Anti-diabetic activity (ADA) of *Sida veronicefolia* on Diabetic Mellitus (DM) induced by Alloxan in rats.

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Abstract

One of the most significant illnesses that has a negative impact on the standard of living of people all over the globe is diabetes mellitus (DM). There is currently no drug on the market that can completely treat diabetes and all of the issues that come with it, much alone keep a person in excellent health. Traditional medicine, which is mostly derived from medicinal plants (MPs), is used to treat a variety of illnesses and conditions, including diabetes and the consequences of the disease. There are several papers that give evidence that many MPs exhibit anti-diabetic activity (ADA), and yet there are still many TMs that have not been researched about their ADA. Therefore, the purpose of this research was to assess the anti-diabetic activity (ADA) of *Sida veronicefolia* on diabetic mellitus that was caused by Alloxan in WISTAR albino rats. The phytochemical examination performed on the extracts of *Sida veronicefolia* indicates the existence of a variety of distinct phytochemical components. These components include sterols, terpenoids, alkaloids, flavanoids, and so on. The anti-diabetic effect of the methanolic extract was dosage dependent, similar to that of the standard medication metformin. The extract considerably regulated the amount of glucose in the blood, led to a reduction of body weight, and caused changes in a variety of enzymes involved in lipid metabolism. The findings of the current study provide scientific credence to the traditional use of *Sida veronicefolia*, and more studies are now being conducted to investigate its wide range of pharmacological applications and to determine which of its constituents contain bioactive compounds.

Key words: ***Sida veronicefolia***, Diabetes mellitus, Traditional medicine, Medicinal Plants, Alloxan.

Introduction

Diabetes mellitus (DM) is a chronic condition that is characterised by poor metabolism of glucose and other energy-yielding fuels, as well as the late development of vascular (including small and large blood vessels), and neuropathic consequences (Harris, 2004). Diabetes mellitus (DM) is a disorder that is characterised by impaired metabolism of glucose and other energy-yielding fuels. The condition is accompanied with a common hormonal defect known as insulin insufficiency. This shortfall may be complete, partial, or relative when examined in the context of coexisting insulin

resistance. This hormonal defect is present regardless of the origin of the disease. Worldwide, it is estimated that there are 177 million individuals afflicted with diabetes, which is over five times as many as the figures from 10 years ago. According to studies from the World Health Organisation (WHO), diabetes mellitus is one of the big killers of our time, with individuals in south-east Asia and western pacific being most at risk (Bommer et al., 2018). This figure may certainly double by the 2030, and WHO records show that DM is one of the great murderers of our time. In recent years, India has been witness to a DM pandemic that has swiftly been growing. indeed India now has the highest number of diabetes patients of any nation in the world, and it is projected that there will be 57.2 million diabetic patients in India by the year 2025, which is equivalent to one sixth of the world's population. Because of this, it is now required to search for a therapy for DM that is both cost-effective and beneficial from a therapeutic standpoint (Marn-Pealver et al., 2016).

On a worldwide scale, the contribution that traditional medicines provide towards the resolution of health issues is of incalculable value. This is even more surprising when we take into account the fact that nearly 80 percent of the population around the globe relies only on conventional medicine to meet all of their health care requirements. 9 Jamshidi-Kia, 2018). Since ancient times, India has been using various plant species in a variety of preparations as herbal medicines (Modak et al., 2007; Choudhury et al., 2017). As a result, India certainly holds the leading spot for the usage of herbal pharmaceuticals. According to the ancient medical practises of Ayurveda and Unani, which have been studied and recorded in great detail, the vast majority of people in India have relied on unrefined substances for the treatment of a variety of illnesses. In addition to Indian traditional medicine, there are many more traditional medicines accessible, such as traditional medicine from China, Korea, Iran, Africa, and the Siddha tradition, amongst others. According to Pang et al. (2019), all of these TMs rely mostly on various types of medicinal herbs. According to Mohanraj et al. (2018), the scientific community knows very little about the therapeutic applications of several plants traditionally used for treatment. According to this point of view, the present study was carried out to assess phytochemical components and alloxan-induced diabetes anti-diabetic activity.

Malvaceae is the family that includes the tropical plant *Sida veronicefolia*, which may be found all throughout India's tropical and subtropical areas. It has been used in the treatment of a variety of respiratory conditions, including but not limited to asthma, nasal congestion, and phthisis, among others. Additionally, it has been used in traditional indications in the treatment of dysentery, rheumatism, fever, and facial paralysis, as well as various mental illnesses (Franzotti et al., 2000; Kushagra et al., 2010; Kalaiarasan and Ahmed John, 2011).

Materials and Methods

Chemicals and Instruments

All of the solvents that were employed in this investigation were of an analytical grade. Coral clinical systems in Verna Goa, India, was the vendor for the acquisition of the testing kits. For the purpose of determining the concentration of glucose in the blood, an accu check glucometer (one touch), an incubator, a digital balance, a rotary flash evaporator (Superfit, Rotary Vaccum Digital Bath), a deep freezer, albino rats of the Wistar strain, and a Shimadzu UV Spectrophotometer model 1800 were employed.

Collection and extraction of plant material

Fresh entire plants of *Sida veronicefolia* were gathered from the Western Ghats in the Nilgiri district of Tamil Nadu. These plants were then sent to the survey of medicinal plants and collecting unit to be validated. Following the drying process in the shade, the plant material was further crushed into a powder form. The Soxhlet equipment was used to bundle the plant material that had been coarsely pulverised (500g). The plant material was first packed, then extracted in a series with alcohol (methanol), after which the extract was filtered and the filtrate was concentrated by evaporating the solvent at room temperature. Finally, the residue was concentrated in a hot air oven, and the final residue was kept in desiccators.

Preliminary phytochemical analysis

The standard procedures were used to know the phytochemical constituents in prepared extract (Doss,2009).

Experimental animals

We obtained albino-Wister rats of any gender, weighing between 150 and 200 grammes each. The animals were allowed to adjust to the laboratory environment for a period of one week, during which time the room temperature in the animal housing was maintained at 37 degrees Celsius. The mice were given a rat pelleted food that was available for purchase elsewhere. Under very sanitary circumstances, free access to water was permitted at any time. In order to acclimatise the rats to the circumstances of the laboratory, a week's worth of their usual diet was given to them before the trials began. The institutional ethics committee gave its stamp of approval to the whole research.

Acute toxicity studies

Studies on the extracts' acute toxicity were carried out using the toxic classic technique in accordance with the rules 423 specified by OECD, 2001 (OECD Guideline for testing of Chemicals, 2001). In the acute toxicity investigation, female albino rats served as the subjects. The animals were only allowed to drink water all through the night while they were maintained in a fasting state. These were separated into groups, with each group consisting of three different creatures. After that, methanolic extracts of *Sida veronicefolia* were given to each of these groups at a

dosage of 5 mg/kg body weight given orally, 50 mg/kg body weight given orally, and 500 mg/kg body weight given orally, respectively. Following the administration of the initial dosage, the animals were monitored continuously for a period of thirty minutes, followed by observation at regular intervals during the first twenty-four hours, with a focus on the first four hours, and then on a daily basis for a total of fourteen days. The observations, which may include drowsiness, convulsions, tremors, salivation, lethargy, and death, are meticulously documented alongside the specific records of each animal. Due to the fact that there was no mortality seen at the dosage levels of 5 mg, 50 mg, or 300 mg, the operation was carried out again with a greater dose of 5000 mg/kg b.w. in fresh animals.

Alloxan induced diabetic model

Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6- pyrimidinetetrone) Studies on the extracts' acute toxicity were carried out using the toxic classic technique in accordance with the rules 423 specified by OECD, 2001 (OECD Guideline for testing of Chemicals, 2001). In the acute toxicity investigation, female albino rats served as the subjects. The animals were only allowed to drink water all through the night while they were maintained in a fasting state. These were separated into groups, with each group consisting of three different creatures. After that, methanolic extracts of *Sida veronicefolia* were given to groupIII at a dosage of 5 mg/kg body weight given orally, 50 mg/kg body weight given orally, 550 mg/kg body weight given orally, respectively. Following the administration of the initial dosage, the animals were monitored continuously for a period of thirty minutes, followed by observation at regular intervals during the first twenty-four hours, with a focus on the first four hours, and then on a daily basis for a total of fourteen days. The observations, which may include drowsiness, convulsions, tremors, salivation, lethargy, and death, are meticulously documented alongside the specific records of each animal. Due to the fact that there was no mortality seen at the dosage levels of 5 mg, 50 mg, or 300 mg, the operation was carried out again with a greater dose of 5000 mg/kg b.w. in fresh animals.

1. Results and Discussion

The investigation into the phytochemicals Extracts of *Sida veronicefolia* were put through a variety of conventional phytochemical testing protocols, and the results indicated that the extracts contained the relevant bioactive compounds. However, the amount of a wide variety of phytochemicals was found to vary significantly amongst the extracts that were evaluated. There was evidence of the presence of sterols, terpenoids, glycosides, tannins, polysaccharides, alkaloids, and phenols in the methanolic extract. Methanolic extract has been shown to contain flavanoids.The investigation into the phytochemicals Extracts of *Sida veronicefolia* were put through a variety of conventional phytochemical testing protocols, and the results indicated that the extracts contained the relevant bioactive compounds. However, the amount of a

wide variety of phytochemicals was found to vary significantly amongst the extracts that were evaluated (Table 1). There was evidence of the presence of sterols, terpenoids, glycosides, tannins, polysaccharides, alkaloids, and phenols in the methanolic extract. Methanolic extract has been shown to contain flavanoids.

Table 1. Phytoconstituentsin different extracts of ***Sida veronicefolia***

Name of the Phytochemicals	methanol
Phytosterols	+
Terpenoids	+
Glycosides	+
Saponins	-
Flavonoids	+
Tannins	+
Carbohydrates	+
Alkaloids	+
Amino acids	-
Oils	+
Quinones	-
Phenols	+
+= Present, – = Absent	

After the preliminary phytochemical analysis, the extract were tested to know about their toxicity. The selected plant extract showed neither visible sign of toxicity nor mortality. The results clearly indicated non-toxicity of the extracts at a dose of 5000 mg/kg. From this, $1/20^{th}$ $1/10^{th}$, and $1/5^{th}$ and doses were selected for the experimental study. Hence there is no LD_{50} and methanolic extract tested are considered safe and nontoxic.

The current investigation, anti-diabetic activity of ***Sida veronicefolia*** was carried on alloxan induced diabetic albino rats. Intraperitoneal administration of alloxan (120 mg/kg of body weight) effectively induced DM in normal rats as reflected by glycosuria,hyperglycaemia, polyphagia, polydipsia and body weight loss compared with normal rats.

The variations in body weight due to the alloxan were observed before the treatment and after the treatment. Alloxan diabetic control significantly reduced the body weight, then treatment with standard and plant extract which gained significant weight. The methanol extract treated diabetic albino rats showed a significant dose dependent beneficial effect when compared with the reference drug ***Metformin*** (Fig 1).

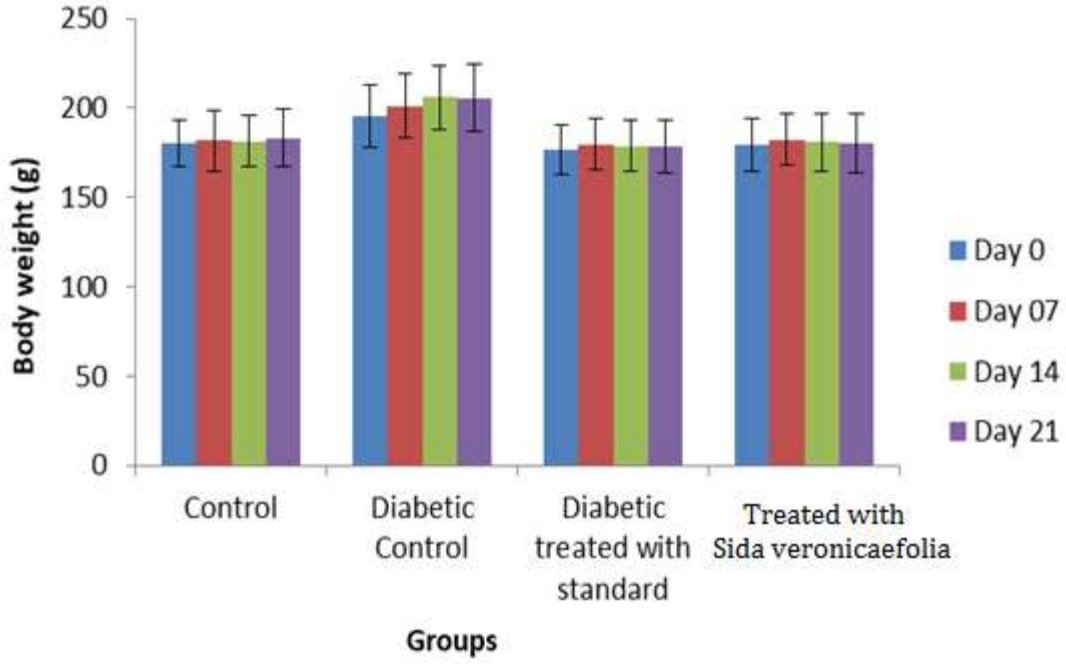

Fig 1. Effect of ***Sida veronicefolia*** on body weight in alloxan treated diabetic rats.

The blood glucose levels (BG) of the animals which are treated with ***Sida veronicefolia*** (Groups IV & the standard drug ***Metformin*** (Group-III), were observed on 1st, 7th, 14th, and 21st day. The diabetic rats which treated with ***Sida veronicefolia*** and ***Metformin*** showed a significant decrease in blood glucose level on 1st, 7th, 14th, and 21st day. On 21st day BG of Group-III decreases nearly too normal range. When compared with untreated group, the ***Sida veronicefolia*** at the dose of 550 mg/kg significantly reduces the hyperglycemia (Fig 2).

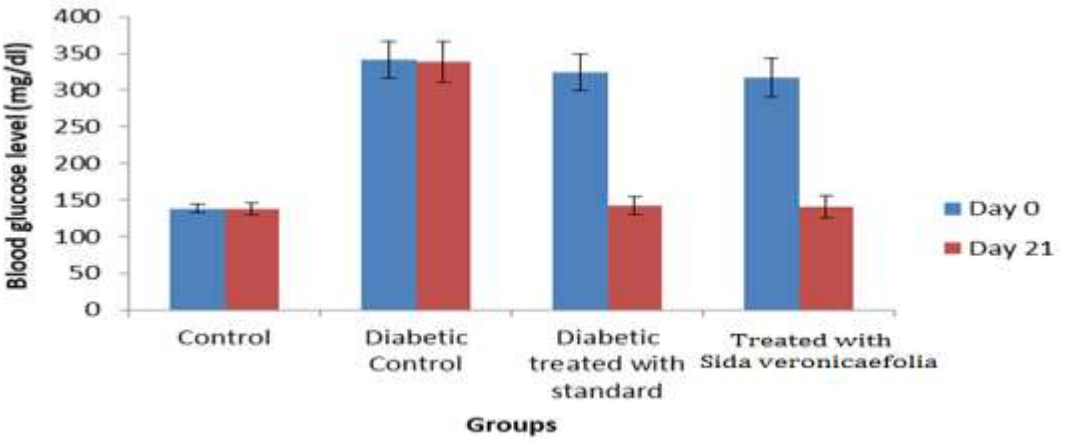

Fig 2. Effect of ***Sida veronicefolia*** on blood glucoselevel in alloxan treated diabetic rats.

Diabetes mellitus is also associated with hyperlipidemia with profound alteration in the concentration and composition of lipid and changesin the concentrations of the lipid contribute to the development of vascular disease (Parhofer, 2015; Athyros *et al.*, 2018). (Figs 3-5). The abnormally high concentration of serum lipids in DM is mainly due to an increase in the mobilization of free fatty acidsfrom the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Erejuwa *et al.*, 2016; Farsani *et al.*, 2016). Excess of fatty acids in plasma produced by alloxan promotes the liver conversion ofsome fatty acids to phospholipids and cholesterol. These two substances, along with excess of TG formed in the liver, may be discharged into lipoproteins in the blood. Administration of ***Sida veronicefolia*** to diabetic rats reversed all the above- mentioned changes and improved the HDL levels. The results of the present investigation clearlyindicate that the ***Sida veronicefolia*** has a glucose lowering effect on alloxan-induced diabetic rats. It was also found to be highly effective in managing the complications associated with DM, such as body weight maintenance and hyperlipidaemia and prevents the defects in lipid metabolism. The observed hypolipidemic effect may be because of decreased cholesterogenesis and fatty acids. Significantly lowering the total cholesterol and raise in HDL Cholesterol is a very desirable biochemical status for prevention of atherosclerosis and ischemic conditions.

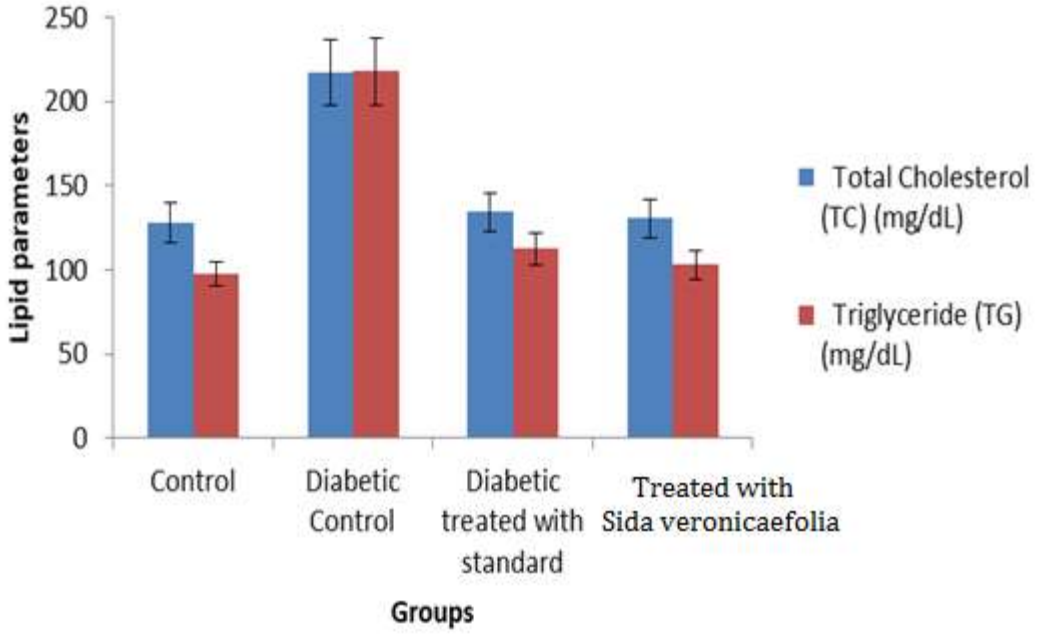

Fig 3. Effect on Serum TG and TC level on alloxan treateddiabetic rats.

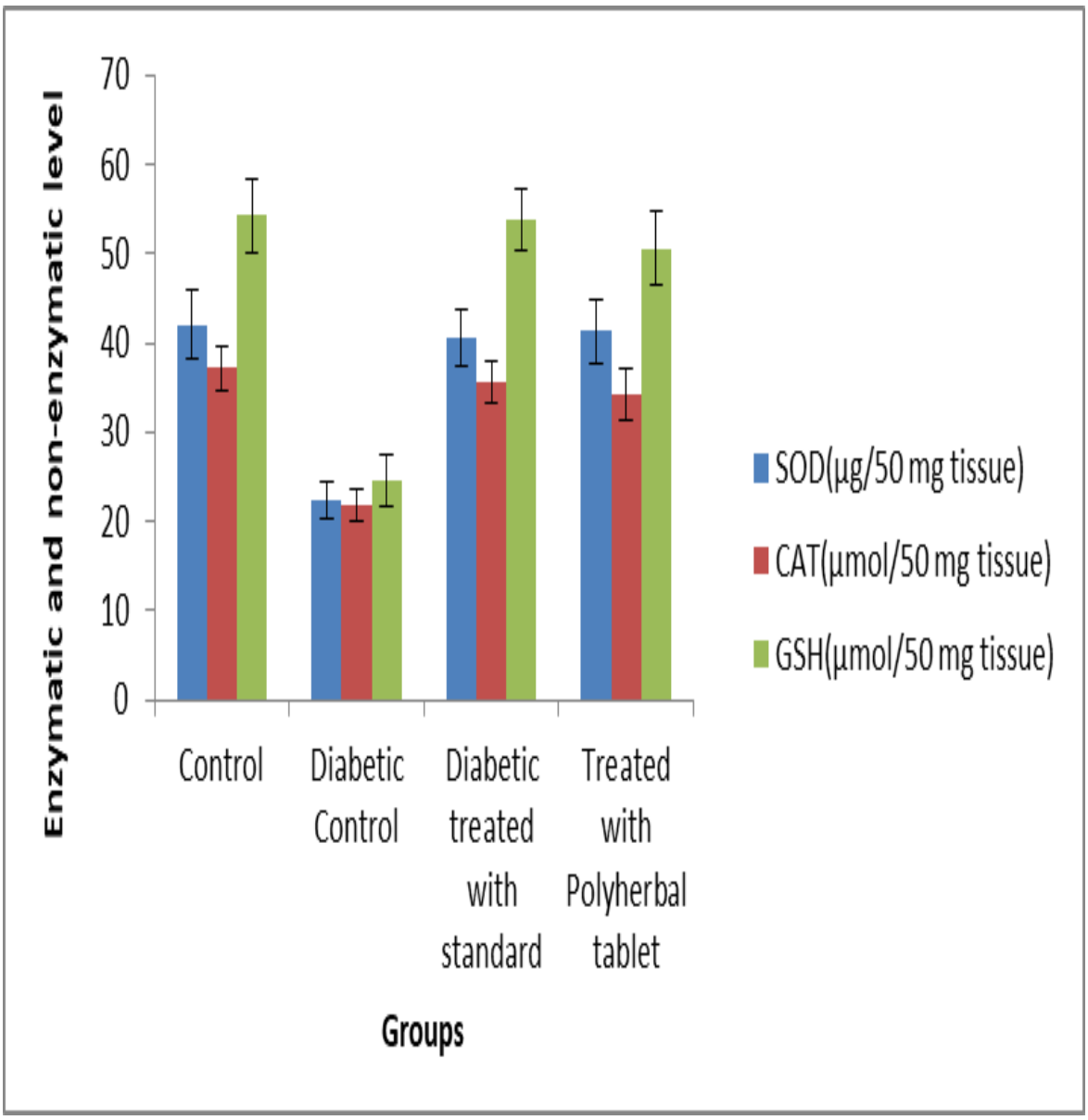

Fig 5. Effect on enzymatic and non enzymatic level on alloxan treateddiabetic rats.

The methanol extract of ***Sida veronicefolia*** was screened to explore the anti-diabetic activity because there have been no studies on it. However, in current study, presence of different phytochemical compounds in tested extract and are possess hypoglycemic activity. The results of present study suggest that ***Sida veronicefolia*** extract possess significant reduction in increased BG, controls the loss of body weight due to DM and control the lipid metabolism complications s as standard drug ***Metformin***.

Conclusion

The results of current research conclude that ***Sida veronicefolia*** having significant bioactive phytochemical constituent which are protective agent against the development and progression of DM and possible related cardiovascular complications in DM.

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