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EVALUATION OF THE ANTI-DIABETIC PROPERTIES OF DROMAIUS NOVAEHOLLANDIAE IN WISTAR RATS WITH EXPERIMENTAL DIABETES MELLITUS

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ABSTRACT
Diabetes mellitus is a chronic disease whose global spread has given it the characteristics of a pandemic. The most frequent form is Type 2 diabetes which represents more than 85% of the cases. Other forms are Type 1 (10%), specific diabetes and gestational diabetes (5%). The current work is to evaluate the beneficial effect of dromaius novaehollandiaeon fructose and Alloxan(125mg/kg.Ip./single dose) induced diabetic wistar rats. All the parameters were studied and histopathological studies also performed.

KEYWORDS
Diabetes mellitus, Dromaius novaehollandiaeon, Alloxan, Wistar rats and Histopathological studies.

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INTRODUCTION[1,2]

Diabetes mellitus is a chronic disease whose global spread has given it the characteristics of a pandemic. The most frequent form is Type 2 diabetes which represents more than 85% of the cases. Other forms are Type 1 (10%), specific diabetes and gestational diabetes (5%). For this, therapies developed along the princIp.les of western medicine (allopathic) are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. Therefore, treating diabetes mellitus with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems

highly attractive. Although, oral hypoglycemic agents and insulin is the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications. As the knowledge of heterogeneity of this disorder increases, there is a need to look for more efficacious agents with lesser side effects. Over the years, various medicinal plants and their doses have been reported to be effective in the treatment of hyperglycemia and diabetes. The hypoglycemic actions of some of these phytochemical constituents have been evaluated and confirmed in animal models suggesting that natural products could serve as a source in the search for effective antidiabetic agents. Indeed, the widely prescribed insulin-sensitizer metformin was derived from guanidine, a molecule isolated from *Galega officinalis* L. Moreover, *Trigonella foenum-graecum* L. (Fenugreek) is a plant long-consumed around the world for its anti-diabetic properties. Considering the significant importance of the antidiabetic agents from natural products against diabetes, development of new effective agents with fewer side effects is a compelling urgency. Our valley of Kashmir being a rich source of medicinal plants possesses a great potential that could be exploited for the welfare of the mankind. More than 50% of plant species described in British pharmacopoeia is reported to grow in Kashmir Valley. Kachroo and Nahvi (1976) have listed a total of 103 medicinal plant species used by early Kashmiris. Guna (2006) has reported 220 medicinal plant species, belonging to 178 genera distributed over 77 families used in Kashmir. There are still many plants which have not been paid due attention.

Chemically-induced diabetes in animals[3]

Chemically induced type I diabetes is the most commonly used animal model of diabetes. Alloxan (125mg/kg.Ip./single dose) (2, 4, 5, 6-tetraoxo hexahydro pyrimidine) was the first agent that was reported to produce permanent diabetes in laboratory animals. Streptozotocin (STZ) has replaced Alloxan (125mg/kg.Ip./single dose) as the principal agent used to produce experimental diabetes (Figure No.1).

The emu (Dromaius novaehollandiae) is a member of the ratite family of flightless birds and is the second largest bird in the world behind its African ratite cousin, the ostrich. Dromaius novaehollandiae is having a significant antidiabetic activity relatively.

MATERIALS AND METHODS

Experimental protocol[4-7]

Healthy male wistar albino wistar rats divided into five groups of five animals each (n=5) as follows:

Group 1: normal control (NC)-Fed with digital water.

Group2: diabetic control (DC)-single dose of Alloxan (125mg/kg.Ip./single dose) monohydrate (125mg/kg,i.p) and Glucose (2g/kg bw.).

Group3: diabetic control +Emu oil low dose (0.2ml/kg.,b.w., p.o.,once daily) for 15 days.

Group4: diabetic control +Emu oil high dose (0.5ml/kg., b.w., p.o., once daily) for 15 days.

Group5: diabetic control + Insulin (10IU/kg,s.c.).

The treatment of grouped animals with the standard /doses of dromaius novaehollandiae was started from 7th day of Alloxan (125mg/kg.Ip./single dose) administration and continued for the next 15 days. On the 15th day, blood samples (Approximately 0.5ml) were collected from overnight fasted wistar rats by puncturing the retro orbital sinus, under mild ether anaesthesia for biochemical estimation. Than wistar rats were sacrificed Pancreas and liver were collected and were kept in 15%v/v formalin solution for histopathological examination.

Biochemical parameters

Bio chemical parameters were analyzed by using auto bio chemistry analyzer manufactured by Robonik Pvt.Ltd, Mumbai.

All the results including biochemical parameters are tabulated in the results.

RESULTS

The Emu oil, which is most preferred in this invention, has the following chemical analysis tabulated in Table No.1. All results are showed in Table No.2-7 and Figure No.1-5.

Histopathological analysis

Histological examination of pancreas showed normal histology in normal wistar rats and wistar rats treated with PC ectract. Pancreas in diabetic wistar rats showed shrinkage of islets and growth of adIp.ose

tissue. Pancreas in diabetic wistar rats treated with Dromaius noviehollandiet(0.2ml/kg,0.5ml/kg)and Insulin showed decreas shrinkage of islets and growth of adipose tissue. The resultant figures are of following:-

Effect of Dromaius novaehollandiae (0.2ml/kg0.5ml/kg;day/15days) on Alloxan (125mg / kg. Ip. / Single dose) (125mg/ kg. Ip./single dose) (125mg/kg. Ip./single dose) treated wistar rats on pancreatic histology after 15 days of treatment (Figure No.6 and 7).

Table No.1: Chemical analysis

S.No	Free Fatty Acid	0.33 - 0.02%
1	Acid Value	0.66%
2	Calculated Iodine value	69.7 - 72.8 m Eq/100g
3	OSI	11.95 Hours@110.0°C.

Table No.2: Where in the fatty acid composition of the Emu oil can be compared to human skin as follows

S.No	Fatty Acid	Ratio	Emu Oil	Human Skin Oil
1	Myristic	C:14:0	0.3%	2.1%
2	Palmitic	C:16:0	20.3%	20.2%
3	Palmitoleic	C:16:1	3.2%	3.8%
4	Margaric	C:17:0	0.2%	-
5	Margaric oleic	C:17:1	0.1%%	-
6	Stearic	C:18:0	10.1%	11.2%
7	Oleic	C:18:1	51.6%	30.8%
8	Linoleic	C:18:2	13.1 %	15.1%
9	Linolenic	C:18:3	0.5%	0.3%
10	Arachidic	C:20:0	0.1%	-
11	Eicosinoac	C:20:1	0.5%	-

Table No.3: Acute toxicity study of Emu oil in wistar rats

S.No	Drug treatment	Dose	Weight of animal in gms Group		Signs of toxicity	Onset of toxicity	Death
		ml/kg	Before treatment (1st day)	After treatment (14th day)			
1	Emu oil	1ml/kg	161	172	No signs of toxicity	Nil	Nil
2	Emu oil	5ml/kg	172	180	No signs of toxicity	Nil	Nil
3	Emu oil	10ml/kg	184	190	No signs of toxicity	Nil	Nil
4	Emu oil	50ml/Kg	171	178	No signs of toxicity	Nil	Nil
5	Emu oil	100ml/Kg	165	171	No signs of toxicity	Nil	Nil

Table No.4: Effect of oil doses of Dromaius novaehollandiae on glucose tolerance test in wistar rats

S.No	Groups	Initial	1st Day	5th Day	10th Day	15th Day
1	Normal	73.38±0.94	71.50±0.92	73.66±0.56	74.26±0.62	77.34±0.85
2	Diabetic	74.20±0.80	264.78±0.94	278.88±0.74	286.56±1.22	300.86±0.77
3	Emu oil (0.2 ml/kg)	73.46±0.33	245.34±1.07	190.20±0.67	174.04±0.58	125.18±0.82
4	Emu oil (0.5 ml/kg)	74.28±0.56	254.78±0.71	171.10±0.48	106.70±0.50	98.18±0.58
5	Insulin (10 IU /kg)	72.28±0.71	244.70±0.85	144.54±0.68	90.24±0.51	72.82±0.56
6	Groups	Initial	1st Day	5th Day	10th Day	15th Day
7	Normal	73.38±0.94	71.50±0.92	73.66±0.56	74.26±0.62	77.34±0.85
8	Diabetic	74.20±0.80	264.78±0.94	278.88±0.74	286.56±1.22	300.86±0.77
9	Emu oil (0.2 ml/kg)	73.46±0.33	245.34±1.07	190.20±0.67	174.04±0.58	125.18±0.82
10	Emu oil (0.5 ml/kg)	74.28±0.56	254.78±0.71	171.10±0.48	106.70±0.50	98.18±0.58
11	Insulin (10 IU /kg)	72.28±0.71	244.70±0.85	144.54±0.68	90.24±0.51	72.82±0.56

Data was analyzed by using two way ANOVA and Tukey's t test.

Table No.5: Effect of Emu Oil on Glucose Levels of Alloxan Induced Diabetes in Wistar Rats

S.No	Blood glucose levels(mg/dl)				
	Groups	**Initial**	**30min**	**90min**	**150min**
1	Control	67.60±0.50	65.80±0.37	70.80±0.37	68.60±0.50
2	Disease	68.60±0.50	111.2±0.58	121.0±0.44	130.4±0.50
3	Emu oil (0.2 ml/kg)	69.20±0.37	88.40±0.50	77.00±0.70	70.20±0.37
4	Emu oil (0.5ml /kg)	68.20±0.37	65.20±0.37	69.80±0.37	67.20±0.37
5	Insulin(10 IU/kg)	68.20±0.37	66.20±0.37	70.80±0.37	68.20±0.37

Data was analyzed by using two way ANOVA and Tukey's t test Values are expressed in mean ± S.E.M.P<0.0001 when compared to diabetic group (by using graph pad prism 6.02 version).

Table No.6: Effect of Emu Oil on Lipid Parameters of Alloxan Induced Diabetes in Wistar Rats

S.No	Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	H.D.L. (mg/dl)	L.D.L. (mg/dl)	V.L.D.L. (mg/dl)
1	Normal	122.62±2.70	107.34± 2.84	53.36± 2.15	51.58± 1.87	31.84± 2.83
2	Diabetic	168.52± 1.22	150.36± 0.56	35.70± 2.11	99.98±0.70	64.08± 2.73
3	Emu Oil Low (0.2 ml/kg)	149.78± 0.56	132.08± 0.97	45.58± 0.89	67.64± 0.93	39.18± 1.29
4	Emu Oil High (0.5 ml/kg)	139.06± 2.88	116.58± 2.77	77.36± 0.86	46.46± 1.32	21.42±1.05
5	Insulin (10IU/kg)	125.02± 1.90	103.30± 1.21	84.10± 1.66	35.96± 1.97	15.90± 1.08

Data was analyzed by using one way ANOVA and Turkey's t test Values are expressed in mean±S.E.M.P<0.0001 when compared to diabetic group (by using graphpad prism 6.02 version).

Table No.7: Mean ± SEM Values for Biochemical Parameters

S.No	Estimations/Groups	SOD(pg/ml)	MDA(ng/ml)	GSH(µM/ml)	TPC(mg/dL)
1	Water	93.75 ±0.641*	6.38 ±0.0478*	56.06 ±0.313*	14.52±0.047*
2	Alloxan(125mg/kg.Ip./single dose)	72.54 ±0.277	9.306±0.1156	30.813±0.279	10.63±0.407
3	Dromaius novaehollandiae (0.2ml/kg)+Alloxan (125mg/ k.Ip./single dose)	79.67 ±0.407*	8.522±0.0764*	43.443±0.343*	11.78±0.641*
4	Dromaius novaehollandiae (0.5ml/kg)+ Alloxan(125mg/kg.Ip./single dose) (125mg/kg.Ip./single dose) (1mg/kg)	88.30±0.340*	8.30±0.0256*	49.312±0.452*	13.188±0.40*
5	Insulin+Alloxan(125mg/kg.Ip./single dose)	91.26±0.551*	7.309±0.0728*	57.565±0.330*	15.21±0.025*

Values are mean±SEM of four samples of six observations. Statistical significant test for Comparison was done by ANOVA, followed Dennett's test. For Comparison between Alloxan (125mg/kg.Ip./single dose) treated and others *p<0.01 significant.

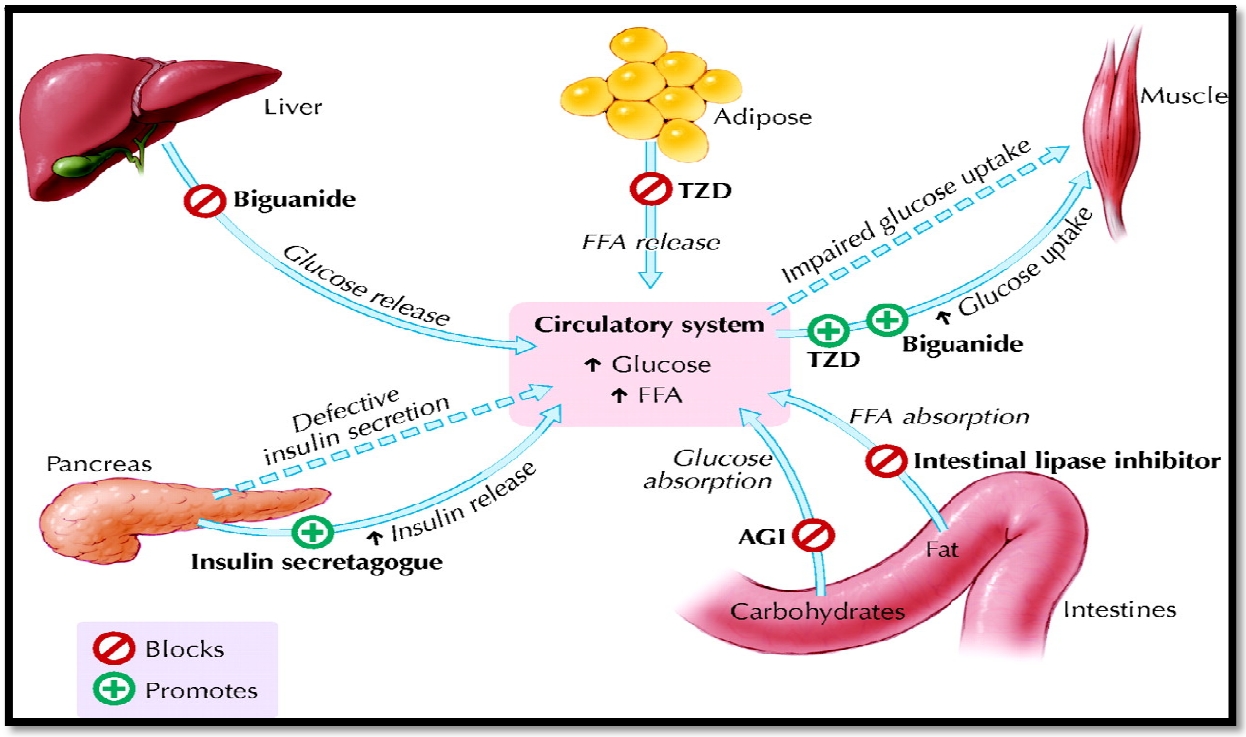


Figure No.1: Schematic representation of the mechanism of pancreatic β-cell destruction by streptozotocin

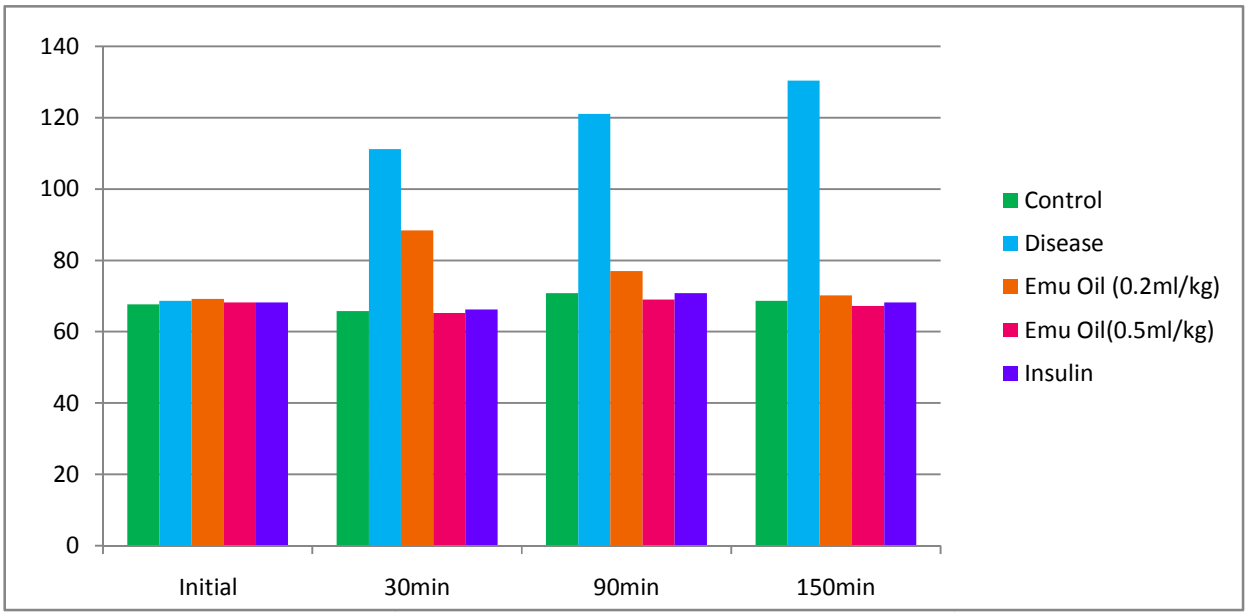


Figure No.2: Effect of oil doses of Dromaius novaehollandiae on glucose tolerance test in wistar rats On X-axis lipid parameters, On Y-axis mean values in mg/dl

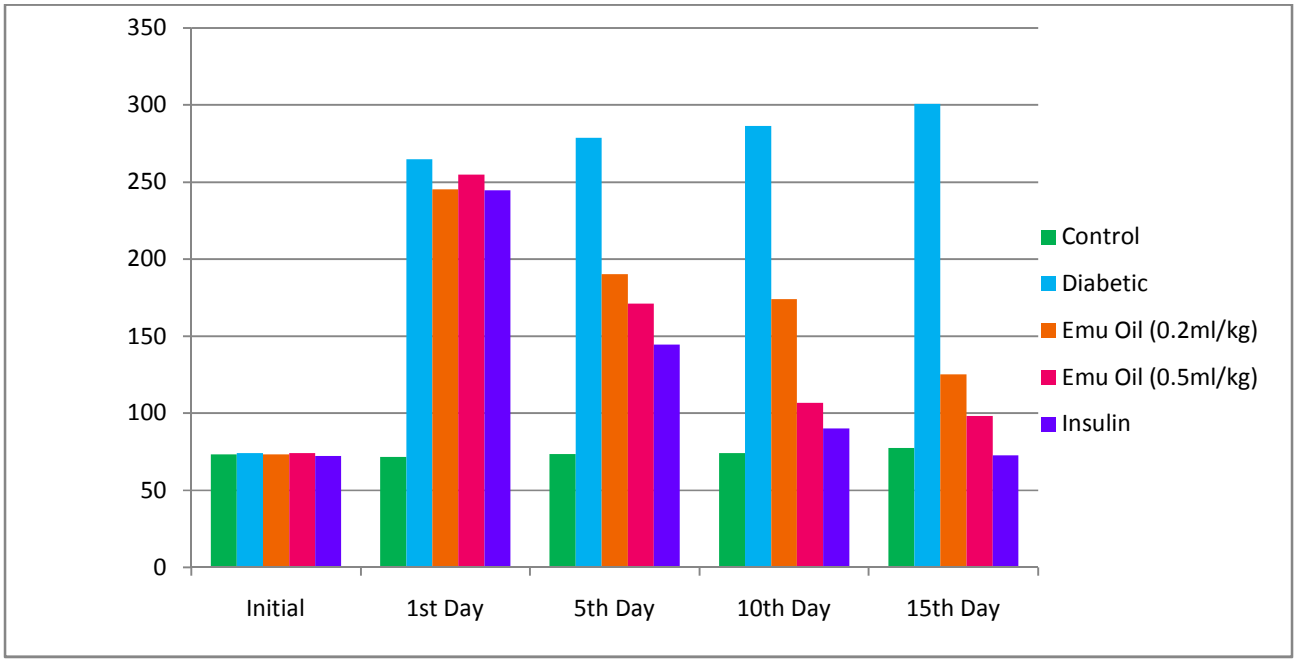


Figure No.3: Effect of Emu Oil on Glucose Levels of Alloxan Induced Diabetes in Wistar Rats On X-axis days, On Y-Axis mean values in mg/dl

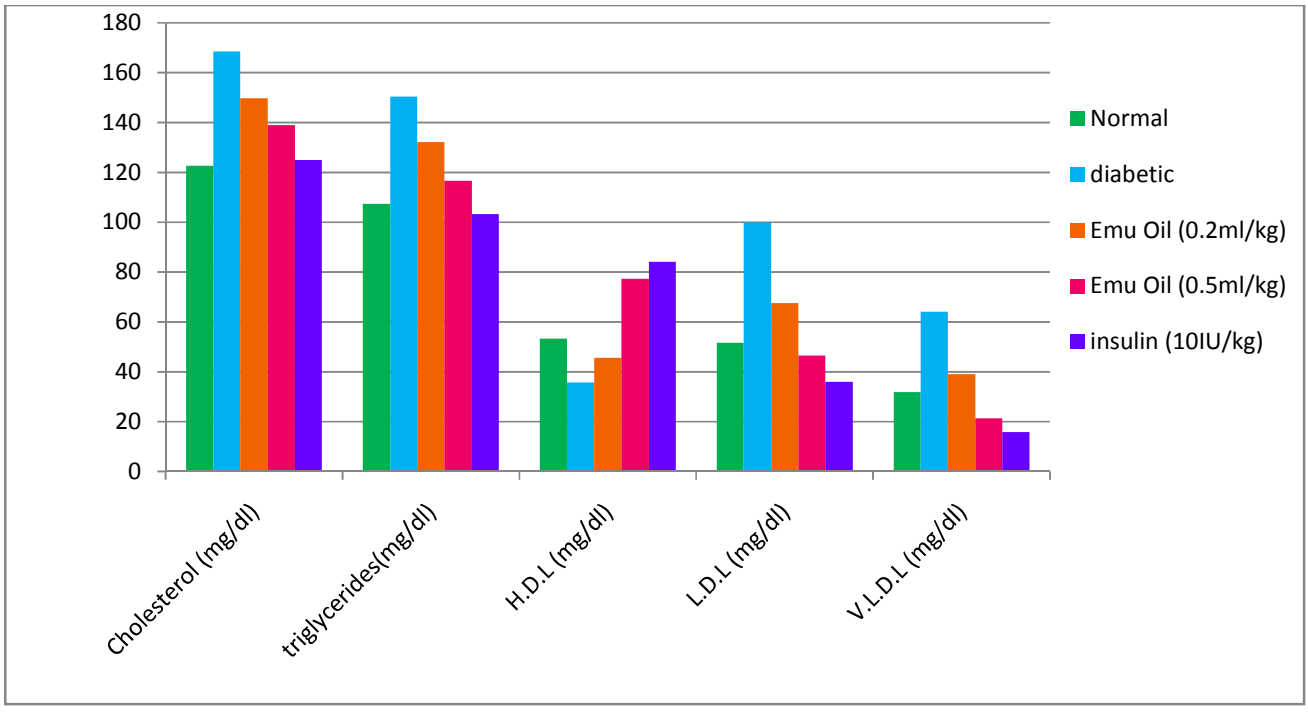


Figure No.4: Effect of Emu Oil on Lipid Parameters of Alloxan induced Diabetes in Wistar Rats On X-axis Lipid parameters, On Y-axis mean values in mg/dl

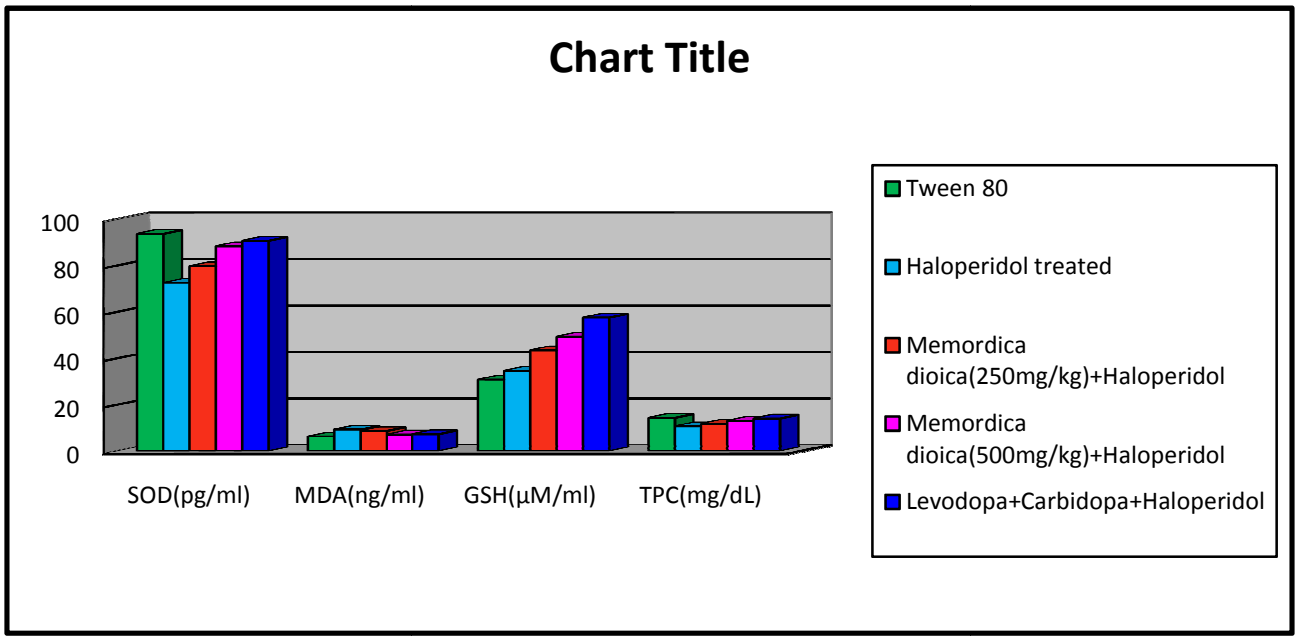
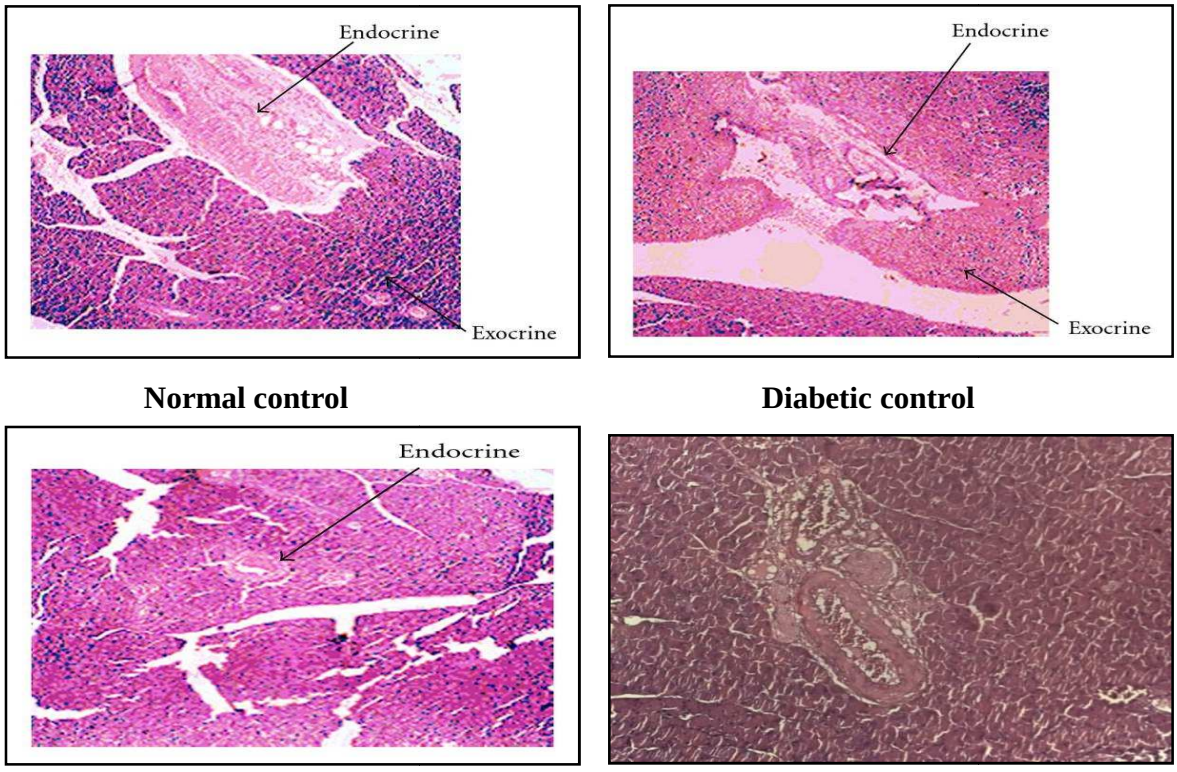


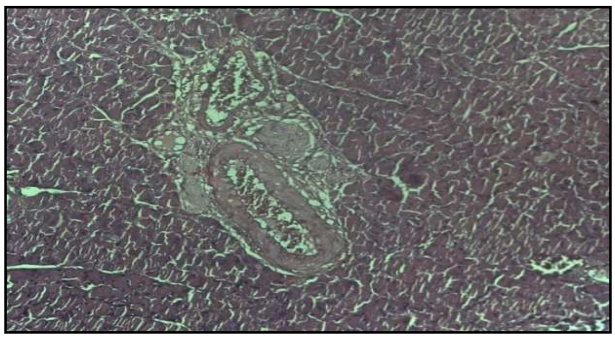
Figure No.5: Comparison chart



Normal control **Diabetic control**

Standard **EMU oil 0.2ml/kg**

Figure No.6: Histopathological studies



Emu oil high dose

Figure No.7: Histopathological studies

CONCLUSION

From this study, we can conclude that the Dromaius novaehollandiae (0.5ml/kg) have beneficial effects on blood glucose levels. However, further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research. Hence there should be a need o carryout the research on the mechanism of anti diabetic activity of Dromaius novaehollandiae in order to overcome the future needs of effective antidiabetic treatment.

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