INTRODUCTION

Medicinal plants are the source of many potent and powerful drugs. The plant derived drugs are healthier and safer alternate to the synthetic drugs [1]. Different parts of medicinal plants like root, stem, flower, fruit, seed etc. are used to obtain pharmacologically active constituents. Medicinal activities of plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins and terpenoids present in these plants. These active principles are isolated for direct use as drugs, lead compounds and or pharmacological agents. Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia [2]. In recent days, herbal medicines are used worldwide even without documentation of their curative effect and there is only little essential to know about the pharmacological evaluation of various plants used in the traditional system of medicines [3]. A number of investigations confirmed that oral anti-hyperglycemic agents derived from plants can be used in traditional medicine and many of the plants were found with good antidiabetic activity [4, 5] Oral administration of the Centrum nocturnum leaves extract for 15 days caused a significant reduction in blood glucose levels and considerable increase in body weight in diabetic rats [6]. Diabetic rats treated with methanolic extracts of Dolichos lablab dose dependably reduced blood glucose levels, total cholesterol, triglycerides, SGPT, SGOT levels when compared to untreated diabetic rats in streptorotocin (STZ) induced diabetic animal model (7). Similarly, acetone extract of whole fruit powder of M. charantia in doses 0.25, 0.50 and 0.75mg/kg body weight lowered the blood glucose from 13.3% to 50.0% in alloxan diabetic rats confirming hyperglycemic diabetic potential of C. dactylon extracts using alloxan induced albino rats.

MATERIALS AND METHODS

Preparation of plant materials and phytochemical analysis

Collection of plants

Plants of Cynodon dactylon (L.) Pers. were collected from different localities near Madurai and maintained in the herbal garden of Saraswathi Narayanan College, Madurai, Tamil Nadu. The plants were identified and authenticated by referring the standard taxonomic characteristic features (keys) according to the Flora of Madras Presidency and the Flora of Tamil Nadu Carnatic [11]. The voucher specimens of the plants are kept in the Department of Botany, Saraswathi Narayanan College, Madurai, Tamil Nadu, India for future reference.

Processing of plant materials

The leaves were washed in running water and cut into small bits to facilitate drying. The pieces of plant material were dried for 12hrs in a hot air oven (Model: HIPL-024A) at 60°C. The dried plant material (leaves) was taken separately and grounded using an electric blender to obtain a fine powder. The powder was further passed through a 2mm sieve to obtain finer particles. The powdered samples were stored in a clean glassware container until needed for analysis.

Extraction of the plant

500mg of powdered plant material was separately dispensed in 1000ml of each water and solvents used. The powdered plant material was defatted with petroleum ether for 24 hours at 20°C and extracted with chloroform, ethanol and methanol in a Soxhlet apparatus for 72hrs at 40°C respectively. The thick mass obtained by evaporating the solvent under reduced pressure at room temperature. It rendered a gummy concentrates of chocolate black color. The gummy concentrate was designated as crude extract. The extract obtained was used for the phytochemical screening.

Keywords: Cynodon Dactylon, Phytochemical Screening, Antidiabetic Activity, Alloxan Induced Rats.
Phytochemical analysis

Qualitative phytochemical tests for the identification of alkaloids, tannins, saponins, carboxydrates, glycosides, steroids, terpenoids and fixed oils were carried out for all the solvent extracts. Phytochemical screening of crude solvent extracts was performed using the following reagents and chemicals: alkaloids with Wagner reagent, tannins with 5% ferric chloride, saponins with ability to produce foam by adding water and olive oil, carbohydrates with Molish reagents and concentrated sulfuric acid, glycosides with glacial acetic acid, ferric chloride and concentrated sulphuric acid, steroids with chloroform, acetic anhydride and concentrated sulphuric acid, terpenoids with chloroform and concentrated sulphuric acid and fixed oil using spot and oil staining methods [12, 13, 14].

Animal studies

Administration of Cynodon dactylon extracts

Male albino rats (Sprague-Dawley strains) with body weight of 160-220 gm were divided into seven groups of five rats each. All experiments were described and approved by the Institutional Animal Ethics Committee (IAEC). (Animal Ethical committee Approval number: MKU/IAEC/KMGP/8b/PY/78/Ph.D/2013). In all experiments normal rats fed with pellet diets served as positive control (Group I) and the alloxan induced diabetic rats without any solvent extracts maintained as negative control (Group II). The alloxan induced diabetic rats were treated in separate groups with petroleum ether (Group III), chloroform extract (Group IV), methyl extract (Group V), ethanol extract (Group VI) and water extract (Group VII). The normal rats (Group I) and alloxan induced diabetic rats (Group II) received normal pellet diets. The duration of the experiment was 21 days. The experimental animals were injected with freshly prepared Alloxan (150 mg/kg body weight) dissolved in normal saline.

RESULTS

Blood Glucose Level

The effect of various extracts of C. dactylon leaves on blood glucose level is given in Table 2. Significant differences in the blood glucose level were observed in methanol extract at the end of 21 days of treatment. The blood glucose level was increased significantly in alloxan induced diabetic rats as compared with the control rats (P<0.001). In diabetic rats, significant decrease in blood glucose level was observed after the treatment with different extracts of leaves of C. dactylon. Among different solvent extracts, methanolic extract significantly decreased the blood glucose level of diabetic rats. Blood glucose level of methanolic extract of C. dactylon treated rats showed 180 ± 10.0 mg/100ml and it was similar to blood glucose level of normal rats. The value is found to be more than 50% less to alloxan induced rats (negative control) which had 345 ± 6.2 mg/100 ml. The other extracts also reduced blood glucose level at significant levels except the extract prepared with petroleum ether.

Plasma Lipids: Difference in the plasma cholesterol level of all groups were observed at the end of 21 days of treatment (P<0.05) as indicated in Table 2. Serum cholesterol level was significantly increased in diabetic rats (142 ± 5.83 mg/100ml) when compared with control groups (72 ± 1.9 mg/100ml). Administration of methanol (87 ± 0.77 mg/100ml) and petroleum ether extracts (87 ± 1.91 mg/100ml) of C. dactylon significantly reduced the plasma cholesterol level in diabetic rats. Similarly, plasma triglycerides levels (111 ± 4.13 mg/100ml) were also found to be lesser in the group V animals which received methanol extract of C. dactylon than all other groups of animals but higher than control (94 ± 1.72 mg/100ml). Moreover, the reduction in triglycerides was by 50% in the diabetic rats treated with methanol extracts. The level of cholesterol and triglycerides was reduced at significant level when the group II animals (diabetic rats) treated with other solvent extracts of C. dactylon.

Plasma Urea: In our experiments, urea level was increased in the group of alloxan induced diabetic animals (102 ± 21.4 mg/100ml) which received normal lab diets. There was a sharp fall in urea level in the group of animals which received petroleum ether (45 ± 1.71 mg/100ml) and chloroform (60 ± 1.9 mg/100ml) extract of C. dactylon. It was observed that petroleum ether extract found to be effective as it reduced the urea level better (by above 50%) than all other extracts. There was 45% reduction in urea level in the diabetic animals treated with extracts of chloroform (60 ± 1.9 mg/100ml) and methanol (68 ± 0.72 mg/100ml).

Table 1: Preliminary phytochemical screening of various extracts of Cynodon dactylon

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytoconstituents</th>
<th>Chloroform extract</th>
<th>Methanol extracts</th>
<th>Ethanol extract</th>
<th>Petroleum ether extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>+</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Glicosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Saponin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Sterol</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Tannin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Fixed oil</td>
<td>-</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Terpene</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ + Presence of compounds - Absence of compounds
to be bioactive for the management of diabetes [29, 30]. Moreover, phenolics, coumarins, iridoids, polysaccharides, glycopeptides and presences of flavonoids, alkaloids, triterpenoids, aminoacids, studies. The marked hyperlipidemia that characterizes the diabetic experiment is in agreement with the findings of the aforementioned excretion [35]. A significant increase in serum cholesterol and with bile acids within the intestine and increasing bile acids decrease in cholesterol absorption from the intestine, by binding reduced the serum cholesterol level. It has been reported that plant plasma of diabetic rats. Further, alloxan induced diabetic rats when concentration in turn increases the beta-oxidation of fatty acids by

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Glucose (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control</td>
<td>10±1.78a</td>
<td>50±2.1a</td>
<td>72±1.9a</td>
<td>94±1.72a</td>
</tr>
<tr>
<td>Group II Alloxan</td>
<td>345±6.12e</td>
<td>102±2.14e</td>
<td>142±5.83d</td>
<td>233±8.49d</td>
</tr>
<tr>
<td>Group III Petroleum</td>
<td>281±4.0d</td>
<td>45±1.71a</td>
<td>87±1.91a</td>
<td>167±1.01c</td>
</tr>
<tr>
<td>Group IV Chloroform</td>
<td>109±5.29a</td>
<td>60±1.9b</td>
<td>96±1.49bc</td>
<td>167±0.27c</td>
</tr>
<tr>
<td>Group V Methanol</td>
<td>108±1.2a</td>
<td>68±0.72c</td>
<td>87±0.77ab</td>
<td>111±4.13b</td>
</tr>
<tr>
<td>Group VI Ethanol</td>
<td>117±3.1b</td>
<td>87±1.10d</td>
<td>101±1.79c</td>
<td>170±1.92c</td>
</tr>
<tr>
<td>Group VII Aqueous</td>
<td>118±2.0b</td>
<td>87±1.10d</td>
<td>101±1.89c</td>
<td>212.529cd</td>
</tr>
</tbody>
</table>

Means sharing within the rows are significantly different (P ≤ 0.05 level). Different letters followed in each row statistically significant based on DMRT (Values are expressed in mg/100ml).

**DISCUSSION**

Recently, many researchers have reported that various plant-derived flavonoids, anthraquinones, and terpenes stimulate glucose uptake in cells [20, 21, 22]. Similarly, certain flavonoids exhibited hypoglycemic activity [23, 9] and are also known for their ability of beta cell regeneration of pancreas [21]. In a study, saponin stimulated the release of insulin and blocked the formation of glucose in the bloodstream and ferulic acid induced insulin secretion. Further, flavonoids suppressed the glucose level, reduced plasma cholesterol and triglycerides significantly and increased hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets [24]. Several workers have carried out phytochemical investigation of *C. dactylon* which revealed the presence of flavonoids, alkaloids, triterpenoids, aminoacids, phenolics, coumarins, iridoids, polysaccharides, glycopeptides and guanidines and sterols [25, 26, 27, 28]. These compounds are known to be bioactive for the management of diabetes [29, 30]. Moreover, *Discora* extract decreased the levels of triglycerides and cholesterol which is responsible for the enhancement of the transcription of lipoprotein lipase similar to that of insulin, since the level of triglycerides and cholesterol increases due to increased production of VLDL [31]. Similarly, dietary fiber present in *Euphorbia neriifolia* might have played a major role in lowering the blood glucose level by slowing the rate of carbohydrate absorption from intestine and are hence beneficial for diabetics [32]. In our study, the solvent extract exhibited differential occurrence of secondary metabolites. There was presence of alkaloids, carbohydrates, saponins, tannins and terpenes in methanolic extracts and there were glycosides in addition to them in ethanolic extracts.

Insulin is potent inhibitor of lipolysis. During diabetes, activity of lipase enzyme increases lipolysis and release more free fatty acids in the circulation because of lack of insulin [33]. Increase in fatty acid concentration in turn increases the beta-oxidation of fatty acids by increasing the activity of HMG-CoA reductase for producing more cholesterol. Insulin also increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes hypercholesterolemia [34]. Moreover, cholesterol is a powerful risk factor for many coronary heart diseases. The degree of hypercholesterolemia is directly proportional to severity in diabetes. In our study, we have observed higher levels of cholesterol in plasma of diabetic rats. Further, alloxan induced diabetic rats when treated with petroleum ether and chloroform extracts significantly reduced the serum cholesterol level. It has been reported that plant extracts exert their cholesterol lowering effect seems to be a decrease in cholesterol absorption from the intestine, by binding with bile acids within the intestine and increasing bile acids excretion [35]. A significant increase in serum cholesterol and triglycerides observed in alloxan induced diabetic rats in our experiment is in agreement with the findings of the aforementioned studies. The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [36]. From our study, it is assumed that the administration of methanolic extracts of *C. dactylon* to surviving diabetic rats might have reduced the pancreatic lipase activity, which is responsible for the hydrolysis of non-absorbable dietary triglycerides into absorbable monoglycerides and free fatty acids, which, in turn, leads to the decrease of plasma cholesterol and triglycerides level [37, 38].

Our study revealed that the chloroform and methanolic extracts of *C. dactylon* had a significant antidiabetic effect in alloxan induced rats. Different solvent extracts used in our study showed differential responses in lowering blood glucose, urea, cholesterol and triglycerides of alloxan treated diabetic rats. Such a phenomenon of antidiabetic activity from common indigenous plants was also observed in *Momordica cymbalaua* [39], *Aegle marmelos* and *Cinnamonum tamala* [40] and *Vinca rosea* [41]. In earlier studies, the aqueous extract of *Cynodon dactylon* had significant antidiabetic potential along with significant hypoglycemic and hypolipidemic effects. Different doses of aqueous extract of *C. dactylon* significantly decreased the blood glucose, urea, serum cholesterol, serum triglycerides, creatinine, total hemoglobin, glycosylated hemoglobin and other biochemical parameters in alloxan induced diabetic rats [42, 43, 44]. But in our study, chloroform and ethanolic extracts of *C. dactylon* decreased the elevated blood glucose, triglycerides and urea level in alloxan induced diabetic rats better than aqueous extracts of *C. dactylon*. Further, a possible mechanism may be involved in enhancing the glycolytic flux and a concomitant decrease in gluconeogenesis. Our results also corroborated with the works done by [45, 46].

Our study revealed the presence of saponins and flavonoids in *C. dactylon* extracts might have played an imperative role in lowering the glucose level and plasma lipids in alloxan induced diabetic rats. Presence of such wide range of phytochemicals in this family may open a new dimension in the field of discovery of new drugs against diabetes.

**CONCLUSION**

Our results suggest that the different solvent extracts of *C. dactylon* may provide a new therapeutic avenue against diabetes and diabetes-related complications. The possible mechanism by which methanolic extracts of *C. dactylon* controls the diabetic condition may be due to biological activity of certain compounds which induce may either the pancreas to secrete insulin from the existing beta cells or involve in reduction of blood glucose by related biochemical mechanisms. The ability of *C. dactylon* extracts to reduce the diabetic conditions in the rats might be attributed to its ability to modulate the immune system leading to the decrease of β-cell damages. It is worth noting that the findings indicate that the curative effects could presumably be attributed to the qualitative and quantitative occurrence of phytochemical compounds present in the extracts. Presently, it is not possible to pin point the exact mechanism of antidiabetic activity of these plant extracts. Therefore, it is important
to characterize the biological compounds in this valuable herbal plant and their mode of action in controlling this multi factorial endocrine disorder for future studies in pharmaceutical application.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

40. Jaraid EE, Joshi SB, Jain DC. Antidiabetic activity of aqueous extract and non polysaccharide fraction of Cynodon dactylon Against Stz Induced Hepatic Injury In
