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ANTIDIABETIC ACTIVITY OF ERYHTROXYLUM MONOGYNUM IN ALLOXAN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Among metabolic disorders Diabetes is widely spread around the world. Lack of exercise, bad food habits, stress, heredity are some of the reasons which may cause diabetes. Medicine available to diabetes is not completely targeting the problem. The plants are well known for their therapeutic nature. The Indian ethics are very good source for the remedies. The present study was carried out to evaluate the antidiabetic activity of *Erythroxylum monogynum* plant extract in alloxan (150mg/kg) induced diabetic rats. The extract was prepared with hydroalcohol solvent (70% ethanol and 30% water) through maceration technique. Alloxan induced rats with above 150 mg/dL blood glucose levels were selected for the study. These rats were divided into 5 groups. Group- I was control, group-II was alloxan induced rats, group-III was alloxan + Metformin 500mg/kg and group-IV was alloxan + Extract 200 mg/kg and group-V was alloxan +Extract 300mg/kg with 8 rats in each group. The treatment was carried out by oral for 14 days. The blood samples were collected from retro orbital plexus on 15th day and centrifuged to separate serum. The serum samples were performed serological tests. The serum glucose levels were decreased in the extract treated rats. The values of SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase) and ALP (Alkaline Phosphotase) were decreased in group-IV and V compare to the group-II. The reduced serum cholesterol, triglycerides, LDL (Low Density Lipoproteins) and VLDL (Very Low Density Lipoproteins) were also seen in group-IV and V rats. The HDL values were increased in the extract treated diabetic rats. The results were significantly comparable with group-III. The reduced glucose levels, SGOT, SGPT, ALP, cholesterol, triglycerides,

VLDL and LDL and increased HDL values were explaining the antidiabetic activity of the extract.

Key words: Diabetes, alloxan, glucose, SGOT, SGPT.

INTRODUCTION

Diabetes is a metabolic disorder spread all over the world. It is estimated that 381 million people are suffering from diabetes in 2013 according to international diabetes federation. Changes in life style and diet have brought changes in the human physiology. The Diabetes is a disorder characterized by the symptoms chronic hyperglycemia, defects in the secretions of insulin and insulin action or both. Long terms of vascular and neuropathic complications also associate with this. Patients who are consuming these synthetic medicines are getting side effects like constipation, hepatitis, weight gain, abdominal pain, gastro intestinal disturbances, drowsy etc. The patients are eager to have the best and alternate remedies to treat the diabetes without any side effects. Researchers have screened different plant species to know their therapeutic nature. Plants such as *Momordica charantia*, *Vinca rosea, Terminalia chebula, Gymnema sylvestra* etc. are proved for antihyperglycemic activity. The present approach is to study the anttidiabetic activity of *Erythroxylum monogynum* leaf extract in alloxan induced diabetic albino rats.

MATERIAL AND METHODS

Erythroxylum monogynum plant was brought from the village of Gudur, Warangal district, Telangana state. The plant is being practiced by the people for treating inflammations, malaria etc. The plant leaves were separated and dried under shade. The dried leaves were powdered with electric grinder. The fine powder was collected from the sieve plate-10. The leaf powder was used to collect extract with maceration technique. The 250 ml of hydroalcholic solvent (70% ethanol and 30% of distilled water) was added to the 50g of leaf powder in stoppard conical flask with random shaking. The filtrate was collected after 24 hrs. Then, the separated marc was mixed in 250 ml of hydroalcoholic solvent and left for 24 hrs. The filtrate was collected after 24 hrs. The both filtrates were combined together and subjected to distillation to get the extract. The extract (EMHE- *Erythroxylum monogynum* hydroalcoholic extract) was kept in the refrigerator prior to use.

Albino rats weighing about 200-250 g were selected for the experimental work. The protocol was approved by Institutional Animal Ethical Committee (IAEC/03/UCPSc/KU/10). The

animals were housed in standard polypropylene cages, and maintained under standard laboratory conditions (12:12 hour light and dark cycle; at an ambient temperature of 25 ± 5^{O}C, 35-60% of relative humidity). They were fed with std. rat pellet diet and water *ad libitum.* The alloxan monohydrate (150mg/kg) was used to induce diabetes in albino rats. The alloxan was dissolved in normal saline (0.2ml) according to weight of the rat [1]. The blood glucose levels were estimated on 3rd day after the alloxan induction and the rats which showed glucose levels above 150 mg/dl were selected for the study.

OGTT (oral glucose tolerance test)

Alloxan induced diabetic rats were used for the study. Overnight fasted albino rats were induced diabetes by alloxan and divided into 4 groups consisting of 8 rats and their fasting blood glucose levels were recorded. Control group (I) received vehicle 0.1% CMC where as group II was administered with Metformin (500mg/kg), groups III and IV were given with EMHE 200mg/kg and 300mg/kg respectively followed by loading of glucose (2g/kg). The administration was done through oral. Blood samples were collected from the rats on 0hr, 1hr, 2hr and 8 hr and 25 hr for determining blood glucose levels.

Experimental procedure to evaluate antidiabetic activity

Alloxan induced rats showing glucose levels above 150 mg/dL were selected for the study. The rats were divided into following groups.

Group- I – Control (given saline for 14 days)

Group-II – Alloxan induced

Group-III– Alloxan + Metformin (500mg/kg)

Group-IV– Alloxan+ EMHE 200mg/kg

Group-V– Alloxan + EMHE 300 mg/kg

The treatment of the EMHE and metformin was carried out through oral for 14 days. On day 15 the blood samples were collected through retro orbital plexus. The blood samples were centrifuged to collect serum. The serum samples were used to perform the serological tests. The pancreas was separated from the dissected rats. They were processed for the histological sections. The commercially available kits were used to study the serological tests. The glycogen and protein levels of liver were also estimated by modified Anthrone and Lowry methods respectively.

The results were expressed in mean± SD and they were analyzed one way ANOVA followed by Dunnett test with the significance value 0.05.

RESULTS

Oral glucose tolerance test: The glucose levels were decreased in the group-II, III and IV compare to group-I. The reduced levels of glucose were significantly more in the group –IV than to the group-III. The results were compared to group-II. The significant reduction in glucose levels of treated groups were increased from 0 hr to 2hr and from 2hr to 8 hr and from 8 hr to 25 hr (table- 1).

The final body weights were decreased in the group-II. The normal growth of body weights were observed in the group-III, IV and V. The significant improvement in the body weights of group-IV and V were seen compare to group-II (table-2).

After 14 days the blood glucose levels of group-II rats were increased compare to other groups. The decrease in the glucose values were seen in the EMHE and metformin treated diabetic rats. The glucose levels were decreased more in the group-V than to group-IV (table-3).

Serum cholesterol levels were increased in the diabetic rats of group-II. These cholesterol levels in serum were normalized in the group-III rats. The lowered values of cholesterol were also seen in the group-IV and V (table-3).

Serum triglycerides, LDL and VLDL were increased in the group-II, whereas, these values were reduced in the group-III, IV and V. The HDL levels were decreased in the group-II. The increased HDL values were observed in the group-IV and V. The increased HDL values were also seen in group-III compare to group-II (table-4).

SGOT, SGPT and ALP levels were elevated in the alloxan induced diabetic rats. These values were decreased in the group-III, IV and V. The reduced values of SGOT, SGPT and ALP were observed more in the group-V than to the group-IV (table-5).

Total protein and albumin levels were decreased in the group-II. The normal values of total protein and albumin were seen in the group –III. The group-IV and V rats were also observed with normal values of total protein and albumin (table-6).

The liver glycogen and protein content values were decreased in the group-II rats. The restored values were observed in the group-III. The normal levels were seen in group-IV and V compare to group-II (table-7).

The damaged beta cells were seen in the pancreas section of group-II (figure 2). Beta cells were reformed in pancreas of group-III rats (figure 3). The arrangement of islets and exocrine part of pancreas was clearly seen in the EMHE treated diabetic rats (figures 4 & 5).

Table-1-Oral glucose tolerance tests in alloxan induced diabetic rats

GROUP	NAME	O hr	1 hr	2hr	8 hr	25 hr
I	ALLOXAN INDUCED	82.72± 2.37	326.36± 10.03	365.61± 10.67	397.48± 10.02	398.18± 10.85
II	ALLOXAN+ METFORMIN	77.48± 3.46*	255.33± 6.35**	227.41± 9.89**	181.94± 6.80**	177.46± 8.84**
III	ALLOXAN + EMHE 200 mg/kg	82.22± 3.33^{ns}	286.29± 4.01**	265.83± 10.37**	219.21± 7.97**	200.58± 8.57**
IV	ALLOXAN + EMHE 300 mg/kg	79.20± 4.00 ^{ns}	274.45± 6.93**	245.95± 8.138**	194.47± 9.10**	187.34± 6.09**

All values were expressed in mean ±SD. The results were analyzed by one way ANOVA followed by Dunett test. **=P< 0.01, *=P<0.05 compare to Group-I, ns= not significant when compare to Group- I.

Table-2- Changes in the body weights

GROUP	NAME	B.WTS 7TH DAY (g)	B.WTS 15TH DAY (g)
I	CONTROL	219.75±5.17	238.5±8.41
II	ALLOXAN INDUCED	215.87±6.12^{c}	212.87±10.98^{a}
III	ALLOXAN+ METFORMIN 500mg/kg	224.87±5.22^{c,f}	243.5±6.34^{c,b}
IV	ALLOXAN + EMHE 200 mg/kg	218.25±8.51^{c,d}	237.37±9.51^{c,b}
V	ALLOXAN + EMHE 300 mg/kg	221.62±7.74^{c,d}	240±10.67^{c,b}

All values were expressed in mean ± SD, the values were analyzed with one way ANOVA followed by Dunnett Test. a=p<0.01compare to Group-I and b=p<0.01 compare to Group- II. c= not significant when compare to Group-I, d=not significant when compare to Group- II. e=p<0.05 compare to Group-I, f=p<0.05 compare to Group-II.

Table-3- Glucose levels in serum of alloxan induced diabetic rats on 15th day, triglycerides and cholesterol levels in serum

GROUP	NAME	Glucose (mg/dl)	Triglycerides (mg/dL)	Total Cholesterol (mg/dL)
I	CONTROL	83.00±2.08	70.93±3.50	117.16±5.074
II	ALLOXAN INDUCED	250.24±3.14[a]	143.05±4.80[a]	170.03±3.85 [a]
III	ALLOXAN+ METFORMIN 500 mg/kg	126.02±5.80[a,b]	84.26±4.38[a,b]	127.40±4.09[a,b]
IV	ALLOXAN + EMHE 200 mg/kg	203.62±7.18[a,b]	109.97±4.79[a,b]	140.65±4.33[a,b]
V	ALLOXAN + EMHE 300 mg/kg	160.70±7.91[a,b]	94.78±3.08[a,b]	126.50±4.15[a,b]

Table- 4 - HDL, LDL and VLDL levels in serum

GROUP	NAME	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I	CONTROL	41.32±3.47	61.61±7.91	14.19±0.72
II	ALLOXAN INDUCED	24.27±4.12[a]	117.24±6.42[a]	28.58±0.93 [a]
III	ALLOXAN+ METFORMIN 500 mg/kg	39.50±3.40[c,b]	71.05±5.25[e,b]	16.85±0.87[a,b]
IV	ALLOXAN + EMHE 200 mg/kg	31.39±3.47[a,b]	87.19±5.59[a.b]	22.01±.95[a,b]
V	ALLOXAN + EMHE 300 mg/kg	40.87±3.86[c,b]	66.43±6.53[c,b]	18.95±0.65[a,b]

Table- 5- SGOT, SGPT and ALP levels in serum

GROUP	NAME	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)
I	CONTROL	28.96±4.07	33.73±3.91	127.41±5.13
II	ALLOXAN INDUCED	116.05±4.20[a]	132.93±4.32 [a]	198.99±5.80[a]
III	ALLOXAN+ METFORMIN 500 mg/kg	46.23±4.41[a,b]	47.58±4.55[a,b]	152.29±5.51[a,b]
IV	ALLOXAN + EMHE 200 mg/kg	77.00±3.24[a,b]	89.61±3.40[a,b]	169.52±3.39[a,b]
V	ALLOXAN + EMHE 300 mg/kg	52.63±4.00[a,b]	61.64±4.07[a,b]	156.58±3.92[a,b]

Table- 6- Serum total protein and albumin levels

GROUP	NAME	Total Protein (g/dL)	Albumin (g/dL)
I	CONTROL	7.00±0.49	3.08±0.58
II	ALLOXAN INDUCED	4.17±0.55[a]	1.48±0.39[a]
III	ALLOXAN+ METFORMIN 500 mg/kg	6.50±0.53[c,b]	2.53±0.62[c,b]
IV	ALLOXAN + EMHE 200 mg/kg	4.99±0.62[a,f]	2.28±0.45[e,f]
V	ALLOXAN + EMHE 300 mg/kg	6.27±0.60[e,b]	2.97±0.59[c,b]

Table-7-Tissue glycogen and protein levels

GROUP	NAME	Liver Glycogen (µg/100mg)	Liver Protein (mg/g)
I	CONTROL	793.47±10.01	183.63±9.57
II	ALLOXAN INDUCED	488.78±11.95[a]	105.83±7.40[a]
III	ALLOXAN+ METFORMIN 500 mg/kg	780.18±10.66[e,b]	154.68±8.31[a,b]
IV	ALLOXAN + EMHE 200 mg/kg	593.75±8.15[a,b]	131.06±8.04[a,b]
V	ALLOXAN + EMHE 300 mg/kg	701.92±10.41[a,b]	162.53±8.58[a,b]

All values were expressed in mean ± SD, the values were analyzed with one way ANOVA followed by Dunnett Test. a=p<0.01compare to Group-I and b=p<0.01 compare to Group- II. c= not significant when compare to Group-I, d=not significant when compare to Group- II. e=p<0.05 compare to Group-I, f=p<0.05 compare to Group-II.

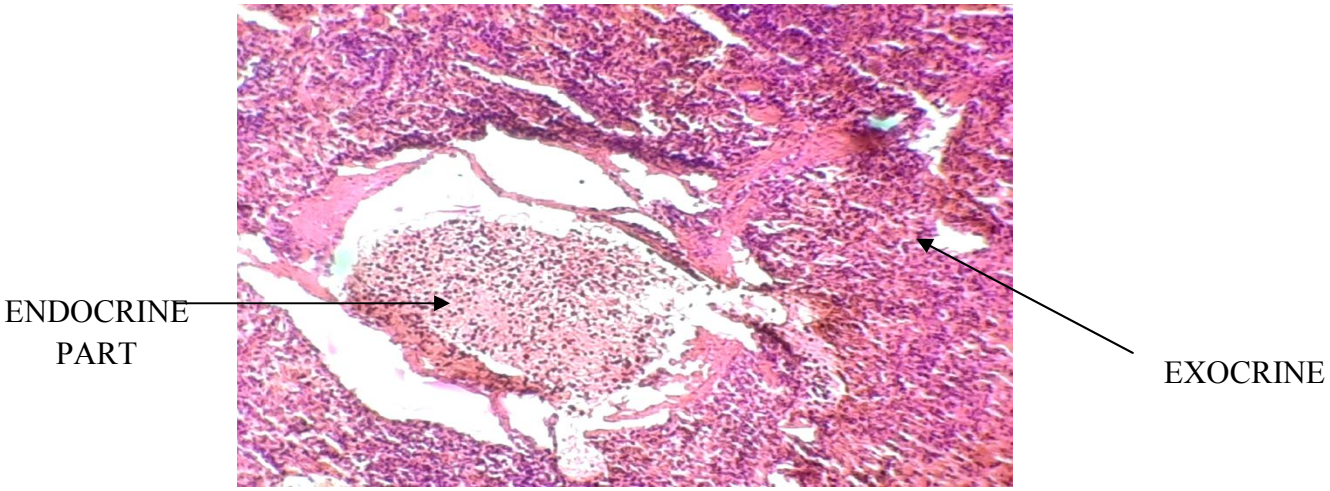
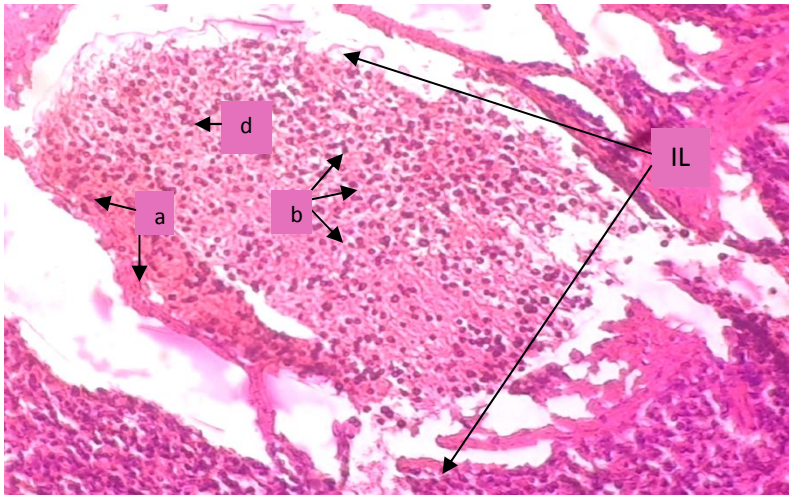


Figure- 1(a) – Cross Section Of Pancreas (Control)



Pancreas Section shows the Islet of Langerhans (IL), alpha (α) cells at peripheral (a), at center beta (β) cells (b) and delta (δ) cells (d) adjacent to beta Cells.

Figure- 1(b) - Cross Section Of Pancreas (Control)

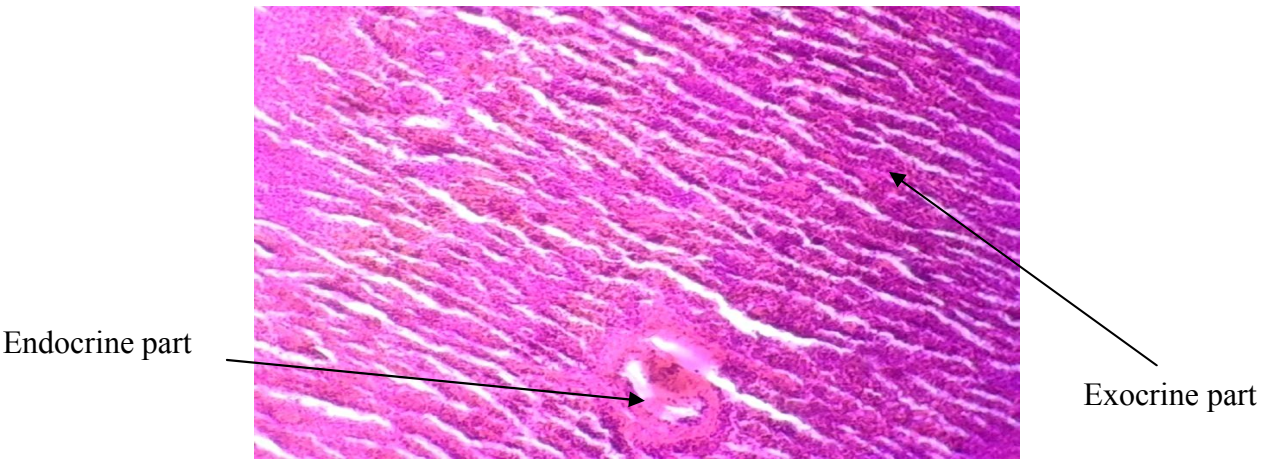
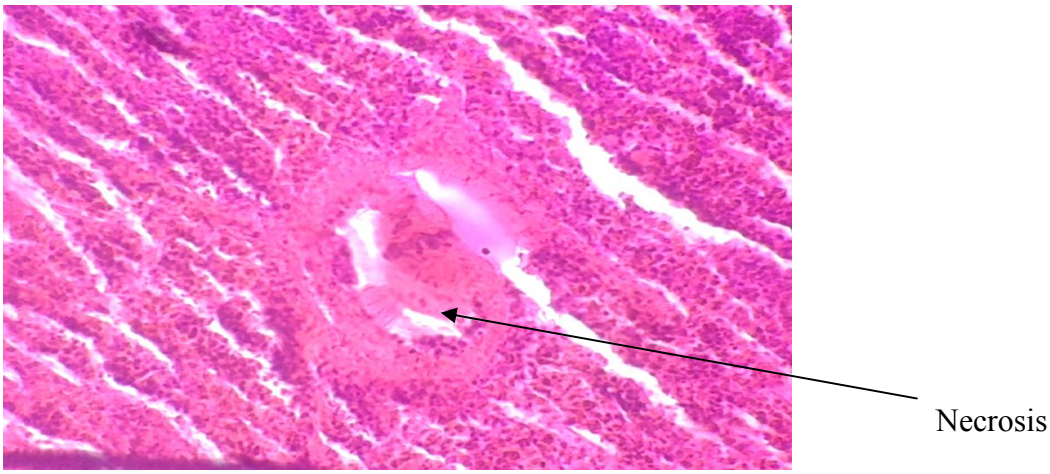


Figure 2(a) – Cross Section Of Pancreas (Alloxan Induced)



The decrease in the size of islet of Langerhans with necrosis and less number of islets cells in the group-II rat's pancreas

Figure 2 (b) - Cross Section Of Pancreas (Alloxan Induced)

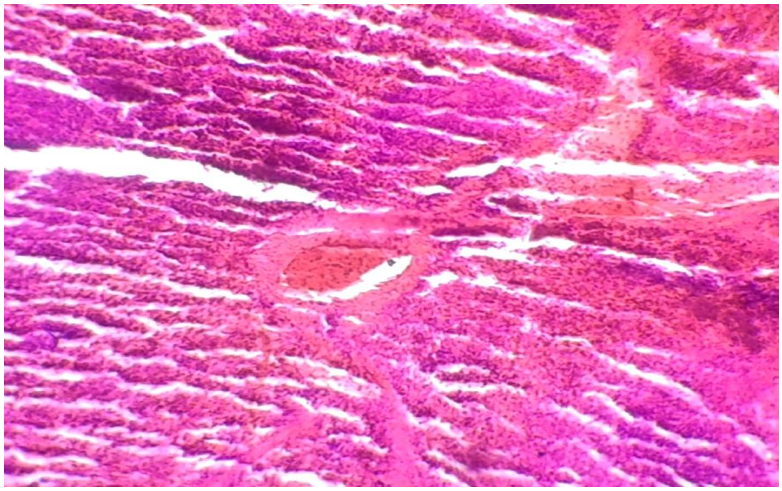
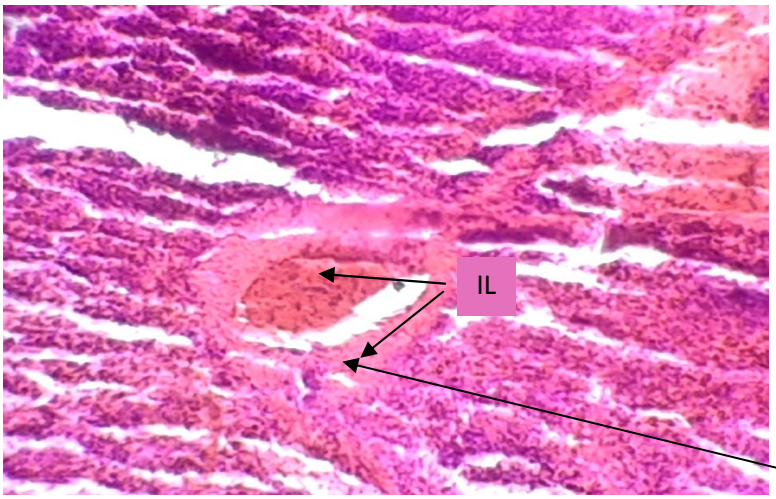


FIGURE –3(a) – Cross Section Of Pancreas (Alloxan+Metformin 500 mg/kg)



The increase in the number of alpha (α), beta (β) and delta (δ) cells were seen in the group-III rat's pancreas

Figure – 3 (b) - Cross Section Of Pancreas (Alloxan+Metformin 500 mg/kg)

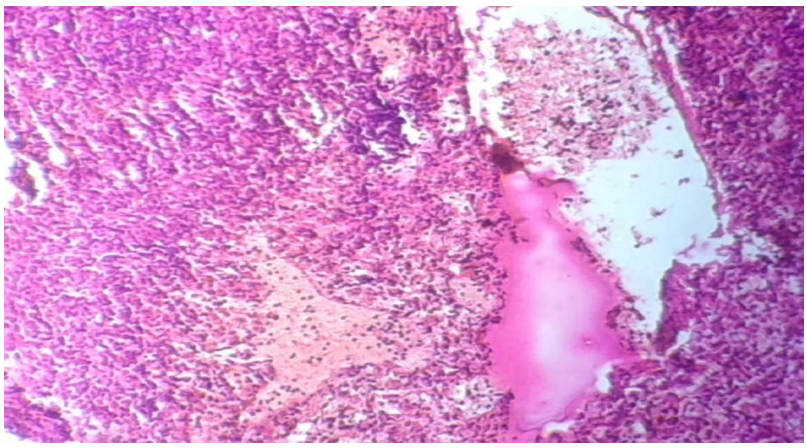
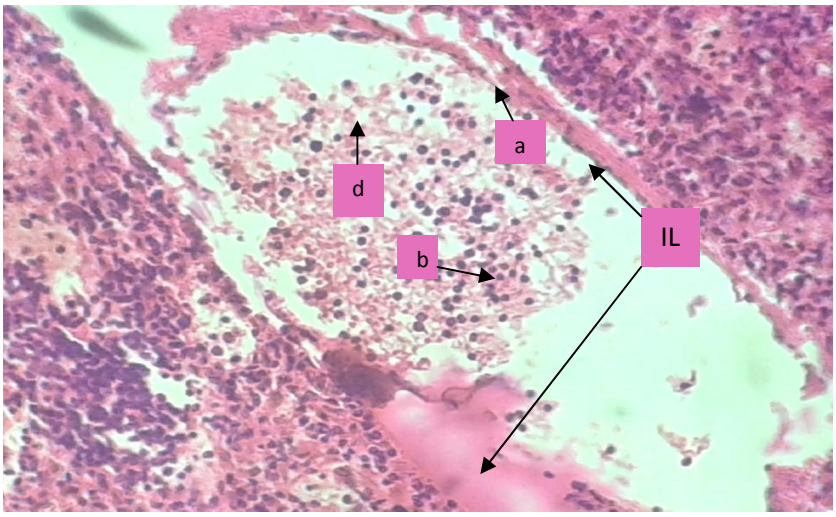


Figure- 4(a) – Cross Section Of Pancreas (Alloxan + EMHE 200mg/kg)



The increase in the number of cells (a-α, b-β and d-δ) in Langerhans with the enlarged size were observed in the pancreas section of group- IV rat.

Figure-4 (b) - Cross Section Of Pancreas (Alloxan + EMHE 200mg/kg)

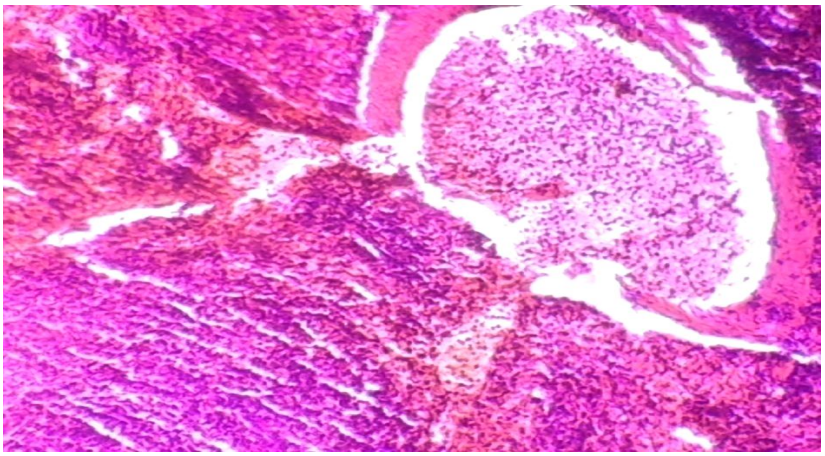
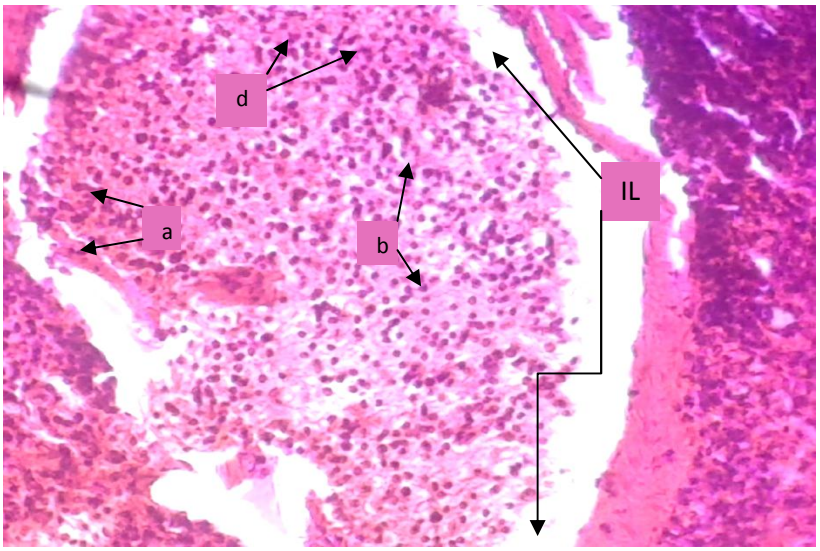


Figure- 5 (a) - Cross Section Of Pancreas (Alloxan + EMHE 300 mg/kg)



The improvement in the number of islet cells (a-α, b-β and d-δ) in Langerhans were seen in section of the pancreas of group-V rat.

Figure- 5(b) Cross section of pancreas (Alloxan+ EMHE 300 mg/kg)

DISCUSSION

The body weights were decreased in the diabetic control animals. It was observed that the increase in average body weight of EMHE treated groups (IV&V). The raised weights of body were also seen in group- III and were very significant.

The reduced body weight of diabetic animals is because of the insufficient insulin levels. The increase of body weights supporting the antidiabetogenic activity of EMHE. The significant improvement of body weight was also studied in the *Ficus carica* leaf extract treated diabetic rats [2] . The fluctuating body weights were observed in diabetics.

The increased serum insulin levels also the reason for the development of total body weight of EMHE, Metformin treated diabetic rats [3].

The reduced glucose levels were observed in the EMHE treated diabetic rats. The *Momordica charantia* extracts were also lowered the blood glucose (antihyperglycaemic) levels in treated diabetic rats [4]. The maximum decreased glucose levels were noticed in the 300 mg/kg EMHE treated diabetic rats (group V) than to the 200mg/kg treated rats. One of the consequences of the diabetes mellitus is hyperlipidemia. This may be attributed to the diabetic animals by the raised tissue cholesterol, phospholipids and free fatty acids. It is also because of the underutilization of glucose and also mobilization of adipose tissue. The elevated levels of triglycerides and total cholesterol in diabetes are in consistent [5].

The altered lipid profile in diabetics leads to atherosclerosis [6]. The total cholesterol and triglycerides were decreased in the EMHE treated diabetic rats. The lowered levels of triglycerides and cholesterol were also seen in the standard drug group. The reduced levels of serum cholesterol also noticed in the investigation of *Gymnema sylvestra* treated diabetic (alloxan induced) rats [7]. The normalized serum cholesterol and triglycerides levels were also analysed in the diabetic rats treated with *Costus pictus* methanol leaf extract [8].

The deficiency of insulin inactivates the lipoprotein lipase which encourages the liver to convert free fatty acids to phospholipids and cholesterol. This results the release of phospholipids into blood [9]. Might be the insulinogenic activity of the EMHE lowered the LDL, VLDL and total cholesterol and triglycerides in the treated group (IV, V). This can also prevent the coronary artery diseases like atherosclerosis. The similar results were observed in the *Caesalpinia bonducella* in alloxan caused diabetic rats [10].

The HDL (High Density Lipoprotein) helps the body, which is considered as good cholesterol. The decreased levels were seen in alloxan induced diabetic rats. But they were restored in the EMHE treated groups. The standard drug also helped the levels of HDL to increase. This reveals the counter action of EMHE against artery diseases. SGOT, SGPT and Alkaline phosphatase are reliable markers for the functional liver. The liver was damaged through necrosis caused by the induction of alloxan.

In the necrosis of liver the vital Enzymes like SGOT and SGPT etc release into circulation. These values generally elevate due to liver dysfunction. They were lowered in the EMHE and Metformin treated diabetic rats.

The study on *Areca catechu* leaf extract for antidiabetic activity also revealed the reduced level of SGOT, SGPT, and ALP in treated diabetic rats [11].

The increased gluconeogenesis and ketogenesis are observed in the diabetes which may be due to high level in the activities of transaminases [12].

The decreased levels of these markers in the EMHE treated rats indicate the hepatoprotective activity of the extract. This may strengthen the antidiabetogenic effect of the extracts.
The antioxidant property of the EMHE extract results the reduction of the oxidative stress caused by the alloxan. The extract may decrease the lipid peroxidation marker malondialdehyde in serum. It may also support the enhancing of the antioxidative enzymes like superoixide dismutase, catalase and glutathione peroxidase.

The glycogen levels of liver were decreased in the alloxan induced diabetic rats. This may be due to the impairing the normal capacity of synthesizing glycogen. The reduced glycogen levels of diabetic rats were because of the reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. The inactivation of glycogen synthase was because of the lack of sufficient insulin [13].

The increased activities of the gluconeogenic enzymes -- glucose-6-phosphatase and fructose 1, 6-biphorphatase induce the synthesis of glucose production during diabetes by the liver. The extract may be regulated these two enzymes and inhibiting the glycolysis and gluconeogenesis. The results were observed similar in the *Terminalia chebula* fed diabetic rats [14].

Studies on *Elytraria acaulis* were also revealed that the reduced liver glycogen levels of diabetic rats and were restored in the *Elytraria acaulis* treated rats [15].

The depleted protein levels were also observed in the liver of diabetic rats. This may be because of the gluconeogenesis. The protein storage was elevated in the EMHE extract treated rats.

The histological changes also supported the antidiabetic activity of the extract. The control rat's pancreas section contains the normal histology, acini and normal islets. The diabetic control observed with the atrophy. The normal islets were observed in the EMHE treated rats. This histology is comparable to the standard drug treated rats' pancreas. The regeneration of β-cells was also seen in the EMHE treated rats (Figure 1 to 5).

CONCLUSION

The present study shows that the reduced glucose levels in the EMHE treated rats. The normal levels of HDL, LDL, Cholesterol etc reveals the potential antidiabetic activity of the extract. Might be the presence of flavonoids, phenols, glycosides cause the improvement of beta cells in the pancreas of extract treated rats. However, further study is necessary to find out the active compounds to confirm the antidiabetic activity of the extract.

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REFERENCES

1. Mohammad FA, Syed Md K, Syed SG, Syed SM, Shaik RA, Shaik M A, Mohammad I. Anti diabetic activity of *Vinca rosea* extracts in alloxan induced diabetic rats. International Journal of Endocrinology, 2010; 6: 1-6.
2. Stalin C, Dinesh Kumar P, Nithyananthan K. Evaluation of antidiabetic activity of methanolic leaf extracts of *Ficus carica* in alloxan –induced diabetic rats. Asian J of Pharmaceutical and Clinical Research, 2012; 5(3): 85-87.
3. Horikoshi H, Hashimoto T, Fujiwara T. Troglitazone and emerging glitazones: new avenues of potential therapeutic benefits beyond glycemic control. Prog. Drug. Res., 2000; 54: 191- 212.
4. Kokate CK, Purohitha AP, Gokhale SB. Text Book of Pharmacognosy. 42nd edition, Nirali Prakashan, Pune, 2008; 1-13.

5. Maiti, Uttam KD, Debias G. Attenuation of hyperglycemia and hyperlipidemia in Streptozotocin induced diabetic rats by aqueous extract of seed of *Tamarindus indica*. Biological and Pharmaceutical Bulletin, 2005; 28(7): 1172-1174.
6. Chattopadhyay RR, bandyopadhyay M. Possible mechanism of hepatoprotective activity of *Azadirechta indica* leaf extract against paracetamol- induced hepatic damage in rats. Indian J Pharmacol, 2005; 37(3): 184-185.
7. Grijesh KM, Pankaj K, Mand Veeru P. Antidiabetic and hypolipidemic activity of *Gymnema sylvestre* in alloxan induced diabetic rats. Global J of Biotechnology & Biochemistry, 2009; 4 (1): 37-42.
8. Nandhakumar J. Sethumathi PP, Malina A, Sengottuvelu S, Duraisamy R, Karthikeyan D, Sivakumar T. Antidiabetic activity of methanol leaf extract of *Costus pictus* rats. J of Health Science, 2007; 53(6): 655-663.
9. Sweety L, Dabapriya G, Dheeraj A, Papiya B, Avtarchand R, Kartik CP, Sanjay KL, Murugan K. Antidiabetic activity of methanolic extracts of stem burk of *Elaeodendron glancumpers* in alloxanized rat model. Advances in Applied Science Research, 2011; 2(1): 47-62.
10. Chakrabarti S, Biswas TK, Seal T, Rokeya B, Ali K, Azadkhan AK, Nahar N, Mosihuzzaman M, Mukharjee B. Antidiabetic activity of Caesalpinia bonducella F.in chronic type -2 diabetic model in Long- Evans rats and evaluation of insulin secretegogue property of its fractions on isolated islets. J. Ethnoparmacol, 2005; 97 (1): 117-122.
11. Suvankar M, Snjib B, Monlisha B. Antidiabetic activity of Areca catechu leaf extracts against steptozotocin induced diabetic rats. J of Advanced Pharmacy Education and Research, 2012; 2(1): 10-17.
12. Felig P, Marliss E, Ohman J, Cahill JF. Plasma amino acid levels in diabetic ketoacidosis. Diabetes., 1970; 19:727-730.
13. David HP, Rangachari B, Harshith RS. Antidiabetic activity of methanol extract of *Acorus calamus* in STZ induced diabetic rats, Asian Pacific Journal of Tropical Biomedicine, 2012; S941-946.
14. Senthel Kumar GP, Arulselvan P, Sathesh Kumar D, Subramanian SP. Antidiabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. J of Health Science, 2006; 52(3): 283-291.
15. Ruby KK, Raj KB, Mohammad A. Anti hyperglycemic activity of *Elytraria acaulis* on streptozotocin induced diabetic rats. Medicine and aromatic Plants, 2012; 1(4): 1-6.