Pancreas section of rat treated with Aavarai kudineer formulation 250mg/kg (Fig.4) showed moderate degenerative changes in endocrine and exocrine pancreas along with disruption of islets of Langerhans observed. Minimal necrosis and mild to moderate atrophy and fibrotic changes were found. Pancreas section of rat treated with Aavarai kudineer formulation 500mg/kg (Fig.5) clearly projects clear restoration of beta cells and exocrine glands. Mild degenerative changes in pancreatic parenchyma were observed. Acini and intercalated ducts are showing normal morphology. No necrosis and mild to moderate atrophy and fibrotic changes were found. Pancreas section of rat treated with 5 mg/kg Glibenclamide (Fig.6) showed that restoration of beta cells and exocrine glands. Acini and intercalated ducts are showing normal morphology. No necrosis and mild to moderate atrophy and fibrotic changes were found.

Histopathological observations of Aavarai kudineer formulation and Glibenclamide treated pancreas in streptozotocin-induced diabetic rats (under low power)

Figure 1
Group I – Normal Control showing presence of normal pancreatic islet cells

Figure 2
Group II- Diabetic control showing reduction in islet cell mass

Figure 2A
Group II- Diabetic control showing necrosis of islets
CONCLUSION

The extract of Aavarai Kudineer formulation exhibited significant anti-hyperglycemic activity in normal and Streptozotocin (STZ) induced diabetic rats. This extract showed improvement in parameters like body weight, biochemical parameters and histopathological changes which might be of great valuable in diabetic treatment.

The results are encouraging and on par with currently used allopathic drug Glibenclamide. This study strongly proves the traditional use of this Siddha, polyherbal, antidiabetic formulation Aavarai kudineer scientifically.

ACKNOWLEDGEMENT

We are expressing our sincere gratitude to the Dean,
REFERENCES


CONFLICT OF INTEREST

The authors are declare that there is no conflict of interest.