Effects of Erythromycin on Beta Cells of Pancreas

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Summary

The study was formulated on 56 albino rats divided into one control and two experimental groups, for the observation of the effects of Erythromycin (2 Gm/70Kg/day for 28 days and 3 Gm/70Kg/day for 28 days) on the beta cells of islets of Langerhans of Pancreas. Group I–A and II–A animals were studied for immediate effects of treatment, while group I–B and II–B animals were studied for the delayed effects after the cessation of the drug. The Erythromycin's activity on beta cells appears to be a stimulating one for the production and release of insulin, but does not prove to be harmful as it did not cause any appreciable damage which could have been demonstrated histologically. It however caused a drop in the blood sugar level which reverted after the cessation of the treatment, indicating that permanent damage did not occur. Observing its stimulating effect on the beta cells, in our opinion, due precautionary measures should be taken for its use along with the drugs which lower the blood sugar level by beta cell stimulation, as Erythromycin may enhance their effects.

Introduction

Erythromycin, a macrolid antibiotic was discovered in 1952. The antibacterial activity of Erythromycin has been studied extensively[10,15,16,17,20] but its effects on the host tissues have not drawn much attention of the workers except a few, who described its toxicity on some of the organs like liver,[3,4,12,18] thyroid[11] and enamel tissue[1,2] and also grouped it among ototoxic antibiotics.[8] Some authors have placed it among the protein-synthesis inhibitors[15,17,19] but its mode of action has not been confirmed yet.[4,5,6,7,13,14,21] Others have placed it

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among the group which effects the intermediary carbohydrate metabolism.[9] Whether this drug interferes in protein synthesis and/or carbohydrate metabolism, this study has been formulated on the beta cells of Islets of Langerhans of Pancreas as target tissue.

Material and Methods

Fifty six male and female albino rats were divided into three groups, one control, consisting of 12 rats and two experimental groups I and II containing 22 rats in each group. The groups were further sub-divided into A and B sub-groups. The experimental sub-group A animals were studied for the immediate effects of Erythromycin and the experimental sub-group B animals were studied for the delayed effects of the drug. The group I animals were injected 2 Gm/70Kg/day Erythromycin and the group II animals were injected 3 Gm/70Kg/day Erythromycin. The animals among the sub-group I–A and II–A had been sacrificed on alternate days, while animals of sub-group I–B and II–B had been sacrificed after the cessation of the drug for the study of the delayed effects of treatment. The tissues were processed, sectioned and stained by H & E Aldehyde Fuchsin and Gomori's Chrom-Alum-Haematoxylin stain for visualization of the islets and beta cells in particular. The blood sugar level had been determined by Orthotoluidine method.

Results

In the control group, the islets appear as more or less spherical masses of pale staining cells, when stained with H & E stain, arranged in the form of irregular anastomosing cords; between the cords are numerous blood capillaries, the endothelial walls of which appear to be closely applied to the adjacent cell cords. The size of the islets is variable. Some are having only a few cells while others contain a large number of them and are therefore quite large. The number of islets varies in different portions of the gland.

The beta cells make up the greater part of the islet mass. These cells are arranged in cords in which the individual cell boundaries are difficult to see. The granules appear as fine dots between the rounded or ovoid nucleus and the cell wall towards the periphery.

With the differential staining techniques, the beta cells were distinctly visualized. They appeared purple with the Aldehyde-Fuchsin stain and blue with Gomori's Chrom-Alum Haematoxylin stain.

In the Experimental group I–A, where Erythromycin had been injected at a calculated dose of 2G/70Kg day, the histological changes were not very

marked. After the first week, the only effect noted was a comparative degranulation without any other conspicuous change. After the second week, there was no remarkable change; but after the third week, the cells were looking a bit more empty, however, complete degranulation did not occur. No other significant change was observed. In few islets the hyalinization was also visible.

In the Experimental group II–A, where Erythromycin had been injected at a calculated dose of 3G/70Kg/day, the histological changes were similar to those of the Experimental group I–A. The degranulation was observed but it was not to the degree of completeness. Occasional hyalinization was also noted in some of the islets.

In the Experimental group I–B, where the animals were given 2G/70Kg/day for four weeks and then the drug was withdrawn and the sacrifice was done after every week, the cells showed almost the normal histology with gradual increase in their granular store.

In the Experimental group II–B, where the dose was 3G/70Kg/day for four weeks and then withdrawn and the sacrifice was done as for group I–B, the cells showed similar recuperation but with slight delay.

Blood Sugar Level

The blood sugar level of the control group, as determined by the Orthotoluidine method, remained within the range of 162.5 ± 5.318 for group A (Table I) and 166.0 ± 5.71 for the group B (Table II), which is taken as the reference level for our experimental work (Fig. 1).

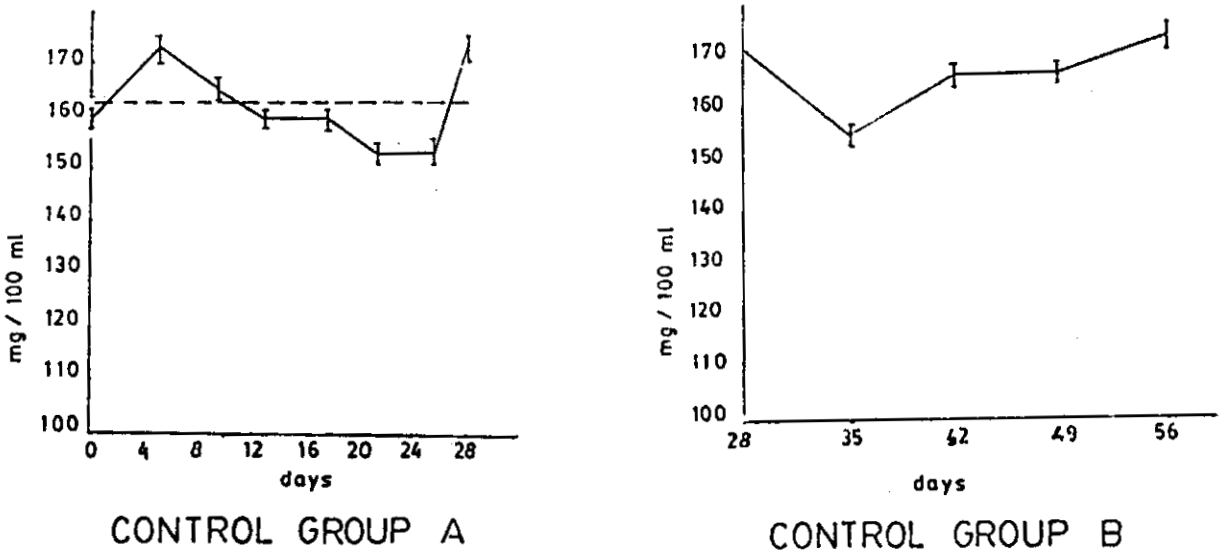

Fig. 1. Blood Sugar Level

TABLE I

BLOOD SUGAR LEVEL
CONTROL GROUP–A

No.	Observations (x)	Deviations $(x-\bar{x})$	Squared Deviations	Calculation of S.D.
1.	160	— 2.5	6.25	$(x-\bar{x})^2 = 396$
2.	173	10.5	110.25	$S.D. = \sqrt{\Sigma(x-\bar{x})^2/(n-1)}$
3.	166	3.5	12.25	$= \sqrt{396/7}$
4.	160	— 2.5	6.25	$= \sqrt{56.571429}$
5.	160	— 2.5	6.25	$= 7.521398$
6.	154	— 8.5	72.25	$S.E. = S.D./\sqrt{n}$
7.	154	— 8.5	72.25	$= 7.521398/\sqrt{8}$
8.	173	10.5	110.25	$= 7.521398/2.828$
				$= 2.6592158$
Total	1300	0.0	396.00	Range $= \bar{x} \pm 2e$
	$\bar{x} = \frac{1300}{8}$			$= 162.5 \pm 2\,(2.659)$
	$= 162.5$			$= 162.5 \pm 5.31843$

The Experimental group I–A animals have shown the effects of 2Gm/70Kg/day of Erythromycin. The drug had produced an appreciable change on the blood sugar level which had dropped gradually day by day as the treatment continued. The mean value for the group I–A animals came to be 130.482, within the range of 130.482 ± 5.88 (Table III). It is very clearly indicated by the graphic record (Fig. 2, 3) that as the days passed by, the blood sugar level showed the progressive fall with an occasional fluctuation.

After the cessation of the drug, as in the case of Experimental group I–B, where the course of the treatment lasted for four weeks, the blood sugar returned to the reference level in a month time, with the mean value of 154.5 and remained within the range of 154.5 ± 11.03 (Table IV).

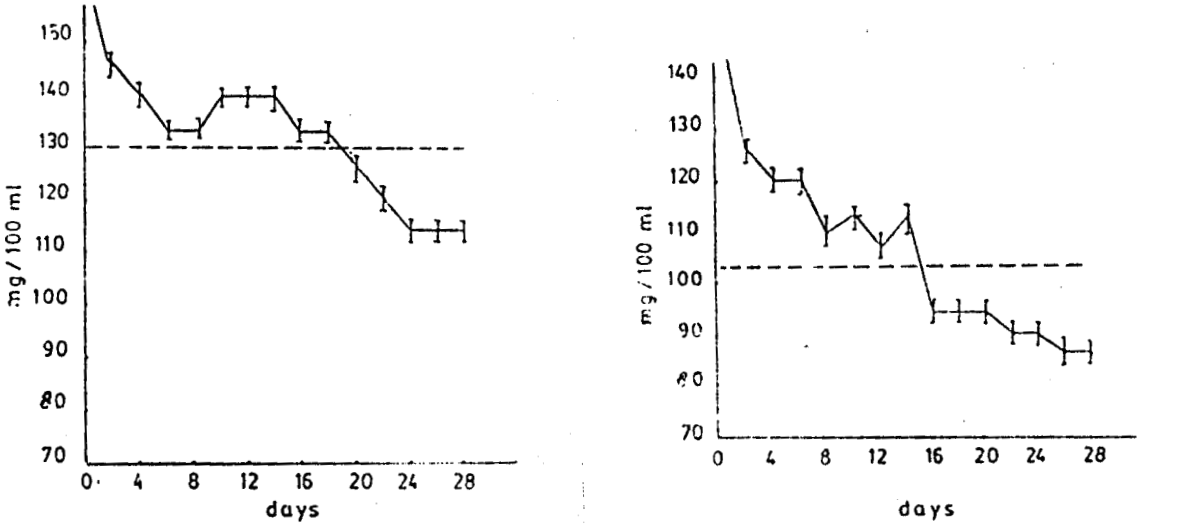

Fig. 2. Blood Sugar Level

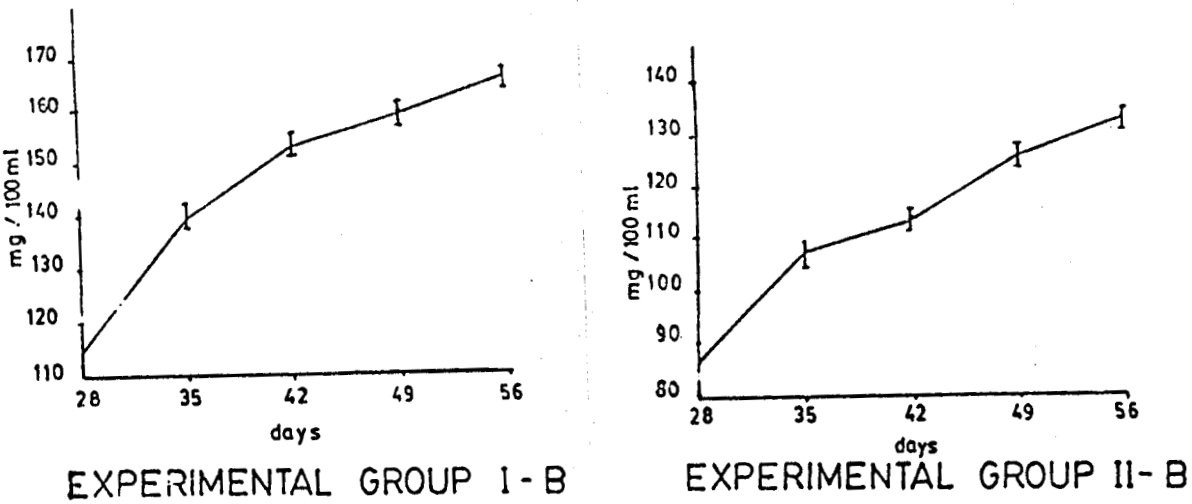

Fig. 3. Blood Sugar Level

The Experimental group II–A animals had shown the effects of 3 Gm/ 70Kg/day of Erythromycin. The drug had produced a remarkable effect on the blood sugar level which dropped steadily. The mean value for the group came to be 103.57 with the range being 103.57 ± 7.579 (Table V). The graph depicts the situation very clearly (Fig. 2, 3).

After the cessation of the drug, as in the case of the Experimental group II–B, where the treatment lasted for four weeks, the blood sugar tended to return to the reference level but could not reach to it after four weeks. The mean value came to be 119.75 with the range being 119.75 ± 11.87 (Table VI).

The significance test, as shown in Tables VII and VIII indicated that the effect was specifically due to use of the drug and not merely due to chance.

TABLE II
BLOOD SUGAR LEVEL
CONTROL GROUP–B

No.	Observations	Deviations $(x-\bar{x})$	Squared Deviations	Calculation of S.D.
1.	159	— 7	49	$\Sigma(x-\bar{x})^2 = 98$
2.	166	0	0	S.D. $= \sqrt{\Sigma(x-\bar{x})^2/n-1}$
3.	166	0	0	$= \sqrt{98/3}$
4.	173	7	49	$= \sqrt{32.67}$
				$= 5.71$
Total	664	0.0	98	S.E. $= S.D./\sqrt{n}$
	$\bar{x} = \frac{664}{4}$			$= 5.71/\sqrt{4}$
				$= 5.71/2$
	$= 166$			$= 2.857738$
				Range $= \bar{x} \pm 2e$
				$= 166 \pm 2(2.857)$
				$= 166 \pm 5.71$

Discussion

The Erythromycin's activity on beta cells has never been studied before, as there is no record available in the literature. This experiment had been postulated to study the Erythromycin's activity on the beta cells by a direct histological demonstration and an indirect study through the blood sugar level determination. As this work is first of its kind, therefore, the question of comparison of the results that are produced in our experiment does not arise.

Keeping the blood sugar estimation as a parameter, it became evident that the blood sugar had been affected by the release of excess amount of Insulin into the circulation, resulting in the lowering of the blood sugar level. The rate of Insulin release by the pancreatic beta cells is controlled mainly by the glucose concentration of the fluid that bathes the beta cells. High blood sugar stimulates, whereas low blood sugar depresses it.

The partial depletion of the granules with-in the beta cells, observed histologically, might have occured as a result of the mechanisms discussed below :-

TABLE III
BLOOD SUGAR LEVEL
EXPERIMENTAL GROUP I-A

No.	Observations	Deviations $(x-\bar{x})$	Squared Deviations	Calculation of S.D.
1.	146	15.57	242.42	$(x-\bar{x})^2 = 1573.36$
2.	140	9.57	91.58	S.D. $= \sqrt{\Sigma(x-\bar{x})^2/(n-1)}$
3.	133	2.57	6.60	$= \sqrt{1573.36/13}$
4.	133	2.57	6.60	$= \sqrt{121.02769}$
5.	140	9.57	91.58	$= 11.001259$
6.	140	9.57	91.58	S.E. $= \frac{11.001259}{3.7416574}$
7.	140	9.57	91.58	$= 2.9402101$
8.	133	2.57	6.60	Range $= x \pm 2e$
9.	133	2.57	6.60	$= 130.428 \pm$
10.	126	— 4.43	19.62	$2(2.94021101)$
11.	120	—10.43	108.78	$= 130.428 \pm$
12.	114	—16.43	269.94	5.8804201
13.	114	—16.43	269.94	$= 136.30842$
14.	114	—16.43	269.94	$— 124.54758$
Total	1826	0.0	1573.36	
	$\bar{x} = \frac{1826}{14}$			
	$= 130.428$			

1. The drug, Erythromycin, might have directly stimulated the beta cells and caused the release of Insulin. The drug might have maintained the situation by a constant direct effect for the continuous synthesis of Insulin and its release into the circulation, resulting in lowering of the blood sugar during the entire period of treatment.

2. The drug might have effected the beta cells indirectly through alteration in the glucose level of the blood, tending to increase the blood sugar by inhibiting the peripheral utilisation of glucose by tissues viz. muscles as was reported earlier by Gershbein[9] (1966) in an experimental study when he showed that in the presence of Erythromycin, the glucose uptake by isolated rat diaphragm muscle

TABLE IV
BLOOD SUGAR LEVEL
EXPERIMENTAL GROUP I-B

No.	Observations	Deviations $(x-\bar{x})$	Squared Deviations	Calculation of S.D.
1.	140	— 14.5	210.25	$(x-\bar{x})^2 = 365$
2.	153	— 1.5	2.25	S.D. $= \sqrt{(x-\bar{x})^2/n-1}$
3.	159	4.5	20.25	$= \sqrt{365/3}$
4.	166	11.5	132.25	$= \sqrt{121.667}$
				= 11.030261
Total	618	0.0	365	S.E. = S.D./$\sqrt{n}$
	$x = 618/4$			$= 11.030261/\sqrt{4}$
				= 11.030261/2
	= 154.5			= 5.515
				Range $= \bar{x} \pm 2e$
				= 154.5 ±2(5.515)
				= 154.5 ± 11.03

showed decrease of a low order and the muscle glycogen was markedly depressed.[11] The resulting inhibition of peripheral utilisation of glucose then directly increases the blood sugar level, increasing the glucose in the tissue fluid bathing the beta cells. As a result, the beta cells are stimulated for continuous synthesis of Insulin and its release into the circulation, tending to normalize the blood sugar level.

3. In this experimental study, probably both the mechanisms might have been operative simultaneously resulting in a continuous fall in the sugar level graph throughout the period of treatment.

After the discontinuation of the drug, the blood sugar level returned to the reference level after about a period of four weeks in those animals treated with the low doses and took a little more time for those animals treated with a higher dose. It is thus evident that the possible mechanisms discussed above had been rectified and the animals had recovered from the transient phase of the cell stimulation.

TABLE V
BLOOD SUGAR LEVEL
EXPERIMENTAL GROUP II-A

No.	Observations	Deviations $(x-\bar{x})$	Squared Deviations	Calculation of S.D.
1.	126	22.43	503.10	$\sum(x-\bar{x})^2 = 2613.92$
2.	120	16.43	269.94	S.D. $= \sqrt{\sum(x-\bar{x})^2/n-1}$
3.	120	16.43	269.94	$= \sqrt{2613.92/13}$
4.	110	6.43	41.34	$= \sqrt{201.07077}$
5.	114	10.43	108.78	= 14.179942
6.	106	2.43	5.90	S.E. = S.D./$\sqrt{n}$
7.	114	10.43	108.78	$= \frac{14.179942}{3.7416574}$
8.	96	— 7.57	57.30	Range = x ± 2e
9.	96	— 7.57	57.30	= Range = x ± 2e
10.	96	— 7.57	57.30	= 103.57 ± 2e
11.	90	—13.57	184.14	= 103.57 ±
12.	90	—13.57	184.14	2(3.789749)
13.	86	—19.57	382.98	= 103.57 ±
14.	86	—19.57	382.98	7.5794981
Total	1450	0.0	2613.92	= 111.1499
	$\bar{x} = \frac{1450}{14}$			— 95.990502
	= 103.57			

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TABLE VI

BLOOD SUGAR LEVEL
EXPERIMENTAL GROUP II-B

No.	Observations	Deviations $(x-\bar{x})$	Squared Deviations	Calculation of S.D.
1.	107	— 12.75	162.5625	$(x-\bar{x})^2$ 422.75
2.	113	— 6.75	45.5625	S.D. $= \sqrt{(x-\bar{x})^2/n-1}$
3.	126	6.25	39.0625	$= \sqrt{422.75/3}$
4.	133	13.25	175.5625	$= \sqrt{140.91667}$
				$= 11.870833$
Total	479	0.0	422.75	S.E. $= S.D./\sqrt{n}$
	$\bar{x} = 479/4$			$= 11.870833/\sqrt{4}$
	$= 119.75$			$= 11.870833/2$
				$= 5.9354163$
				Range $= \bar{x} + 2e$
				$= 119.75 \pm 2(5.935)$
				$= 119.75 \pm 11.87$

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TABLE VII
SIGNIFICANCE TEST
EXPERIMENTAL GROUP I

No.	Blood sugar in mg/100 ml		$(a-\bar{a})^2$	$(b-\bar{b})^2$	Working
	Control	Experimental			
1.	160	146	6.25	242.42	$t = \frac{(\bar{a}-\bar{b})}{(e_a)^2 + (e_b)^2}$
2.	173	140	110.25	91.58	
3.	166	133	12.25	6.60	
4.	160	133	6.25	6.60	$= \frac{32.072}{3.96}$
5.	160	140	6.25	91.58	$= 8.092$
6.	154	140	72.25	91.58	
7.	154	140	72.25	91.58	
8.	173	133	110.25	6.60	
9.		133		6.60	
10.		126		19.62	
11.		120		108.78	
12.		114		269.94	
13.		114		269.94	
14.		114		269.94	
Total	1300	1826	396.00	1573.36	
	$a = \frac{1300}{8}$	$b = \frac{1826}{14}$	$e_a = \pm 2.66$	$e_b = \pm 2.94$	
	$= 162.5$	$= 130.428$	$(e_a)^2 = 7.07$	$(e_b)^2 = 8.64$	
	$(\bar{a}-\bar{b}) = 32.072$				

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TABLE VIII

SIGNIFICANCE TEST
EXPERIMENTAL GROUP II

No.	Blood sugar in mg/100 ml		$(a-\bar{a})^2$	$(b-\bar{b})^2$	Working
	Control	Experimental			
1.	160	126	6.25	503.10	$t = \frac{(\bar{a}-\bar{b})}{(e_a)^2 + (e_b)^2}$
2.	173	120	110.25	269.94	
3.	166	120	12.25	269.94	
4.	160	110	6.25	41.34	$= \frac{58.93}{4.63}$
5.	160	114	6.25	108.78	$= 12.728129$
6.	154	106	72.25	5.90	
7.	154	114	72.25	108.78	
8.	173	96	110.25	57.30	
9.		96		57.30	
10.		96		57.30	
11.		90		184.14	
12.		90		184.14	
13.		86		382.98	
14.		86		382.98	
Total	1300	1450	396.00	2613.92	
	$a = \frac{1300}{8}$	$b = \frac{1450}{14}$	$e_a = \pm 2.66$	$e_b = \pm 3.79$	
	$= 162.5$	$b = 103.57$	$(e_a)^2 = 7.076$	$(e_b)^2 = 14.36$	
	$(\bar{a}-\bar{b}) = 58.93$				

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