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Biochemical and lipid profile evaluation of antidiabetic properties of *Achyranthes aspera* L. seeds in streptozotocin induced diabetic rats

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Abstract

Diabetes mellitus is one of the common metabolic disorders acquiring around 2.8% of the world's population and is anticipated to cross 5.4% by the year 2025. Though drugs are plenty for the treatment of diabetes, none is found to be ideal due to undesirable side effects and diminution after prolonged use. Hence, search for novel drugs, especially from plant origin continues. *Achyranthes aspera* has been long history of medicinal plant, it's found to exhibit a wide range of pharmacological properties. The present study was investigated to evaluate the antidiabetic potential of *A. aspera* seeds in STZ-induced experimental diabetes in rats. Phytochemical analysis of the *A. aspera* seeds extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids. Oral administration of *A. aspera* extract (300 mg/kg b.w./day) to diabetic rats for 28 days significantly reduced the level of blood glucose. The observed decrease in the level of plasma insulin and hemoglobin in the diabetic rats were elevated to normal by the extract treatment. The level of glycogen content was improved upon treatment with the *A. aspera* seed extract. Similarly, TG, LDL, VLDL- Cholesterol, TG and Lipid of serum were significantly decrease in STZ induced diabetic rats but HDL-Cholesterol level remained unchanged when compared to the diabetic control group. The results of the present study indicate that the *A. aspera* seeds extract is nontoxic and possess antidiabetic nature.

Keyword: *A. aspera* L, Diabetes mellitus, Streptozotocin, Biochemical, Lipid profile

INTRODUCTION

Diabetes mellitus is a multisystem endocrine disorder characterized by persistent hyperglycemia resulting from the defects in insulin secretion, action or both. The International Diabetes Federation (IDF) estimated the global burden of diabetes was 366 million in 2011 and it would rise to 552 million by 2030. The chronic hyperglycemia of diabetes associated with long-term damage of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development and progression of diabetes mellitus. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency (ADA, 2012).

Administration of 60 mg/kg streptozotocin dose can initiate an autoimmune process that results in the destruction of the Langerhans islets beta cells, ultimately contributing to the toxicity of beta cells. According to this model, oxidative stress is produced under diabetic conditions which possibly cause various forms of tissue damage in patients with diabetes. Diabetes mellitus is a complex and a multifarious group of disorder that disturbs the metabolism of carbohydrates, fats and proteins. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are currently available to reduce hyperglycemia in diabetes mellitus (Mutalik et al., 2003 and Halberstein et al., 2005).

Herbal medicines are popular remedies for a number of diseases and used by a vast majority of the world's population. Since pre-historic times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Further, most of our marketed medicines are distillations, combinations, reproductions or variations of substances which are abundantly found in nature. Our forefathers recommended some of the substances, which are abundantly found in nature long before their pharmacological actions were demonstrated and understood by scientific validations (Senthil et al., 2006).

In the traditional system of Indian medicine, formulation with extracts of plant parts is used as the drug of choice as antidiabetic, antiulcerative, hepatoprotective and lipid-lowering agents. Several medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases without proper

knowledge of their function. Although phytotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny. One such medicinal plant, which lacks scientific evidence for its folklore use is *A. aspera* Linn. belongs to the family Amaranthaceae. It is an annual, stiff erect herb, and found commonly as a weed throughout India and is one of the important medicinal plants having many therapeutic uses. All the plant parts has been used in traditional systems of medicines. Seeds, roots and shoots are the most important parts which are used medicinally. It is some important medicinal plants having many therapeutic uses against inflammation, microbes, odontalgic, Rheumatism, Bronchitis, skin disease and rabies (Girach et al., 1992). Therefore, the present study was carried out to investigate Biochemical and Lipid Profile Evaluation of Antidiabetic Properties of *A. aspera* Linn seeds in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Collection and authentication of experimental plant

Fresh and mature *A. aspera* Linn. Were collected from Kandhari, Thiruvarur District, Tamil Nadu. The plants were identified and authenticated by a taxonomist and an exemplar specimen was deposited at the Department of Botany, St. Joseph's College, Tiruchirappalli, India. The herbarium number of the plant is RVR001.

Preparation of extract

The dried and powdered seed of *A. aspera* (500 g) was extracted using soxhlet by evaporating with 75% ethanol.

Preliminary phytochemical screening

Ethanolic extract of *A. aspera* Linn seeds were subjected to preliminary phytochemical screening (Harborne, 1998 and Kokate 2001).

Experimental animals

The male albino wistar rats (150-200 g) obtained from Venkateswara Enterprises, Bangalore were used in this study. They were kept in polypropylene cages (47cm x34cm x 20cm) lined with husk. It was renewed every 24 hours under 12:12 hour light: dark cycle at around 22ºC and had free access to water and food. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental design

Animals were divided into five groups 6 animals in each

Group 1: Normal Control,

Group 2: Negative Control (STZ 60mg/kg),

Group 3: Positive Control (Glibenclamide, 5 mg/kg)

Group 4: Ethanolic Extract of *A. aspera* (300mg/kg)

Group 5: Ethanolic Extract of *A. aspera* (600mg/kg)

Induction of diabetes mellitus

Streptozotocin was used to induce diabetes mellitus in normoglycaemic male albino wistar rats. A freshly prepared solution of STZ (65 mg/kg body weight) in 0.1M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1mL/kg body weight to overnight fasted rats. After 48 hours of STZ administration, rats with moderate diabetes having glycosuria and hyperglycemia were selected for the experiment.

Examination of body weight

Body weight of rats from each group was measured on initial stage to 28 days. Weight was measured using standard digital weight balance to get accuracy.

Sample collection

At the end of the treatment period, all rats were fasted for 12 hours and sacrificed by cervical decapitation. The blood was collected into heparinized tubes and plasma and serum was separated by centrifugation and used for biochemical analysis.

Biochemical parameter

Blood glucose level was estimated by using o-toluidine reagent (Zlatkis et al., 1953). Plasma insulin was assayed using the Ultra-sensitive ELISA kit for rat insulin (Linco Research, St Charles, MO, USA). Hemoglobin level was estimated.

Estimation of lipid profile

Total cholesterol and triglyceride of serum were estimated by using standard method (Varley 1988 and Lyons 1992). HDL cholesterol was determined by phosphotungstate/mangnesium method (Lyons, 1992). VLDL cholesterol was calculated as Triglyceride /5.LDL cholesterol was calculated by the equation: LDL cholesterol = Total serum cholesterol - (HDL+VLDL)

Statistical analysis

All the results are presented as mean ± SEM data were analyzed by the standard deviation method with help of SPSS software. Results were considered statistically at $P < 0.001$.

RESULTS AND DISCUSSION

Traditional herbal medicine is used for treatment of diabetes in developing countries. India has the richest plant based traditional medicine system because of its rich biodiversity. Like India, in other developing countries, herbal plants constitute a very important national resource of health sector. These herbals constitute a very important national constitute a very important national resource of health sector. These herbal medicines are mainly used for health care due to

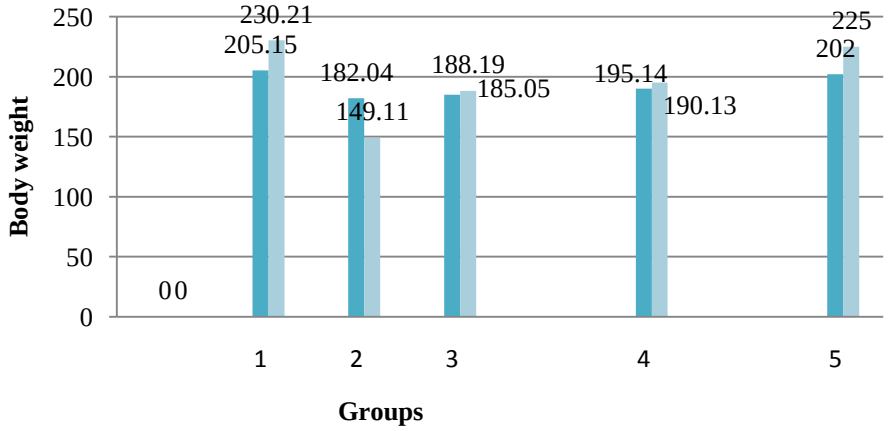

Fig 1: Estimation of Body Weight

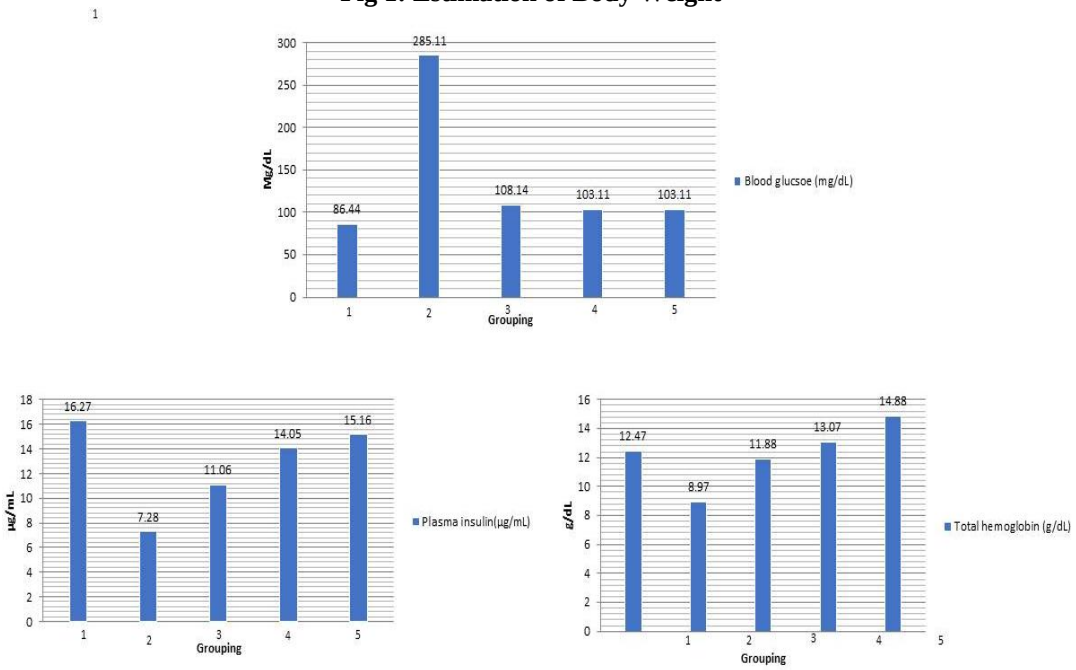

Fig 2: Estimation of Biochemical Parameter

their cost value, effectiveness and lesser side effects on human body (Sekar et al., 2010). Herbal medicine or phytomedicine is recognized as the most common form of alternative medicine (Patrick-Iwuanyanwu et al., 2012). WHO estimates that 80% of the world population currently use herbal drugs for major healthcare. Herbal drug is a chief constituent in traditional medicine and a common constituent in ayurvedic, homeopathic, naturopathic and other medicine systems (Maiti et al., 2011). However, another consideration was the fact that plants having antidiabetic activity have been ascertained to be rich in alkaloids, flavonoids and Saponin (triterpenoid+ steroidal glycosides) (Mishra, 2010), which are known to be bioactive against diabetes. The phytochemical screening of *A. aspera* exhibited the presence of alkaloid, flavonoid, steroid and triterpenoid Induction of triterpenoid Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins (Swanston-Flat et al., 1990). Diabetic rats treated with *A. aspera* seeds showed significant improvement in body weight (Fig.1.). Hence *A. aspera* exhibited a marked effect in controlling the loss of body weight of diabetic rats. The oral administration of aqueous leaves extract of *P. guajava* at the dose of 500mg/kg b.w for 15 days have shown beneficial effect not only on blood glucose but also on bodyweight, glucose and ketone level of urine and tissue of pancreas in streptozotocin induced adult albino diabetic rats (Fig. 2.). Methanolic extract (51%) of *P. guajava* leaves showed hypoglycemic effect in type 2 Diabetes (Joseph et al., 2011). Aqueous extract of *A. squamosa* root (at a dose of 250 mg/kg and 500

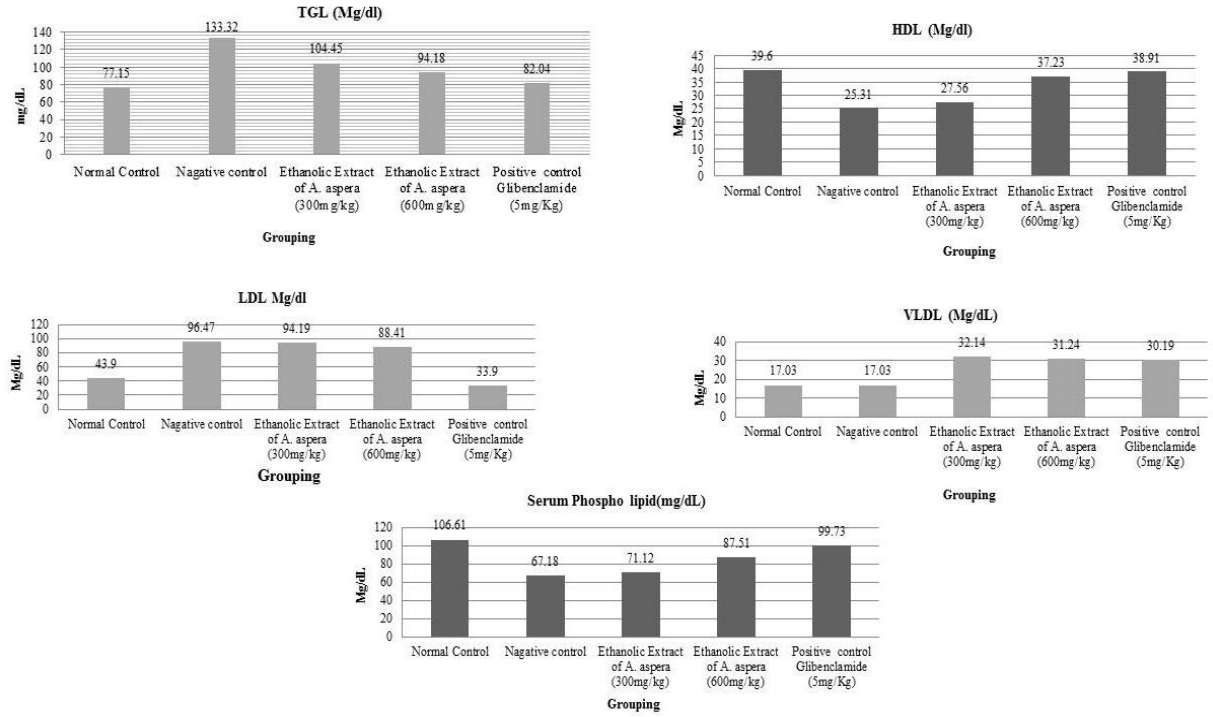

Figure 3: Estimation of Lipid Profile

mg/kg bw) when given to STZ- induced diabetic rats reduced the blood glucose level from 285.52 to 208.81mg/dl, 6 hours after oral administration of extract. It further decreases the hepatic and renal lipid. peroxidation with a concomitant increase in the activities of antioxidative enzymes, such as Catalase and Superoxide dismutase (Mohd et al., 1969). The present study reported that oral administration of *A. aspera* herbal extract produces a significant dose related hypoglycemic effect in normal as well as in diabetic rats. Oral administration of *A. aspera* seeds extract (300 mg/kg b.w. /day) to diabetic rats for 28 days significantly reduced the levels of blood glucose. The observed decrease in the levels of plasma insulin and hemoglobin in the diabetic rats were elevated to near normal by the extract treatment. The level of glycogen content was improved upon treatment with the extract. Streptozotocin results elevation of triglycerides, total cholesterol and decrease HDL cholesterol. Hypercholesteremia and hyper-triglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of Diabetes (Ananthan et al., 2003). Dyslipidaemia is characterized by high plasma levels of total cholesterol, LDL-cholesterol and triglycerides, with low plasma levels of HDL cholesterol. A variety of dysfunction in metabolic and regulatory mechanisms, due to insulin deficiency, is responsible for accumulation of lipids (Rajalingam et al., 1993).

A. aspera significantly reduced serum triglycerides, total cholesterol and increase level of HDL in STZ induced diabetic rats (Fig 3.). Hence, the present investigation revealed the antidiabetic activity of the *A. aspera.* L seeds on streptozotocin induced diabetic rats.

CONCLUSION

The present study indicates that the treatment with *Achyranthes aspera* Linn has favorable effect not only on biochemical and lipid parameters but also body weight. These findings point out the promising effect of *Achyranthes aspera* Linn as a useful antidiabetic agent. Our findings could be targeted for the promising potential applications including drug formulation and biomedical applications in future.

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