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PATHOMORPHOLOGICAL EVALUATION OF ANTIDIABETIC EFFICACY OF *TRIGONELLA FOENUM GRAECUM* SEED EXTRACT IN STREPTOZOTOCIN INDUCED DIABETES IN RATS.

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Abstract***:*** *The alcoholic extract of Trigonella foenum graecum seed extract (1 gm/kg b w / day) and Trigonella foenum graecum seed extract with glibenclamide half dose were tested for its pathomorphological effects in streptozotocin induced diabetes in rats. Streptozotocin produced diabetes effectively in all the animals at 45 mg/kg in citrate buffer intraperitonially with a significant increase in beta cell whereas, Trigonella foenum graecum seed extract alone and in combination with glibenclamide half dose treated rats showed significant improvement in structure of beta cell its morphology. Trigonella foenum graecum seed extract alone and in with glibenclamide half dose also alleviated the damage caused by STZ morphologically in beta cells of islets of Langerhans and hepatocytes. The efficacy of Trigonella foenum graecum in rats individually as well as in combination with the half dose of glibenclamide was evaluated and observed synergistic effect between Trigonella and glibenclamide.*

Key words: Diabetes, *Trigonella foenum graecum*, streptozotocin,

INTRODUCTION

Diabetes mellitus (DM) 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 [1]. Oral hypoglycemic agents such as biguanides, sulphonylureas and thiozolidinediones or insulin therapy are the mainstay of treatment of diabetes and are effectively used in controlling hyperglycemia, but they fail to significantly alter the course of complications and side effects caused by them [2]. The Trigonella plant constituent phytochemicals such as (4-hydroxyisoluecine, flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanin, tannin) which can help in either repair or regeneration of islets of Langerhans in diabetes [3]. To establish a systematic scientific support over the effect of *Trigonella foenum graecum* on the diabetic pathology, the study was designed and the results were compared with glibenclamide, a sulphonylurea antidiabetic drug.

MATERIAL AND METHODS:

Animals: Genetically 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 extract of *Trigonella foenum graecum* seed was procured from PLANTEX, Vijayawada, India. The plant was identified by HPTLC finger printing and assayed by Gravimetric method. The extract was administered at the dose rate of 1g/kg body weight as aqueous solution.

Glibenclamide solution

Glibenclamide (Daonil®, 5 mg) an oral hypoglycaemic drug was administered orally at a dose of 600 µg / kg (Babu *et al*., 2003)

Administration of plant extract and glibenclamide

The plant extract, glibenclamide and combined plant extract with glibenclamide 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 animals treated with glibenclamide (600 µg/kg b w), group-IV was diabetic rats supplemented with alcoholic extract of *Trigonella foenum graecum* seeds (1g/kg b w) and the group-V was diabetic rats supplemented with alcoholic extract of *Trigonella foenum graecum* seeds(1g/kg b w) and glibenclamide half dose (300 µg/kg b w).

Experimental induction of diabetes

Freshly prepared streptozotocin at the dose 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 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, liver, kidney, lungs, heart, intestine, brain and muscle 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

In the present study the efficacy of *Trigonella foenum graecum* was evaluated in STZ induced diabetes in rats individually as well as in combination with the half dose of glibenclamide as diabetic patients very commonly consumes herb with antidiabetic effect along with antidiabetic drugs. The prime purpose of evaluation of combined effect was to know whether there is any synergistic or antagonistic effect between Trigonella and glibenclamide and also to check whether Trigonella could be used as an adjunct in diabetic therapy with oral antidiabetic agents. Hence the results of Group IV and V with regard to pathomorphological effects are discussed together.

Fenugreek (*Trigonella foenum graecum*) is an annual crop from the family Fabaceae, extensively 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 and hence, it is used in the prevention and treatment of diabetes and coronary heart diseases.

Fenugreek consists of three important chemical constituents with medicinal value which are steroidal sapogenin, galactomannans and isoleucine. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products. Fenugreeks seeds are also are rich in saponins, including diosgenin, gitogenin and tigogenin ([6-7].

The beneficial effect of fenugreek could be attributed to several bioactive compounds present in it. Amino acid 4-hydroxyisoleucine (4-OH-Ile) of fenugreek has been shown to have insulinotropic activity and to stimulate insulin secretion [8-10]). It was also shown to exert hypoglycaemic effect by stimulating glucose dependent insulin secretion from beta cells as well as by inhibiting the activities of alpha amylase and sucrose [11].

In the group V rats with combined treatment, a better hypoglycaemic effect was observed compared to Trigonella alone treatment which indicated that Trigonella and glibenclamide half dose have a synergistic effect. However, the hypoglycaemic effect was not on par with that of the glibenclamide complete dose. The possible reason for a reduced hypoglycaemic effect in combination group compared to glibenclamide alone could be the low dose of glibenclamide used which could have failed to elicit the desired antidiabetic effect in the present study.

The treatment with Trigonella alone (Group IV) in diabetic rats increase in lipid profile may be a result of increased breakdown of lipids and mobilization of free fatty acids from the peripheral deposits. The controlled mobilization of serum triglycerides, cholesterol and phospholipids by *T. foenum graecum* in diabetic animals could be due to controlled tissue metabolism and improvement in the levels of insulin secretion and action.

[13-14] who attributed the improved insulin level to regeneration or repair of damaged beta cells. Attainment of normal architecture of islets of Langerhans in the present study, a finding observed microscopically also strengthens the positive effect of *T. foenum graecum* on insulin levels.

The 4-hydroxyisoleucine of fenugreek is reported to be insulinotropic and powerfully stimulate insulin secretion at all levels of cellular organization and increase the number of insulin receptor sites to burn cellular glucose at high fibre diet [15-16].

Pathology

In both the groups (IV and V) there was a gradual decrease in the severity of clinical signs such as polyuria, polydypsia, polyphagia and weight loss in diabetic animals treated with the alcoholic extract of *T.foenum graecum* alone as well as in combination with glibenclamide. Earlier workers also have reported the similar kind of improvement in the condition could be attributed to the hypoglycaemic, hypolipidaemic, hepatoprotective and insulin secretagogue effects of the plant extract in diabetic subjects [Plate 8].

Gross pathological changes observed in the Groups IV and V reduced progressively with advancement in time on treatment with *T.foenum graecum* alone as well in combination with glibenclamide half dose.

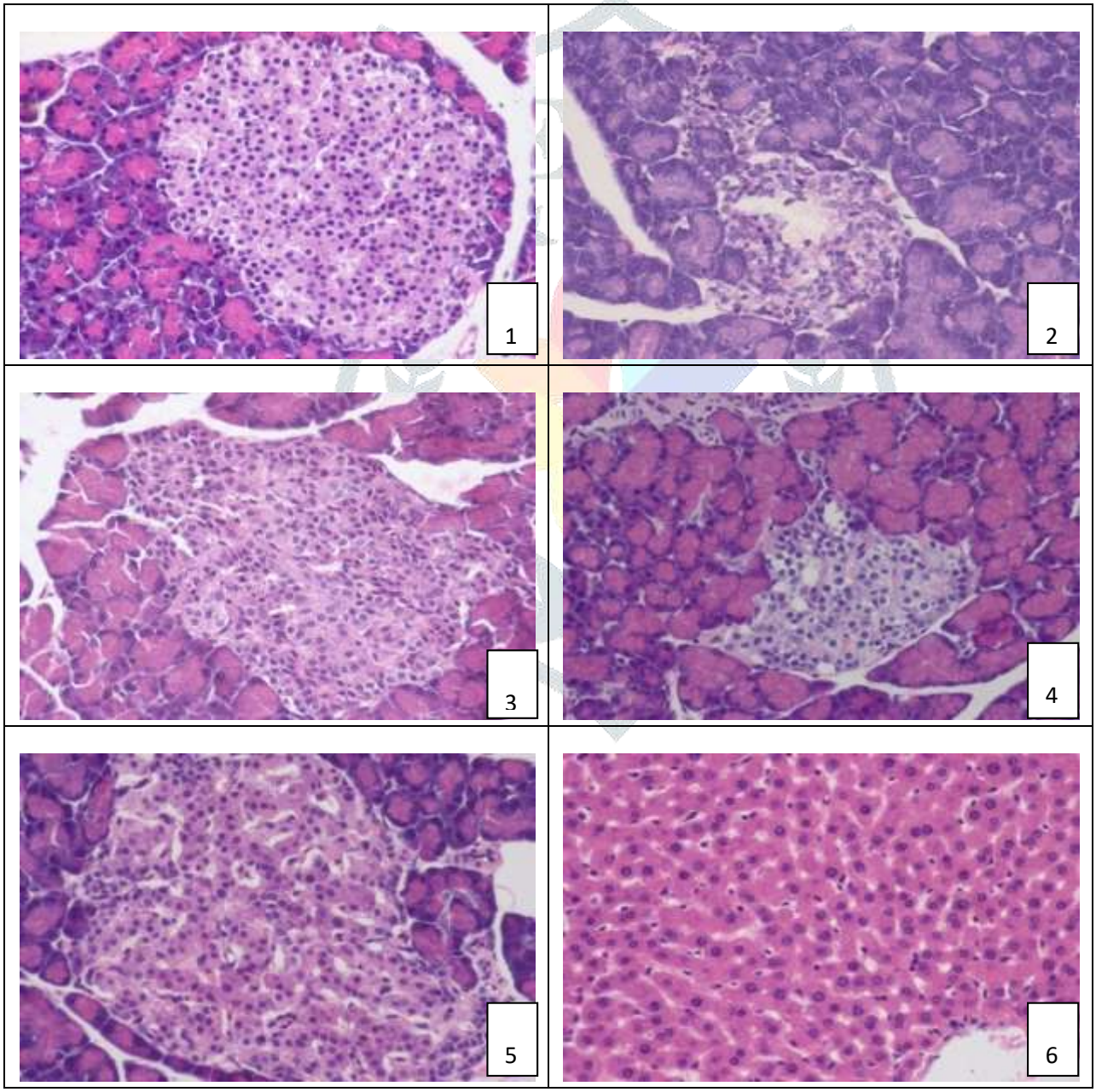
Histopathologically, in pancreas there was a progressive reconstruction of normal architecture of islets from Day 15 to Day 90 post treatment in *T.foenum graecum* alone as well as in combination with glibenclamide half dose in the present study[Plate 1-2]. In comparison, the improvement in the pancreatic architecture was better in combination group suggesting a synergistic effect between Trigonella and glibenclamide in modifying the tissue architecture[Plate 3,4].

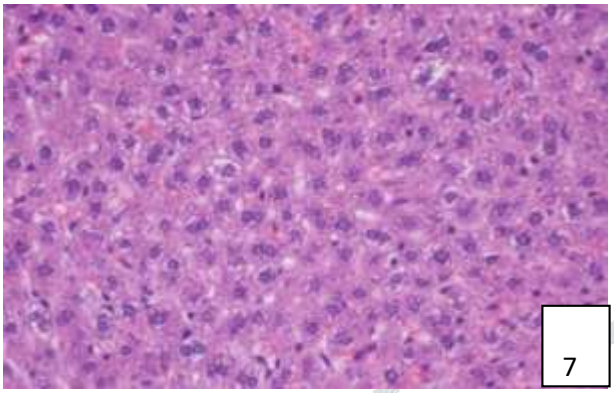
During 15^{th} day observation, there was persistence of STZ induced damage in the form of highly vacuolated to necrotic beta cells with affection of exocrine pancreatic component. However, on Day 30^{th} and onwards the improvement in endocrine and exocrine component was observed in the form of increase in the number of islets, increase in the number both beta and alpha cells in the islets and improvement in the shape and size of the islets[Plate 5-6]. They attributed the restoration of islets to regeneration and repair of damaged beta cells by the stimulating effect of 4-hydroxyisoleucine of *T.foenum graecum* extract.

Microscopically in the present study, the liver revealed progressive improvement in the architecture from STZ induced liver damage from 15^{th} day onwards in both *Trigonella foenum graecum* alone and combination group-V[7-8]. The hepatoprotective effect of *Trigonella foenum graecum* has been attributed to the presence of tannin and flavonoids by [12].

Conclusion

Diabetes mellitus can be effectively induced by using streptozotocin at the dose rate of 45 mg/kg intraperitonially in laboratory rats. *Trigonella foenum graecum* along with half dose of glibenclamide has a synergistic effect in increase in the improvement in the number of beta cells in bringing about regeneration of beta cells and in increasing the insulin production and secretion. Lastly the present study high lights that the indigenous medicinal plants can be used successfully as an alternative treatment in the management of diabetes with or without antidiabetic drugs.





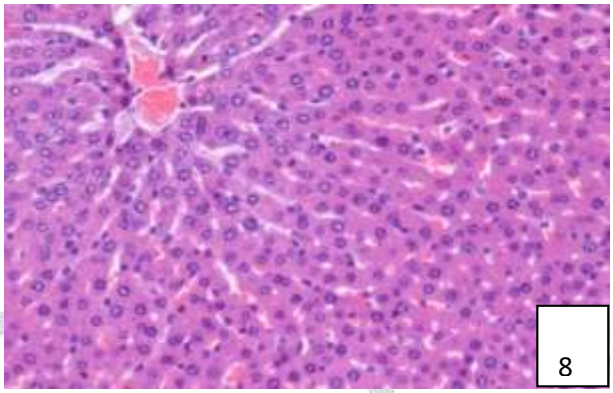


Plate 1 Section of pancreas of normal control showing a normal islet with round to oval shape, compact arrangement of beta cells at the centre and alpha cells at the periphery.

H&E X 200

Plate 2 Pancreas of diabetic animal showing loss of normal architecture, hypertropic and vacuolated acinar cells with vacuolated and degenerating Islet cells on 15th day of the experiment.

H&E X 200

Plate 3 Section of pancreas from glibenclamide treated animal showing compact arrangement of islet with increase in cellularity on Day 90 of the study.

H&E X 200

Plate 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 occational swollen and highly vacuolated beta cells were observed.

H&E X 200

Plate 5 Pancreas from a diabetic rat treated with combination of *Trigonella foenum graecum* and glibenclamide showing large and well formed islet on Day 90 of the treatment.

H&E X 200

Plate 6 Section of liver from control rat on 45th day showing central vein and well formed hepatic cords with normal appearing hepatocytes.

H&E X 100

Plate 7 Section of liver from diabetic control animal showing highly swollen hepatocytes with cytoplasmic vacuolation on Day 15. H&E X 200

Plate 8 Section of liver from a diabetic rat treated with the combination of *Trigonella foenum graecum* and glibenclamide showing almost normal architecture on 90th Day of treatment.

H&E X 200

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