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Research Article

Antihyperglycemic Effect of crude Extract of Spermacocehispidain alloxan Induced Diabetic Rat

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ABSTRACT

One of the world's widespread and well-known disorder is diabetes mellitus. It is being emerged as an epidemic as well as one of the most common health issue. And also it is really a big challenge to manage diabetes. Therefore, this leads to the need for research on the products that should be natural along with having the anti-diabetic properties.

Aim: In this study, diabetic rat models were used for evaluating the effect of antihyperglycemicofcertainplants.

Materials and methods: These rats were allowed to fast overnight and after that, according to 150 mg/kg body weight a fresh solution of alloxanis prepared and injected in rats to induce diabetes.

Results:In the result, before and after supplementing the plant extracts on the diabetic rats, the lipid profile has been studied. Furthermore, the plant extract's ameliorative effect is also studied. As compared to the normal group, in the diabetic group the GPx, GST, CAT, and SOD levels were decreased, however, these levels have risen up to nearby normal due to the plant extract supplementation.

Conclusion: The male albino rat has been affected by a strong antidiabetic effect that includes the *Spermacocehispida'*saqueous leaf extract.

Keywords: Hyperglycemic, Oxidative stress,Plants, Diabetic, Alloxan.

INTRODUCTION

In today's world, one of the main health issues that are related to human is diabetes mellitus. It is the major endocrine as well asa chronic disorder that can be either inherited or may be caused either by insulin's ineffectiveness or by the pancreas's deficiency for producing the insulin. In most of the countries,diabetes mellitus is considered as the health issue that is growing day by day with around 4% to 5% worldwide. Various complications that are associated with diabetes like neuropathy, kidney disease, retinopathy, and heart diseases are caused by chronic hyperglycemia. In the world's working population the chronic disability and morbidity are also caused by it. In the present times, hyperglycemia is reduced through various drugs like a-glycosidase, metformin, and sulfonylurea's inhibitors. It seems like that in the medical field, diabetes and its associated complications are still significant medical problems, despite using several hypoglycemic agents [1]. The diabetes mellitus'complications and pathology are associated with excessive oxidative stress. The superoxide anions were produced by the increased blood glucose levels in diabetes through a Haber-Weiss reaction is initiated that generates the hydroxyl radicals which results in protein glycation and lipids peroxidation of the lipid membrane. It results in oxidativedestruction of the cell membrane. Various other significant bio-molecules are being damaged by these radicals that include DNA proteins, and carbohydrates [2]. It is believed that the oxidative stress's elevated state is being associated with the hyperglycemia which actually has a significant part in process of progression and onset of complications that are caused due to late-diabetic via the gene product formation and initiation of stress-sensitive intracellular signaling pathways that actually results in damaging the molecule cells [3]. The normal cellular metabolism produces the biological free radicals and further antioxidants are used for maintaining these radicals at steady-state level, and these antioxidants actually work as free radical scavengers. At the elevated concentrations, the cellular antioxidant system's detoxification capacity is overwhelmed by the free radicals production rate which then results in oxidative stress and cell structure damage [4]. Also, the diabetic animal models' blood has been reported with the various altered antioxidant enzyme activities. Also, it is observed that the protein membrane's oxidation and hyperglycemia

are intensely related to the several pathological consequences along with the rise in haemolysis of RBC [5]. The oxidative stress is caused by hyperglycemia through various mechanisms, a few are glucose autoxidation, non-enzymatic proteins glycation and superoxide anion's increased production in the mitochondria [6]. Additionally, due to metabolic stress, one can experience the increased inflammatory mediator's levels, reduced antioxidant defense and changes in energy metabolism [7]. Among various companions of oxidative stress altered antioxidant defense and hyperlipidemia are of main concern. For the macro and micro-vascular complications, there are various risk factors and among them, the hyperlipidemia induced diabetes is described as of main concern [8].For preventing the damage that is caused by the oxidative stress one of the major mechanism is to maintain the balance between antioxidants and ROS (Reactive Oxygen Species), thus; during the process of treating diabetes, the antioxidants dietary supplementation can be proved as one of the promising approaches.

The current study focuses on studying the "antioxidant and anti-hyperglycemic effects of aqueous leaf extract of *Spermacocehispida* on alloxan-induced diabetic male *albino* rats".

MATERIALS AND METHODS

Plant material

From the Vellore District, Tamil Nadu, India six Indian traditional plant species (*Spermacocehispida, Cocciniagrandis*, *Mimosa pudica, Andrographispaniculata, Pongamiapinnata, and Mimusopselengi*) were collected. In the initial tests performed on these six plants and it was found that against the diabetic rats that were induced with alloxan,*Spermacocehispida'*scrude extract has the strongest anti-diabetic activity. Also, from the areas near "Vellore District, Tamil Nadu, India," the *Spermacocehispida'*sfresh leaves were gathered. After that, at the room temperature, these leaves were wiped and dried in shade. Furthermore, after the authentication of these specimen of the plant they were deposited in the college.

Extract preparation

For preparing the extract, we have used 100 g powder of plant leaf and 500mL distilled water which is then mixed with the powder. This mixture was then put in at room temperature and stirred magneticallyovernight. After that, the filtration process is used for removing the residue and then to yield the 10% solid the concentration of obtained aqueous extracts were performed under vacuum. Then, the aqueous solution is used for administrating the plant extract to the animals.

Animals

We have bought the Wistar strainadult male albino rats from "Tamilnadu Veterinary and Animal Sciences University, Chennai, India" that weighs around 180-190grams. Polypropylene cages were used to keep these rats (each cage containing three rats) with relative humidity at 55%-65% and room temperature set to (25±2) ^{0}C. Until the animals adapt for the specific laboratory conditions they were kept in the house with a maintained (12±1) hour dark and light schedule. These rats were provided with free water access and commercially accessiblerat food (Hindustan Lever Limited plantlocated in Mumbai). Furthermore, as per the guidelines provided by the institution, the experiment was designed and performed.

Alloxan induced diabetic animal

In normal saline, the Alloxan monohydrate's intraperitoneal injection (150 mg/kg body weight,) which was provided by the SD Fine Chemical Limited located in Mumbai, were used for induction of diabetes in selected animals [9]. Afterward, their tail's tip was considered for taking the blood samples and with the help of Gluco Check Glucose estimation kit, the blood glucose is measured (Aspen diagnostic Private Limited situated in Delhi) after 48 hours.After the blood glucose level has risen up to 250 -350 mg/dl, then it has been used for further study. Every animal under study put in polypropylene cageas well as theywere provided with pellet food and water access and room temperature is maintained.

Acute Toxicity Study:

According to the guiding principle provided by the "Organization for Economic Co-operation and Development, 2001 (OECD)", we have performed the acute oral toxicity study. Initially, a 50mg/kg b.wt.thedose was given to animals which are then increased to of 5000 mg/kg b. wt. so as to evaluate the toxicity.

Experimental design

In our experimental design, saline is used for 45 days for administrating the rats. Forfurther experimentation, these animals were categorized into 3 groups. Group-I: Normal.Group-II: Alloxanis used for rat administration (150 mg/kg body weight) intraperitoneally.Group-III: The *Spermacocehispida'*saqueous extract (250 mg/kg body weight) is orally administrated to diabetes-induced animals for 45 days with the help of intragastric tubes.

Tissue Homogenate, Plasma andSerumPreparation

After performing the experiments, the cervical decapitation was used for sacrificingthese animals. After that blood was gathered and then serum is separated through the centrifugation. Further for the plasma, the blood was gathered along with anticoagulant and then it was centrifuged for 20 minutes at a speed of 2000 rpm.

Biochemical analysis

Following methods were used in the laboratory for biochemical estimation such as serum VLDL, HDL, LDL [12], TG [11], and TC [10]. The enzymatic antioxidant's tissue level viz. GST, GPX, CAT, and SOD is also calculated with the help of methods introduced by Habig et al. [16], Rotruck et al. [15], Sinha [14], and Kakkar et al. [13] respectively.

Statistical Analysis

The Mean ±Standard deviations were used for expressing our experimental values. For carrying out the statistical analysis one way ANOVA method is used in the standard statistical Software Package of Social Science (SPSS).

RESULTS

The 250 dosage level of *Spermacocehispida'*saqueous plant extractwas used for oral administration to the animals that were induced withdiabetics. In comparison to single plant extract treated groups levels, the reduction in the lipid profile and blood glucose was observed to be greater in plant extract treated groups. In the animals that were administrated with the plant extract, it was observed that their serum insulin decreased level was elevated extensively as compared to the control animal's serum insulin levels as shown in Figure 1 and figure 2. In comparison to the individual plant extract treated groups the rise in insulin levels is substantially greater. Figure 3 represents the creatinine, uric acid and urea levels in the investigationalas well as controlled rats group. It was also observed that in comparison to the normal control group, the diabetic rats were having the extensively increased renal markers creatinine, uric acid, and urea. Whereas in comparison to the diabetic control group when diabetic rats were administrated with 250 mg/kg body weight dose of *Spermacocehispida* extract it provides the almost normalcreatinine, uric acid, and urealevels.

In comparison to the control animals, the GST, GPx, CAT, and SOD activity levels were extensively raised after *Spermacocehispida*aqueous plant extract's administration as presented in figure 4.

DISCUSSION

An introduction of diabetes in the form of experiment among rodents by making use of chemicals that can selectively damage the pancreatic β-cells was simple in use and proved to be convenient. Alloxan [17] is the most commonly utilized agent of diabetogenic for rodents in screening the effect of anti-diabetic by making use of medicinal herbs. Using GLUT2: a Glucose Transporter [18] the Alloxan was taken by pancreatic β cells. This research was aimed to find the impacts of *Spermacocehispida*aqueous plant extract up on the insulin serum and antioxidants liver enzymes of diabetic rats (model) after the effect of chronic administration. The amount of serum lipids is usually increased in diabetes that also give rise to a higher factor of risk for any coronary cardiovascular diseases. The insulin enhances the enzyme lipoprotein lipase, under normal conditions which in turn hydrolyzes triglycerides. But, because of the deficiency of insulin, the lipoprotein lipase does not activate in sufficient quantity in the diabetic state giving rise to hypertriglyceridemia [19]. The oral processing of aqueous plant'sextract in the amount of 250 mg kg-1 bodyweight in white Wistar albino rats enhanced the levels of inulin serum as well as resulted in decrease in VLDL, LDL levels, triglycerides, and cholesterol level, which means that such a formulation can help in reducing the issues of lipid metabolism and other related risk factors related to cardiovascular issues in a diabetic patient.

The *Spermacocehispida*plant extract resulted in being an effective measure in enhancing the lipid metabolism in this research as opposed to using glibenclamide. Hyperlipidemia is directly linked to diabetes. It is already been discussed in previous research that lipid serum concentration increases in diabetes. The serum lipids have a crucial role in increasing problems of biological progress of diabetes mellitus. The decrease in HDL serum levels cholesterol and increase in serum cholesterol levels, in a diabetic patient, increases the chances of developing complications of microvascular giving rise to atherosclerosis that in turns give rise to heart problems.The unusually high amount of serum lipids in a diabetic patient is because of the rising mobilization of extra fatty acids through peripheral fat deposit through the adipose tissue, the hormone-sensitive lipase occurs after the insulin inhibits. The research stated the diabetic rats treated with alloxan usually shows abnormal lipid profile, but the group treated using plat extracts

showed comparatively better results in lipid profile as compared to the control animals.The plant extracts has a potential of anti-diabetic properties because of the presence of one or more elements of bioactive anti-hyperglycaemia like the synergistic impacts of flavonoids, and has found a high level of plasma insulin levels in the group treated by plant extracts which further confirmed that plant extracts has stimulated insulin secretion by surviving the pancreatic β-cells. The research also tested the antioxidant feature of *Spermacocehispida* aqueous plant extract in 250 mg kg-1 beta weight among the alloxan diabetic rats.

Diabetes Mellitus is known to be a major disorder that adversely affects uric acid, urea, kidneys, and creatinine are known to be the makers of renal function. In initial periods of diabetic nephropathy, there exists a hyperfiltration along with a rise in creatinine clearance giving rise to no effective change in levels of creatinine serum, but with the increase in disease, the serum levels of creatinine start to increase.

In this research,diabetic rats were noted with the rise in creatinine, uric acid, and urea levelsand such increase in levels, further reduced through of *Spermacocehispida* plant extract'soral administration as opposed to that is using Alloxan in the diabetic rats. The results of this study werealso linked to the results of other experiments [20,21] as well as it is also stated that extract ofthe aqueous plant can help in protecting the protein catabolism in the muscles and it also improved the conditions of kidneys in diabetic rodents.

Another potential reason for the increase in complications of diabetic conditions is Oxidative stress. No or less response of antioxidant defense or rise in the production of free radicals are the conditions which take place in diabetes that further results in higher oxidative stress.

The results of such oxidative stress are cell injury which means damaged antioxidant system for enzymes, damage to lipids, and destruction of cellular homeostasis along with the accumulation of damaged molecules. A decrease in levels of antioxidant like GST, SOD, CAT, and GPxactivities is due to a significantalterationof oxidative in the enzymatic proteins through the generationof extreme ROS. Further the activities reduction in above-mentioned enzymes give rise to a lower rate of synthesis.The study also discovered that SOD played a very important role in removing ROS derived through peroxidative development of xenobiotics in the tissue of the liver. This observation of SOD activity rise proposes that in response to ROSan enhanced protective activity is shown by the *Spermacocehispida's*aqueous plant extract. Such outcomes also show that *Spermacocehispida*aqueous plant extract is linked to a decrease in oxidative stress and also reduces the radical damage to tissues.

The main element of the antioxidant defense system is called CAT. It is a protective method which gives rise to an increase in sensitivity to reduce the radical-induced damage to cells. Increase in the free radical production gives rise to change in cellular macromolecule'sbiological activity. Hence with the decrease in the activity of such enzymes results in various deleterious impacts because of the accumulation of hydrogen peroxide and superoxide radicals.Through *Spermacocehispida* plant extract'soral administration, the catalase effect in diabetic rat induced with alloxan was increased to stop free radicalsexcessivegrowth.

The substantial increase in the level of activities of GST and GPx increases the free radicals scavengingmechanism. An equal lipid peroxidation reduction further enhanced the antioxidant enzyme's activity levelswhen the plant extract supplementation was observed. The outcomes prove to be evidence showing an increase in antioxidants enzyme's activity levels as well asa decrease in lipid peroxidation in animals consideredwith plant extracts as stated by Ramakrishna et al. [22].

As a result, this research shows a considerable antioxidant/anti-hyperglycemic impact of *Spermacocehispida* aqueous leaf extract. The results also supported the use of *Spermacocehispida*plant extract for treating diabetes mellitus.

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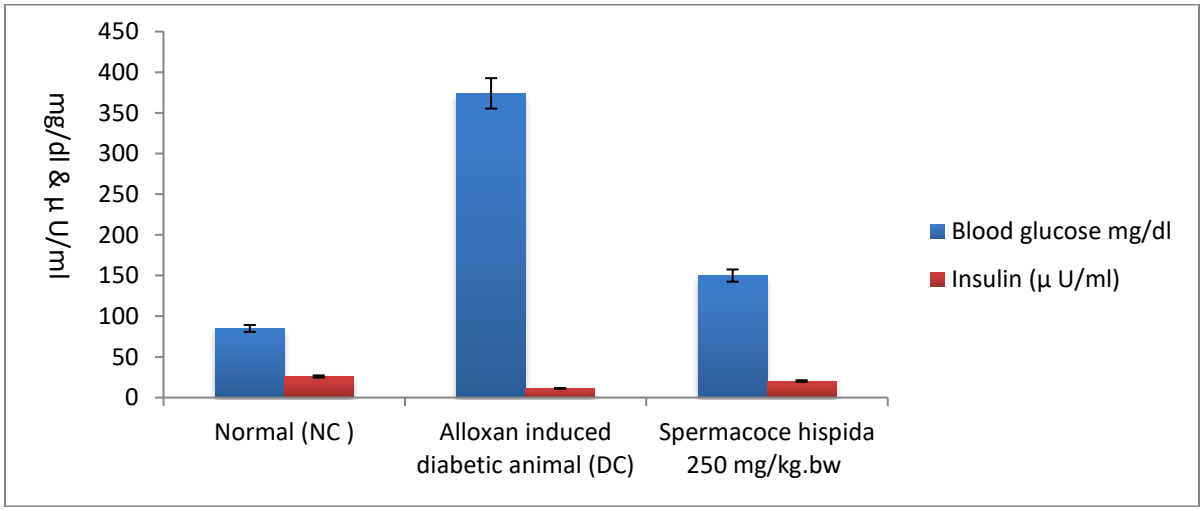

Fig.1: Effects of aqueous plant extract of Spermacocehispida against the alloxan-induced diabetic male *albino* rat. Levels of blood glucose and insulin in serum

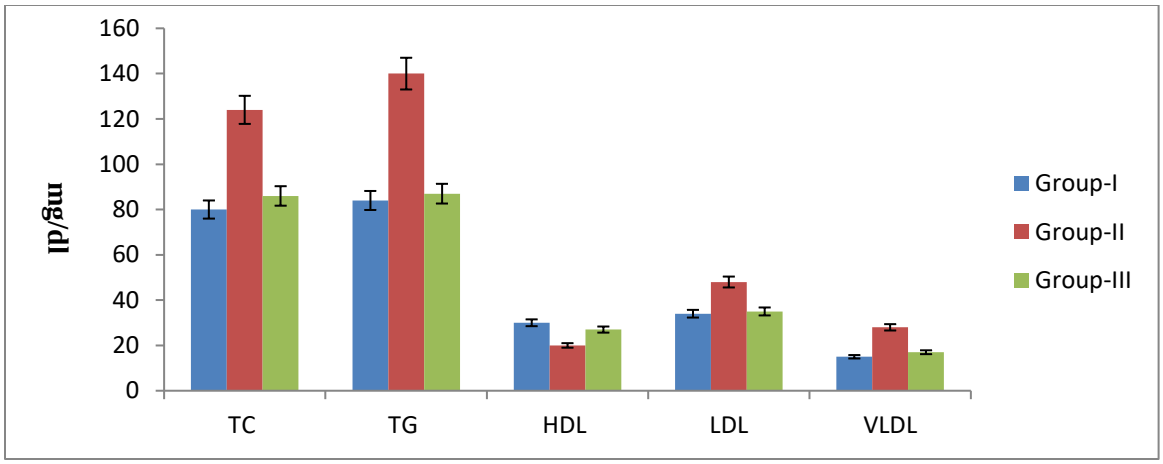

Fig.2: Effects of aqueous plant extract of Spermacocehispida against the alloxan-induced diabetic male albino rat. Levels of cholesterol, triglycerides, HDL, LDL and VLDL in serum

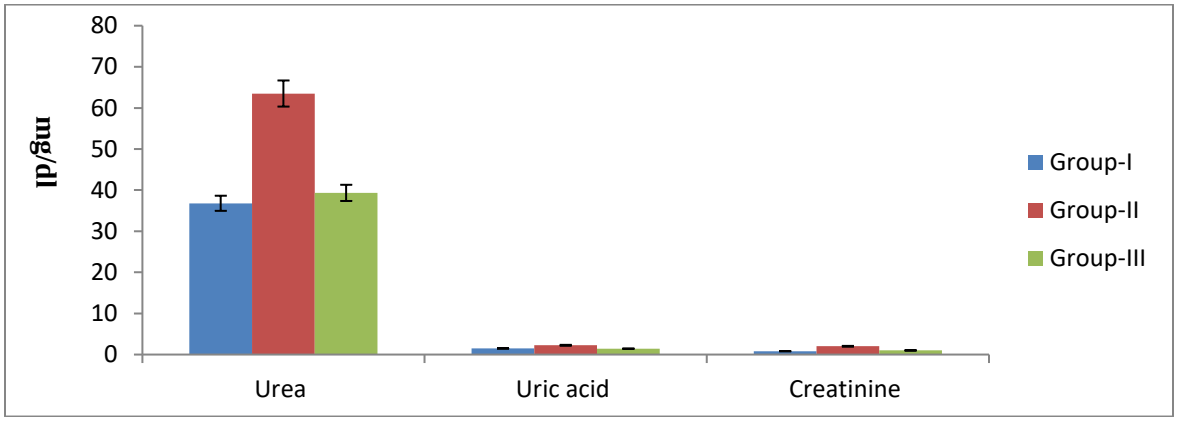

Fig.3: Effects of aqueous extract of *Spermacocehispida*on urea, uric acid, and creatinine of control and alloxan-induced experimental diabetes in rats

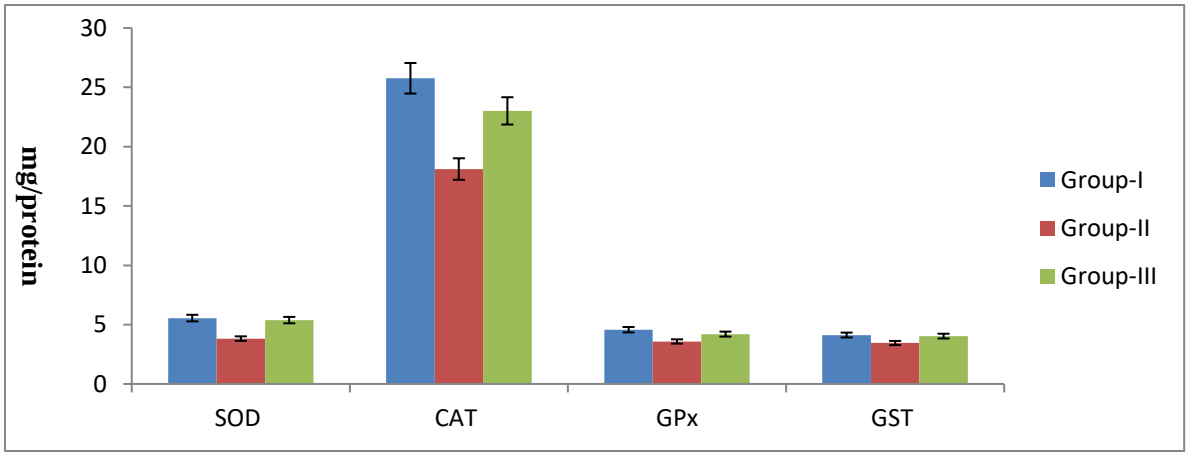

Fig.4: Effects of aqueous extract of *Spermacocehispida*against alloxan-induced diabetic male *albino* rat - Levels of SOD, CAT, GPx, and GST in the liver tissue of control and experimental animals.