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Diabetes and Hypercholesterolemia Impair the Cytological Structure of the Anterior Pituitary Gland

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Increase consumption of high fat diet was found to alter blood sugar level similar to diabetes and contributed to the development of obesity and affected the reproductive function of both sexes. The study aimed to clarify the influence of diabetes and or hypercholesterolemia on the cytological picture of cells of the anterior lobe of pituitary gland of male albino rats. Eighteen male albino rats weighing approximately 120 gram body weight were divided into three main groups; control, diabetes, hypercholesterolemia, diabetes (single i.p. 40 mg streptozotocin/ kg B.wt plus 100mg. nicotinamide /kg body weight) and hypercholesterolemia (diet containing 3% cholesterol). Dietary feeding on cholesterol and diabetes were carried out for 12 weeks. At the end of treatment, animals were sacrificed, and pituitary glands were separated and their anterior lobe was processed for cytological investigations by transmission electron microscopy. The present study revealed that the rats subjected to experimental diabetes and/ or hypercholesterolemia exhibited a decrease of the secretory granules within the gonadotroph cells somatotroph and corticotrophin cells. There was a detected intracellular accumulation of fat globules in both the gonado- and sommatotroph cells. The authors reported that the altered cytological structures of the secretory function of the anterior pituitary gland led to marked impairment of the male hormonal level and causing infertility.

Keywords: Diabetes, Hypercholesterolemia, Pituitary, TEM, Biochemical markers.

Diabetes is a public health problem associated with increased blood sugar level and decrease of insulin production from the damaged beta cells of theIslet of Langerhans of pancreas which developed metabolic dysfunction of the body organs (Yoon and Jun, 2005).

Diabetes was found to increase blood sugar level and ketone bodies which involved in the reduction of pituitary hormones may be linked in the development of diabetic ketoacidosis (Barnes *et al*., 1978).

Previous studies reported ovarian failure of hypercholesterolemic rat associated with altering the anti-oxidative enzymes and decreased reproductive hormonal levels (El-Sayyad *et al*., 2018 and 2019). Also, diabetes was associated with male infertility through reduction of insulin production in the spermatogenic cells through

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disrupting of the hypothalamic-pituitary gonadal axis. Streptozocin-induced diabetic rats produced approximately half of the insulin protein in testis (Schoeller *et al*., 2012) reflecting its importance in spermatogenesis. Diabetic patients were found to exhibit low plasma level of testosterone (Gluud *et al*., 1982) associated with decreased sperm production and development of infertility (Agbaje *et al*., 2007).

It is known that the spermatogenesis need the production of lactate from glucose by the Sertoli cell. The transport of glucose from blood to the spermatogenic cell and the recovery of the metabolic intermediates is controlled by the blood-testis-barrier (Riera *et al.*, 2009). The glucose transporter Glut1, Glut2, Glut3,Glut 4 and Glut8 have been demonstrated in the testis (Carosa *et al.*, 2005; Verma and Haldar, 2016). Diabetes was found to alter the GLUT8 immunoreactivity in the acrosomic system of spermatids, and at low levels in Leydig cells as well as reduced testicular insulin levels which involved in retarding spermatogenesis(Gómez *et al*., 2009) STZ-induced hyperglycemic rats was found to upregulate the pro-apoptotic factors Bad, Bax and c-Jun N-terminal kinases parallel with an increase in germ cell death (Koh, 2007).

This work was carried out to exhibit the cytological structure of hormonal secreting cells of the anterior pituitary gland in relation to hypercholesterolemia and/ or diabetes.

MATERIALS AND METHODS

Biochemical investigations

The serum levels of high density lipoproteins (HDL) (Grove, 1979) and total cholesterol (TC) (Deeg and Ziegenhorn, 1982) were investigated. The level of low density lipoproteins (LDL) was calculated from the total concentration of triglycerides, total cholesterol (TC) and HDL-cholesterol (Friedewald *et al* , 1972). The blood glucose levels were measured by blood glucometers one touch ultra (Life Scan Milipitas, CA, USA).

Application of hypercholesterolemia

Hypercholesterolemia was induced by feeding the experimental group on diet containing

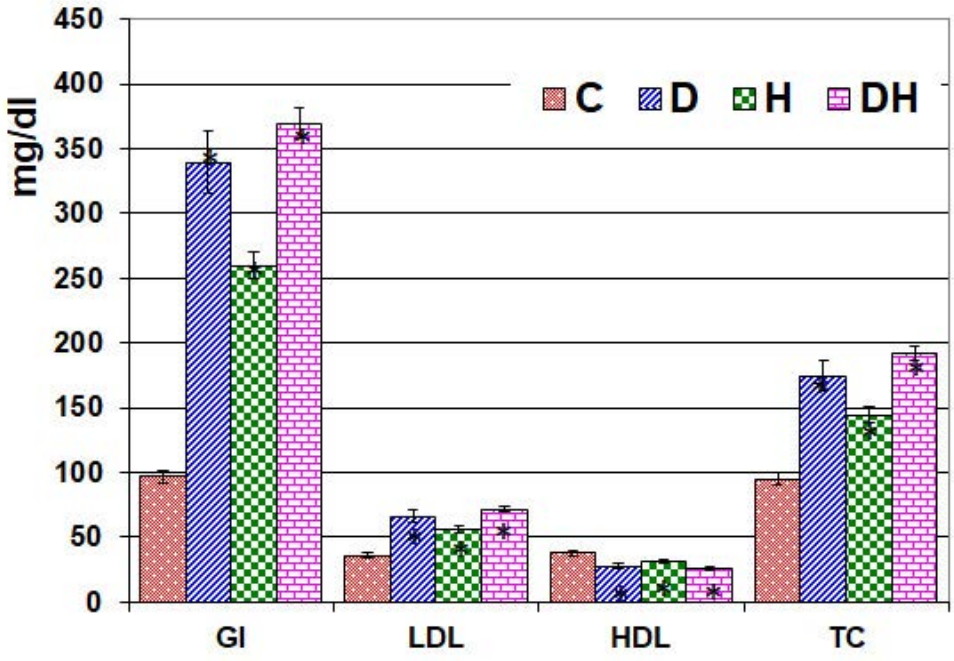

Fig. 1. Chart illustrating the blood glucose level and sera levels of LDL-C, HDL and total cholesterol levels of diabetic mother supplemented watery cinnamon extract. Each result represent the mean ± SD of n = 5. Star means significant at $P < 0.05$. Abbreviations; GL, glucose, HDL, high density lipoprotein; LDL, low density lipoprotein; TC, Total cholesterol

3% cholesterol beside 7% fat , 1% cholic acid and 0.3% thiouracil for 12 weeks according to Enkhmaa *et al*.(2005). The control group was fed on a standard diet free from hypercholesterolemic components

Induction of diabetes

It was occurred by intraperitoneal injection of a single dose of streptozotocin (60 mg/kg) in the citrate buffer (pH 4.5) plus nicotinamide 100mg/kg body weight (Povoski *et al*., 1993). The control received saline solution as a vehicle. Hyperglycemia was maintained by assaying the blood glucose with approximately 250-280 mg/dL.

Experimental Work

The study was carried out on 24 male Wistar albino rats weighing almost 100g body weight, obtained from Farm of Ministry of Health, Giza, Egypt. The animals were housed in aerated room with approximately12-hour light and dark cycle. Free access of standard diet and water was allowed *ad-libitum.* The rats were arranged into four groups (n=6) such as control, hypercholesterolemic group, diabetic group, hypercholesterolemic and diabetic group. At the end of treatment, the studied groups were anesthetized, sacrificed and blood was collected, and glucose levels were estimated. Sera were separated and subjected for estimating total cholesterol and LDL-c. The heads were removed, and brain dissected, and the pituitary glands were separated and fixed in cacodylate buffered 2% glutaraldehyde (pH 7.4) and post fixed in 1% osmium tetroxide at 4°C. The specimens were dehydrated in ascending ethyl alcohol (70-100%0, cleared in propylene oxide and embedded in epoxy resin. Ultrathin sections were cut with a LKB Ultratome IV, mounted on grids and stained with uranyl acetate and lead citrate, and examined on a Joel 100CXl transmission electron microscope of Mansoura University Lab.

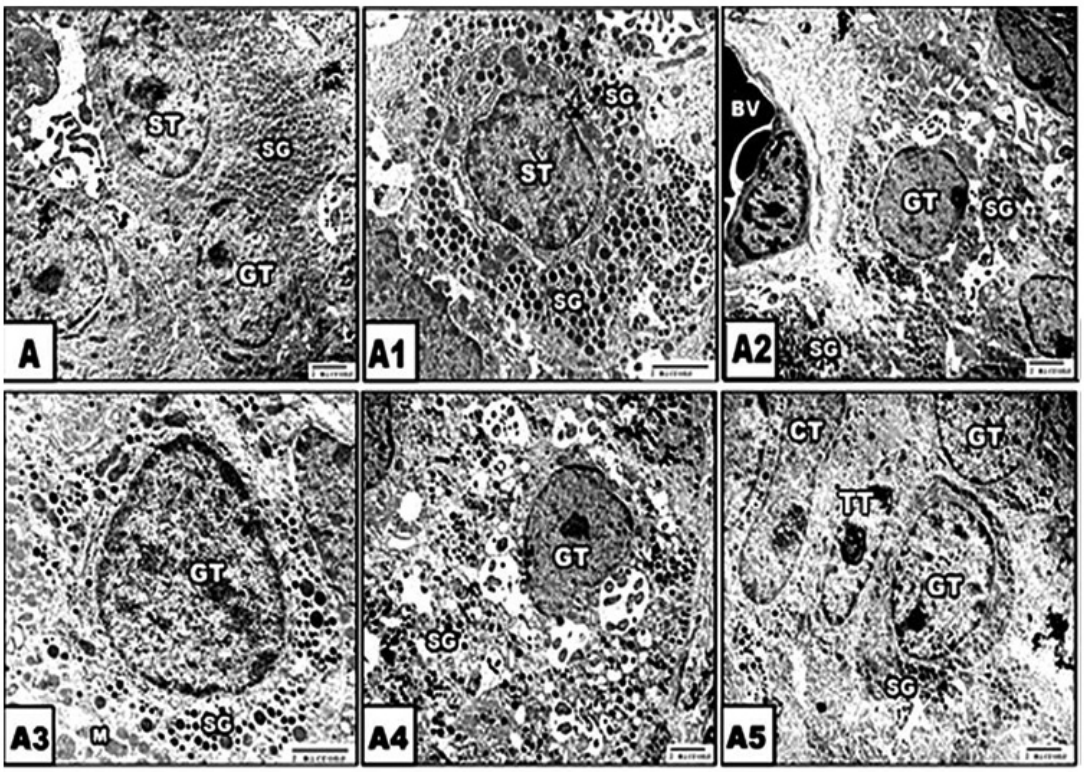

Fig. 2. (A-A5): Transmission electron micrographs of anterior lobe of control pituitary gland. A. Showing gonadotroph cells with large cytoplasmic secretory granules. Note sommatotrophs with finely granulated cytoplasm. A1. Showing sommatotrophs with dense finely granulated cytoplasm.A2. Showing gonadotroph cell adjacent to blood capillary. A3 & A4. Showing gondotroph cells. A5. Showing gondotroph, corticotroph and thyrotroph cells. Abbreviations; BV, blood vessel; CT, corticotroph cell; GT, gondotroph cells; N, nuclei ; ST, somatotroph cell. Lead citrate & uranyle acetate

RESULTS

Biochemical markers

Diabetes increased blood levels of glucose, and serum HDL, LDL and total cholesterol levels in comparison with the control groups (Fig.1).

Cytological observations

In control, the gonadotroph cells appeared aligned near to the blood capillaries. It takes large round or polyhedral structure. The nuclei possessed peripheral marginated heterochromatin on the nuclear envelope and euchromatin forming the bulk of the nucleoplasm. The cytoplasm is rich of electron-dense secretory granules of varying sizes. The somatotroph cells possessed irregular-shaped nuclei with abundant euchromatin and thin coat of peripheral heterochromatin. Mitochondria are detected dispersed in the cytoplasm. Rough endoplasmic reticulum and their ribosomes are distributed throughout the cytoplasm. Secretory granules are abundant around the nucleus and take their homogenous size. The corticotroph cells appeared elongated with eccentric nuclei. Rough endoplasmic reticulum and polysomes are detected within the cytoplasm. Mitochondria and rough endoplasmic reticulum are more abundant in the cytoplasm of the sommato-and gonadotroph cells (Fig.2 A-A5).

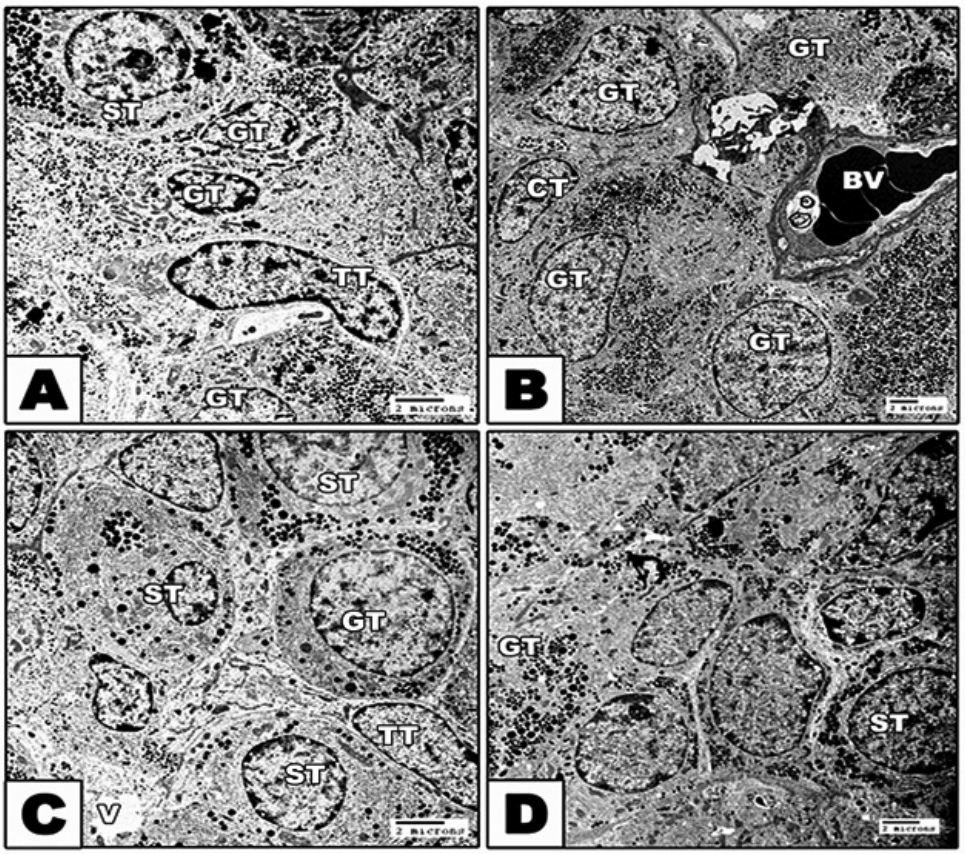

Fig. 3. (A-D). Transmission electron micrographs of anterior lobe of pituitary gland of diabetic rat. A. Showing atrophied gonadotroph cells with irregular nuclear envelop and and comparatively decreased secretory granules. Sommatotroph cell appeared with moderate cytoplasmic secretory granules. Note thyrotroph cell with decreased cytoplasmic granules. B. Showing gonadotrophs with altered nuclear envelope and degranulated cytoplasmic granules.C. Showing atrophied sommatotrophs with decreased cytoplasmic secretory granules. D. Showing degenerated gondotroph and sommatotroph cells. Abbreviations; BV, blood vessel; CT, corticotroph cell; FG, fat globule; GT, gondotroph cells;N, nuclei PN, pyknotic nuclei; RER, rough endoplasmic reticulum; RBC, red blood cell; ST, somatotroph cell; V, vacuoles. Lead citrate & uranyle acetate

In diabetic group, the nuclei of the pituitary cells including gonadotroph, somatotroph, and throtroph cells showed different pattern of karyolysed chromatin materials and reduction of the cytoplasmic granules. Some somatotroph and gonadotroph cells exhibited atrophied nuclei with compacted chromatin materials (Fig. 3A-D).

In hypercholesterolemic group, both gonadotroph and somatotroph cells exhibited electron-dense nuclear chromatin materials associated with vesiculated cytoplasmic materials and reduction of secretory granules. Many pituitary cells appeared with pyknotic nuclei (fig. 4 A-D).

In diabetic and hypercholesterolemic group, there was a detected massive degeneration of the anterior pituitary cells especially gonadotroph and somatotroph cells. The cells possessed pyknotic nuclei, depletion of secretory granules and increase of lysosomes. Fibrotic pattern of somatotroph cells were detected. The endothelial cells lining the blood vessels were damaged. Increased deposition of fat globules was detected within the pituitary cells illustrating the most pathological observation (Fig. 5 A-D). In some other specimens, there was apparent increase of gonadotroph and somatotroph cell exhibited convoluted nuclear enveloped and possessed compacted nuclear chromatin materials. Gonadotroph cells with pyknotic nuclei with vesiculated cytoplasm and decreased secretory granules and fragmented rough endoplasmic reticulum (Fig. 6 A-D).

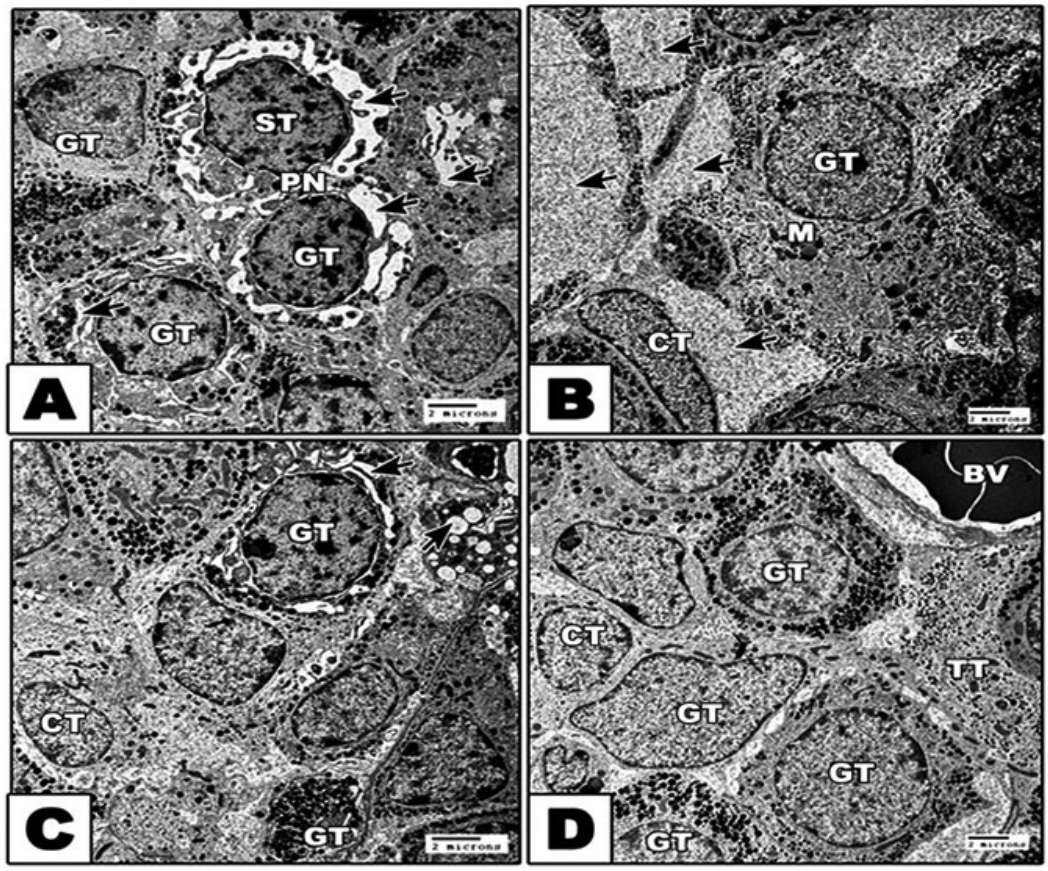

Fig. 4. (A-D): Transmission electron micrographs of anterior lobe of pituitary gland of hypercholesterolemic rat. A. Showing damaged gonadotroph and sommatotroph cells with degranulated cytoplasm. B. Showing damaged gonadotroph cells with electron-dense mitochondria. C. Showing damaged and fibrotic gonadotrophs and degenerated corticotrophs. D. Showing varying degree of damaged gondotrophs, corticotroph and thyrotroph cells. Abbreviations; BV, blood vessel; CT, corticotroph cell; FG, fat globule; GT, gondotroph cells;N, nuclei PN, pyknotic nuclei; RER, rough endoplasmic reticulum; RBC, red blood cell; ST, somatotroph cell; TT, thyrotroph cell. Lead citrate & uranyle acetate

DISCUSSION

The present findings reported that diabetes and or hypercholesterolemia are linked to marked increase of blood levels of glucose, LDL and total cholesterol level. Similar findings of the association of diabetes with altering lipid profiles were reported by El-Sayyad *et al*. (2010, 2011, 2014). Hypercholesterolemic diet was found to develop type 2 diabetes via increase blood sugar level and glycosylated haemoglobin (HbA_{1c}) after 30 weeks (Morris *et al*., 2016) as well as increase plasma level of triglycerides and decreased HDL-cholesterol (Ramalho *et al*., 2017).

The mammalian reproductive cycles are tightly regulated by the hypothalamus (gonadotrophin-releasing hormone)-pituitary (follicle-stimulating hormone) and ovarian axis (17â- estradiol) (Nett *et al*., 2002; Kermath and Gore, 2012). FSH represents an important indicator of both normal and abnormal testis function. It is known that follicle stimulating hormones promoted de novo biosynthesis of the steroid hormone from the circulating lipoprotein and cholesterol in the theca and granulosa cells (Miller and Auchus, 2011).

Also, the gonadotroph cells are important for secreting two kinds of hormones; follicle

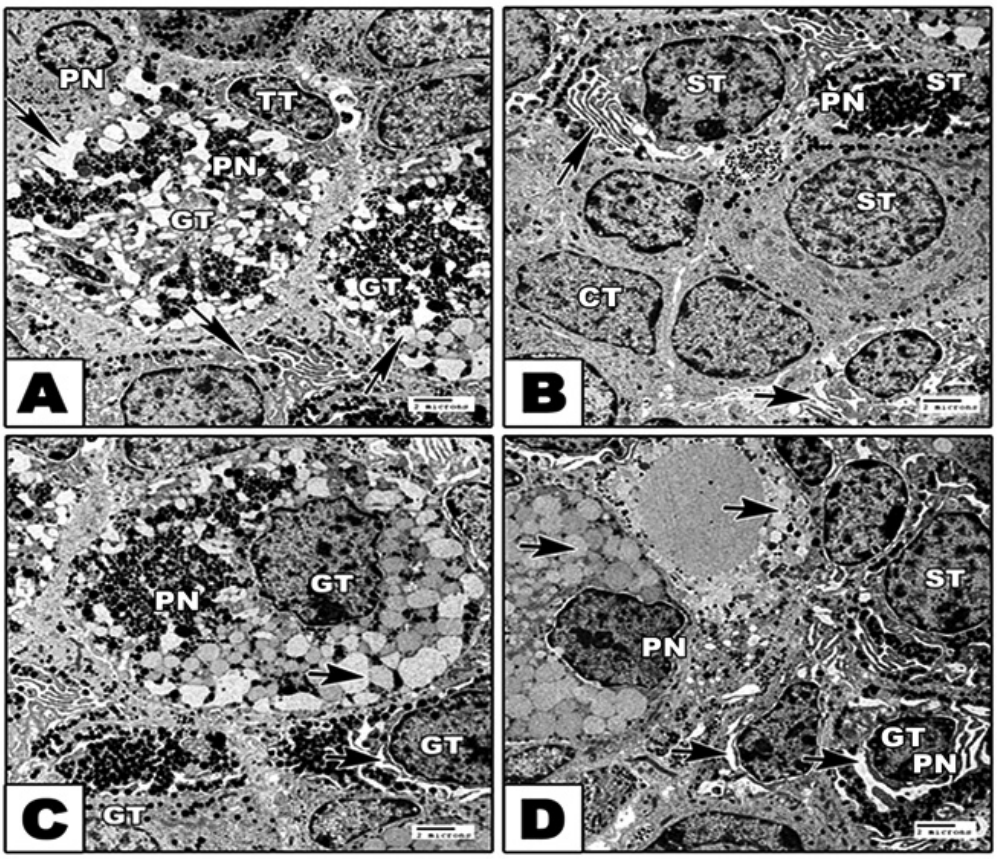

Fig. 5. (A-D): Transmission electron micrographs of anterior lobe of pituitary of diabetic and hypercholesterolemic rats. A. Showing gonadotroph cells having pyknotic nuclei and vesicuolated cytoplasm with decreased secretory granules. B. Showing somatotroph with pyknotic nuclei and degranulated cytoplasmic granules and corticotroph with karyolitic nuclei and degranulated cytoplasm. C &D. Showing gonadotrophs with pyknotic nuclei and degranulated cytoplasm having dense accumulation of fat globules. Note also fibrotic appearance of sommatotrophs. Abbreviations; BV, blood vessel; CT, corticotroph cell; FG, fat globule; GT, gondotroph cells; N, nuclei PN, pyknotic nuclei; RER, rough endoplasmic reticulum; RBC, red blood cell; ST, somatotroph cell; TT, thyrotroph cell. Arrow head revealed dense deposition of fat globules.Lead citrate & uranyle acetate

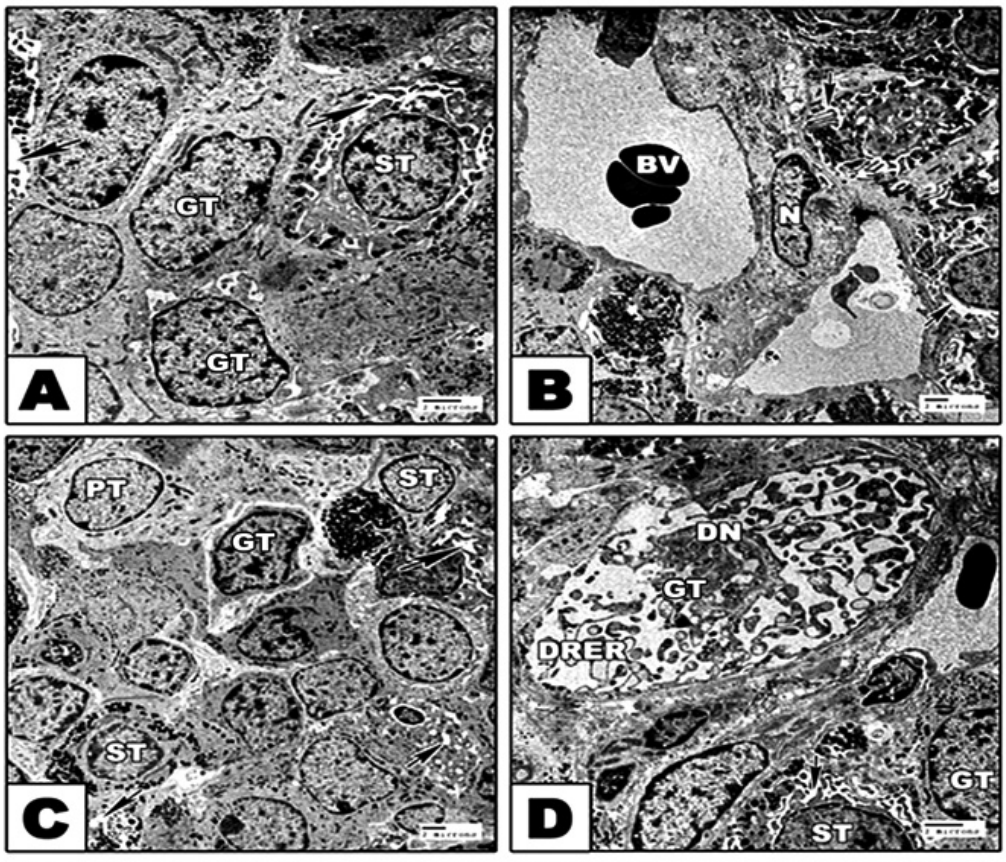

Fig. 6. (A-D): Transmission electron micrographs of anterior lobe of diabetic and hypercholesterolemic pituitary gland. A. Showing gnadotroph cells with karyolytic nuclear chromatin and comparatively decreased secretory granules. Also, decreased secretory granules of sommatotrophs. B. Showing swollen blood vessel and degenerated pituitary cells. C. Showing pyknotic gonadotrophs and decreased secretory granules of sommatotrophs. D.Showing gonadotrophs with pyknotic nuclei and cytoplasm with missing secretory granules and degenerated rough endoplasmic reticulum. Abbreviations; BV, blood vessel; DRER, degenerated rough endoplasmic reticulum; GT, gondotroph cells;N, nuclei; PN, pyknotic nuclei; RER, rough endoplasmic reticulum; RBC, red blood cell; ST, somatotroph cell

stimulating hormone and luteinizing hormone. The follicle stimulating hormone is involved for managing spermatogenesis, meanwhile luteinizing hormone activated the Leydig cells for testosterone secretion (Nicholson and Ricks, 2011).

The observed hypofunction of gonadotroph secretion which was assessed by the observed reduction of the gonadotroph secretory granules in diabetic and or hypercholesterolemic group impaired testosterone section which disrupt testicular structure and function. Also, depletion of testosterone secretion impair the prostatic function and damaging the prostate (Gründker and Emons, 2017). Rat and rabbit fed on a hypercholesterolemic diet was found to damage Leydig cells associated depletion of serum free testosterone level (Dupont *et al*., 2014) and consequently depleted semen volume, sperm cell count, and percentage of sperm motility (Saez Lancellotti *et al*., 2013; Raad *et al*., 2017) and developed infertility(McPherson and Lane, 2015).

It is known that under oxidative stress of increased blood sugar level from diabetes (El-Sayyad *et al*., 2010, 2011, 2014), the damaged cell organelles released reactive oxygen species (Dumollard *et al*., 2007) which enhanced further dysfunction of mitochondrial and endoplasmic reticulum (Luzzo *et al*., 2012). These was involved

in depletion of the ATP associated decrease of the oxidative phosphorylation , liberation of oxygen free radical and activation of caspases leading to DNA fragmentation (Wu *et al*., 2011; Yang *et al*., 2012).

GH is one of the glucose counter-regulatory hormones. Low IGF-I and growth hormone levels linked directly to deficient insulin levels or lack to circulating binding protein. In type 2 diabetes, growth hormone is decreased due to opposite alterations in hypothalamic somatostatin (Giustina and Wehrenberg, 1994) or to type 2 diabetes as in 17-year-old non-obese female due to disrupting glucose and lipid metabolism (Henry and Menon , 2018).

The observed damage of corticotroph cells supported the findings of Rhyu *et al*. (1994) whom reported mpairing of cortisol and/or growth hormone secretion due to severe hypoglycemia.

Type 2 diabetes and alterations of thyroid hormone secretion is of great importance. Gene polymorphism, abnormal gene expression and regulation, enhanced absorption of dietary glucose from intestine, decreased its utilization by tissues and aberrations in hepatic tissues similar to T2DM. Hypo-and hyperthyroidism have been associated with impaired glucose metabolism in T2DM (Wang, 2013; Jayanthi and Srinivasan *et al*., 2019).

Finally the author concluded that diabetes is associated with degranulation of the secretory function of gonadotrophs, sommatotroph, thyrotroph and corticotroph cells, disrupting hormonal secretion and development of diseases.

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