Additions in green

Deletions in red

Comments in blue

Where is list of abbreviations?

Abstract needs correction as shown, structure, grammar, and shortage

Introduction needs corrections as shown, grammar, references, and shortage

Methods and results: need shortage

Discussion needs correction regarding shortage, and references

Conclusion needs shortage

References need correction as shown

Where is the Ethical committee of the study?

Where is the registration of the study?

Where is the animal experimental approval document?

GLUCOCORTICOID-INDUCED MORPHO-FUNCTIONAL ALTERATIONS IN PANCRAETIC BETA CELLS OF WISTAR RATS

Abstract:

Background and objectives: Though prolonged use of glucocorticoids has been reported to promote adverse effects, traditionally, high-dose glucocorticoids have been implicated in immune-suppression following organ transplant. With Cortisone being a well-known artificial glucocorticoid, this study investigated the histo-architectural and functional changes in pancreatic beta cells due to Cortisone administration. Methods: Forty Two (42) wistar rats (140 – 200kg) were assigned into seven groups of six (6) rats each with group A acting as control. While groups B and C were respectively treated with

0.1mg/kg and 0.3mg/kg of Cortisone, groups D and E received 0.1mg/kg and 0.3mg/kg of Cortisone respectively plus 33mg/kg of Ketoconazole; whereas, groups F and G were respectively given 0.1mg/kg and 0.3mg/kg of Cortisone alongside 150 mg/kg of Vitamin E each for twenty-eight (28) days. After 28 days of administration, rats were euthanized and blood samples collected for insulin assay. Pancreatic tissues were also harvested and observed for histo-morphological changes. Results: Analysis of variance (ANOVA) found Cortisone to have significantly ($p < .05$) increased glucose level in a dose dependent manner. This was however attenuated following co-administration of Ketoconazole and Vitamin E as Ketoconazole showed more potency in this ameliorating effect. Also, Cortisone was observed to significantly decrease (in dose dependent fashion), pancreatic β-cell functions, with attenuating effect seen following co-administration of Ketoconazole. Conclusion:It is recommended that caution be applied with the intake of glucocorticoids, especially in polypharmacy while treating certain ailments.

Keywords: Pancreas, Cortisone, Glucocorticoids

Where is list of abbreviations?

INTRODUCTION

The pancreas is a major accessory organ in the digestive system. It is both an endocrine gland and an exocrine gland[1&2]. Through its secretion of substances that help in food digestion, the organ functions in the control and metabolism of blood sugar within the body. Classically, the "endocrine" function of the pancreas relates to its secretion of insulin (and other hormones); a function mediated through its islet beta cells of Langerhans[3].

Approximately 3 million clusters of islets cells are present in the pancreas[4]. Within these islets there are four different cells that are involved in the regulation of blood glucose levels, with each secreting different types of hormones [alpha (α) cells secrete glucagon which increases glucose in blood, beta (β) cells secrete insulin which decreases blood glucose, delta (δ) cells secrete somatostatin which regulates/stops α and β cells, and PP cells, or gamma (γ) cells, secrete pancreatic polypeptide, which act to control blood glucose through secretion of glucagon to increase glucose levels, and insulin to decrease it[4-6].

Diseases of the pancreas are relatively rare. Cancer of the pancreas is rare but deadly. It is the fourth leading cause of cancer deaths in the United States and the fifth leading cause worldwide. The mortality rate is high because pancreatic cancer produces few (if any) symptoms and so is often not detected until it has spread to other organs. (ref)Hemorrhage in the pancreas and acute pancreatitis are also serious conditions. Several factors and disease conditions including gallstone, autoimmune diseases, sedentary lifestyle, alcohol, smoking, and Hepatitis B infections have been reported to predispose one to high risk of getting pancreatic ailments. (ref) Drugs may be considered a potential cause of pancreatic diseases in patients who take medications like anti-biotics (like Tetracycline), ACE inhibitors, Alkenyl Succinic Anhydride Inhibitors, etc.(ref)

Cortisone is a well-known anti-inflammatory and anti-allergic drug used in immunosuppression. It is an artificial drug affiliate of the glucocorticoid hormones with an activity that is 20 - 30 folds greater than cortisol[7]. Previous studies show that Cortisone has some antioxidant properties based on reduction of malondialdehyde (MDA) and elevation of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX). (ref)

Interestingly, glucocorticoids stimulate mass growth of β-cell while also promoting severe insulin resistance with the former being a significant

adaptive response to the latter[8]. The direct relationship between glucocorticoids and β-cell failure remains a controversial area of research[8]. Increase in circulating and/or tissue specific glucocorticoids have been linked with the development of obesity and Type II Diabetes Mellitus in humans and rodents. However, the progression from insulin resistance to overt Type II Diabetes Mellitus is highly disputed with respect to the in vivo and in vitro effects of glucocorticoids[9]. Paradoxically, both alternating physical stress and regular exercise ease insulin resistance and help to preserve β-cell mass, possibly by lowering glucocorticoid levels[10-12].

In response to GC-induced peripheral insulin resistance (IR) and in an attempt to maintain normoglycaemia, pancreatic β-cells undergo several morphological and functional changes that may result in high insulin level in the blood[13-14]. Failure of β-cells to compensate for this situation favours glucose homeostasis disruption, which can result in hyperglycaemia, particularly in susceptible individuals. (ref)The use of Cortisone as potent glucocorticoid, as well as an anti-inflammatory agent has become popular. However, when Cortisone is used in pharmacological doses, adverse side-effects are observed as high as fifty percent (50%) of the cases. (ref)The major risks of serious adverse neuro-developmental effects remain after antenatal, pre-natal and post-natal glucocorticoid administration[11]. There is paucity of information on the role of Cortisone on pancreatic β-cell functions; hence it became necessary for a study of this nature.

Aim of Study

This study aimed at ascertaining in albino wistar rats, the morpho-functional alterations in pancreatic β-cells due to exogenous glucocorticoid administration. Specifically, study investigated the effect of cortisone administration on total body and some organ weight changes. Study also examined the effect of cortisone administration on blood glucose and insulin

levels. Lastly, study examined the effect of cortisone administration on histo-architecture of pancreatic beta cells.

Methodology

Scope of Study

Study was conducted in the Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria. Due to the sensitive and invasive nature of the study, wistar rats were used as choice of experimental model. Study was restricted to the analysis of pancreatic β-cell histo-architecture and functions.

Study Design

Study design is experimental. Forty-two (42) adult male rats of the albino wistar strain were used for the study. Animals were randomly divided into seven groups of six (6) rats each (n = 6):

Group A: Control Rats fed with rat chow and water for twenty-eight (28) days

Group B: Treated with 0.1 mg/Kg body weight of Cortisone for twenty-eight (28) days

Group C: Treated with 0.3 mg/Kg body weight of Cortisone for twenty-eight (28) days

Group D: Administered with 0.1 mg/Kg body weight Cortisone and 33 mg/Kg body weight of Ketoconazole for twenty-eight (28) days

Group E: Administered with 0.3 mg/Kg body weight Cortisone and 33 mg/Kg body weight of Ketoconazole for twenty-eight (28) days

Group F: Administered with 0.1mg/Kg body weight Cortisone and 150 mg/Kg body weight of Vitamin E for twenty-eight (28) days

Group G: Administered with 0.3mg/Kg body weight Cortisone and 150 mg/Kg body weight of Vitamin E for twenty-eight (28) days

Materials and sources

Animal Procurement

Forty-two (42) albino wistar rats were raised in the animal house unit of the Faculty of Basic Medical Sciences, Delta State University, Abraka. The rats were kept in clean and well-ventilated cages, at the Animal House. The rats were allowed acclimatization for fourteen (14) days prior to experiment. They were then fed daily with clean tap water and chow while experiment lasted for twenty-eight (28) days where in the animals were administered with Cortisone, Ketoconazole and Vitamin E

Cortisone Administration

Cortisone was purchased from local Pharmacy stores in Abraka. Cortisone solution were prepared fresh daily in saline and the animals were injected subcutaneously at 0.1mg/kg body weight (low dose) and 0.3mg/kg body weight (high dose) daily between 6:00 A.M. to 7:00 A.M. for twenty-eight days prior to euthanasia.

Vitamin E Administration

Vitamin E in form of α-tocopherol tablets were freshly dissolved in distilled water at 150mg/kg and administered orally via orogastric cannula between 6.am and 7.am once daily for twenty-eight days.

Ketoconazole Administration

Ketoconazole was dissolved daily in distilled water administered orally at a dose of 33mg/kg body weight via orogastric cannula for twenty-eight days (28) experimental period.

Procedure

Sample Collection

Using a digital electronic balance (CAS ED Digital Weighing Scale), Body weights of animals were measured prior to commencement, and at the last day of the experiment. Pancreatic weights were also measured after euthanasia. By gently nipping the rats' tail with sterilized blade, blood samples were also collected for insulin assay using the ACCU check glucose meter, while the pancreas was harvested for histo-morphometric analysis.

Preparation of Tissues for Histological Analysis

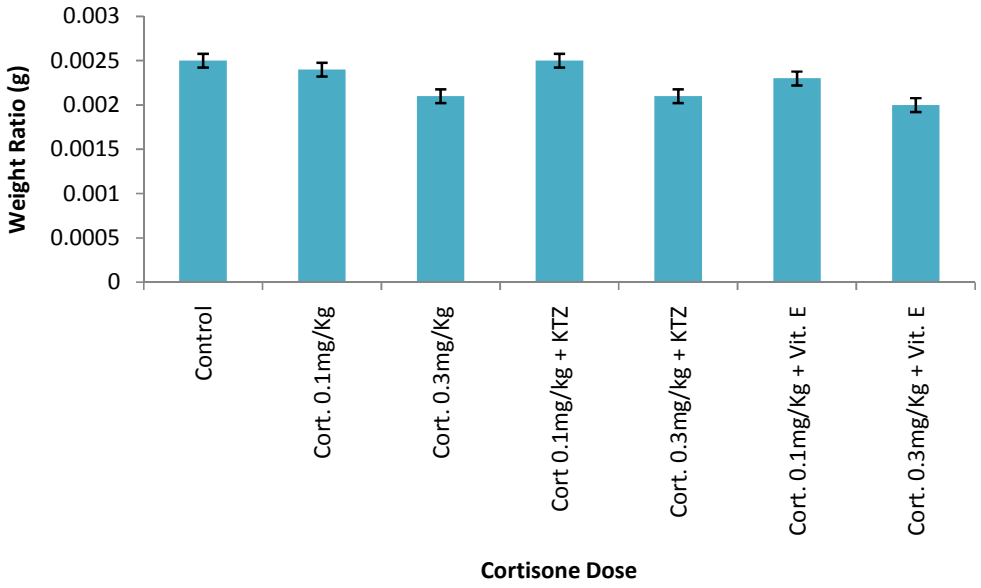
This was done to provide a solid medium for tissue sectioning and assist in microscopic examinations. Here, normal saline was used as fixative; the pancreas specimens were fixed immediately after harvest to prevent autolysis and putrefaction. The tissues were left in the fixative for 24 hours. Dehydration was done gradually to remove inherent water content in the pancreas specimen by gradually placing in alcohol as follows; 70% alcohol for 2 hours; 90% alcohol for 2 hours; 95% alcohol for 1 hour; Absolute alcohol – 2 charges for 2 ½ hours and Absolute alcohol overnight. Clearing was done using two changes of xylene for 1 ½ hour each. Tissues were put in three changes of paraffin wax for 1 hour each, this was to enable the paraffin wax permeates the tissue, filling up the vacuoles left by dehydration. Processed infiltrated tissues were positioned in molten paraffin wax and left to solidify. Tissues were then cut into blocks and held firmly in position by paraffin wax. Sectioning was done with the aid of a microtome at a precise thickness of 3 microns. Sectioned tissues were then stained with haematoxylin and eosin (H and E).

Statistical Analysis

Evaluation of data for statistical significance was done with One-Way Analysis of Variance (ANOVA). Obtained Data were expressed as Mean ± Standard Deviation (SD). Graph Pad prism – statistical software was used for analysis of obtained data. P-value < .05 was accepted as statistically significant.

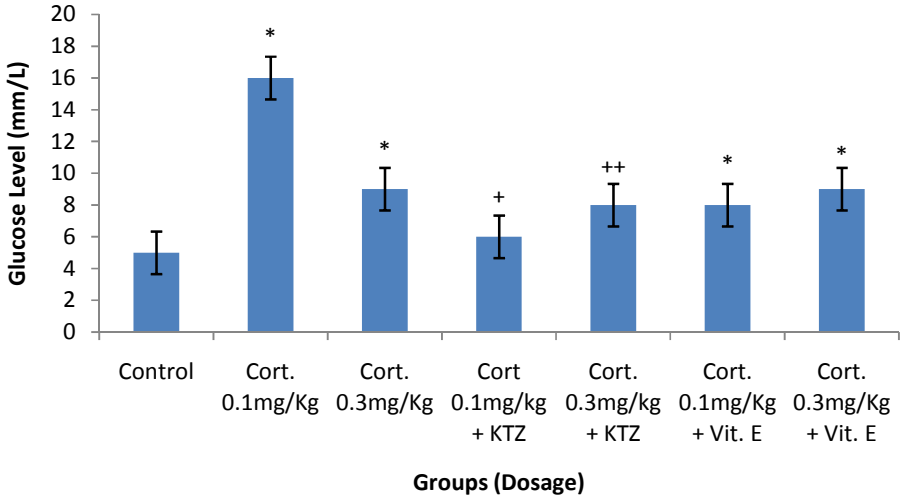
Results

Figure I: Pancreas to Body Weight Ratio of Cortisone Treated Rats Administered with Vitamin E and Ketoconazole



Treatment with Cortisone caused insignificant decrease in pancreatic organ/body weight ratio in comparison with control ($p < .05$). However, administration of KTZ and Vitamin E caused a minimal attenuation of the pancreatic/body weight ratio. Here, KTZ = Ketoconazole.

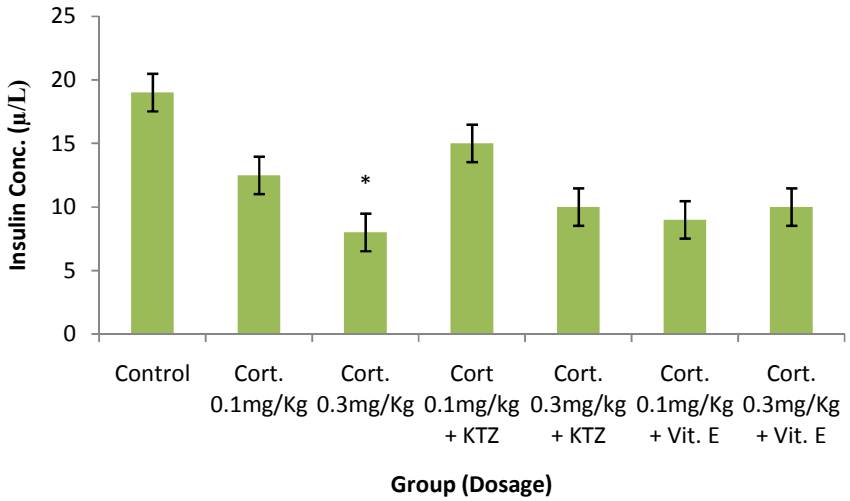
Figure II: Glucose Levels of Cortisone Treated Rats Administered with Vitamin E and Ketoconazole



*: significance ($p<0.05$) when compared to control;
+: Significance ($p<0.05$) when compared to 0.1mg/Kg Cortisone;
++: significance ($p<0.05$) when compared to 0.3mg/Kg Cortisone

Cortisone significantly increased ($p<0.05$) glucose level in dose dependent manner. These changes were however attenuated with co-administration of Ketoconazole (KTZ) and Vitamin E. Ketoconazole showed more potency in this ameliorating effect, with significance ($p<0.05$) compared to those of rats treated with their corresponding doses of 0.1mg/Kg and 0.3mg/Kg of Cortisone.

Figure III: Changes in Insulin Concentration of Cortisone Treated Rats Administered with Vitamin E and Ketoconazole



*: significance ($p < 0.05$) when compared to control;

Cortisone (Cort.) caused a dose dependent decrease in insulin concentration with statistical significance ($p<0.05$) at the highest dose (0.3mg/Kg Cortisone) of administration. Furthermore, Ketoconazole antagonized the actions of Cortisone by limiting the insulin decreasing effect. The antagonistic effect of Vitamin E was minimal on Cortisone treated rats as the insulin level shared similar range with the insulin level of rats treated with 0.1mg/Kg Cortisone and 0.3mg/Kg Cortisone. Despite these changes, statistical significance was not recorded.

Figure IV: Effect of Cortisone and co-administration of Vitamin E and Ketoconazole on the histology of the pancreas

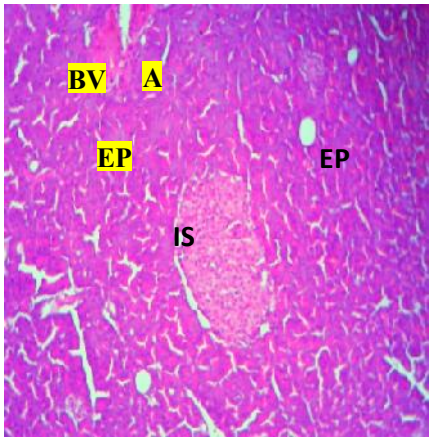


Plate A: Control rat pancreas (H & E x100)

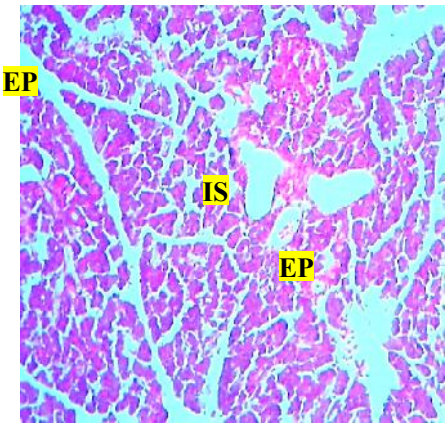


Plate B: Pancreas of rats treated with 0.1mg/Kg CORTISONE (H & E)

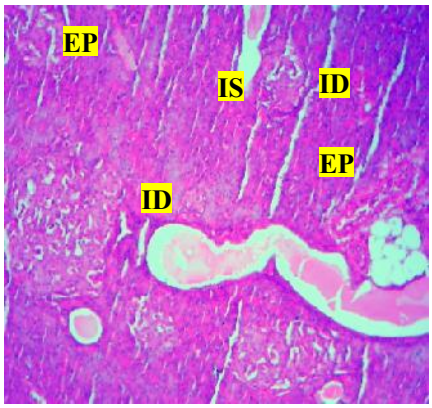


Plate C: Pancreas of rats treated with 0.3mg/Kg

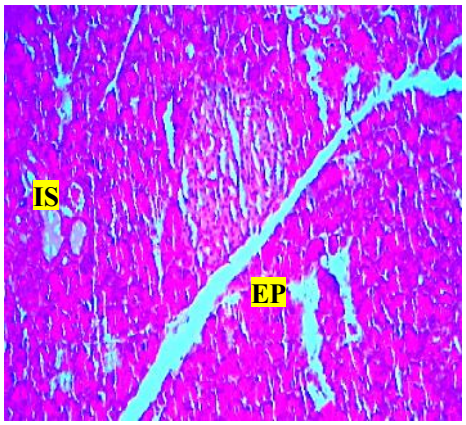


Plate D: Pancreas of rats treated with 0.1mg/Kg Cortisone + Ketoconazole

EP

Discussion

Cortisone is potent synthetic glucocorticoid that has a long history of use in veterinary and human medicine especially in the treatment of metabolic diseases and inflammatory disorders. Clinically, cortisone is administered for the suppression of inflammation in addition to alleviation of other disease ailments[16].

In this study, the observed significant ($p < 0.05$) dose dependent increase in glucose concentration in cortisone treated rats may be due to activation of hepatic gluconeogenic enzymes and stimulation of gluconeogenesis by cortisone. Pasternak *et al*. (2004) and Lukins *et al*., (2005) also had similar findings as cortisone was reported to have raised blood glucose concentrations in non-diabetic patients[17&18].

In cases of normal physiological conditions, glucocorticoids function to prevent hypoglycaemia during period of acute stress and/or reduction in energy intake.(ref) Glucocorticoids raise circulating glucose concentrations through several mechanisms; specifically, they increased production of glucose in the liver[19], decreased peripheral glucose uptake[20], and promote the breakdown of muscle and fat to provide substrates for gluconeogenesis.(ref)

However, study from Rhee *et al*. (2004)[21] on patients undergoing surgery further confirmed that cortisone, even with a single dose of administration, elevated blood glucose. Matsumoto *et al*. (2006)[22] reported that this effect may be related to an increase in gluconeogenesis and the development of insulin resistance, which have been demonstrated in both animals and humans.(ref)

Cortisone also reduced the organ-body weight of the rats, this decrease in organ-body weight was significant ($p<0.05$). According to Katagiri *et al*. (2001), treatment with cortisone at a dose of 1 mg/kg/day improved or increased the density of 5-HT (dopamine) receptor in the rat frontal cortex and decreased body weight. In previous studies, using cortisone administration on mid-trimester has resulted in a reduction in fatal weight[22] which is identical to the reduction in the present study. The possible drop in organ-body weight by graded doses of cortisone could be due to prolonged elevation in plasma leptin level which would cause drastic decrease in food consumption and weight gain, in accordance with previous findings[18]. It was reported that cortisone causes increase in the synthesis and secretion of leptin in the adipose tissue[23]. Together, it is suggested that chronic cortisone treatment may induce lengthy increase in plasma leptin level, most likely, in a dose dependent manner, and the prolonged elevation in plasma leptin may contribute in cortisone-induced diminution in food consumption and weight gain. Additionally, it is hypothesized that plasma leptin may take more significant role in increasing energy usage than decreasing energy intake during cortisone-induced anorexia. This is assertion was supported by previous report that glucocorticoids limited the ability of leptin to signal satiety[19]; however, further studies are required to verify our hypothesis.

The decrease in pancreas-body weight induced by Cortisone can be explained by the alteration of the histo-architecture of the pancreas. It was observed that Cortisone caused a dose-dependent destruction of pancreatic tissues. The altercation of pancreatic beta-cell by Cortisone is responsible for the dose dependent decrease in beta-cell count, hence the decrease in insulin

secretion. This assertion was supported by the findings of Lee *et al*. whose study reported that proliferation is very much involved in the modulation of the beta cell mass. Glucocorticoids have already been shown to induce reduced proliferation in various cell types[22], this was further confirmed by the dose dependent derangement of beta cells following exposure to Cortisone in this study, a model of type II diabetes, hence the beta-cell count and function were impaired, together with an alteration of the islet vascular integrity as is consistent with report of Lee *et al*. From the findings of this study, it is proposed that vascular alteration by Cortisone also played a role in the beta-cell deficiency.(ref)

Ketoconazole, a wide spectrum antifungal agent inhibits adrenal and gonadal steroids reversibly. Several studies have reported that ketoconazole treatment results in 30–90% remission in Cushing's disease. As a known Cortisone receptor blocker, it is not surprising by the data generated in this study showed the attenuating effects of Ketonazole in Cortisone activities in blood glucose level, insulin secretion, insulin resistance, beta cell function, beta cell count and diameter.(ref) The change in blood glucose level and beta-cell count induced by Ketoconazole in Cortisone treated rats was significant ($p<0.05$) when compared to parameters of rats solely treated with Cortisone. The significance observed showed that though Vitamin E had similar reversal effect as Ketoconazole, but the antifungal drug was more potent in its ameliorating action. In few experiments like changes in insulin secretion, insulin resistance, beta cell function, Vitamin E offered minimal ameliorating effects. The reparative effects observed in the pancreatic-body weight gain and glucose level increase in co-administration of Vitamin E and Cortisone could be attributed to decrease in oxidative stress level associated with hyperglycemic conditions[4].

Ketoconazole showed that it is more effective in ameliorating the adverse effects of Cortisone compared as to Vitamin E. A possible explanation for this

could be that the whilst the Ketoconazole act as a blocker to Cortisone receptor or other forms of glucocorticoids receptors, Vitamin E on the other hand attenuates the detrimental effects of Cortisone through its curative activities, hence requiring a longer period of time to exert its effect.

5.2 Conclusions:

Results from this study suggest that administration of graded doses of cortisone caused increased level of glucose metabolism, peripheral insulin resistance and pancreatic beta-cell diameter. Cortisone also caused a dose dependent decrease in insulin level, pancreatic beta-cell count, and beta-cell function. Cortisone also caused distortions in the islets cells of the pancreas by inducing apoptosis, and vascular congestion. Ketoconazole and Vitamin E caused ameliorating effects in the changes induced by Cortisone.

Benefit of Study

Data obtained from this study would be of immense benefit through its b positive contribution to already existing data on the pancreatic β-cells function and structure. The results will add to the body of knowledge on the effect of glucocorticoid on pancreatic beta cell function as it relates to the development of type II diabetes mellitus[24]. Since insulin is produced by the β-cells of the pancreas, the morpho- functional changes may give an insight into the mechanism of glucose intolerance, insulin level/ resistance and possible effects

Recommendations

We recommend applied caution to the intake of glucocorticoids, especially when treating certain ailments. From the data generated, Ketoconazole appears to play a major role in blocking glucocorticoid receptors; hence its co-administration with glucocorticoids is advised. It is also recommended that

similar study of this nature be carried out in a longer experimental duration as the minimal ameliorating effects of Vitamin E could be down to the duration of the present study.

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