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Transmission electron microscopic (TEM) studies on interaction of *Gymnema sylvestre* with glimepiride and insulin in experimental diabetes mellitus in *Sprague dawley* rats

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Abstract

An experimental study was conducted to evaluate the interaction of *Gymnema sylvestre* extract with insulin and glimepiride in diabetic *Sprague dawley* rats. Rats were randomly divided into seven groups of six rats in each and blood glucose was estimated to ascertain group differences. Group I was kept as normal control. Remaining six groups were induced diabetes by intra peritoneal injection of streptozotocin at the rate of 40 milligram per kilogram body weight. After 72 hours, rats with blood glucose value of more than 200 milligram per decilitre were included in the study. Treatment protocols were initiated 48 hours post-confirmation of diabetes and continued for 2 months. Group 1 is non-diabetic control, group 2 is streptozotocin (40 milligram per kilogram intra peritoneal single dose)-induced diabetic control, group 3 is insulin treatment (4 Units/ kilogram body weight subcutaneously once daily), group 4 is glimepiride treatment (4 milligram per kilogram b.wt orally once daily), group 5 is *Gymnema sylvestre* methanolic leaf extract treatment (400 mg/kg body weight orally once daily), group 6 Insulin + *Gymnema sylvestre* methanolic leaf extract treatment (once daily) and group 7 is glimepiride + *Gymnema sylvestre* methanolic leaf extract treatment (once daily). A detailed necropsy was conducted on each rat and kidney, pancreas and aorta were collected for ultra structural studies. The electron microscopy of kidney, pancreas and aorta revealed marked alterations in group 2, while group 6 and 7 revealed better architecture.

Keywords: Tem studies, *Sprague dawley* rats, *Gymnema sylvestre*, glimepiride, diabetes mellitus

Introduction

Diabetes mellitus is one of the most frequent chronic diseases worldwide, being the top five main causes of death in developed countries. This endocrine disease is also becoming epidemic in developing countries (World Health Organization, 2012). The world prevalence of Diabetes mellitus in 2010 was 6.6%, with an estimated number of 285 million carriers; by 2030, this number may reach 552 million carriers (International Diabetes Federation, 2012). The evident increase in disease incidence and the higher frequency of chronic complications due to micro vascular (e.g., nephropathy and retinopathy) and macro vascular (stroke, macro vascular coronary and peripheral artery diseases) alterations, as well as the difficulties for controlling it, makes Diabetes mellitus a great challenge when considering health in the 21st century [1]. Glimepiride belongs to the third generation sulphonylureas and chemically it is a carboxamido phenyl pyrroline drug used in the treatment of type II diabetes. It has the ability to enhance insulin release and action and then to lower blood glucose level [2]. Glimepiride has antioxidant activity, it increases antioxidant defences like SOD, catalase and GPx, and decreases lipid peroxidation [3]. Any herb or natural product may interact with a drug by altering any one or a combination of these systems only to result in a clinical response other than expected [4]. Hence, the present investigation was undertaken to evaluate the interaction of *Gymnema sylvestre* extract with insulin and glimepiride in diabetic *Sprague dawley* rats.

Materials and Methods

Male *Sprague dawley* rats of uniform age (3 months) and weight were procured for the study. Feed and water was provided *ad libitum* throughout the experiment. Animals were housed in polypropylene cages in a air-conditioned animal house with 12h – 12h light – dark cycles. Acclimatization period of 2 weeks was observed before start of the experiment. The experimental protocol was approved by the Institutional Animal Ethics Committee (Lr.No.:I/6/2012; Dated: 06/01/2012).

Experimental Design

After induction of diabetes, all the groups were maintained as per the following treatment schedule for 8 wks.

Group-1: Non-diabetic control

Group-2: Streptozotocin (40 mg/Kg i/p single dose)-induced diabetic control

Group-3: Insulin (Insuman 25/75; 4 U/kg once daily for 8 wks) treatment in diabetic rats

Group-4: Glimpiride (4 mg/Kg orally once daily for 8 wks) treatment in diabetic rats

Group-5: *Gymnema sylvestre* methanolic leaf extract treatment (400 mg/kg orally once daily for 8 wks) in diabetic rats

Group-6: Insulin + *Gymnema sylvestre* methanolic leaf extract treatment (once daily for 8 wks) in diabetic rats

Group-7: Glimpiride + *Gymnema sylvestre* methanolic leaf extract treatment (once daily for 8 wks) in diabetic rats

Transmission Electron Microscopy (TEM): For electron microscopic studies, small samples were transferred to vials and fixed in 3% gluteraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 h at 4 °C and post-fixed in 2% aqueous osmium tetroxide in the same buffer for 1 h. After the post-fixation, samples were dehydrated in a series of graded alcohol and infiltrated and embedded in Araldite 6005 resin. Ultra thin sections (50-70 nm thickness) were cut with a glass knife on a Leica Ultra cut UCT-GA-D/E-1/00 ultra microtome and mounted on grids. Then the sections were stained with saturated aqueous uranyl acetate and counter stained with 4% lead citrate and were observed at various magnifications under a transmission electron microscope (Model: Hitachi, H-7500) at RUSKA Lab, College of Veterinary Science, Hyderabad, India.

Results and Discussion

The electron microscopy of kidney of group 2 showed disintegration of chromatin material, condensed mitochondria with loss of cristae and gyri and mild dilatation of intertubular spaces. There was variation in shape and size of nucleus (Fig: 1). The electron microscopy of kidney of group 6 showed uniform mitochondria, normal cell junctions and eccentrically placed nucleolus. Restoration of epithelium and rough endoplasmic reticulum was also noticed (Fig: 4).

The electron microscopy of aorta of group 2 showed mild disruption of smooth muscle bundles. Tunica intima, tunica media, tunica adventitia appear to be intact. Mild thickening of endothelium, nucleolemma filled with electron dense granular material (Fig: 2). The electron microscopy of aorta of group 6 showed normal endothelium. Tunica intima, Tunica media and Tunica adventitia appear to be normal.

The electron microscopy of pancreas of group 2 showed swollen nucleus, increased volume of nucleolus, eccentrically placed nucleolus and numerous vacuolation. Dilatation of cisternae of rough and smooth endoplasmic reticulum and degenerating mitochondria was also noticed (Fig:3). The electron microscopy of pancreas of group 6 showing uniform size of nucleus, eccentrically placed nucleolus with numerous varied shape and size of secretory granules and intact secretory acinar epithelial cells.

Addition of *Gymnema sylvestre* leaf extract to insulin and glimepiride had positive pharmacodynamic interaction in improving the ultra-structural alterations due to streptozotocin-induced diabetes mellitus in rats, which was evident from greate improvement in organ parameters in the

groups that were treated using a combination of *Gymnema sylvestre* with either insulin or glimepiride as compared to individual agent treated groups.



Fig 1: TEM of kidney showing disintegration of chromatin material, condensed mitochondria with loss of cristae and gyri and mild dilatation of inter tubular space. 3860X (Group 2)



Fig 2: TEM of Aorta showing mild thickening of endothelium. Nucleolemma is filled with electron dense granular material. Mild disruption of smooth muscle bundle. 3860X (Group 2)

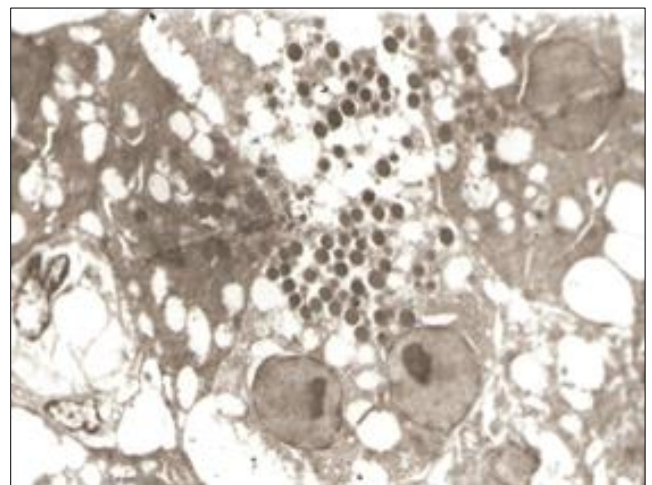


Fig 3: TEM of pancreas showing vacuolation, granular mitochondrial matrix and swollen nucleus with eccentrically placed nucleolus. 3860X (Group 2)

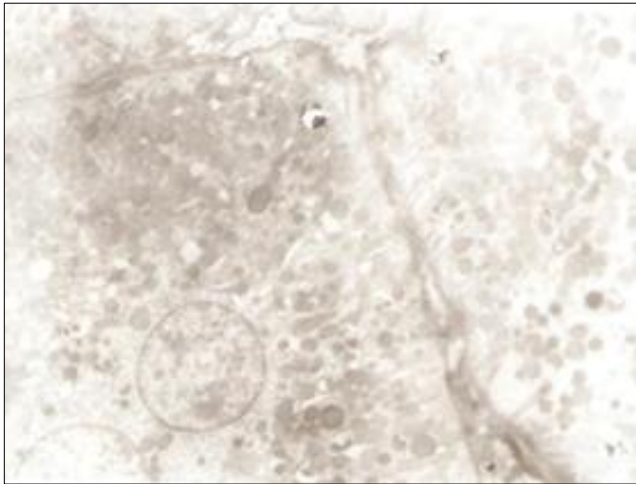


Fig 4: TEM of kidney showing uniform mitochondria, normal cell junctions and normal nucleus with eccentrically placed nucleolus. 3860X (Group 6)

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