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Evaluation of Hypoglycemic and antihyperglycemic effects of alcoholic extract of *Chonemorpha fragrans* root in normal and alloxan induced diabetic rats

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ABSTRACT

In light of traditional claim of *Chonemorpha fragrans* in the treatment of diabetes were carryout evaluate its effect on rats. Alcoholic extract of *Chonemorpha fragrans* root (CF.alc.extract) administered orally at dose 100 mg/kg and 200mg/kg in different condition such as normal, glucose over loaded in normal rats, and alloxan induced diabetic rats. At dose 200 mg/kg per oral (p.o.) was produce significantly reduce blood glucose level in fasted normal rat and against glucose over loaded rats and alloxan induced diabetic rats at single dose as well as twelve day treatment. Histopathology studies on pancreas alloxan induced diabetic rats inflammatory changes were detected in pancreatic islets results from selectively destroy of insulin producing β-cells. These changes are dose-dependently inhibited by CF.alc.extract and gliclazide

KEYWORDS: Diabetes, Hypoglycemic, *Chonemorpha fragrans* and alloxan.

INTRODUCTION

From ancient time diabetes mellitus was known to mankind and yet with the tremendous scientific advances witnessed in this century medical science cannot claim that it known all that needs to be known about this disease, including its management (1). Prevalence of diabetes in adults worldwide was estimated to be 4.0 % in 1995 and will to rise 5.4 % by the year 2025. It is higher in developed than in developing countries. The number of adults with diabetes in world will rise from 135 million in 1995 to 300 million in the 2025. The major part of this numerical increase will occur in developing countries (2, 3). At present, the oral anti-diabetic agent belongs to sulphonylureas, biagunides α- glucosidase inhibitors and thiazolidinediones and meglitinide derivatives. The major limitations of these drugs are their side effects. The high cost of modern treatment of diabetes indicates a great need for the development of alternative strategies for prevention and treatment of diabetes (4). Nature always stands as golden mark to exemplify the outstanding phenomenon of symbiosis. The plants are indispensable to man for his life. Nature has provided a complete storehouse of remedies to cure all ailments of mankind (5). Over 50 % of all modern clinical drugs are of natural products. Origin and natural products play an important role in drug development programs in the pharmaceutical industry. There has been a revival of interest in herbal medicines. This is due to the increased awareness of the limited products to control major disease and the need to discover new molecular structures as lead compounds from plant kingdom. Plants are the basic source of

knowledge of modern medicine (6). The traditional knowledge derived empirically should be supported by scientific testing when traditional diabetic remedies have been tested for antidiabetic activity. Plants with a traditional indication for diabetes are more likely than randomly selected plants to show activity in standard hypoglycemic assays (7). *Chonemorpha fragrans* (Apocynaceae) vernacularly called garbedero is a perennial lactiferous clambing shrub grow on bushesh and hedges. It is distributed on southern part of India. Although traditional liteture its claim as antidiabetic (8, 9) and it is phylogenetic relationship with *Holarrhena antidysentrica* (Apocynaceae) (10, 11) antidiabetic activity reported in reputed journal. Therefore the present study was reports the hypoglycemic and antihyperglycemic as effect of alcoholic extract of root *Chonemorpha fragrans in* normal and alloxan induced diabetic rats.

MATERIALS AND METHODS

CHEMICALS

Alloxon monohydrate obtained from S.D. fine-Chem. Ltd., Mumbai. And solvent for extraction obtained from Otto chemical Biochemica reagents, Mumbai.

EXTRACTION

Yellowish brown colored root of *Chonemorpha fragrans* collected from Saptagiri herbs Malappuram district Kerala during august 2007 and were authentificated by the authority by Dept. of Agriculture, Govt., of Kerala. The roots were dried in shade. Then the dried roots were powdered mechanically to get the coarse powder. About 1kg of dry powder was extracted with petroleum ether at 50°–60°C by hot percolation using a soxhlet apparatus. The extraction was continued for 72 hour, for defatting purpose. The marc left after the petroleum extract was taken and subsequently extracted with alcohol 95 % at (60°–70°C) up to 72 hours in soxhlet apparatus. A brown residue was obtained after concentrating the alcoholic extract (12 g).

Preliminary Phytochemical Screening

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects (12, 13).

ANIMALS

Healthy adult Wistar albino rats of either sex weighing about 180–220 g respectively were used for study. The animals were housed in polypropylene cages, maintained under standard condition (12 h Light /12 h dark cycle 25 ± 3° C: 35 % to 60 % humidity) water *ad libitum*. The study was approved by institutional animal ethics committee of Ultra College of pharmacy. Madurai, India. (UCP/IAEC/2007/011)

ACUTE ORAL TOXICITY STUDY (14)

The acute oral toxicity study was done according to OECD 423 guidelines. Administration of the stepwise doses of alcoholic extracts of *Chonemorpha fragrans* from 5 mg/kg up to the dose 3000 mg/kg cause no considerable signs of toxicity in the tested animals, while dose of 4000 mg/kg resulted in 100 % mortality consequently one tenth and one twentieth of the half dose of the lethal dose were selected as the levels for examination of antidiabetic.

PREPARATION OF DIABETIC RATS

Rats were induced diabetes by single intraperitoneal injection of alloxan monohydrate dissolved in sterile normal saline at a dose 120 mg/kg, after 18h fasting to induce hyperglycemia. After 1 hour alloxan administration the animals were fed on standard pellets and water *ad libitum*. The blood glucose level was monitored after alloxanization in blood sample collected by tail tipping method using glucometer. After 72 h, the rats having blood glucose level above 150 mg/dl of blood were selected for the study and divided in to four Groups, each of six rats.

BLOOD GLUCOSE ESTIMATION

Before testing the blood glucose level, the rate were kept fasting overnight at least 12 hrs, but were allowed free access to water. Blood samples for glucose determination were obtained from the tail tips of the fasting animals. The Blood glucose level was determined by using glucometer blood glucose test strips (Advance micro-dra). The animal was anesthetized with ether anesthesia and the blood was collected from the tail vein by making a small incision on the tail tip and a drop of blood was placed an glucose test strip and was inserted in to glucometer. Blood glucose test results are displayed on the glucometer as milligrams of glucose per deciliter of blood (mg/dl) (15).

HISTOPATHOLOGICAL STUDIES

At the end of the study, all the surviving animals of the respective groups were sacrificed by an overdose of ether anesthesia. After exsanguinations of the animals pancreas were removed immediately and washed with ice-cool saline. The pancreas was fixed in 10 % of neutral formalin. The sections of 3–5 mm thickness were stained

with hematoxylin and eosin for histopathological studies (16).

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA) followed by Dunnet's t-test, was carried out and $P<0.05$ was considered as significant.

PHARMACOLOGICAL INVESTIGATION

Effect of alcoholic extract of Chonemorpha fragrans on Oral glucose tolerance (17)

To perform glucose tolerance test, overnight fasted rats were used. Rats were divided into four groups, each of six animals. Group I was kept as control which received 1 ml of 2.5 % Tween 80 p.o. and Group IV received gliclazide (25 mg/kg) p.o. suspended in vehicle. A dose 100 mg/kg and 200 mg/kg of alcoholic extract of *Chonemorpha fragrans* suspended in vehicle was administered orally, to the Groups II & III respectively. All the animals were given glucose (3 g/kg p.o.) 30 min after dosing. Blood glucose levels measured by using glucometer 0 min and at 30 min, 90 and 150 after drug administration.

Effect of alcoholic extract of Chonemorpha fragrans on blood glucose level in normal fasted rats (18)

Fasted rats were divided into four groups of six rats of each. Group I received only vehicle (Tween 80 in distilled water 2.5 % v/v) p.o. Group IV received Gliclazide (25 mg/kg p.o) Group II & III received C.F. alc. extract suspended in vehicle was administered 100 mg/kg, 200 mg/kg to the animals.

Blood glucose levels were measured by using glucometer just prior to and at 1, 2, and 3 h after dosing.

Effect of alcoholic extract of Chonemorpha fragrans on alloxan induced diabetes in rats. (19, 20)

Rats were induced diabetes by single intraperitoneal injection of alloxan monohydrate dissolved in sterile normal saline at a dose 120 mg/kg, after 18h fasting to induce hyperglycemia. After 1 hour alloxan administration the animals were fed on standard pellets and water *ad libitum*. The blood glucose level was monitored after alloxanization in blood sample collected by tail tipping method using glucometer. After 72 h, the rats having blood glucose level above 150 mg/dl of blood were selected for the study and divided in to four Groups, each of six rats. The extract 100 & 200 mg/kg was administered orally to Group II & III respectively. The standard drug gliclazide (25 mg/kg) and vehicle also administered orally to animals of Group IV. The blood glucose level was monitored for 1, 3, 6, & 12h of administration of single dose of extract. The above mentioned treatment schedule was followed for the respective group of animals for 12 days. Blood samples were collected from over night fasted animals in morning one hour after drug administration on 1, 3, 6 and 12 days to estimate the blood glucose level using electronic glucometer.

RESULTS AND DISCUSSION

Alcoholic extract of *Chonemorpha fragrans* roots (CF. alc.extract) was prepared by continuous hot extraction method using soxhlet apparatus and the yield was 1.2 % w/w. The color of the extract was yellowish brown. As the extract was observed to be deliquescent and showed the presence of various phytochemical constituents like alkaloids, tannins, saponins, phytosterol

The CF. alc. extract at 200 mg/kg and standard drug gliclazide (25 mg/kg) p.o. produced significant ($p < 0.01$) hypoglycemic effect in the fasted normal rat after 2 hour and 3 hour of oral administration, when compared with normal group. The CF. alc extract at 100 mg/kg p.o. dose of extract did not exhibit any significant hypoglycemic effect. (Table No-1) Present study produces direct evidence of the stimulation of insulin secretion by CF. alc. extract. The activity was comparable to that of the standard drug which reinforces the insulin secretogogue action of the extract. The CF. alc. extract reduced the blood glucose level, (Hyperglycemia due to glucose load 3 g/kg p.o.) significantly by 200 mg/kg extract and gliclazide (25 mg/ kg) after 60 min of oral administration, when compared to control group. (Tablel-2) These results may be obtained due to promotion of disposal of glucose by enhancing translocation of glucose transporter to the plasma membrane as the sulfonylurea acting in similar manner. Alcoholic extract of *Chonemorpha fragrans* reduced elevated blood glucose level in dose dependent fashion in alloxan induced diabetic rats. At single dose as well as during testing period of 12 days. Similar results also have been found with gliclazide. (Table 3 & 4)The alloxan induced diabetic rats inflammatory changes were detected in pancreatic islets results from selectively destroy of insulin producing β-cells. These changes are dose-dependently inhibited by CF.alc. extract and gliclazide. The anti-diabetic activity of numerous herbal extract observed based on these histopathology of the pancreas. The histopathological investigation along with the biochemical evaluation suggests the possibility of the islets regeneration and recovery of normal carbohydrate metabolism in treated group.

In conclusion, alcoholic extract of *Chonemorpha fragrans* at 200 mg/kg dose was found antihyperglycemic effect in

Table–1: Effect of *Chonemorpha Fragrans* on oral glucose tolerance test on normal rats.

Group	Treatment	Blood Glucose (mg / dl)				
		0 min	30 min	60 min	90 min	120 min
I	Vehicle	88.5 ± 3.10	144.5 ± 2.83	136.1 ± 1.95	126.3 ± 1.99	122.0 ± 2.55
II	CF.Alc Extract 100 mg/kg	87.0 ± 2.43	135.8 ± 1.86	132.2 ± 1.90	124.8 ± 2.05	116.5 ± 1.74
III	CF.Alc Extract 200 mg/kg	86.17 ± 4.36	128.6 ± 3.56*	117.5 ± 2.39**	104.2 ± 2.84**	98.5 ± 2.23**
IV	Gliclazide 25 mg/kg	87.17 ± 3.02	115.5 ± 3.91**	103.0 ± 2.46**	94.6 ± 2.84**	85.5 ± 4.05**

Value are given as mean ± SEM (n = 6).

*Values are statistically significant compared to normal Group at $p < 0.05$, $p < 0.01$ respectively.

**Values are statistically significant compared to normal Group at $p < 0.05$, $p < 0.01$ respectively.

Table–2 Effect of *Chonemorpha Fragrans* on blood gluose level in normal fasted rats.

Group	Treatment	Blood Glucose (mg / dl)			
		0 Hours	1 Hours	2 Hours	3 Hours
I	Vehicle	75.0 ± 3.80	80.2 ± 3.18	78.8 ± 2.93	75.8 ± 3.16
II	CF.Alc Extract 100 mg/kg	80.3 ± 3.94	79.5 ± 2.501	77.8 ± 3.21	76.1 ± 2.15
III	CF.Alc Extract 200 mg/kg	75.5 ± 2.20	77.5 ± 2.07	60. 6 ±1.93*	58.5 ±1.05*
IV	Gliclazide 25 mg/kg	75.8 ± 2.89	63.1 ± 1.15*	54.2 ± 2.07*	48.6 ± 1.86*

Value are given as mean ± SEM (n = 6).

*Values are statistically significant compared to normal Group at $p < 0.01$ respectively.

Table–3. Effect of Chonemorpha Fragrans on blood glucose level of alloxan induce diabetic rats.

Group	Treatment	Blood Glucose (mg / dl)				
		0 Hours	1 Hours	3 Hours	6 Hours	12 Hours
I	Diabetic Control	242.± 51.7	245.0 ± 4.30	247.7 ± 7.48	240.5 ± 7.72	242.0 ± 11.82
II	CF.Alc Extract 100 mg/kg	225 ± 6.36	193.5 ± 8.99* (21.02)	186.3 ± 8.9** (24.78)	196.2 ± 7.15* (18.41)	212.7± 6.68 (12.10)
III	CF.Alc Extract 200 mg/kg	227.5 ± 8.9	186.0 ± 7.26** (23.95)	161.0 ± 6.86** (35.00)	185.0 ± 12.92** (23.04)	209.2 ± 4.64 (13.53)
IV	Gliclazide 25 mg/kg	229.5 ± 7.3	160.2 ± 2.39** (34.61)	136.0 ± 2.27** (45.09)	171.2 ± 6.25** (28.81)	198.5 ± 5.10** (17.97)

Value are given as mean ± SEM (n = 6).

*Values are statistically significant compared to control Group at $p < 0.05$, $p < 0.01$ respectively.

**Values are statistically significant compared to control Group at $p < 0.05$, $p < 0.01$ respectively.

Value expressed in bracket indicates percentage reduction in blood glucose levels compared to Diabetic Control

Table–4 Effect of *Chonemorpha Fragrans* on blood glucose level of alloxan induced diabetic rats.

Group	Treatment	Blood Glucose (mg / dl)				
		0 Day	1 Day	3 Day	6 Day	12 Day
I	Diabetic Control	242.5 ± 1.7	240.6 ± 6.39	235.7 ± 7.37	224.7 ± 5.17	220.0 ± 8.91
II	CF.Alc Extract 100 mg/kg	225.0 ± 6.36	196.2 ± 7.15* (18.41)	182.5 ± 3.01** (22.57)	166.2 ± 9.89** (26.03)	134.0 ± 4.39** (39.09)
III	CF.Alc Extract 200 mg/kg	227.5 ± 8.99	185.2 ± 10.9** (23.16)	168.2 ± 1.79** (28.63)	136.2 ± 3.59** (39.38)	102.0 ± 2.27** (53.63)
IV	Gliclazide 25 mg/kg	229.5 ± 7.38	171.2 ± 6.22** (28.81)	152.5 ± 6.14** (35.29)	100.2 ± 2.49** (55.40)	88.7 ± 3.22** (59.68)

Value are given as mean ± SEM (n = 6).

*Values are statistically significant compared to control Group at $p < 0.05$, $p < 0.01$ respectively.

**Values are statistically significant compared to control Group at $p < 0.05$, $p < 0.01$ respectively.

Value expressed in bracket indicates percentage reduction in blood glucose levels compared to Diabetic Control

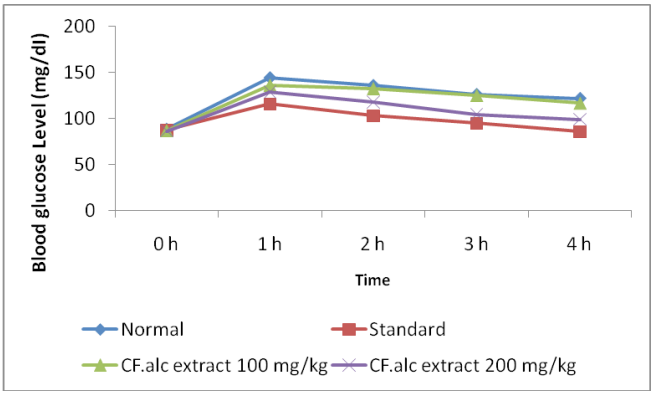

Figure 1: Effect of *Chonemorpha Fragrans* on oral glucose tolerance test on normal rats.

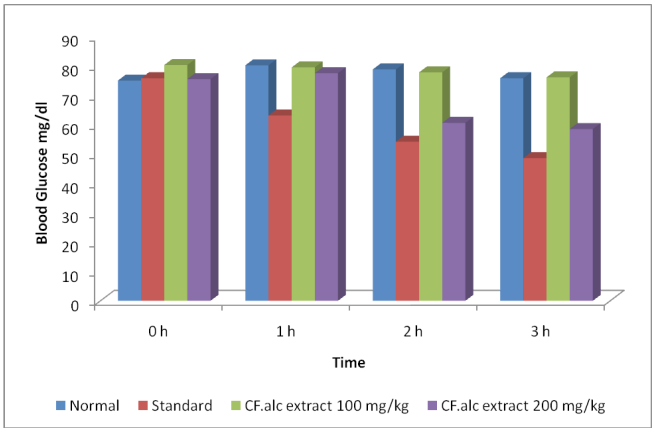

Figure 2: Effect of *Chonemorpha Fragrans* on blood gluose level in normal fasted rats

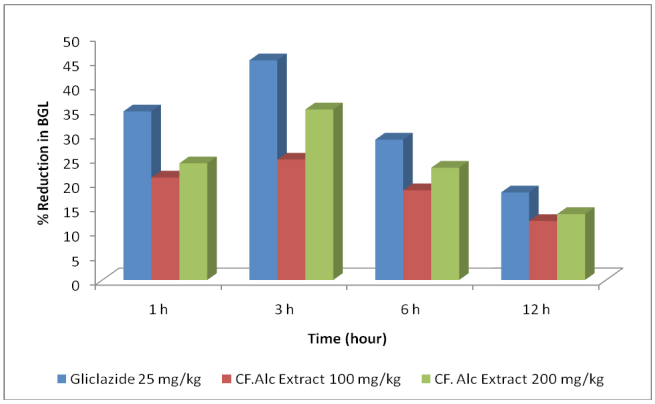

Figure 3: Effect of *Chonemorpha fragrans* on blood glucose level of alloxan induce diabetic rats

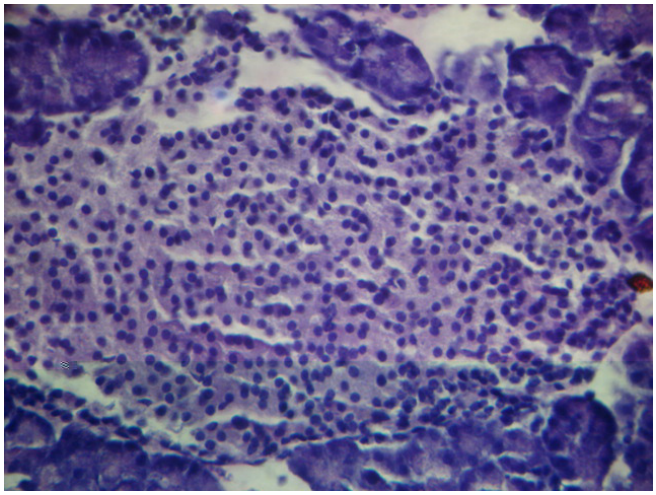

Figure 6: CF.ALC 100 mg/kg 45 x

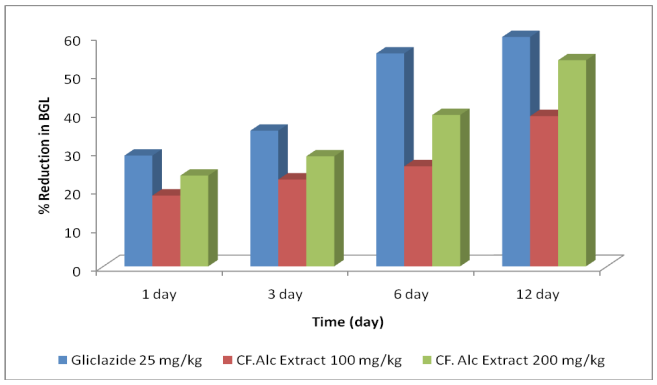

Figure 4: Effect of *Chonemorpha fragrans* on blood glucose level of alloxan induced diabetic rats

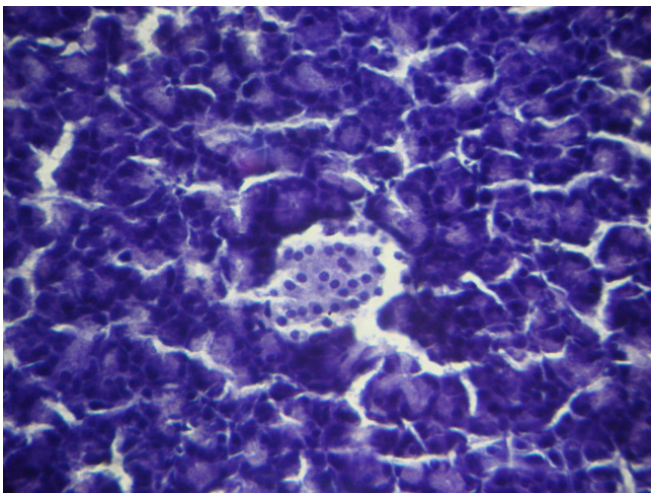

Figure7: CF.ALC 200 mg/kg 45 x

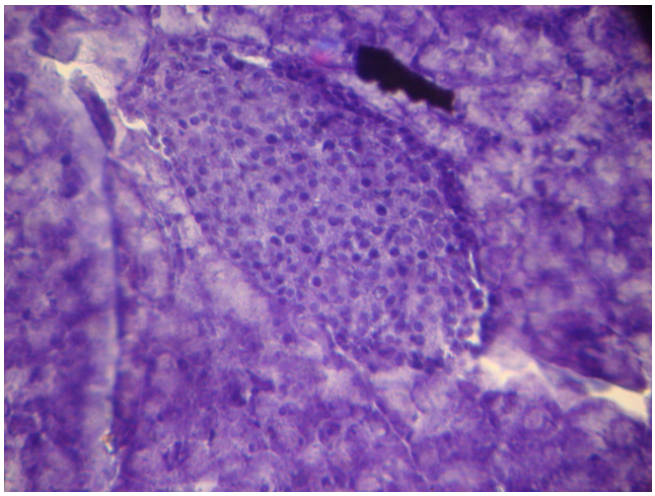

Figure 5: Normal 45 x

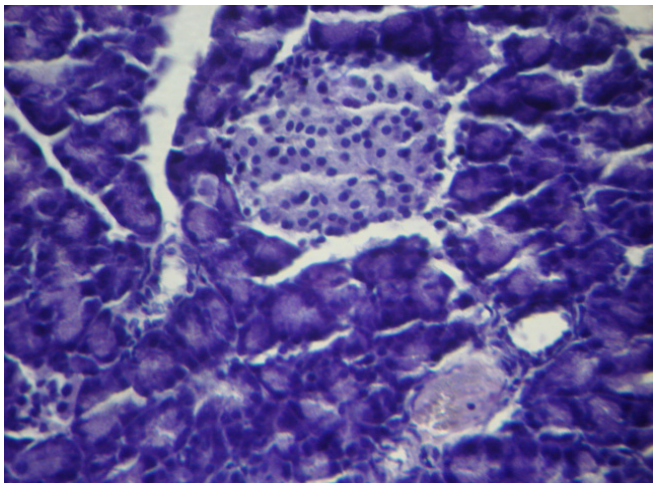

Figure 8: GLICLAZIDE 25 mg/kg 45 x

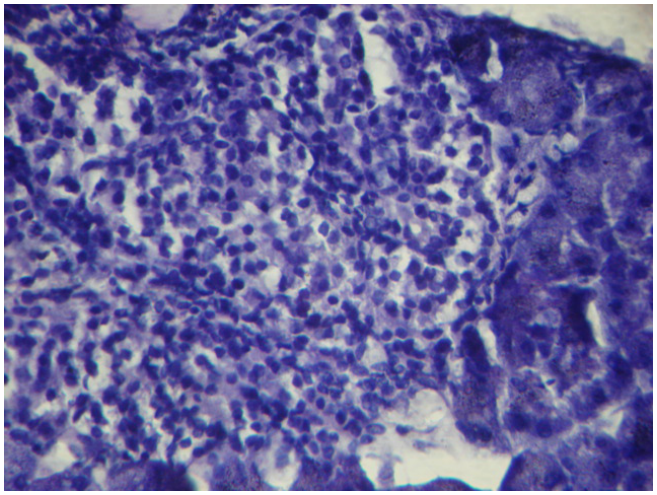

Figure 9: Diabetic Control 45 x

alloxan induced diabetic rats and oral glucose tolerant rats, and also hypoglycemic effect was found in fasted rats.

The antidiabetic plant extract may involve one or more compound to decrease blood glucose suggesting that natural constituents could act separately or synergistically to induce an hypoglycemic effect, further studies are being undertaking in explain more fully the mechanism of hypoglycemic effect of *Chonemorpha fragrans*.

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