

## Effect Of Gelucire Based Gastroretentive Multiparticulates Of Metformin Hydrochloride And Glibenclamide On Histology Of Diabetic Rat Pancreas

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### Abstract

The objective is to observe the effects of Gelucire based gastroretentive multiparticulates of metformin hydrochloride and glibenclamide on histology of diabetic rat pancreas. Diabetes was induced in wistar albino rats of either sex weighing 150-200g by streptozotocin. They were divided into different groups, Control group was given saline buffer only while other groups are orally administered with standard drugs (metformin hydrochloride and glibenclamide) separately, their combinations, Gelucire based gastroretentive multiparticulate formulations and their combination. Blood glucose level and physical health of rats were monitored regularly. After seven days of treatment, rats were sacrificed to isolate the pancreas for histopathological observation. The histopathological observation of pancreas shows that combination formulation of metformin hydrochloride and glibenclamide with Gelucire has remarkable effect on regeneration of pancreatic  $\beta$ -cells without any side effects.

The formulations shown promising results and it is safe for diabetes type II treatment.

KEY WORDS: HISTOPATHOLOGY, PANCREAS, STREPTOZOTOCIN, DIABETES, GLIBENCLAMIDE, METFORMIN HYDROCHLORIDE, GASTRORETENTIVE MULTIPARTICULATES

## Introduction

Diabetes is one of the major causes of death and disability in the world. The global figure of people with diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025. Most cases will be of type II diabetes, due to sedentary lifestyle and obesity [1].

In order to increase the bioavailability of such drugs, the residence time of the orally administered dosage form in the upper GIT needs to be prolonged. The main approaches to prolonging the gastric residence time of pharmaceutical dosage forms include bioadhesive drug delivery system, which adhere to mucosal surface; devices that rapidly increase in size once they are in stomach to retard the passage through the pylorus; and density control delivery system, which float on gastric fluid [2,3,4,5]. Gastric floating drug delivery system (GFDDS) is able to prolong the retention time of a dosage form in the stomach, thereby improving the oral bioavailability of the drug [5].

Recently, much attention has been focused on the use of fats and fatty acid as carriers in drug delivery systems [6,7,8]. The use of amphiphilic lipid glyceryl monooleate for the design of floating matrix system [9]. Gelucire is the family of vehicle derived from mixtures of mono-, di- and triglycerides with poly ethylene glycol (PEG) esters of fatty acids. These are available with range of properties depending on their hydrophilic lipophilic balance (HLB) and melting point range (33-65°C). Gelucire containing only PEG esters are generally used in the preparation of fast release formulation. Owing to their extreme hydrophilicity and low density, Gelucire 50/13 may be considered an appropriate carrier for designing fast release floating drug delivery system [10]. Gelucire containing only glycerides or a mixture of glycerides and PEG esters (Gelucire 39/01, 43/01) are used in the preparation of sustained release formulation. Owing to their extreme hydrophobicity and low density, Gelucire 39/01 and 43/01 are considered as appropriate carriers for designing sustained release floating drug delivery system [11, 12, 13]. The GFDDS is

particularly useful for drugs that are primarily absorbed in the duodenum and upper jejunum segments [14].

A plethora of antidiabetic drugs are used, of which Glibenclamide and Metformin hydrochloride is a very widely accepted combination of drugs [15]. The rationale of combinations of the Sulfonylureas and the Biguanides is both are major oral antidiabetics. The sulfonylureas, such as Glibenclamide act by stimulating the secretion of insulin. Their targets are insulin-producing pancreatic  $\beta$  cells and the biguanides, such as Metformin, inhibit glycogenesis and increase the peripheral use of glucose. The biguanides can only be active in the presence of endogenous insulin. Since the introduction of the various antidiabetic medicaments, doctors prescribe in particular oral treatments of diabetes which combine these various products, that forces patients to take these combinations of medicaments several times per day. Unavoidably, low compliance is then observed on the part of the patients, who are often elderly persons.

Under these conditions, oral treatments do not have the expected effects and the patients suffer serious complications. Thus, compliance is a fundamental parameter for the efficacy of the treatment (prevention of serious disorders caused by hyperglycemia and survival of the patient). By improving compliance, dosage errors and their deleterious effects would be limited. Since sulfonylureas are capable of stimulating insulin release, but are not capable of acting on insulin resistance, and biguanides are able to act on insulin resistance, whereas they are not able to stimulate insulin secretion, the therapeutic rationale suggest the use of combined formulations of medicaments capable of finding a remedy for both the deficiency in insulin secretion and the insulin-resistance condition. At present 4 combinations are marketed which use a combination of Metformin with Glibenclamide i.e. Glucophage - Glibenclamide & Metformin (2.5 mg) (500 mg), Glibomet Guidotti-Glibenclamide & Metformin (2.5 mg) (400 mg), Suguan M Hoechst - Glibenclamide & Metformin (2.5 mg) (400 mg) and Bi-Euglucon M Boehringer M- Glibenclamide &

Metformin (2.5 mg) (400 mg)[16].

The present histopathological study is aim to explored the possible effects of Gelucire based gastro retentive formulation on streptozotocin induced diabetic rat pancreas. Both formulations can be explored individually as well as in combination for improved efficacy in wistar albino rats.

## Methods

### Chemicals

Metformin hydrochloride (MH) was obtained as gift sample from Sohan Healthcare Pvt. Ltd. (Pune, India) and Glibenclamide (GLB) was kindly supplied as gift sample by Prudence Pharma Chem (Ankleshwar-393002, INDIA). Gelucire 39/01, 43/01 and 50/13 was procured as gift sample from Gattefosse (St. Priest, Cedex, France). Streptozotocin procured from Sigma (USA). Ultra-pure water (HPLC Grade) was supplied by Qualigens (Delhi, INDIA).

### Animals

Albino wistar rats were handled as per CPCSEA Guidelines of Good Laboratory Practice and were housed in polypropylene cages with free access to standard laboratory diet and water. The research protocol of the animal experimentation (Registration no: 837/ac/04/CPCSEA; Resolution no: 20/PhD/2008-2009; Dated 08/03/2010) was approved by the 'Institutional Animal Ethical Committee' of College of Pharmacy, IFTM, Moradabad-244001, Uttar Pradesh, India.

### Application

Effect of Gelucire based optimized gastroretentive multiparticulates formulation of metformin hydrochloride and glibenclamide on diabetic rat pancreas were determined in comparison with pure drugs in streptozotocin (STZ) induced diabetic wistar rats of either sex weighing 150-200 g. Naive albino wistar rats 48 in number housed in cages in groups of six and kept fasting for 24 hr with water *ad libitum*. The dosage of drugs administered orally

was decided upon human therapeutic dose extended to animals.

### Experimental protocols

The animals were classified into 8 groups, each containing 6 rats.

1. Sham or Control Group I (n=6): were given 1ml saline p.o.
2. Diabetic Control Group II (n=6) were given streptozotocin 35mg/kg body weight of rat /i.p injection.
3. Diabetic standard control Group III (n=6) were given GLB (0.25mg/Kg) pure drug in aqueous solution p.o through oral cannula.
4. Diabetic standard control Group IV (n=6) were given MH (50 mg/Kg) pure drug in aqueous solution p.o through oral cannula.
5. Diabetic standard control Group V (n=6) were given GLB (0.25mg/Kg) and MH (50 mg/ Kg) pure drug in aqueous solution p.o through oral cannula.
6. Diabetic treated control Group VI (n=6) were given GIV formulation [17] optimized batch containing GLB reduced to equivalent of drug dose in 0.25mg/Kg body weight of rat /p.o with 1ml of 1% Sodium CMC through oral cannula.
7. Diabetic treated control Group VII (n=6) were given MII formulation [18] optimized batch containing MH reduced to equivalent of drug dose in 50mg/Kg body weight of rat /p.o with 1ml of 1% Sodium CMC through oral cannula.
8. Diabetic treated control Group VIII (n=6) were given combination of GIV and MII formulation optimized batch containing both GLB and MH reduced to equivalent of drug dose in GLB (0.25mg/Kg) and MH (50 mg/Kg) body weight of rat /p.o with 1ml of 1% Sodium CMC through oral cannula.

The animals in groups II to VIII were rendered diabetic by a single intraperitoneal injection of streptozotocin (STZ; 35 mg/kg) freshly prepared

in citrate buffer (pH 4.4). The animals in groups I and II were injected with the buffer alone. Next, 72 h after the injection, blood was drawn from the tail of conscious rats in order to estimate their glucose levels using a glucometer. Blood glucose was similarly estimated every week until autopsy. Ten days after the STZ injection, animals in groups III to VIII began to receive the formulation daily. Doses were administered orally for 7 days. Body weight was recorded at the beginning and end of the experiment in every group. At the end of the experimental period, animals were fasted overnight and autopsied under light ether anesthesia.

### Histopathological studies

Animals were sacrificed on 7th day during prolonged treatment. Pancreas were removed, washed with cold saline and preserved in 10% formalin in buffered form. Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxylin and eosin for histomorphology evaluation of pancreas on confocal microscope at 5x magnification.

### Statistical Analysis

The experimental data was carried out by Student's t-test and one way analysis of variance (ANOVA) at a significance level of probability ( $p < 0.05$ ) using SPSS 12.0 software (IBM). Numerical values in tables & figures were expressed as mean  $\pm$  standard deviation (SD). Means were assumed to be statistically significant when  $p < 0.05$ .

## Results

### Animal Physical observation

see Figure 1.

As per Figure 1, Prior to STZ administration, there was no significant difference in the average weights of all the rats in the eight groups. By the end of the one week after diabetes mellitus was experimentally induced, the weights of diabetic rats in groups II and VIII were significantly reduced despite the increase in food and fluid intake in these animals. This weight loss continued for ten days after STZ administration. The animals manifested alopecia and polyurea, shown by marked wetness of the ventral body surface of the animals. However, the weight of the animals in group III to VIII gradually increased with treatment with formulation over the period of seven days. In addition, there was an improvement in the physical outlook of the formulation treated animals over time. At the end of the experiment, there was a significant increase in the body weights of formulation treated diabetic rats when compared to the untreated diabetic rats; while no significant difference existed between the weights of the treated rats and the control.

### Changes in the blood glucose level

see Figure 2.

As per Figure 2, Prior to STZ administration, the fasting blood glucose levels (BGL) did not differ significantly between the eight groups of experimental animals. Twenty-four hours after administration of STZ, the blood glucose level was significantly higher in animals from groups

II to VIII. The blood glucose levels of animals in group III to VIII gradually decreased with treatment with formulation over the period of seven days. Control rats treated with citrate buffer were euglycaemic throughout the period of the experiment. At the end of the experiment there was a significant reduction in the blood glucose levels of groups III to VIII rats compared to those of group II rats.

### **Histopathology of Pancreas (Photomicrographs):**

see Figure 3.

see Figure 4.

see Figure 5.

see Figure 6.

STZ-induced hyperglycemia in rats is considered a good model for the preliminary screening of agents active against type 2 diabetes and is widely used. Generally, destruction of  $\beta$ -cells starts three days after STZ administration and reaches its peak at three to four weeks in rats. Streptozotocin-induced diabetes in laboratory animals has been widely used for research on diabetes and its long-term complications. Control animals in these studies are usually injected with citrate buffer solution. However, STZ is known to possess pharmacological effects other than its diabetogenic properties.

### **Histopathological observations**

Photomicrographs of pancreas in slide A showed normal acini, and normal cellular population in the islets of Langerhans (IL) in pancreas of vehicle-treated rats. The histological appearance of the pancreatic islet cells of the control was normal (Fig.3).

In slide B, extensive damage to the islets of Langerhans and reduced dimensions of islets. Microscopic examination of the pancreatic sections of the untreated STZ induced diabetic group revealed a breakdown of micro-anatomical features including degenerative and necrotic changes, and shrunken in the pancreatic islets of Langerhans, decreased islets cellular density, and severe vacuolation (Fig.3) in the islets, as well as a severe reduction in the number of cells in the islets; though ductal and connective tissues (CT) appeared normal.

In slide C and D restoration of normal cellular population size of islets with hyperplasia by standard pure drug MH and GLB 50 mg/kg b.w and 0.25mg/kg b.w respectively (Fig.4). The morphology of the pancreas of Standard and formulation treated diabetic rats as shown in Slide E and F revealed remarkable improvement in the islet of Langerhans. There was an increase in the islets cellular density, with an increase in granulation, but vacuolation was reduced or absent in many islets (Fig.5). Slide G and H were also shown the partial restoration of normal cellular population and enlarged size of  $\beta$ -cells with hyperplasia by treatment with combination of standard drugs and optimized drug formulation (Fig.6). Histopathology of pancreas in control animals showed normal pancreatic parenchyma and islets cells. In diabetic control, pancreas sections showed moderate hyperplasia of islets cells, severe congestion in pancreatic parenchyma, and mild infiltration of inflammatory cells. In diabetic animals, treated with GLB and MH, pancreas show mild hyperplasia of islet cells and congestion of pancreatic parenchyma. In normal animals pancreas section showed normal pancreatic parenchyma structure.



### Islets of langerhans examination

Fig.3 (A) and (B) represent two slides of islets of Langerhans from a normal and a STZ-induced diabetic rat respectively. Comparison of these two slides clearly indicates the reduction in the number of pancreatic islets as well as their number of  $\beta$ -cells in the diabetic rats. As it is evident from slide B, the islets are irregularly shaped and relatively small and atrophic. Most cells of the islets are small, degranulated and dark with scanty cytoplasm. Severe vacuolation and degranulation are present in the  $\alpha$ -cells of a number of islets. However, compared to the untreated diabetic rats, histopathological examination of the GIV formulation and MII and GIV combination treated diabetic rats revealed an increase in the number of pancreatic islets and the number of  $\beta$ -cells (Fig.5F and Fig.6H). In other words, the combination formulation treated diabetic samples resembles close to healthy pancreatic samples that confirms no side effects of formulation treatment as well as positive increase in  $\beta$ -cells. The regeneration of the  $\beta$ -cells of the STZ-destroyed islets is probably due to the fact that pancreas contains stable (Quiescent) cells which have the capacity of regeneration. Therefore, the surviving cells can proliferate to replace the lost cells.

In conclusion to our histopathological investigation suggests the possibility of the islets regeneration upon formulation treatment. The results of this present study indicate that the decrease in the blood glucose concentration of diabetic rats by GIV and MII combination formulation treatment is due to the regeneration/proliferation in the pancreatic  $\beta$ -cells.

In this present study, almost all the insulin-producing  $\beta$ -cells were degranulated, degenerated, or necrosed in the streptozotocin-treated rats, leading to a decrease in insulin secretion

and an increase in blood glucose concentration.

However, treatment with GIV and MII combination formulation showed a significant antihyperglycaemic activity in STZ-induced diabetic rats at the end of the experiment.

Therefore, the surviving cells can proliferate to replace the lost cells. The majority of islets cells are formed by  $\beta$ -cells which are responsible for producing insulin. Depletion of  $\beta$ -cells will therefore result in insulin deficiency which will lead to a disorder in carbohydrate, protein and fat metabolism with a resultant hyperglycemia.

### Discussion

It was concluded that the experiment showed the regenerative effect of Gelucire based gastroretentive multiparticulates of metformin hydrochloride and glibenclamide on diabetic rat pancreas. Histopathology studies of diabetic pancreas and drug treated diabetic pancreas do not show any side effects of drug and formulation. Remarkable increase in number of  $\beta$ -cells suggests the possibility of islets regeneration on combination formulation treatment.

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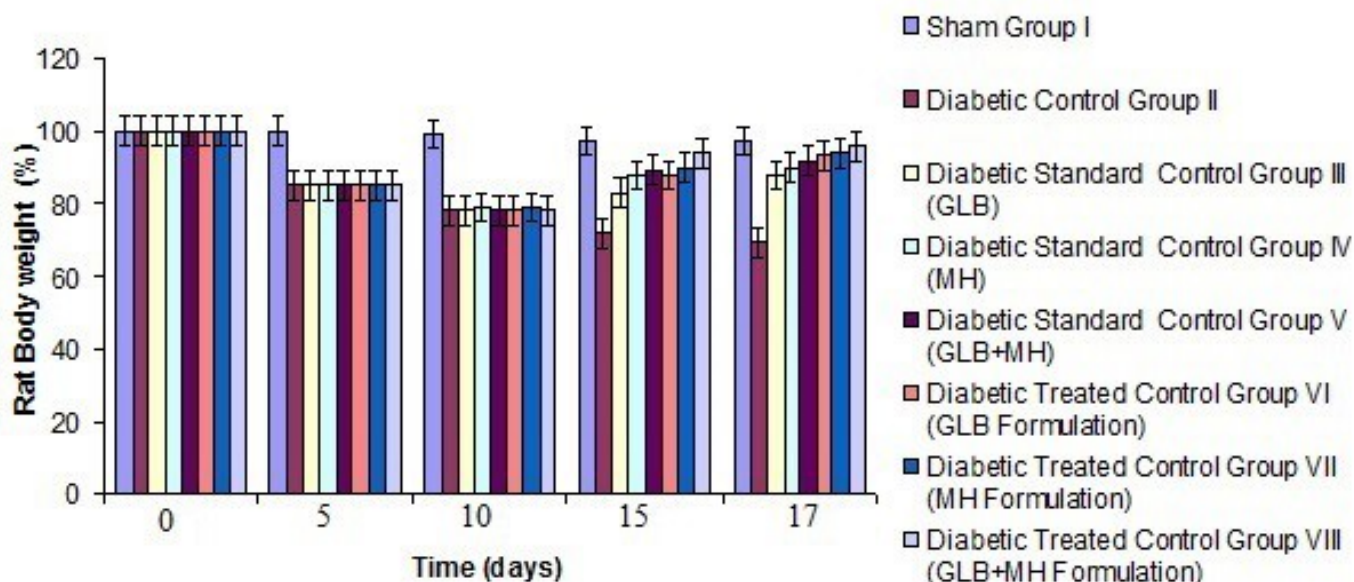


Figure 1: Percent rat body weight variation with days on treatment

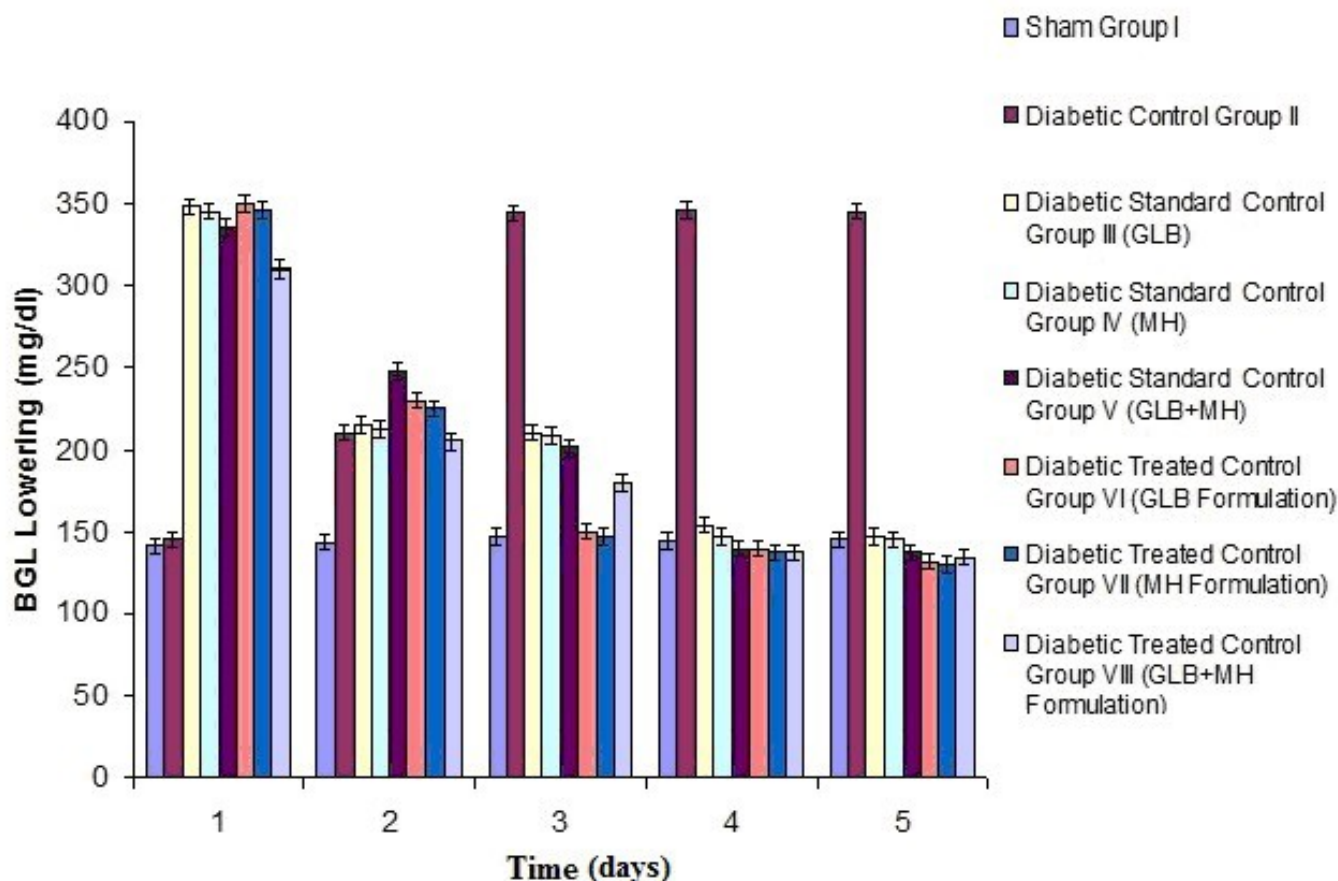


Figure 2 : BGL lowering with time on treatment



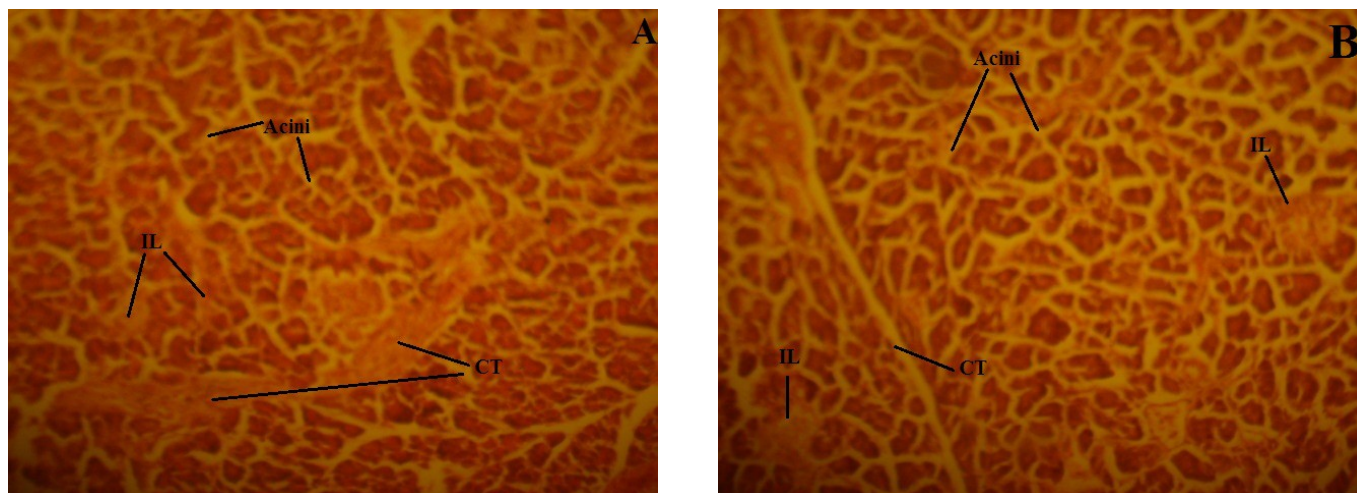


Figure 3: (A) Islets of Langerhans from normal rat pancreas; (B) Islets of Langerhans from STZ induced diabetic rat pancreas.

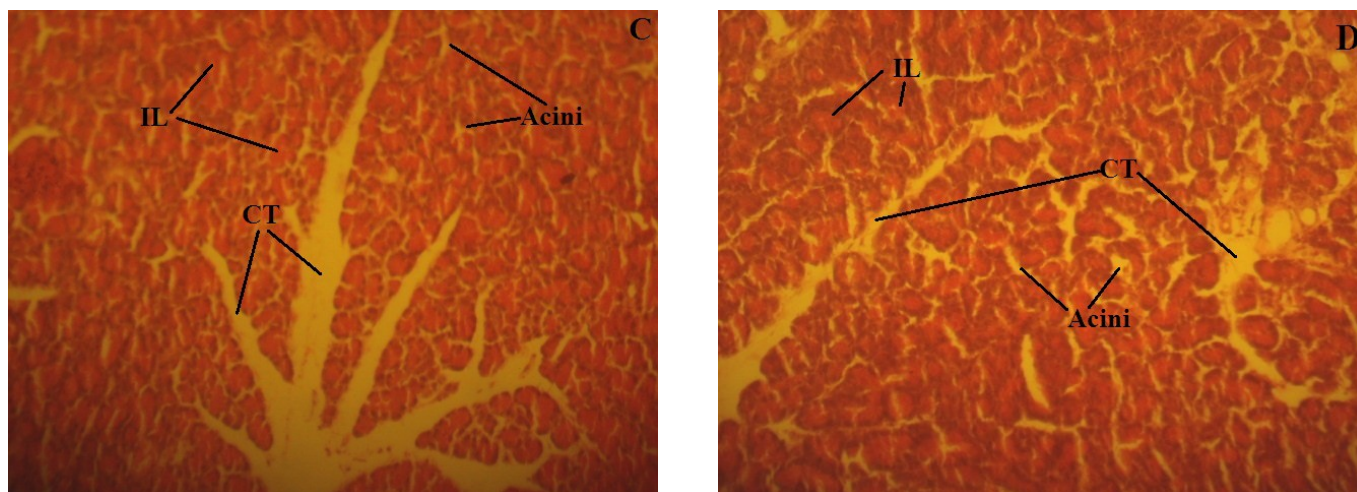


Figure 4: (C) Islets of Langerhans from standard MH treated diabetic rat pancreas; (D) Islets of Langerhans from standard GLB treated diabetic rat pancreas.

Where, IL= Islets of Langerhans and CT= Connective Tissues.

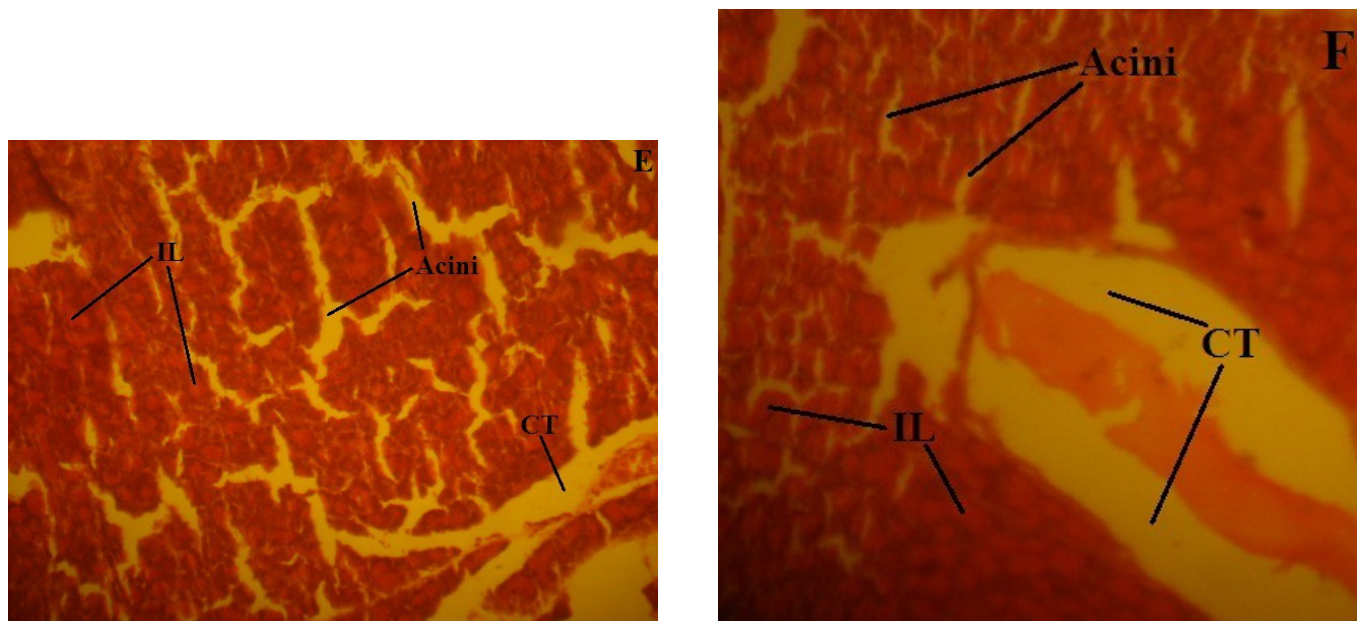


Figure 5: (E) Islets of Langerhans from Optimized MII treated diabetic rat pancreas; (F) Islets of Langerhans from Optimized GIV treated diabetic rat pancreas.

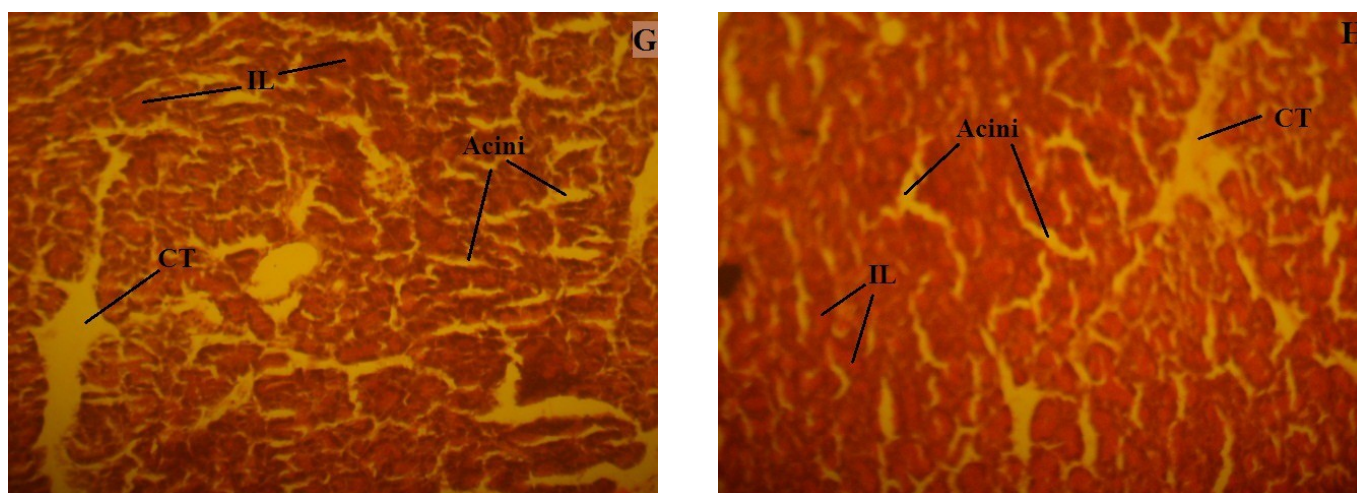


Figure 6: (G) Islets of Langerhans from Standard MH-GLB combination treated diabetic rat pancreas; (H) Islets of Langerhans from Optimized MII-GIV combination formulation treated diabetic rat pancreas. Where, IL= Islets of Langerhans and CT= Connective Tissues.