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## EVALUATION OF AQUEOUS LEAF EXTRACT OF *SAPINDUS SAPONARIA* ON CARBOHYDRATE METABOLIZING ENZYMES IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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### Keywords:

Diabetes mellitus, *Sapindus saponaria*, Hypoglycemia, Phytomedicine, Phytochemicals

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**ABSTRACT:** Diabetes mellitus (DM), a longtime metabolic illness characterized with the high concentration of glucose residue in the blood, is one of the key global health issues because of the prevalence and chronic outcomes. The traditional plant remedies in tackling the condition have attracted attention as an alternative to the conventional therapies, which are usually associated with undesirable side effects. The current study grasps the growing momentum in the scientific testability of the medicinal plants considering their portentous biochemical depth and the advisability of regulating key processes of metabolism. To elaborate the peculiar enzymatic activities inhibited by *Sapindus saponaria* leaf extract and to comprehend about the antidiabetic activities of the extracts, the study investigated the effects of the extract on carbohydrates metabolism in this research. The study demonstrates that the extract has a substantial effect on carbohydrate metabolism-related enzymes, and can propose its potential use as a diabetes treatment. The capacity of an aqueous extract of *Sapindus saponaria* leaves to affect the antidiabetic properties was explained by measuring the influence of these extracts on the enzymes responsible of breaking down the carbohydrates of the model being streptozotocin-induced diabetic rats. Further research of the topic should be aimed at isolating and characterizing the bioactive molecules through isolation and characterization and elucidation into the mechanism of action by which they act. Thereafter, clinical testing done to ascertain the safety and the efficacy of the extract in treating diabetes mellitus in human beings.

**INTRODUCTION:** One cannot underestimate the significance of carbohydrate metabolism in keeping the energy level in the cells and adjusting the level of glucose in living beings<sup>1</sup>. The process consists of numerous enzymes to control the formation, degradation, and transformation of carbohydrates<sup>2</sup>. In case of any disruptions in such enzyme pathways, it may result in severely complicated metabolic disturbances, impacting the general health and even causing other disorder<sup>3</sup>.

Thus, it is relevant to learn the enzymes that participate in the carbohydrate metabolism to resolve the metabolic disorders and associated health disorders<sup>4</sup>. The quest of finding safe and effective drugs against high blood sugar has contributed to more interest in natural products and in particular the extracts of plants owing to the potential of containing multi-beneficial properties and owing to their probably good safety<sup>5</sup>.

Plant-based secondary metabolites have the potential to touch on the pathways of insulin signaling and assist with transferring GLUT 4 towards the surface of the cell. In this process, glucose uptake in muscle and fat tissues is enhanced (improving insulin sensitivity)<sup>6</sup>. Besides this, phytochemicals can protect pancreatic beta-

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cells (help prevent harm) and restore normal cellular insulin signaling, confined to insulin production<sup>7</sup>. Different chemical groups such as alkaloids, flavonoids, anthocyanins, terpenoids, phenolic, and glycosides are related to the antidiabetic effects of phytochemicals<sup>8</sup>. Among the previously academically studied plants that have gained popularity through traditional purposes and the avenues of containing saponins, bioactive molecules with widely systemic pharmacological potential, including the possible effect of antidiabetics the *Sapindus saponaria*, or soapberry. Research revealed that this plant has triterpenoid saponins, which have an ability to affect key enzyme pathways including inhibition of alpha-glucosidase and alpha-amylase that are critical to managing post-meal blood sugar<sup>9</sup>.

Hypoglycemic, hypocholesterolemia, and antioxidant activities exist in amphiphilic structures, e.g. saponins with carbohydrate structures coupled with triterpenoid or steroid aglycone groups. All this renders saponins a promising agent to manage diabetes<sup>10,11</sup>.

Carbohydrate-gearing enzymes, such as glucokinase, play an important role in regulating blood glucose, so they present promising therapeutic value. Modifying the activity of these enzymes, in particular, by inhibiting the enzymes that digest carbohydrates, like alpha-glucosidase, is the way to lower postprandial high blood sugar<sup>12</sup>. In light of the effects of carbohydrates on running, the increased occurrence of hyperglycemia worldwide and evidence of the curative properties of natural compounds, it is proposed that this study will investigate the impact of an aqueous leaf extract of *Sapindus saponaria* on important carbohydrate-metabolizing enzymes in rats with streptozotocin-induced hyperglycemia. By doing so, we hope to be in a better position to explain what exactly changes in enzymes the extract works and how it contributes to controlling hyperglycemia, as well as to steer future treatment approaches.

## MATERIALS AND METHODS:

**Plant Preparation and Extraction:** Leaves of *S. saponaria* were collected close to the Acharya Nagarjuna University at Guntur and tested by one of the Botanist of this university department of

Botany. Before the process of extraction, the leaves were crushed mechanically into fine powder and dried in a shaded condition. Weigh 200g of powdered SS leaf that is soaked in distilled water into a glass beaker within a period of two days at room temperature. A mixture resulting was filtered to extract aqueous and this filtration was repeated three to four times until a clear filtrate was obtained. After this, the filtrate was freeze-dried following the concentration and distillation of the filtrate using a Buchi rotavapor R-200 under the conditions of reduced pressure. All extracts were refrigerated until the time that they were needed to undergo additional testing.

**Reagents/Chemicals:** The reagents and all chemicals involved in the experiments were of an analytical grade.

**Experimental Animals:** Male Wistar of 180-200g were used in the experiments. The feeding of these rats used a standard pellet diet whereby they had access to water *ad libitum*. They were kept in clean, dry polypropylene cages in a well-ventilated facility and kept to a 12-hour cycle of light-dark experiment was conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC), with no.05/IAEC/CLPT/2023-24.

**Induction of Diabetes:** Streptozotocin (STZ), suspended in 0.01M citrate buffer with pH of 4.5, was injected at 50 mg/kg body weight into male Wistar albino rats, to induce diabetes mellitus disease. In reducing the risk of excessive hypoglycemia caused by the hyper insulin secretions and b-cell dysfunction induced by STZ, a 15% glucose solution was administered at *ad libitum* to 24 hours, beginning eight hours post-STZ injection. The rats with remarkable hyperglycemia, i.e., fasting blood glucose levels were 250 mg/dL or higher, were included in the experiment 48 hours after the injection.

**Experimental Design:** Total of 60 rats, grouped as, 30 normal and 30 STZ-diabetic rats. Then split them into six groups, often rats each. SS leaf extracts were dissolved in a vehicle solution

(Dimethyl sulfoxide [DMSO], 0.5%; 1 ml/kg body weight) and orally administered during a period between 15-30 days treatment.

**Animals Sacrifice and Tissue Preparation:** After 15 days of the experiment, the 12-hour starved rats were anesthetized (ketamine, 24 mg/kg body weight, intramuscularly) followed by the dissection process of the animal. The liver tissues were removed, rinsed in cold saline, stripped off all residing lipids, and at the same time subjected to weighing after immersed in ice-cooled containers. These tissues were set to be evaluated on the measurement of the particular carbohydrate-metabolizing enzymes.

**Statistical Analysis:** Systematic analysis of the data was done using the SPSS/11.5 program. ANOVA was used and followed by Tukey test, to evaluate the result. Results are presented in mean  $\pm$  SD, for six rats each group.  $P < 0.05$  is considered significant.

#### **Carbohydrate Metabolic Enzymes:**

**Assay of Hexokinase D (Glucokinase) Activity:** Hexokinase D assay was determined referring to the method of Brandstrup *et al.* (1957)<sup>13</sup>. Enzymatic activity of Hexokinase D was measured by adding 1 ml of 0.005 M glucose, 0.5 ml of 0.05 M MgCl<sub>2</sub> (magnesium chloride), 0.5 ml of 0.125 M K<sub>2</sub>HPO<sub>4</sub> (dipotassium hydrogen phosphate solution), 2.5 ml of tris HCL buffer, 0.4 ml of 0.1 M KCl (potassium chloride), 0.1 ml of 0.5 M NaF (sodium fluoride) making the mixture a total volume of 5 ml. Subsequently, the mixture was incubated five minutes at 37°C.

The reaction was started upon the addition of tissue homogenate of 1.3 ml. Then immediately 1 mL of the reaction mixture combined with 1 mL of 10% TCA. After 30 minutes incubation at 37°C 1ml of the reaction mixture was collected in one tube that already contain 1 ml of 10% TCA. Once the proteins were precipitated when centrifuged using centrifuge, the supernatant was tested on glucose by Sasaki and Matsui o-toluene method<sup>14</sup>. A control blank was prepared against sample. The difference was measured in order to know the amount of glucose phosphorylated. Enzyme activity was given as  $\mu$ /mol of phosphorylated glucose/h1mg of protein.

**Assay of Glucose 6-phosphatase Activity:** The test of glucose 6-phosphatase was determined referring to the method of Koide and Oda *et al.* (1959)<sup>15</sup>. Incubation mix was a combination of 0.2 mL of tissue homogenate, 0.5 mL of 0.01 M glucose 6-phosphate, and 0.3 mL of maleic acid buffer of pH of 6.5 at 0.1 M. One hour incubation was done at 37°C. Enzymes activity was brought to an end by the addition of 1 ml of 10% TCA to the reaction tubes. The reaction tubes were then Centrifuged, the phosphate concentration of the supernatant determined by the Fiske and Subbarow method (1925)<sup>16</sup>.

To analyze the phosphate concentration, 1 mL of supernatant, 0.4 ml of Aminonaphthol Sulphonic Acid ANSA (500 gms of ANSA dissolved in 195ml of 15% sodium bisulfite and 5ml of 20% sodium sulfite), 1ml of ammonium molybdate (prepared with 2.5 g ammonium molybdate dissolved in 100 ml of 3 N sulfuric acid) were combined. After 20 min a blue color emerged and was read at 620 nm. A control tube free of enzyme activity was also prepared. The same steps were applied to the analysis of a blank having only reagents and a set of standards including 8-40  $\mu$ g of phosphorus. Enzyme activity was expressed as  $\mu$ mol inorganic phosphorus released/min/mg of protein.

**Assay of Fructose 1, 6-Bisphosphatase Activity:** Fructose 1, 6-bisphosphatase activity was examined based on the procedure that was created by Gancedo and Gancedo (1971) method<sup>17</sup>. The assay solution, measuring 2.0 mL, consisted of 0.2ml of enzyme source, 0.4 mL of the substrate, 1.0 of Tris-HCl buffer (0.1 M pH7.0), 0.05 mL of fructose 1, 6-bisphosphate (0.05M), and 0.1 M magnesium and potassium chloride each and 0.1 mL of EDTA (0.001 M). This mixture was incubated at 37°C for 15 minutes. This was allowed to proceed until the enzymatic assay was stopped by addition of 1 mL of 10% TCA. The mixture was then centrifuged, and the phosphorus concentration of the supernatant was assessed by Fiske and Subbarow (1925) method<sup>16</sup>. According to this method, 0.5 mL of ammonium molybdate (2.5% of ammonium molybdate solution in 3 N sulfuric acid) and 0.3 mL of distilled water were put in the 1 ml aliquot of the supernatant.

After few minutes 0.2 ml ANSA (aminonaphthol sulfonic acid) was added. Letting the reaction mixture incubated for 10 minutes following which the tubes were mixed constantly which gives blue colour. The resultant blue color has been quantified after 20 minutes at 620 nm. Results were in the form of  $\mu\text{mol}$  of inorganic phosphorus/h1/mg protein. The phosphorus stock standard was made by dissolving 35.1 mg of potassium dihydrogen phosphate in 100 mL of distilled water until the stock contained concentration of 80  $\mu\text{g}/\text{ml}$ .

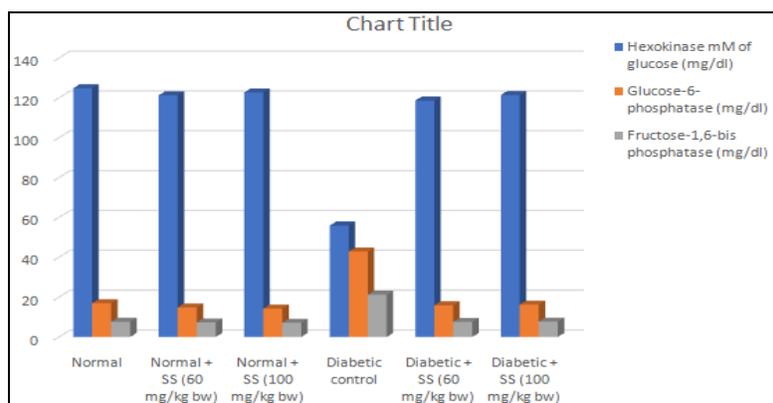
**RESULTS:** In diabetic rats, hexokinase may become dysfunctional causing decline in glycolysis, and therewith reduced usage of glucose to provide energy. It was found that there is a significant reduction of hexokinase activity in diabetic rats as compared to normal controls. STZ induced diabetic rats treated with SS-leaf extract showed significant hexokinase restoration in the liver and in serum **Table 1**. Glucose-6-phosphatase is an enzyme in the process of gluconeogenesis, which is very important in the maintenance of glucose homeostasis, as it catalyzes the last step in glycogenolysis and gluconeogenesis. We found that the STZ induced

diabetic rats had an elevated concentration of fructose-1, 6-bisphosphatase and glucose-6-phosphatase activities when compared to the control. These diabetes changes in enzyme activities in diabetic rats were reversed by interventions of *Sapindus saponaria* extract 60/kg bw and 100/kg bw. When diabetic rats were provided with SS-leaf extract after 15 days, hexokinase activity was restored in the diabetic rats to the levels of normal rats, and glucose-6-phosphatase and fructose-1, 6-bisphosphatase activities were reduced both in serum and tissues (liver and kidney) compared to that of diabetic rats. The positive effect can be attributed to the elevation of insulin concentration, which could indicate that biologically active products of the extract provoke the secretion of insulin **Table 1**. Covers the contrary, the SS-leaf extract failed to exert any real effects on these enzyme activities on normal rats. The positive glucose-lowering effect of the extract together with the metabolic linkage between improved glycolysis and the reduction in metabolic gluconeogenesis is indicative of possible biochemical mechanisms of controlling glucose balance.

**TABLE 1: EFFECT OF SAPINDUS SAPONARIA SS - LEAF EXTRACT ON SERUM CARBOHYDRATE METABOLIZING ENZYMES LEVELS IN CONTROL AND STZ INDUCED DIABETIC RATS**

Groups	Hexokinase mM of glucose (mg/dl)	Glucose-6-phosphatase (mg/dl)	Fructose-1,6-bis phosphatase (mg/dl)
Normal	124.85±9.40	16.95±1.18	7.62±0.52
Normal + SS (60 mg/kg bw)	121.30±8.95 <sup>b</sup>	14.85±1.09 <sup>b</sup>	7.28±0.60 <sup>b</sup>
Normal + SS (100 mg/kg bw)	122.75±9.85 <sup>b</sup>	14.25±1.11 <sup>b</sup>	7.10±0.55 <sup>b</sup>
Diabetic control	55.95±6.80 <sup>a</sup>	42.85±1.25 <sup>a</sup>	21.20±0.64 <sup>a</sup>
Diabetic + SS (60 mg/kg bw)	118.65±8.70 <sup>b</sup>	15.90±1.16 <sup>b</sup>	7.55±0.58 <sup>b</sup>
Diabetic + SS (100 mg/kg bw)	121.45±9.30 <sup>b</sup>	16.25±1.20 <sup>b</sup>	7.72±0.57 <sup>b</sup>

Each value is mean  $\pm$  SD for 6 rats in each group. a:  $p < 0.05$  by comparison with normal rats. b:  $p < 0.05$  by comparison with STZ diabetic rats. Non-significant



**FIG. 1: GRAPHICAL REPRESENTATION EFFECT OF SS SAPINDUS SAPONARIA EXTRACT LEAF EXTRACT ON SERUM CARBOHYDRATE METABOLIZING ENZYMES LEVELS IN CONTROL AND STZ INDUCED DIABETIC RATS**

**DISCUSSION:** The enzyme Hexokinase is the first enzyme in the Glycolysis pathway transforming glucose into glucose-6-phosphate. Thus, decrease in the hexokinase activity among diabetic rats largely implies loss in glucose metabolism. When the functions of hexokinase become weak, cells lose the abilities to absorb and utilize glucose, which causes high blood sugar levels. In contrast, the corresponding increase in the activity of fructose-1, 6 -bisphosphatase and glucose-6 -phosphatase among diabetic rats indicates the intensified rate of gluconeogenesis in the liver, where glucose is made out of non-carbohydrate energy sources. These enzymes are important regulators in the gluconeogenic pathway, and increase in their activities causes excess glucose to be produced by the liver that worsens hyperglycemia. The most striking result of the present study is that aqueous leaf extract/extraction of *Sapindus saponaria* was able normalized the essential carbohydrate-metabolizing enzymes activities in STZ-induced diabetic rats.

Replenishment of hexokinase activity indicates that *Sapindus saponaria* extract induces glucose metabolism and therefore glucose clearance in the bloodstream. Simultaneously, the significant decreases in glucose-6-phosphatase and fructose-1,6-bisphosphatase activity demonstrate that the extract suppresses the production of hepatic glucose level. Notably, the fact that the extract failed to induce adverse effects on the enzyme activities in normal rats can also imply a good safety profile on dosage of exercise within the target experimentation. Hypoglycemia was not observed in the non-diabetes subjects. Future studies that isolate and identify individual bioactive compounds of the extract and describe their action mechanisms would be of great importance.

**CONCLUSION:** This study offers strong findings that *Sapindus saponaria* aqueous leaf extract has strong antidiabetic properties with positive effects in modifying essential carbohydrate-metabolizing enzymes. The results indicate the viability of *Sapindus saponaria* as an aesthetic remedy for the treatment and management of diabetes.

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**CONFLICTS OF INTEREST:** Nil

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