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Estimation of Liver Glycogen in Normal Control, Diabetic Control and *Tinospora Cordifolia* Extract Treated Albino Rats

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Estimation of Liver Glycogen in Normal Control, Diabetic Control and *Tinospora Cordifolia* Extract Treated Albino Rats

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Abstract- **Investigations have been carried out to examine the presence of liver glycogen in normal control, diabetic control and extract treated albino rats. Estimation of liver glycogen was carried out by taking liver samples from normal control, diabetic control and extract treated albino rats. The rats weighing 150-190gm were administered intraperitonealy with 180mg/kg body weight dose of alloxan monohydrate for the induction of diabetes, with alcoholic leaf extract of *Tinospora cordifolia* with a oral dose of 20 ml/kg body weight from day 2 to 30 half an hour prior to feeding twice a day. It produces significant decrease in liver glycogen level.**

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I. Introduction

In the past 200 years, dramatic advances in our understanding of the regulation of normal glucose metabolism have been made. Beginning in the mid-19th century, Claude Bernard showed that blood glucose levels are regulated not just by the absorption of dietary carbohydrate but also by the liver, which plays a central role in producing glucose from non-glucose precursors [1]. Other investigators built on this discovery to identify the enzymes responsible for the synthesis and breakdown of glycogen [2], the role of anterior pituitary hormones in glucose metabolism and the onset of diabetes [3], the role of reversible protein phosphorylation by a protein kinase [4] and the discovery of cyclic AMP and its role in hormonal action, particularly that of epinephrine and glucagon, both of which elevate the blood glucose concentration and contribute to diabetic hyperglycemia [5].

Number of researches have been accomplished experimental diabetes induce in various mammalian species [6, 7, 8, 9, 10].

It is investigated that the whole plant extract of *Tinospora cordifolia* significantly decreases the blood glucose towards the normal blood [11]. Although several therapies are in use for the treatment, there are certain limitations due to high cost and side effects such as development of hyperglycemia, weight gain, gastrointestinal disturbances, liver toxicity etc.[12]. Based on recent advances and involvement of oxidative stress in complicating diabetes mellitus, efforts are on to find suitable antidiabetic and antioxidant therapy. Present investigations were carried out on albino rat *Rattus norvegicus* due to its metabolic relatedness with human.

Medicinal plants are being looked upon one again for the treatment of diabetes. Many conventional drugs have been derived from prototypic molecules in medicinal plants. Metformin exemplifies an efficacious oral glucose –lowering agent. Its development was based on the use of *Galiga officinalis* to treat diabetes. *Galiga officinalis* is rich in guanidine, the hypoglycemic component. Because guanidine is too toxic for clinical use, the alkyl biguanides synthalin A and Synthalin B were introduced as oral antidiabetic agents in Europe in the 1920s but these were discontinued after insulin became more widely available. However, experience with guanidine and biguanides prompted the development of metformin. Upto now, over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization (WHO) Expert Committee on diabetes has recommended that medicinal herbs be further investigated. Based on this recommendation and need for conducting clinical research in herbal drugs, developing simple bioassays for biological standardization, pharmacological and toxicological evaluation, current study is performed to evaluate the antidiabetic potential of *Tinospora cordifolia*.

Tinospora cordifolia (Guduchi) is an evergreen perennial climber. This deciduous and dioecious plant belong to the family Menispermaceae which consists of about 70 genera and 450 species that are found in tropical lowland regions. They are generally climbing or twining, rarely shrubs. This family is rich source of alkaloid and terpenes [13]. In Hindi the plant is commonly called Giloe [14] which is Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young. In Ayurveda, it is designated as Rasayana drug

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recommended to enhance general body resistance, promote longevity and as anti-stress and adaptogen [15,16].

II. Material and Methods

The plant material *Tinospora cordifolia* was collected from hygienic places from in and around the Nagpur city. The plant material was washed with water in order to make it free of dirt and other impurities and was shade dried. Shade dried whole Plant material was grind with mortar and pastel into the fine powder and alcoholic and aqueous extract of *Tinospora cordifolia* was prepared according to the standard procedure.

Healthy albino rats (9 months old) of both the sexes, weighing 150-190gm were used for the experiment. Animals were free to access drinking water and food. Animals were cared for and used in accordance with the Institutional Animal Ethics Committee (IAEC), P.G.T. Department of Zoology, RTM Nagpur University, Nagpur (Registration No.-478/01/a/ CPCSEA).

Four batches of experiment was carried out for standardization of dose. Each batch includes three groups of rats (n=6). Group I included young rats of less than 6 month old, group II included the rat of age group 6-12 month old and group III was included rats of more than 12 month age group. Diabetes was induced in 16hrs fasted albino rats with single intraperitoneal dose of alloxan monohydrate. Alloxan injection was prepared in 0.9%normal saline. Rats with fasting blood glucose more than 220 mg/dl was considered for study. The batch I was injected with 120mg/kg bw, batch II with140mg/kg, bw, batch III with 160mg/kg bw and batch IV with180 mg/kg bw. During dose standardization study it was found that 180mg/kg intraperitoneal dose of alloxan monohydrate was suitable for diabetes induction with the 6-12 month old rats.

For experimental induction of diabetes alloxan monohydrate (A74/3 Sigma Aldrich was used).

For this study the animals were divided into three groups (n=6),

Group-I (NC): Kept as normal control, the animals of this group was free to access drinking water and food; they were neither injected by alloxan nor feed on plant extract.

Group- II (DC): These group of animals were injected with alloxan monohydrate (180 mg/kg bw) and kept as diabetic control. They were not feed on extract.

Group-III (DC+TCE): This group was injected with alloxan monohydrate (180mg/kg bw) and from day 2 to 30 half an hour prior to feeding, orally administrated with TCE (20ml/kg bw) twice a day.

a) Estimation of liver glycogen

Liver sample was dissected out from 16 hrs fasted rat and digested in hot 30% KOH. Liver glycogen was precipitated with alcohol and the precipitate was dissolved in 10% TCA. The sample was processed for centrifugation to sediment the proteins, after centrifugation supernatant was precipitated once again with alcohol. After suitable dilution of the sediment with water, estimation of liver glycogen was carried out with anthrsone reagent [17].

III. Results and Discussion

In the present study we observed the very significantly ($P<0.005$) increased liver glycogen in diabetic control group compared to the normal. This abnormally increased glycogen content is reversed by the *Tinospora cordifolia* whole plant extract. Although almost all the liver glycogen of the normal rats disappeared after only 24 hours of fasting, rather large amount of liver glycogen was reserved in alloxan diabetic rats when they were fasted for 16 hours before testing. The amount of the remaining liver glycogen was affected by the blood glucose level. Several factors which suppress liver glycogen in normal control by 16 hrs fasting may be pointed out; first one of them is the decrease of the blood glucose which may be followed by marked suppression of glucokinase. Since, glucokinase is the key enzyme catalysing glucose phosphorylation in liver. Impairment of glucokinase activity suggests the impaired oxidation of glucose via glycolysis causes its accumulation resulting in hyperglycemia. Secondly, the suppression of glycogen synthetase together with the stimulation of phosphorylase may be related to the suppression of liver glycogen. The high liver glycogen level in diabetic rats may be due to either increase in gluconeogenesis or hyperglycemia due to 16 hr fasting before testing. In *Tinospora Cordifolia* whole plant extract treated group the significant ($P<0.005$) reversion of the liver glycogen towards the normal may be due to it's activating effect on the glucokinase and glycogen synthetase.

Table 1 : Liver glycogen Estimation.

Groups	Liver glycogen (mg/g wet wt)
NC	5.2±0.87
DC	11.7±1.2[a]
DC+TCE	6.3±0.45[a]

NC- Normal control

DC- Diabetic control

TCE- *Tinospora cordifolia* extract

(Values are expressed as Mean ± SEM (n=6), paired t-test was performed to compared between groups. [a]P<0.005 when DC compared with NC and DC+TCE compared with DC).

After absorption into a cell, glucose can be used immediately for release of energy to the cell, or it can be stored in the form of glycogen, which is a large

polymer of glucose. All cells of the body are capable of storing at least some glycogen, but certain cells can store large amounts, especially liver cells, which can be stored upto 5 to 8 per cent of their weight as glycogen, and muscle cells, which can be stored up to 1 to 3 per cent glycogen.

The importance of the liver in the regulation of carbohydrate metabolism is recognized by its ability to store carbohydrates in the form of glycogen (glycogenesis) and to release them in the form of glucose (glycogenolysis) when needed. These processes are regulated by 2 key enzymes: glycogen synthase and glycogen phosphorylase.

According to Dewalkar *et al.,* [18] the fasted liver glycogen concentration was found very significantly higher ($P<0.001$) in diabetic control (DC) than normal control (NC). This may be due to the increase rate of glycogen synthase enzyme in alloxan treated group, which in turn account for accumulation of glycogen in diabetic rat liver. Fresh etiolated wheat grass and fruit squash of *Lagerstroemia speciosa* treated groups show statistically significant difference in glycogen concentration like NC. This reversal of glycogen concentration to normal indicates their preventive effect on alloxan induced increase rate of glycogen synthase. They found fresh etiolated wheat grass posses significant ($^{*}P<0.05$) effect on lowering of glycogen concentration than fruit squash of *Lagerstroemia speciosa*.

According to Daisy and Rajathi, [19] the aqueous extracts of *Clitoria ternatea* leaves and flowers decrease in glycogen content of liver and skeletal muscle in diabetic rats is probably due to lack of insulin in the diabetic state. Prevention of glycogen depletion in the liver and muscles, following the administration of the extracts, could therefore have been achieved by stimulation of insulin release. Administration of *Clitoria ternatea* leaves and flowers to the diabetic animals increased the activity of glucokinase in liver. The extract-induced decrease in the concentration of blood glucose in alloxan-treated rats may be the result of improved glucose uptake. Similar observations have been made by Singh *et al.* and Shanmugasundaram *et al*. in respect of the extracts of *Catharanthus roseus*, and *Gymnema sylvestre* respectively [20, 21]. The activity of the gluconeogenic enzyme, glucose-6- phosphatase, is usually enhanced during diabetes. following extract administration, blood glucose level falls; while liver glycogen content rose. This may be due to the mobilization of blood glucose into the liver glycogen reserve [22, 23, 24].

IV. Summary and Conclusion

Beside blood glucose, quantitative analysis of liver glycogen content was carried out to interpret the relation between blood glucose and liver glycogen here we observed the significantly increased liver glycogen in diabetic control group compared to the normal. This abnormally increased glycogen content is reversed by the *Tinospora cordifolia* whole plant extract. Although almost all the liver glycogen of the normal rats disappeared after only 24 hours of fasting, rather large amount of liver glycogen was reserved in alloxan diabetic rats when they were fasted for 16 hours before testing. The amount of the remaining liver glycogen was affected by the blood glucose level. Several factors which suppress liver glycogen in normal control by 16 hrs fasting may be pointed out; first one of them is the decrease of the blood glucose which may be followed by marked suppression of glucokinase. Since, glucokinase is the key enzyme catalysing glucose phosphorylation in liver. Impairment of glucokinase activity suggests the impaired oxidation of glucose via glycolysis causes its accumulation resulting in hyperglycemia. Secondly, the supression of glycogen synthese together with the stimulation of phosphorylase may be related to the suppression of liver glycogen. The high liver glycogen level in diabetic rats may be due to either increase in gluconeogenesis or hyperglycemia due to 16 hr fasting before testing. In *Tinospora cordifolia* whole plant extract treated group the reversion of the liver glycogen towards the normal may be due to its activating effect on the glucokinase and glycogen synthetase.

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