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HISTOPATHOLOGICAL AND BIOCHEMICAL STUDIES ON THE EFFECT OF *TRIGONELLA FOENUM GRAECUM* AND *COCCINIA INDICA* EXTRACTS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The present study was taken up to evaluate the efficacy of *Trigonella foenum graecum* and *Coccinia indica* individualy and in combination in streptozotocin induced diabetic rats for a period of 90 days. The various groups in this study included normal control (Group-I), diabetic control (Group-II), diabetic rats treated with *Trigonella foenum graecum* (Group-III), diabetic rats treated with *Coccinia indica* (Group-IV), diabetic rats treated with *Trigonella foenum graecum* and *Coccinia indica* (Group-V) respectively. There was significant variation in biochemical and pathomorphological parameters of diabetic rats when compared to normal control rats. The alleviation of the diabetic and its complications induced by streptozotocin was observed in all the treatment groups with variable degree of improvement. *Trigonella foenum graecum* and *Coccinia indica* extracts were effective in alleviating streptozotocin induced diabetes. Combination of *Trigonella foenum graecum* and *Coccinia indica* showed better improvement compared to individual extract alone and improvement was statistically significant. However the combined treatment of *Trigonella foenum graecum* and *Coccinia indica* revealed a very good antidiabetic effect with reference to improvement in insulin level and beta cell number which indicated a synergistic effect between *Trigonella foenum graecum* and *Coccinia indica*

KEY WORDS: **Diabetes, *Coccinia indica, Trigonella foenum graecum* Streptozotocin,**

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. These metabolic abnormalities result either from a deficiency of the blood sugar-lowering hormone insulin or from insulin resistance, a defect in the body's capacity to respond to insulin (1). Thus diabetes mellitus is a complex, multi factorial disease associated with progressive deterioration of beta cell function and insulin resistance. India, a country with a population over 1.2 billion has been reported to have currently 62.4 million people with diabetes according to Indian Council of Medical Research and expected to increase to over 100 million by 2030 (2). Type-2 diabetes mellitus (T2DM) is the most common type of diabetes affecting majority of people with higher incidences in older population in developed countries. However in developing countries like India, diabetes incidence is higher in younger middle aged population who are in the prime of their working lives there is also increase in the prevalence of secondary complications, which is alarming. Hence there is an urgent need for the prevention and effective control of diabetes in India. More than 50 per cent of population with Type-2 DM remain undiagnosed and cost of the long term treatment is adversely affecting the economy of the country. The estimated global healthcare expenditure to treat and prevent diabetes is projected to exceed USD 490 billion by 2030 (3). On the other hand, modern oral hypoglycemic agents like (glibenclamide) is costly which showed some adverse effects, expensive and also develop resistance to diabetes, so that it was attempted to develop an alternative source of drug for controlling diabetes mellitus. Plant sources of hypoglycemic agents are easily available, cost-effective and presumably devoid of any side effects. Among the indigenous plants that are noteworthy for their hypoglycemic and antihyperglycemic principles include *Trigonella foenum graecum* and *Coccinia indica* commonly known as methi and Little gourd (kovai) respectively which are reported to possess hypoglycaemic effect (4). No precise reports on the herbal extracts on diabetic are available. The study was undertaken to compare the antidiabetic activity of *Trigonella foleum graecum* seed extract and *Coccinia indica* leaves extract alone or in combination.

MATERIALS AND METHODS

Animals

Normal adult healthy female Wistar albino rats weighing 170-180 g were procured from RRL Instruments and Animals supplier, Bangalore for the study purpose. They were maintained under standard laboratory conditions and offered *ad lib* of standard commercial rat feed (Amruth Feeds, Bangalore) and clean drinking water. The experiment was carried out for a period of 90 days upon permission from Institutional Animal Ethics Committee.

SOURCES

Streptozotocin

To induce diabetes in rats, streptozotocin (Sigma Chemicals, St.Louis, USA) was used intraperitonially in ice-cold citrate buffer (pH 3.5-4.5) at the dose of 45 mg/kg.

Trigonella foenum graecum

The alcoholic seed extract of *Trigonella foenum graecum* used in the present study was obtained from Plantex Herbal Drug Company, Vijaywada. The extract was administered at the dose rate of 1g/kg body weight as aqueous solution.

Coccinia indica

The alcoholic extract of *Coccinia indica* leaves was procured from PLANTEX, Vijayawada, India. The extract was administered at the dose rate of 200mg/kg body weight as an aqueous solution.

Administration of plant extract

The plant individual extracts, and combined plant extract were administered orally to their respective groups by using clean rat gavaging needle attached to an appropriate disposable syringe every day for a period of 90 days.

Experimental design

The rats were divided into five different groups of twelve animals each based on body weight.

Care was taken to maintain the intra group weight variation to be less than 25g and inter-group weight variation by 30 g. Group-I was normal control, group-II- diabetic control, group-III was diabetic rats supplemented with alcoholic extract of of *Trigonella foenum graecum (*1g/kg b w)*,* group-IV was diabetic rats supplemented with alcoholic extract of *Coccinia indica* leaves (200mg/kg b w) and the group-V was diabetic rats supplemented with *Trigonella foenum graecum (*1g/kg b w) and *Coccinia indica* leaves extract (200mg/kg b w) .

Experimental induction of diabetes

Freshly prepared streptozotocin at the dose rate of 45 mg/kg intraperitonially was injected to the rats fasted for 16 hours (5). The normal control animals received citrate buffer alone. The diabetic state was confirmed by estimating the serum glucose level at 72 hours post STZ injection using Span Diagnostic kit with Semi-Automatic Biochemical Analyser (ARTOS, Bangalore). The animals that showed the serum glucose level above 200 mg/dl were considered diabetic and selected for the study. Rats of all the groups were observed clinically for the feed and water intake, general behaviour, alertness, urine output, diarrhoea and for the development of clinical symptoms and recorded.

Collection of serum samples

About 2 ml of blood from the retro-orbital plexus of the rats of all the groups was collected under light ether anaesthesia separately in clean test tubes at different time intervals of the study such as 3^{rd}, 15^{th}, 30^{th} , 45^{th} and 90^{th} day post STZ injection. The collected blood was allowed to clot for 30 min and then centrifuged at 3000 rpm for 10 min. The serum samples collected at various intervals were subjected to biochemical estimation of glucose, cholesterol, triglycerides, ALT and AST using Semi-Automatic biochemical analyzer with commercial biochemical kits. For the estimation of serum insulin concentrations, radio-immunoassay was performed using iodine labelled insulin assay kit (RIAK-1) obtained from Board of Radiation and Isotope Technology (BRIT), BARC, Mumbai, India.

Collection of tissue samples

To study the progressive effects of the treatments given to different groups, two rats from each group were sacrificed under light ether anaesthesia on Day 15 and 30, 6 animals on Day 45 and remaining two rats on Day 90 of the experiment. Sacrificed animals were subjected for detailed post mortem examination and gross change if any, were recorded. Further, representative tissue samples from pancreas and liver were collected in 10 % neutral buffered formalin (NBF) for the pathomorphological evaluation.

Statistical analysis

Statistical analysis was performed using the statistical software Graph pad Prism, version 5. Mean values and standard error of mean were calculated and all values were expressed as Mean (± SE). The data were analysed by Two Way ANOVA.

RESULTS AND DISCUSSION

The leaves of *Trigonella foenum graecum* are employed as an herbal medicine in many parts of the world for their cooling properties and its seeds for their carminative, tonic and aphrodisiac effects. Fenugreek seeds, which are described in the Greek and Latin Pharmacopoeias, are widely studied for their reputed antidiabetic, hypocholesterolaemic, antifertility and hypolipidemic effects The plant *Coccinia indica* has been used extensively in Ayurvedic and Unani practice in the Indian subcontinent since ancient time (6). It has long tuberous fleshy roots and smooth and green fruits. The fruits, leaves, roots are used for medicinal purpose by folklore like fresh juice of roots to treat diabetes, tincture of leaves to treat gonorrhoea and the paste of leaves to the skin diseases. Dried bark has been reported to be a good cathartic, leaves and stem as antispasmodic and expectorant and green fruits to cure sores on the tongue (7). The mean (± SE) serum glucose values in Groups III, IV and V treated with *Trigonella foenum graecum, Coccinia indica* and in their combination respectively revealed a progressive reduction from Day 3 onwards. The reduction was statistically significant ($P \leq 0.001$) when compared to diabetic control animals Group-II (Table.1). However, the

values were significantly higher compared to that of control group. The mean glucose values in group V though not statistically different was numerically better than all other groups. The results of the present study indicated that combined herbal treatment has better hypoglycaemic effect compared to individual herbal treatments (8,9). The hypoglycaemic effect of combined treatment could be attributed to the respective bioactive compounds like 4-hydroxy isoluecine and triterpene of herbal extracts. The mean serum cholesterol and triglyceride values in the present study in group V significantly improved compared to diabetic control from 15th day onwards and on 90th day the values were statistically did not differ between the groups and were comparable to normal control group. The mean values though significantly did not differ but the values were numerically lesser compared to individual herbal and combined treatment group. The significant reduction in the mean cholesterol and triglyceride values in group V indicated a synergistic effect and was found to be better in alleviating the increase in the cholesterol and triglyceride levels when a combination of herbal extracts was used (9) & (Tabale.2,3). The ALT and AST mean values in combined treatment groups (Groups III, IV and V) were significantly improved from 15th day onwards in comparison with diabetic control group. On 90th day the mean ALT and AST values between the groups III, IV and V were comparable and did not differ significantly. The mean values were comparable with that of control group and better compared to individual herbal extract and combined treatment groups. The mean value in Group V was numerically lesser compared to any other group. The results of the present study indicated that there was a synergistic effect between *Trigonella foenum graecum* and *Coccinia indica* was observed (9). Decreased ALT and AST values indicate directly the hepatoprotective effect of Trigonella and Coccinia plant extracts which could be due to the antioxidants of the plants in prevention of ROS and NO induced lipid peroxidation (Table 4,5). The mean serum insulin values in combined treatment group (Group V) was significantly higher on all the intervals of observation compared to diabetic control group. On 90th day of observation the mean insulin values were higher and comparable to that of control group (Table.6). The mean insulin values of groups V were significantly higher than individual plant extracts (III, IV) group. This clearly indicated that *Trigonella foenum graecum* and *Coccinia indica* have synergistic effect in improving the insuli n level with their respective bioactive components (9). As also evidenced in microscopical examination a significant increase in the number of beta cells with insulin production observed in combined treatment groups (V) was responsible for an improved insulin level comparable to that of control group in the present study (10). Gross pathological changes observed in various organs in treatment groups reduced progressively from Day 15 to Day 90 of the study. An appreciable improvement in the morphological appearance of the pancreas was observed wherein there was an increase in the mass of the pancreas in the treatment groups but was more significant in combined treatment group with plant extracts alone or in combination. Histopathologically, there was a progressive improvement in the architecture of pancreas and liver from Day 15 to 90 of the study. Pancreas of combined treatment groups showed better improvement in terms of architecture of endocrine and exocrine pancreas compared to individual treatment groups. The islets of Langerhans in combined treatment groups showed better cellularity as the treatment progressed. At 90th day, the combined treatment groups revealed almost normal architecture with bigger islets and more number of islets per lobule, with a morphological architecture similar to that of normal islets. There was an increase in cellularity with more number of cells with beta cell morphology with granular cytoplasm which were comparable with that of normal control. In addition an improvement in the exocrine portion of pancreas was also observed (11). The improvement in Group V was higher compared to individual plant extract groups. Between groups III and IV the improvement was better in group V which indicated a synergistic effect between plant extracts in alleviating hypoinsulinaemia in diabetes. This was well substantiated by the Gomori's stain which revealed better regeneration of β-cell

population compared to any other treatment groups indicating the synergistic action compared to individual treatment groups. Based on the observation it could be concluded that β-cell regeneration capacity of combined treatment groups is better than individual treatment groups of *Trigonella foenum graecum* and *Coccinia indica* and a synergism exists between plant extracts in elevation of diabetic effect(Fig.6&7).

Table 1
The Mean (± SE) serum glucose (mg/dL) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	89.25±2.86^{c}	86.00±4.21^{d}	84.20±5.67^{e}	85.87±4.22^{e}	77.50±3.50^{d}
Group II	458.58±40.52^{ab}	526.00±21.74^{a}	483.80±48.80^{a}	565.37±45.65^{a}	572.50±28.50^{a}
Group III	464.83±19.78^{ab}	409.59±18.14^{bc}	367.11±17.48^{bc}	290.02±18.87^{b}	237.75±19.05^{b}
Group IV	475.69±18..05^{ab}	405.95±17.18^{bc}	239.90±21.06^{bc}	273.97±19.50^{bc}	230.10±15.40^{bc}
Group V	493.32±11.19^{a}	443.41±10.83^{bc}	381.00±11.97^{b}	276.66±12.66^{bc}	215.40±3.20^{bc}

Table 2
The Mean (± SE) serum cholesterol (mg/dL) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	80.88±3.58^{b}	81.15±3.63^{c}	84.65±5.52^{c}	81.68±3.07^{c}	79.70±2.39^{b}
Group II	136.21±7.61^{a}	155.96±5.28^{a}	178.29±6.69^{a}	194.67±6.64^{a}	217.80±12.40^{a}
Group III	143.40±8.19^{a}	121.30±5.09^{b}	114.40±3.92^{b}	108.72±3.73^{b}	105.40±3.10^{b}
Group IV	133.43±4.81^{a}	128.77±3.77^{b}	120.37±3.16^{b}	116.80±3.20^{b}	107.95±4.75^{b}
Group V	136.53±6.35^{a}	128.26±3.35^{b}	115.49±3.47^{b}	106.03±4.48^{b}	102.30±2.90^{b}

Table 3
The Mean (± SE) serum triglyceride (mg/dL) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	95.95±0.99^{c}	96.70±1.07^{d}	96.03±1.13^{f}	96.20±1.23^{g}	97.95±1.45^{d}
Group II	207.18±1.34^{b}	249.47±2.13^{a}	283.59±2.67^{a}	341.60±2.39^{a}	364.20±0.10^{a}
Group III	209.26±1.65^{b}	197.09±0.79^{b}	180.41±1.37^{b}	159.53±1.50^{b}	149.55±2.85^{bc}
Group IV	215.27±1.05^{a}	197.70±0.64^{b}	181.18±1.47^{b}	159.17±1.14^{b}	155.40±0.90^{b}
Group V	216.18±1.36^{a}	191.71±0.97^{b}	169.45±1.46^{c}	144.80±1.36^{c}	125.80±2.60^{bcd}

Table 4
The Mean (± SE) serum alanine aminotransferase (ALT) (IU/L) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	53.07±1.64^{c}	51.81±1.70^{b}	51.52±1.90^{c}	52.61±1.83^{c}	56.40±2.10^{e}
Group II	130.33±7.34^{b}	163.02±8.74^{a}	206.58±13.60^{a}	228.80±21.51^{a}	228.05±6.44^{a}
Group III	137.96±2.00^{ab}	118.70±1.24^{bc}	107.43±2.17^{b}	101.17±2.16^{b}	89.65±1.84^{bc}
Group IV	139.47±1.59^{ab}	126.64±8.57^{b}	109.15±2.46^{b}	100.60±2.82^{bi}	94.45±1.95^{b}
Group V	141.85±1.93^{a}	106.89±1.76^{c}	94.95±1.90^{b}	86.36±1.53^{bc}	76.39±1.79^{dc}

Table 5

The Mean (± SE) serum aspartate aminotransferase (AST) (IU/L) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	67.06±2.24^{b}	67.91±2.60^{c}	67.53±1.80^{c}	69.78±2.22^{dc}	71.70±3.10^{dc}
Group II	170.10±10.20^{a}	208.01±9.23^{a}	247.82±21.23^{ac}	286.87±23.35^{a}	301.15±5.35^{a}
Group III	180.05±2.31^{a}	158.05±2.56^{b}	137.83±1.37^{b}	104.67±2.47^{bc}	88.50±3.80^{bc}
Group IV	182.17±2.87^{a}	160.32±2.15^{b}	136.56±1.68^{b}	125.27±2.48^{b}	90.73±2.48^{b}
Group V	177.25±3.19^{a}	150.11±2.02^{b}	124.32±1.40^{b}	93.91±2.05^{bcd}	83.75±1.95^{bcd}

Table 6

The Mean (± SE) serum insulin (µU/ml) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	53.85±3.21^{a}	52.85±3.16^{a}	54.15±2.78^{a}	52.08±5.35^{a}	53.95±4.51^{a}
Group II	17.58±1.70^{b}	15.66±0.87^{d}	13.61±1.37^{c}	14.05±1.81^{d}	12.76±0.94^{c}
Group III	14.79±1.03^{b}	17.43±1.83^{cd}	24.38±1.82^{b}	29.75±2.92^{bc}	37.47±8.87^{ab}
Group IV	17.96±1.36^{b}	19.87±1.99^{bc}	21.76±2.60^{b}	25.04±2.53^{c}	32.03±9.15^{b}
Group V	18.76±1.06^{b}	21.03±1.24^{bc}	27.63±1.093^{b}	33.11±1.83^{b}	46.09±5.90^{ab}

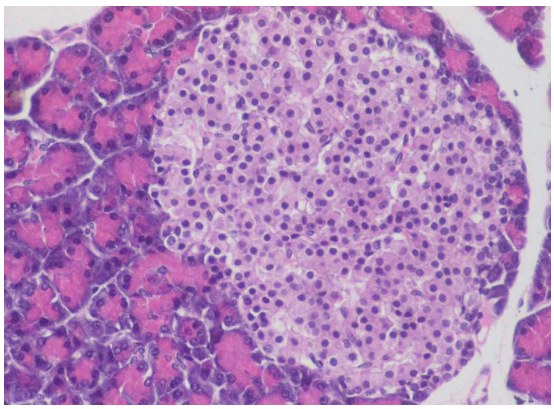

Figure 1

Section of pancreas of normal control showing a normal islet with round to oval shape and compact arrangement of beta cells at the centre and alpha cells at the periphery. H&E X 200

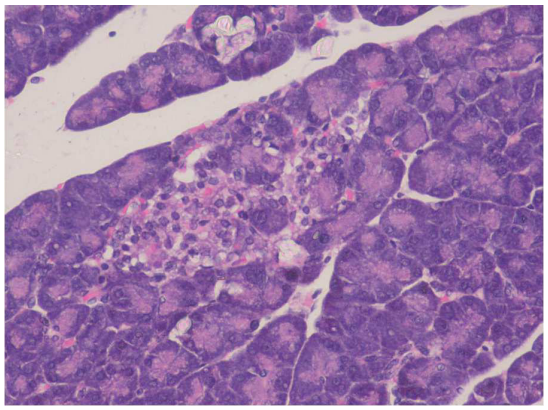

Figure 2

Pancreas of diabetic control animal showing loss of normal architecture, degeneration and necrosis of islet cells.H&E X 200

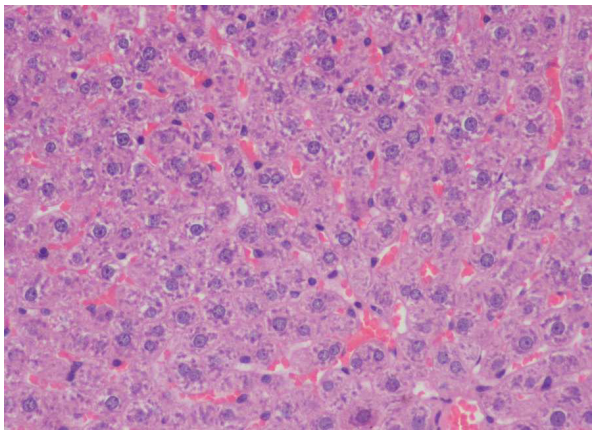

Figure 3
Section of liver from diabetic control animal showing highly swollen hepatocytes with cytoplasmic vacuolation on Day 15.H&E X 200

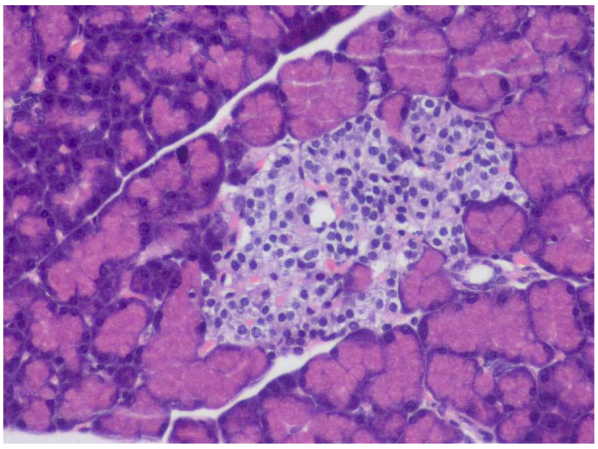

Figure 4
Pancreas from a diabetic rat treated with Trigonella foenum graecum on 45th day post-treatment showing improvement in the architecture of islet with hypercellularity however with occasional swollen and highly vacuolated beta cells. H&E X 200

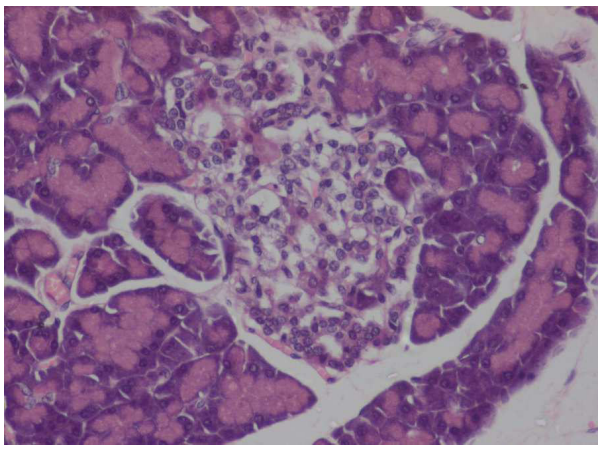

Figure 5
On Day 45, diabetic rat treated with Coccinia indica showing improvement in the overall architecture of islet of Langerhans. Note the arrangement of alpha and beta cells, granularity as well as amount of cytoplasm. H&E X 200

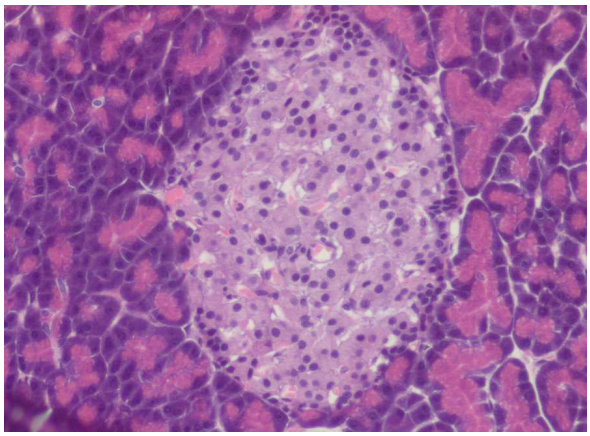

Figure 6
Section of pancreas on Day 90 from a diabetic rat treated with combination of Trigonella foenum graecum and Coccinia indica showing well formed large islet of Langerhans. Note more number of cells with beta cells morphology. H&E X 200

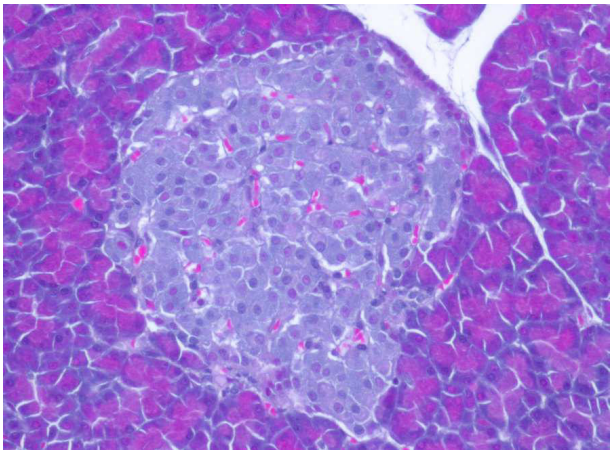

Figure 7
Pancreas from a diabetic rat treated with combination of Trigonella foenum graecum and Coccinia indica showing increase in number of beta cells on Day 90 of the study. Note distribution of beta cells at the centre. Gomori's X 200

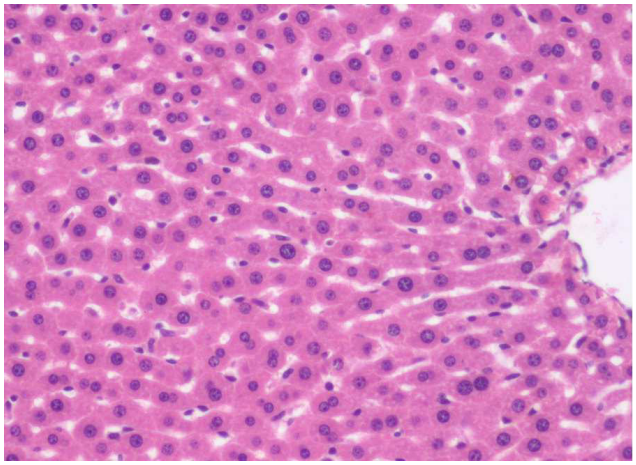

Figure 8
Normal appearance of liver from a diabetic rat treated with the combination of Trigonella foenum graecum and Coccinia indica on 90th day of treatment H&E X 200

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