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Effect of EFPTT/09, a herbal formulation, on blood sugar of normal and alloxan induced diabetic rats

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ABSTRACT

The herbal formulation, EFPTT/09, elicits hypoglycemic or anti-diabetic effects in both normal and experimentally induced hyperglycemic (alloxan induced) rats. The EFPTT/09 also elicited a significant antioxidant effects in alloxan diabetic rats as reflected by its ability to inhibit lipid peroxidation and to elevate the enzymatic antioxidants in pancreatic tissue. The histopathological studies during the long term treatment have shown to ameliorate the alloxan induced histological damage of islets of langerhans. The inhibitory effects on biochemical and histological parameters induced by herbal formulation at a dose of 500 mg kg were almost comparable (p<0.01) to that of standard drug, Glibenclamide (5mg/Kg). It is possible that the herbal formulation may act through both, pancreatic and extra-pancreatic mechanism(s).The present study demonstrates that herbal formulation exhibits promising anti-diabetic activity and helps to maintain good glycemic and metabolic control.

Key words: Antidiabetic activity, Alloxan monohydrate, Herbal formulation, Antioxidant enzymes.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, protein and fat metabolism and it is divided into Type 1diabetes (Insulin dependent) and Type 2 diabetes (Non-insulin dependent). Systems for both may include high level of sugar in the blood, unusual thirst, frequent urination, extreme huger, less of weight, blurred vision, tiredness, irritability, mood changes etc.,[1]. DM is known to ancient Indian physicians as "Madhumeha". Many herbal products including several metals and minerals have been described of the care of DM in ancient literature. Since the allopathic medicine has large number of side effects, in recent years there has been an increase in the use of herbals by a majority of population throughout the world [2].

Herbal medicines are popular remedies for diseases used by a vast majority of the world's population. Herbal formulations, which have attained widespread acceptability as therapeutic agents in India, include nootropics, anti-diabetics, hepatoprotectives and lipid lowering agents [3]. EFPTT/09, a polyherbal anti-diabetic formulation containing five ingredients of herbal origin that is used in medicine to treat type 2 diabetes individually. It contains both anti-diabetic and antioxidant principles. *Eugenia jambolana* and *Tinospera Cordifolia* are proven antidiabetic drugs. They increases peripheral utilization of glucose, inhibits hepatic glucose release caused by adrenaline [4]. *Terminalia arjuna* is a proven cardiotonic as well as antioxidant drug [5]. *Piper nigrum* has stomachic and carminative properties which enhances the bioavailability [6]. *Ficus religiosa* reduced the oxidative stress that protects the heart and blood vessels from oxidative stress of radicals [7]. Keeping the above information in view, EFPTT09 a polyherbal anti-diabetic formulation was made and this formulation has been evaluated its anti-diabetic activity and its probable mechanism(s) of action.

MATERIALS AND METHODS

Animals

Swiss albino mice (15-22g) and adult albino Wistar rats (150-200g) of either sex were obtained from King's institute-Guindy, Chennai, India. They were housed under standard conditions of temperature 24^0C, 65 ±10 % RH, 10:14 L;D Cycle and fed with standard pellet diet and water *ad libitum*. Experimental protocols were approved by Institutional Animal Ethical Committee, SRM University (No: IAEC/91/SRM/2009).

Drugs, Chemicals and Reagents

All the five crude drugs used in EFPTT/09 formulation were purchased from local market, Chennai, India and they were authenticated by Prof. P. Jayaram, Director, Plant Anatomy Research Centre (PARC), Chennai. India (Reg.No: PARC/2010/533). Alloxan

monohydrate was obtained from Loba chemie, Mumbai, India. All other reagents and chemicals used were of analytical grade and purchased locally.

Treatment

The various constituents and their quantity of EFPTT/09 are outlined in the Table 1. Suspensions of finely powdered EFPTT/09 and glibenclamide were prepared in 1% w/v sodium carboxy methyl cellulose (CMC) solution and administered orally. Alloxan monohydrate solution was prepared in ice-cold normal saline and administered intraperitoneally.

Acute toxicity studies

The acute toxicity study was performed according to OECD guideline 423, acute toxic class (3 animals used). The adult Swiss albino mice 15-22g were randomly divided into 5 different groups containing 3 animals in each group. The animals were fasted overnight and the formulation EFPTT/09 were administered orally of various dose levels (250,500, 1000 and 2000 mg/kg, BW) dissolved in 1% CMC. One group was maintained as control and administered vehicle only. The animals were observed continuously for two hours and then occasionally for further 24 hours and finally any mortality. Behavior of the animals and any other toxic symptoms also observed for 72 hrs and they were kept under observation up to 14 days.

Hypoglycemic activity screening in normal fasted rats

Overnight fasted normal rats were randomly divided into 5 groups of 6 rats each. The group I served as control which received vehicle i,e 1% CMC solution (1ml/kg orally), group II,III and IV were treated orally with EFPTT/09 at a dose of 200,400 and 600mg/kg orally, respectively. Group V received glibenclamide, 5mg/kg orally. Blood samples were collected from tail vein prior and 1, 2, 4 and 6 hr after treatment. Fasting blood glucose (FBG) was determined by the glucose oxidase method using Hypogaured's micro draw test strips. The % fall in blood glucose level was also calculated at peak hour of effect [8].

Induction of experimental diabetes

Overnight fasted albino rats were made diabetic by injecting Alloxan monohydrate (in the ice cold normal saline intraperitoneally) at a dose of 150mg/kg body weight [9]. Diabetes was confirmed in alloxan injected rate by measuring the fasting blood glucose concentration, 72 hr after the alloxanization. Rat with blood glucose level above 250 mg/dl were considered to be diabetic and were used in these study.

Anti diabetic activity screening in experimentally induced diabetic rats

The diabetic rats were divided into 5 groups of 6 rats each. Group I and II served as normal and diabetic control respectively and received vehicle (1 ml/kg, PO). Group III and IV

were treated with EFPTT/09at a dose of 250 and 500mg/kg/p.o, respectively. Group V was received glibenclamide 5mg/kg/p.o on 3rd day after alloxanization. In single dose, short term study, FBG was estimated from the tail vein prior and 1, 3 and 6 hr after administration of test drugs and vehicle. In multi-dose long term study, the same animals were continued with same dose of vehicle EFPTT/09 and Glibenclamide once daily for 15 days. FBG of the blood was collected at and measured 24 hr after the previous dose on 3,6,9,12 and 16th day.

Bio chemical determination

After 15 days of treatment, overnight fasted rats were sacrificed and blood was collected. The serum was separated and analysed for lysosomal enzymes such as SGOT [10] (serum glutamate oxaloacetate transaminase), SGPT [11] (serum glutamate pyruvate transaminase) and ALP [12] (alkaline phosphatase) by colorimetric method. The pancreas was dissected out and washed with ice-cold saline immediately. A portion of pancreatic tissue was homogenized and the extract was used for the estimation of enzymatic peroxidase (Catalase, CAT, glutathione peroxidase, GPx and lipid peroxidation, LPO) to see the effect of 15 days treatment with EFPTT/09 [13, 14].

Histopathological study of pancreas

Pancreas were isolated and preserved in 10% formalin. Histopathological observation of the tissues were carried out at SRM Medical college and Research centre, Pathology Laboratory, Chennai, India.

Statistical analysis

The results are expressed as the mean SE. Statistical evaluation was carried out using ANOVA followed by Dunnett's test. The herbal formulation and Glibenclamide treated groups were compared with the corresponding normal and diabetic control. P<O.O1 was considered to be significant.

RESULTS AND DISCUSSION

Acute toxicity

In the acute toxicity study, EFPTT/09 upto the dose level of 2000 mg/kg of body weight did not exhibit any lethality or toxic symptoms. Further dosing to estimate the LD_{50} of the drug was not performed. According to OECD guidelines for acute oral toxicity, an LD_{50} dose of 2000mg/kg and above is categorized as **unclassified and hence the drug is found to be safe** [15].

Blood glucose levels

Normal fasted rats: The onset of hypoglycaemic activity of EFPTT/09 at 200, 400 and 600 mg/kg was evident between 1-2 hr, the peak was found to be at 4 hr [16]. The rats receiving 600 mg/kg of EFPTT/09 showed the onset of effect at 1 hr with a peak effect at 4 hr. The hypoglycaemic effect of EFPTT/09 at 600 mg/kg (18.0% fall) was found to be nearly comparable to that of Glibenclamide (5 mg/kg) i.e., 24.5% fall. (Table 2).

Alloxan induced diabetic rats: A single-dose administration of EFPTT/09 (250 and 500 mg/kg, p.o) on 3rd day after alloxanization, showed a significant (*p<0.01)* reduction in blood glucose level (BGL) after 1 and 3 hr interval [17]. Maximum reduction in BGL to 136.0±2.88 mg/dl was seen at 3 hr after administration of 500 mg/kg of EFPTT/09. Glibenclamide (5 mg/kg, p.o) also showed maximum reduction to 116.5±1.87 mg/dl at 3 hr. At 6 hr the BGL slightly increased as compared to 3 hr values (Table 3). On repeated administration of vehicle, EFPTT/09 or Glibenclamide for 15 days, a sustained and significant (*P<0.01*) decrease in the blood glucose of the diabetic rats was observed at a dose of 250 (39.9 % fall) and 500 mg/kg (42.7% fall), in a dose dependent manner as compared to the vehicle treated group. Glibenclamide also showed a significant (*P*<0.01) decrease in blood glucose (42.9% fall) at a dose of 5mg/kg, as compared with vehicle treated group (Table 4).

Bio-chemical parameters

Serum SGOT and SGPT levels were elevated significantly (*P*<0.01) in alloxan induced diabetic rats as compared to normal rats [18]. In alloxan diabetic rats when treated with the EFPTT/09 and Glibenclamide, there was a significant (*P*<0.01) reduction in the elevated levels of SGOT and SGPT. Similarly, elevated ALP level in serum during alloxan induced diabetes were found to be significantly (*P*<0.01) lowered by EFPTT/09 and glibenclamide treatment.

Superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH*) are the major reactive oxygen species generated during oxidative stress. Free radical decreases the insulin receptor substrate (IRS), tyrosine phosphorylation and in turn the activity of phosphatidyl inositol (PI) 3- kinase. Altered insulin signaling pathway exerts insulin resistance, a state of type 2 diabetes. Aerobic cells are endowed with extensive anti-oxidant defense mechanism including both low molecular weight scavengers such as reduced glutathione, ascorbic acid, vitamin E and enzyme system namely SOD, CAT and GPx. Decrease in CAT activity could be possibly due to less availability of NADPH or gradual decrease in erythrocyte CAT concentration by excessive generation of O_2** that inactivates the enzyme. Since the activity of an enzyme depends upon its substrate, depletion of Glutathione may be the reason for decreased glutathione peroxidases activity. In other words levels of both enzymatic antioxidants (GPx and CAT) decreased and lysosomal enzymes increased in diabetic rats.

It is important to know in EFPTT/09 treated rats; there were increased levels of the antioxidant enzymes (GPx and CAT). EFPTT/09 (500) increased 38.8% of both CAT and GPx with

respect to Diabetic control where as Glibenclamide (5mg/kg) increased 43.8% of CAT and 33.2% of GPx. This shows that EFPTT/09 can reverse all these abnormalities either by pancreatic or hepatic mechanism. Lipid Peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is analyzed by the formation of MDA (Marker). MDA conjugate with amino group of protein to form intra and inter molecular cross-links. These cross-links inactivate the membrane bound enzymes and receptors. Type 2 diabetic rats showed elevated plasma MDA due to peroxidation of lipids [19, 20]. Decrease in MDA by EFPTT/09 showed the ability of drug to prevent oxidative damage. The % Lipid peroxidation value decreased with EFPTT/09 at the dose of 250, 500 mg/kg and Glibenclamide (5mg/kg) were 25.6%, 34.68% and 35.07%, respectively (Table 5).

Histopathological Studies

Microscopically examined pancreas section shows the following features: Pancreas section of rat of normal group **(fig.1)** showed that normal architecture of pancreas with acne of serious epithelial cells along with nest of endocrine cells separated by fibrocollaoenous, stroma into lobules. No fibrosis or inflammation was found. Histopathological findings of pancreas of the diabetic rats showed necrosis, atrophy and fibrotic changes (**fig.2)**. But, the pancreas of the rats treated with EFPTT/09 and glibenclamide showed minimal necrosis and mild to moderate atrophy and fibrotic changes **(fig.3, 4, 5)**.

CONCLUSION

The present study demonstrates that herbal formulation exhibits promising antidiabetic activity and help to maintain good glycemic and metabolic control. The herbal formulation, EFPTT/09, elicit hypoglycaemic/antidiabetic effects in both normal and experimentally induced hyperlycemic (alloxan induced) rats. The herbal formulation under acute toxicity studies by OECD guideline shows it is non-toxic upto 2000mg/kg BW, so it can be recommended for human conception after a safe clinical trial. It is possible that the herbal formulation may act through both, pancreatic and extra-pancreatic mechanism(s). The EFPTT/09 also elicited a significant antioxidant effect in alloxan diabetic rats as reflected by its ability to inhibit lipid peroxidation and to elevate the enzymatic antioxidants in pancreatic tissue. The histopathological studies during the long term treatment have shown to ameliorate the alloxan induced histological damage of islets of Langerhans. The inhibitory effects on biochemical and histological parameters induced by herbal formulation at a dose of 500 mg/kg were almost comparable to that of standard drug, glibenclamide (5mg/kg).

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Table 1: Composition of polyherbal formulation (EFPTT/09)

Herbs	Quantity (g/100g)
Eugenia jambolana (seed)	20
Ficus religiosa (bark)	20
Piper nigrum (fruits)	20
Terminalia arjuna (bark)	20
Tinospora cordifolia (leaves)	20

Table 2: Effect of EFPTT/09 on blood glucose level in normal fasted rats:

Group Treatment (dose mg/kg, p.o)	Blood Glucose level (mg/dl)				
	0 hr	**1 hr**	**2 hr**	**4 hr**	**6 hr**
Normal control	64.3±2.73	63.0±3.16	61.0±2.82	60.6±2.09	59.6±2.82
EFPTT/09 (200mg/kg)	66.0±4.89	65.0±3.94 (1.5)	63.0±5.17 (4.5)	59.0±2.89 (10.6)	60.3±3.77[a] (8.6)
EFPTT/09 (400mg/kg)	67.1±2.85	65.0±1.78 (3.1)	63.5±2.66[a] (5.3)	60.3±2.19[b] (10.3)	62.0±1.47[b] (5.1)
EFPTT/09 (600mg/kg)	72.0±4.09	68.0±2.68[a] (5.5)	64.1±1.72[b] (10.9)	59.0±6.22[b] (18.0)	63.0±2.16[b] (12.5)
Glibenclamide (5mg/kg)	61.1±3.18	57.0±1.41 (6.7)	51.3±2.25[b] (16.0)	46.3±2.36[b] (24.2)	50.3±2.06[b] (17.6)

Values are mean ± SD from 6 animals in each group. Figure in parenthesis indicates % fall in BGL as compared to 0 hr. *P* value: [a] <0.05, [b] *P*<0.01

Table 3: Effect of EFPTT/09 on blood glucose level in alloxan-induced diabetic rats (Single-dose short term study)

Group Treatment (dose mg/kg, p.o)	Blood Glucose Level (mg/dl)			
	0 hr	**1 hr**	**3 hr**	**6 hr**
Normal control	63.8±2.63	61.6±2.06	63.5±1.64	63.1±1.47
Diabetic control	295.8±0.98[a]	306.1±4.89[a] (-3.7)	299.6±3.26[a] (-1.5)	300.3±1.63[a] (-1.7)
EFPTT/09 (250mg/kg)	304.3±1.03	240.0±1.26[b] (21.1)	204.3±1.21[b] (32.8)	200.0±2.13[b] (34.2)
EFPTT/09 (500mg/kg)	302.0±1.41	212.1±2.63[b] (29.7)	136.5±2.88[b] (54.8)	153.1±2.31[b] (49.3)
Glibenclamide (5mg/kg)	292.0±2.28	206.0±1.41[b] (29.4)	116.5±1.87[b] (60.1)	133.8±2.13[b] (54.1)

Values are mean ± SD from 6 animals in each group. Figure in parenthesis indicates % fall in BGL as compared to 0 hr. **P** value: <0.01; compared to [a] normal group, [b] diabetic group

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Table 4: Effect of Multidose administration of EFPTT/09 on blood glucose level in alloxan-induced diabetic rats (long term study of 15 days)

Group/Treatment (dose, mg/kg/p.o)	Blood glucose level (mg/dl)				
	Day 3	Day 6	Day 9	Day 12	Day 16
Normal control	58.1±1.72	60.0±1.41	60.0±0.89	62.8±1.16	59.0±2.09
Diabetic control	313.5±1.37 [a]	308.1±0.75 [a] (1.7)	292.0±1.41 [a] (6.8)	281.0±0.75 [a] (10.3)	276.0±1.41 [a] (11.96)
EFPTT/09 (250mg/kg)	291.3±1.21 [c]	272.6±1.63 [c] (6.4)	230.8±1.47 [c] (20.7)	189.0±1.78 [c] (35.1)	175.0±0.89 [c] (39.9)
EFPTT/09 (500mg/kg)	286.5±1.51 [c]	256.0±1.26 [c] (10.6)	221.5±1.04 [c] (22.6)	182.1±0.98 [c] (36.4)	164.1±1.47 [c] (35.7)
Glibenclamide (5mg/kg)	281.5±1.37 [c]	249.0±1.26 [c] (11.5)	218.1±1.47 [c] (22.5)	180.5±1.87 [c] (35.8)	160.5±1.04 [c] (42.9)

Values are mean ± SD from 6 animals in each group. Figure in parenthesis indicates % fall in BGL as compared to Day 3. P values: <0.01, as compared to [a] normal group; [c] diabetic control group [b]<0.05 compared to diabetic group.

Table 5: Effect of formulation EFPTT/09 on biochemical parameters in alloxan induced diabetic rats.

Group/Treatment (dose- mg/kg, po)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	% LPO	CAT (U/mg)	GP_X (U/mg)
Normal control	70.25± 0.078	40.10± 0.363	136.92± 0.251	-	3.160± 0.140	2.58± 0.107
Diabetic control	138.20± 0.178 [a]	100.16± 0.544 [a]	254.23± 0.232 [a]	100± 1.059	1.988± 0.169 [a]	1.936± 0.175 [a]
EFPTT/09 250	105.23± 0.096 [b]	64.66± 0.360 [b]	194.20± 0.143 [b]	74.36± 0.165 [b]	2.46± 0.069 [b]	2.26± 0.044 [b]
EFPTT/09 500	90.36± 0.066 [b]	55.34± 0.045 [b]	169.20± 0.127 [b]	65.32± 0.058 [b]	2.76± 0.031 [b]	2.688± 0.147 [b]
Glibenclamide (5 mg/kg)	83.33± 0.198 [b]	48.30± 0.224 [b]	155.30± 0.040 [b]	64.93± 0.336 [b]	2.86± 0.233 [b]	2.58± 0.135 [b]

Values were expressed as Mean ± SD of 6 rats in each group. P value: <0.01; compared to [a] normal group [b] diabetic

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Fig 1: Normal control

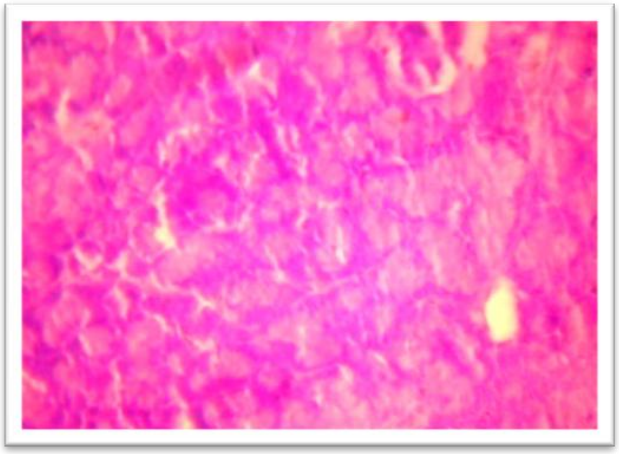

Fig 2: Diabetic Control

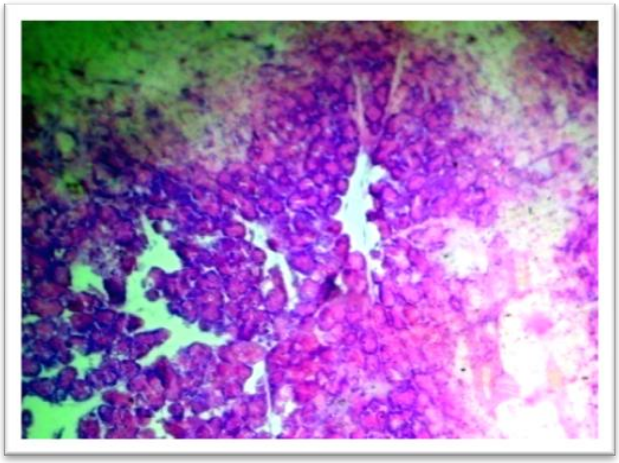

Fig 3: EFPTT/09 at 250mg/kg,BW.

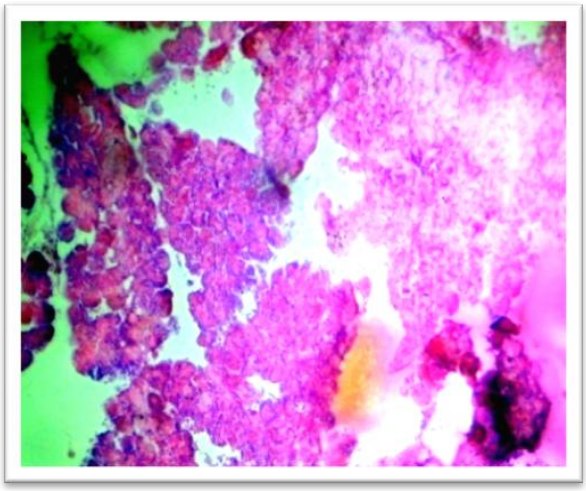

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Fig 4: EFPTT/09 at 500mg/kg,BW.

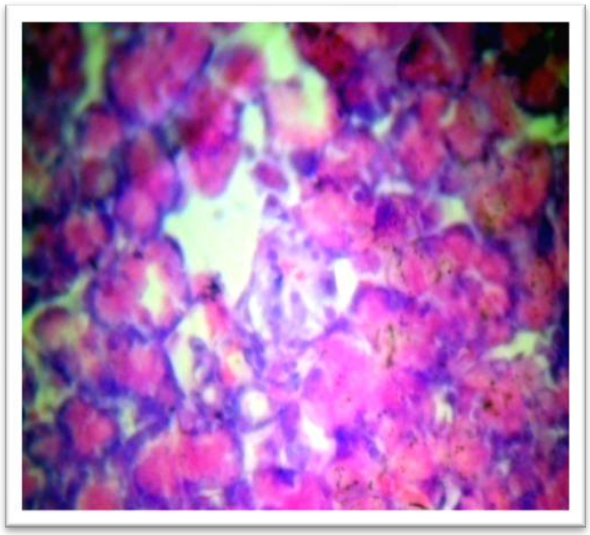
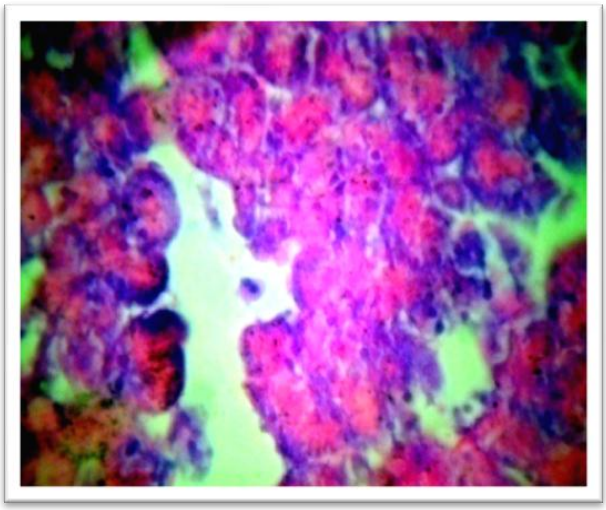


Fig 5: Glibenclamide 5mg/kg,BW.



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