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Electrophoretic pattern of serum protein in Streptozotocin induced diabetic rats treated with palm oil and *Tinospora cordifolia* leaf extract

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Abstract
The present experiment was carried out to study the effect of palm oil and *Tinospora cordifolia* leaf extract to experimentally induced diabetic rats on serum electrophoretic pattern. 24 male Wister rats weighing 180-200g were divided into four groups of six animals in each group. Diabetes was induced by the administration of (40 mg / kg body weight) through intra-peritoneal route. The experimental rats were fed diet that had similar composition except for fat source, which consisted of 8% ghee and 8% palm oil. The first group served as control given diet containing 8% of ghee. The second group was diabetic group fed diet supplemented with ghee. The third group was diabetic and fed diet supplemented with palm oil and the fourth group was also diabetic and fed diet supplemented with ghee and treated with *Tinospora cordifolia* leaf extract. The experiment was carried out for 60 days. At the end of the experiment, the rats were sacrificed and blood was collected. Serum was separated and total protein and albumin were estimated. The serum protein level was significantly reduced and albumin concentrations were not significantly differed in all the treatment groups compared to control group. Electrophoretic pattern of serum proteins has revealed the absence of proteins having the molecular weight of 24.9KDa in diabetic and treatment groups as observed in control group.

Keywords: diabetes mellitus, palm oil, *Tinospora cordifolia*, serum protein

Introduction
Diabetes mellitus is a metabolic disorder in which there is hyperglycemia over a prolonged period. It is accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins [1], resulting from defects in insulin secretion, insulin action or both [2].
Dietary composition could play a significant role in improving insulin sensitivity and reducing the risk of diabetes and its complications [3]. The role of dietary fat in type 2 diabetes is of clinical interest for many decades and the type of fat consumed could influence insulin action in human [4].
Many studies have proved that diet can affect fatty acid composition of cell membrane in human beings and other animals. Dietary fat induced alteration in membrane composition has been shown to influence the function of membrane associated proteins [5]. The dietary fat quality mainly affects cell membrane fatty acid composition and cell membrane functions such as membrane fluidity, ion permeability, insulin receptor binding/affinity and glucose transporters interaction with second messengers [6, 7].
In many instances, vegetable oils in diet could play a significant role in improving insulin sensitivity and reducing the risk of diabetes and its complications. Palm oil contains equal amount of saturated and unsaturated fatty acids, which contains mainly 45% palmitic acid and 40% oleic acid. Three weeks of palm oil supplementation significantly [8] reduced blood glucose in mice and it is due to palm oil-induced hypersinsulinemia) [9]. Tocotrienol fraction of palm oil lowers blood glucose level and improves dyslipidemia [10].
Tinospora cordifolia, belonging to the family Menispermaceae is a widely used shrub in folk and ayurvedic systems of medicine. It is distributed throughout Indian subcontinent. The root, stem and leaves of *Tinospora cordifolia* have antidiabetic effect and administration of root extract of *Tinospora cordifolia* to diabetic rats caused an increase in body weight, total haemoglobin and hepatic hexokinase activity.

The root extract also lowers hepatic glucose-6-phosphatase and serum acid phosphatase, alkaline phosphatase, and lactate dehydrogenase levels. Thus, *Tinospora cordifolia* root has hypoglycemic effect [11].

Treatment of diabetic rats with *Tinospora cordifolia* plant extract showed anti-hyperglycemic activity. Decreased enzyme activity of glucokinase, hexokinase and phosphofructokinase in diabetic animal was partly restored on treatment with *Tinospora cordifolia* extract [12].

Aqueous extract of stem of *Tinospora cordifolia* when given to streptozotocin induced diabetic rats at the dose rate of 200mg/kg bodyweight decreased serum glucose, cholesterol, triglycerides, creatine kinase, and free fatty acids to near normal level when compared to that of standard drug. The crude stem ethylacetate, dichloromethane, chloroform and hexane extracts of *Tinospora cordifolia* inhibited salivary and pancreatic amylases and glucosidase thus decrease the postprandial glucose level and has potential application in the treatment of diabetes mellitus [13]. Diabetic rats treated with aqueous stem extract of *Tinospora cordifolia* showed comparatively less degeneration of Islets of Langerhans and degranulation [14]. *Tinospora cordifolia* leaves extract increased the uptake of glucose in rat L6 myotubes. These findings suggested that *Tinospora cordifolia* possesses antioxidant as well as glucose uptake potential and has complimentary potency to develop as an antihyperglycemic agent for the treatment of diabetes mellitus [15].

Several studies have indicated that the serum protein levels are reduced in diabetic rats. The present study was undertaken to assess the influence of palm oil and *Tinospora cordifolia* leaf extract on serum protein profile in diabetic rats.

Materials and Methods

Materials

All the chemicals required for performing SDS-PAGE were purchased from Bio-rad Inc, USA. The molecular weight marker (Broad range) was purchased from Bio-Rad, Inc, USA. All the plastic ware used for the present study viz., centrifuge tubes, microcentrifuge tubes, microtips (different graduation) were procured from Thermo Scientific, USA.

Preparation of leaf extract

40g of fresh leaves of *Tinospora cordifolia was taken in a bowl and washed thoroughly in tape water and leaves* were chopped well to which 200ml of distilled water was added and boiled for 10min. It was then cooled and filtered. The filtrate was taken in a standard flask and made up to 200ml. From this aqueous extract 2ml was given to each experimental rat.

Methods

Experimental Design

Table 1: Twenty four rats were randomly divided into four groups as follows

Groups	Treatments	No. of rats
Group – I	Normal control (Normal rats fed with ghee supplemented feed)	6
Group – II	Diabetic control (Diabetic rats fed with ghee supplemented feed)	6
Group – III	Diabetic rats fed with palm oil supplemented feed	6
Group – IV	Diabetic rats fed *Tinospora cordifolia* leaf extract	6

The experimental rats were fed with isocaloric mash type during the experimental period. The diet is designed to support growth with similar energy, protein and fat content. The diets had similar composition except for fat source, which consists of 8% of ghee and 8% palm oil. The rats will be given *ad libitum* access to food and water.

Diabetes was induced experimentally by using streptozotocin at the rate of 40 mg/kg body weight through intra peritoneal route. Experimental trials were carried out for a period of sixty days.

The present experiment was carried out to study the effect of palm oil and *Tinospora cordifolia* leaf extract when given to experimentally induced diabetic rats either alone or in combination on serum electrophoretic pattern. 24 male Wister rats weighing 180-200g were divided in to four groups of six animals in each group. Diabetes was induced by the administration of (40 mg / kg body weight) intraperitoneally. The first group served as control given diet containing 8% of ghee. The second group was diabetic group fed diet supplemented with 8% of ghee. The third group was diabetic and fed diet supplemented with 8% palm oil and the fourth group was also diabetic and fed diet supplanted with 8% of ghee ghee and treated with *Tinospora cordifolia* leaf extract. The experiment was carried out for 60 days. At the end of the experiment the animals were sacrificed, blood was collected and serum was separated.

Estimation of serum protein

Serum protein was estimated by Biuret method [16]. Albumin was estimated by BCG method [17].

Protein extraction and SDS Page

The serum proteins were dissolved in the appropriate volume of Laemmli buffer and stored at -20 °C for later use. Protein quantity was estimated by method described by Lowry [8] and 40 µg of denatured protein samples were loaded into each well for SDS- PAGE as per protocol described by Laemmli [19]. Molecular weight marker was used as standard for molecular weight determination. The separated fractions within gels were stained with Coomassie brilliant blue-R250 (Bio Rad Ltd, USA). After proper de-staining, the molecular weight of proteins in the SDS-PAGE was determined using Image Lab software (Bio-Rad, Inc, USA).

Statistical Analysis

Statistical analysis was carried as per method described by Snedecor and Cochran [20]

Results

Serum protein concentration

The serum protein level was significantly reduced in all the treatment groups as compared to control. But, there was no change in the level of albumin concentration between control and treatment groups.

SDS-Page

The electrophoretic separation of serum protein by SDS-PAGE in our study showed that the globulin fraction with the molecular weight of 24.9 KDA was absent in the diabetic and treatment groups when compared to control.

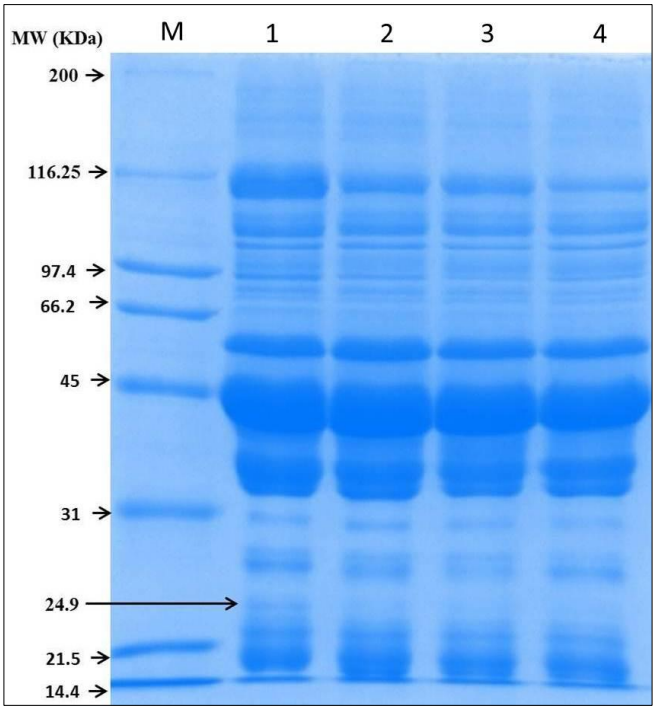

Fig 1

SDS-PAGE of serum proteins

Molecular weight marker (Lane M), Control rats (Lane 1), Streptozotocin induced Diabetic rats (Lane 2), Palm oil treated (Lane 3) and Tinospora leaf extract treated (Lane 4).

Discussion

STZ induced incomplete destruction of β-cells of pancreas but the rats became permanently diabetic [21]. The reduction of serum insulin concentration may be attributed to progressive loss of β-cells by the action of STZ [22]. Insulin generally has an anabolic effects on protein metabolism in that it stimulates protein synthesis and retards protein degradation [23]. During diabetes, there is an increase in protein catabolism, with flow of amino acids in to the liver, which feeds gluconeogenic process. Accelerated proteolysis occurs in uncontrolled diabetes as a result of deranged glucagon mediated regulation of cAMP formation in insulin deficiency [24, 25]. The reduced total protein concentration observed in our experiment without any alteration of albumin level indicates the reduction may be due to the reduced levels of globulin fractions, which may be due to loss of particular protein fractions in urine or it may be also due to catabolism of protein. Plasma proteins may be reduced due to increased catabolism of proteins in untreated diabetes mellitus.

We observed the reduction of total protein with no alteration in the level of albumin, which indicated a reduction in the level of globulin.Hence, we wanted to find out which fraction of the globulin was reduced, for which electrophoresis was carried out using serum. Electrophoresis is widely used to show changes in serum proteins particularly in the globulin fractions. Electrophoresis is used for the qualitative assessment of the changes in the relative amounts of the globulin fraction present and to roughly quantify the relative amounts of the various globulin fractions.

Treatment of diabetic rats with palm oil and *Tinospora cordifolia* leaf extract did not show any significant improvement in the protein levels in the treatment groups. Further works are required to find out whether the reduction of protein may either be due to reduction in the synthesis. Lack of insulin also reduces RNA and mRNA, which is another factor for the reduction of total protein [26] or increased catabolism or due to loss of the protein fraction through urine. There are several proteins with the molecular weight of 24.9 KDa. Hence, further works are required to identify the protein fraction responsible for the reduction.

Conclusion

The serum protein level was significantly reduced in all the treatment groups. There was no change in the level of albumin concentration. Electrophoretic pattern of serum protein has revealed the absence of proteins having the molecular weight of 24.9 KDa, which may be one of the reasons for the reduction of serum total protein concentration. Treatment was carried out for sixty days which did not improve the protein levels in the experimental groups and further elaborative research is required to study the effects on the diabetic rats.

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