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DEMONSTRATION OF ANTI-DIABETIC ACTIVITIES OF ALLOXAN IN POTENTISED DILUENT STATE—AN EXPERIMENTAL APPROACH

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Abstract

Dynamised and undynamised preparations of Alloxan viz. 6x, 30x, and 200x were examined for its anti-diabetic activities in Alloxan–induced diabetes mellitus albino rats. Oral administration of dynamised potencies of Alloxan 6x, 30x and 200x at a dose level of 50μ/100gm. b.w. daily for 30 days regularly exhibited slow and steady fall in blood sugar level i.e. $p<0.01$ (less significant) and $p<0.001$ (significant) respectively when compared to dynamised and undynamised control groups as well as undynamised Alloxan fed groups under identical conditions. Histological and histomorphometric studies also revealed that ß-cell counts were functional to 30-40% population and protects the ß-cells against necrotic effect especially in dynamised dilution of Alloxan in 30x and 200x potencies. It was noticed that the dynamised dilutions of alcohol fed control group is more toxic and lethal to animals than dynamised and undynamised dilutions of Alloxan and undynamised alcohol fed control groups. Furthermore, it was also discernible that blood sugar levels were stabilised mildly on withdrawal of dynamised test drug in its 30x and 200x potencies.

These observations clearly indicate that mechanical potentisation decreases the material quantity of solute while potentising the energy supply by agitation/vigorous shock, activates the solvent system/diluent medium to acquire and mimic the chemical specificity of original drug molecules of Alloxan and then act as Therapeutic agent. The present probe confirms the Homoeopathic principle of "SIMILIA SIMILIBUS CURENTUR" in having therapeutic as an anti-diabetic agent in dynamised dilutions of 30x and 200x of Alloxan in diabetised rats and also demonstrates the phenomenon of minimum dose. Further probe in this area would be rewarding in order to locate the mechanism of action of Homoeopathic dilution beyond Avogadro's number.

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Introduction

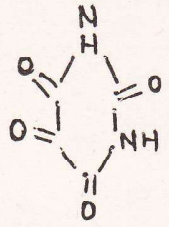
Diabetes is not a disease but includes a variety of related disorders of metabolism, having in common, an increase in blood sugar, usually accompanied by glycosuria. In many of these there is also a greater and lesser tendency to ketosis which is an important immediate danger and an increased liability to various forms of vascular degeneration i.e. a long-term risk.

The management of Diabetes mellitus by replacement therapy with Insulin and oral anti-diabetic drugs has revolutionised the concept of disease. However, the use of these drugs during the last three decades has exposed more intricate problems. The problems of insulin resistance, insulin insensitivity and insulin antibodies are intriguing. In view of these findings it has been conceivable that besides the existing anti-diabetic drugs, other modalities might offer more rational approach (Mukherjee et al 1979 a).

Homoeopathic medicines are prepared by successive reduction of (1:100) of the material quantities of the medicine (solute) in a solution with vigorous shaking/agitation at every stage. This procedure is termed as potentisaion. The solvents normally used are water, ethyl alcohol (liquids), sucrose and lactose (solids). Beyond 12th potency (n) the presence of solute is 10^{-24} parts in 1 part of the solution. According to Avogadro's hypothesis, there are 6.03×10^{23} molecules in a gram molecule of any substance. Hence, it is quite evident that physically there is no existence of solute in a solution,

beyond this potency (high dilution). Despite this Homoeopathic potencies/preparations are therapeutically active even for n»1000. The centesimal potencies of 30,200, 1M and above upto CM, MM are frequently employed in Homoeopathic practice.

The present experiment was designed with a view to locate the therapeutic efficacy of dynamised and undynamised dilutions of Alloxan, a chemical, its commercial name is 2,4,5,6 (1H, 3H) pyrimidinetetrone: –2;4,5,6 tetraoxohexahydropyridine, Mesoxalyurea of molecular formula $C_4H_2N_2O_4$ with molecular weight 142.07. It is represented diagrammatically, as,



It is chiefly used in its ability to produce diabetes mellitus in experimental animals.

Keeping in view the Homoeopathic principle of "Similia Similibus Curentur", an attempt has been made to discern the curative characteristics/therapeutic potentiality of potentised diluent medium of Alloxan in its 6x, 30x, and 200x potencies, with special reference to biological aspects and phenomenon of minimum dose.

Materials and Method

To study the hypoglycaemic activity of dynamised and undynamised drugs, vehicle and normal saline, albino rats of either sex weighing 230 ± 25gms. were acclimatised to standard laboratory conditions for 15 days. Water was allowed ad-libitum. Photo-period L/D (10 light hours/14 dark hours) was also maintained. The acclimatised animals were subjected for quantitative analysis of blood sugar estimations adopting the Folin-Wu method, by taking 0.5ml. blood sample from the tail vein or through cardio-puncture and measuring absorbance at 620 nm wavelength in a Beckmann model 35 spectrophotometer.

Diabetes mellitus was induced in the albino rats whose blood sugar level were within 80-120mg/dl. through intraperitoneal injections. Three doses of 10-12 mg/gm. b.w., at 7 days interval of Alloxan dissolved in distilled water, were administered after 12 hours fasting. Blood sugar estimations were done to confirm the establishment of diabetes mellitus. The diabetised animals were divided into following groups for in-vivo and in-vitro studies. Each group consisted of 10 diabetised animals for experimental analysis. The long term experiment was conducted over 45 days but the drug was administered for the first 30 days once in a day to each animal. After that the drug, vehicle, saline administration was stopped and the animals were assessed for blood sugar stabilization.

The potentised form of Alloxan, 6x, 30x and 200x as well as equivalent concentration of vehicle i.e. 90% v/v alcohol, dynamised and undynamised preparations were made as per formulations of Homoeopathic Pharmacopoeia Laboratory, Ghaziabad.

The blood sugar estimation was done at 12 hours fasting on the first 15th and 30th days. The histopathological studies were also conducted on a 25% sample of the experimental animals. The brain, pituitary gland, pancreas, liver, kidney, adrenal glands were isolated by decapitation of animals, after which the entire retroperitoneal fat containing pancreatic tissue was dissected out and fixed in freshly prepared Bouin's fluid. The tissues were cut into 2 to 4 μm/thick sections stained in Haematoxylin—Eosin and Gomori's Aldehyde Fuchsin stain with aqueous light green 0.6gm. + chromotroph 3R 0.5gm + orange G 1.0gm+Glacial acetic acid 1ml/100ml of water as counterstain. The ß cells per islet area in cross-section (2mm×350) were counted under microscope.

Experimental Groups for Diabetised Rats

GP. I	NORMAL CONTROL 0.9% (w/v) saline	— Fed on 50μl/100gm. b.w. of saline once in a day for 30 days orally.
	DYNAMISED DILUTIONS	
GP. II	CONTROL 90% (v/v) alcohol	— Fed on 50μl/100gm. b.w. of alcohol once in a day for 30 days orally.
	TEST DRUG	
GP. III	Alloxan 6x (v/v)	— Fed on 50μl/100gm. b.w. of Alloxan 6x once in a day for 30 days orally.
GP. IV	Alloxan 30x (v/v)	— Fed on 50μl/100gm. b.w. of Alloxan 30x once in a day for 30 days orally.
GP. V	Alloxan 2000x (v/v)	— Fed on 50μl/100gm. b.w. of Alloxan 200x once in a day for 30 days orally.
	UNDYNAMISED DILUTIONS	
GP. VI	CONTROL 90% (v/v) alcohol	— Fed on 50μl/100gm. b.w. of alcohol once in a day for 30 days orally.
	TEST DRUG	
GP. VII	Alloxan 6x (v/v)	— Fed on 50μl/100gm.

b.w. of Alloxan 6x once in a day for 30 days orally.

GP. VIII Alloxan 30x (v/v) — Fed on 50μl/100gm. b.w. of Alloxan 30x once in a day for 30 days orally.

GP. IX Alloxan 200x (v/v) — Fed on 50μl/100gm. b.w. of Alloxan 200x once in a day for 30 days orally.

Results

The experimental data obtained was statistically analysed using Student's "t" test. It is evident from the observations that regular administration of dynamised form of Alloxan in its 6x, 30x and 200x potencies at a dose level of 50μl/100gm. b.w. exhibited a slow and steady fall in the blood sugar level as compared to normal control and undynamised dilutions of alloxan, dynamised and undynamised alcohol fed control groups, as evident from the Table-1 and Histogram-1. Furthermore, it was also observed that hypoglycaemic potentiality of dynamised dilutions of alloxan are more pronounced and perceptible in 30x and 200x as compared to 6x potency.

The acute and sub-acute toxicity studies indicate that dynamised control group of alcohol show more toxic effects and finally lethal to the animals when compared to dynamised and undynamised dilutions of alloxan, vehicle and saline. The revival of degenerated and damaged ß-cells were not achieved perceptibly in any of the group. As such only 30-40% ß cell counts were found in functional state especially in 30x and 200x potencies of dynamised alloxan. Histomorphometric studies of brain also discern non-involvement of Hypothalamo-hypophysial pancreatic axis. The blood sugar stabilization studies of dynamised dilution of 30x and 200x potencies exhibited mild stabilization of blood sugar after withdrawal of test drug (Table-2, Graph-1, Figs 1 to 6).

Discussion

The Histopathological studies of dynamised dilutions of 30x and 200x potencies of alloxan exhibited mitosis in ß-cells which in-turn shows 30-40% of ß-cells count alongwith perceptible decrease in blood sugar level. On the contrary, the documented report of drug induced ß-cell regeneration was observed with Homoeopathic drug, Cephalandra indica Q in diabetised rats (Rastogi et al 1988). Furthermore, Chakraborty et al 1980 and 1981 discerned the similar phenomenon of selective ß-cell regenerative potentiality and protects the ß-cells against necrotic effect with Pterocarpus marsupium roxb. in diabetised rats.

The undynamised dilutions of test drug, vehicle and simple dynamised vehicle did not show any hypoglycaemic potentiality on examination of Histopathological parameters of certain cellular and neuronal components and biochemical estimations of blood. These observations clearly indicate that the mechanical potentization decreases with the material quantity of the solute. While potentising, the energy supplied by the agitation/vigorous strokes, activates the solvent system/diluent medium to acquire and mimic the chemical specificity to original drug molecule and then act as Therapeutic agent. This implies two hypothesis, firstly the action of Homoeopathic potencies will alternate in two opposite directions either "INHIBITORY" or "STIMULATORY" in BIOSYSTEMS depending upon whether the potency imitates the solute or represents the replica of it. In view of this concept and INHIBITORY action was noticed as a result of mechanical potentization of dynamised dilutions of alcohol fed control which in turn brings about maximum toxicity and ultimately the animals were fatal in the corresponding groups. Hence, the dynamised potentization process thus induced the diluent medium to acquire and then mimic the chemical specificity of alloxan. Sharma (1964) has also confirmed the anti-diabetic potentiality through controlled experiments on alloxan induced diabetes in rats with dynamised 30 and 200 potencies of alloxan. The present probe confirms the Homoeopathic principle of "Similia Similibus Curentur" in having the therapeutic potentiality as an anti-diabetic agent in dynamised dilutions of 30x, 200x of alloxan in diabetised rats and also demonstrates the phenomenon of minimum dose. Further probe in this area would be rewarding in order to locate the mechanism of action fo Homoeopathic dilutions beyond Avogadro's number.

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INFLUENCE OF DYNAMISED & UNDYNAMISED STATE OF ALLOXAN ON BLOOD SUGAR LEVEL IN DIABETISED ALBINO RATS.

TABLE- 1 (MEAN ± S.E.VALUE)

Sr. No.	ADMN.OF SALINE, VEHICLE and TEST DRUG.	DOSE ATTEMPTED : 50µl/100gm.BODY WEIGHT ONCE IN A DAY FOR 30 DAYS. I.P.								
		NORMAL CONTROL	DYNAMISED DILUTIONS				UNDYNAMISED DILUTIONS			
		GP-I	GP-II	GP-III	GP-IV	GP-V	GP-VI	GP-VII	GP-VIII	GP-IX
		0.9 % SALINE (w/v)	CONTROL 90-% ALCOHOL (v/v)	TEST DRUG ALLOXAN. 6x (v/v)	TEST DRUG ALLOXAN. 30x (v/v)	TEST DRUG ALLOXAN. 200x (v/v)	CONTROL 90 % ALCOHOL (v/v)	TEST DRUG ALLOXAN. 6x (v/v)	TEST DRUG ALLOXAN. 30x (v/v)	TEST DRUG ALLOXAN. 200x (v/v)
1-	INITIAL BLOOD SUGAR LEVEL (Fasting in mg./dil.)	30 ±3.2	320 ±7.2	290 ±3.3	310 ±5.2	340 ±6.5	300 ±8.0	310 ±5.2	320 ±6.2	300 ±7.1
2-	30th DAY BLOOD SUGAR LEVEL (Fasting in mg./dil.)	310 ±4.0	370 ±5.2 (7D)	160** ±4.2	125* ±4.5	110* ±5.3	370 ±6.5 (3D)	340 ±5.2 (4D)	360 ±5.2 (5D)	370 ±6.2 (5D)

D = Death.

* = P< 0.001 versus control - SIGNIFICANT VALUE.
** = P< 0.01 versus cobtrol - LESS SIGNIFICANT VALUE.

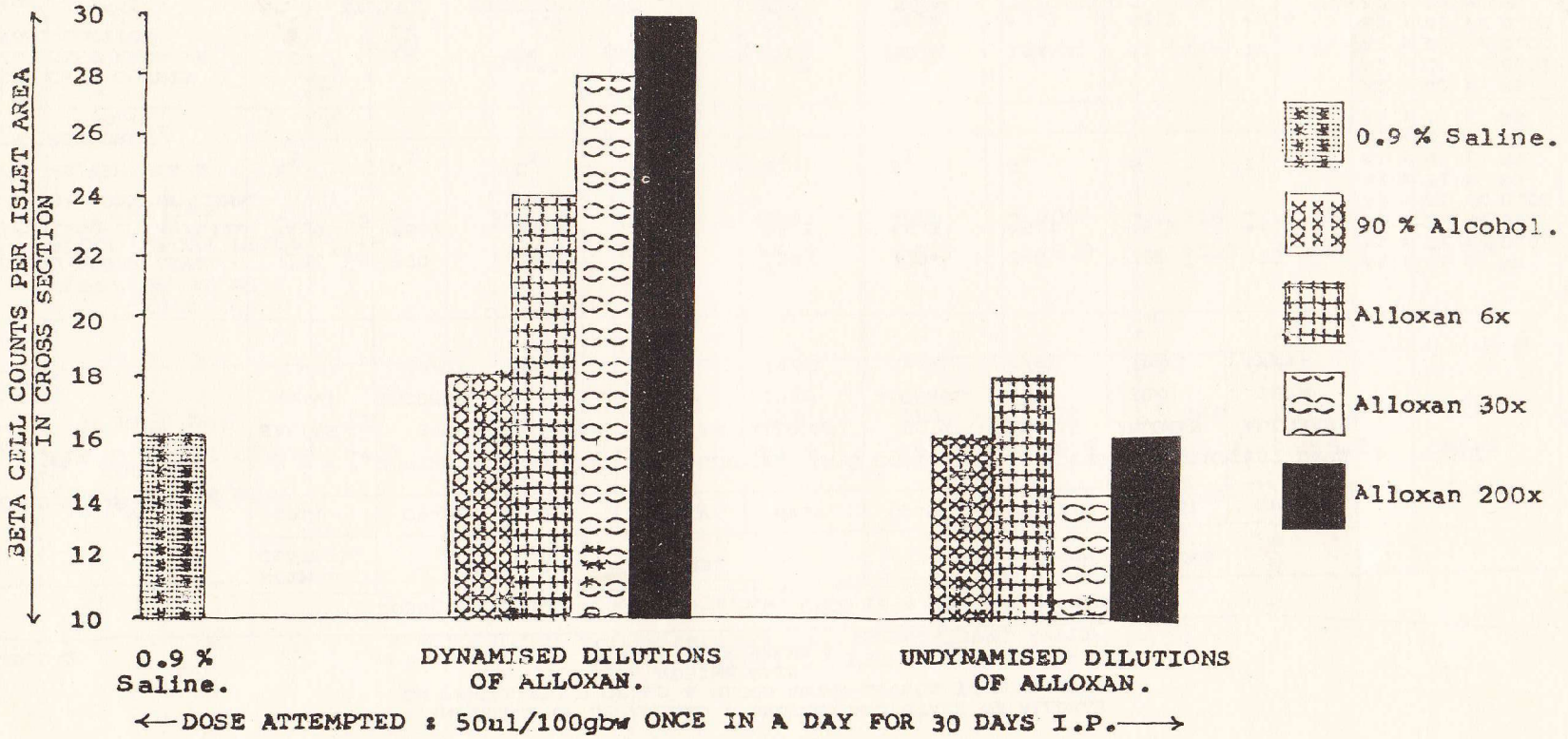
INFLUENCE OF DYNAMISED & UNDYNAMISED STATE OF ALLOXAN ON PANCREATIC β CELLS & BLOOD SUGAR LEVELS IN DIABETISED ALBINO RATS.
(MEAN ±S.E.VALUE)

TABLE-2

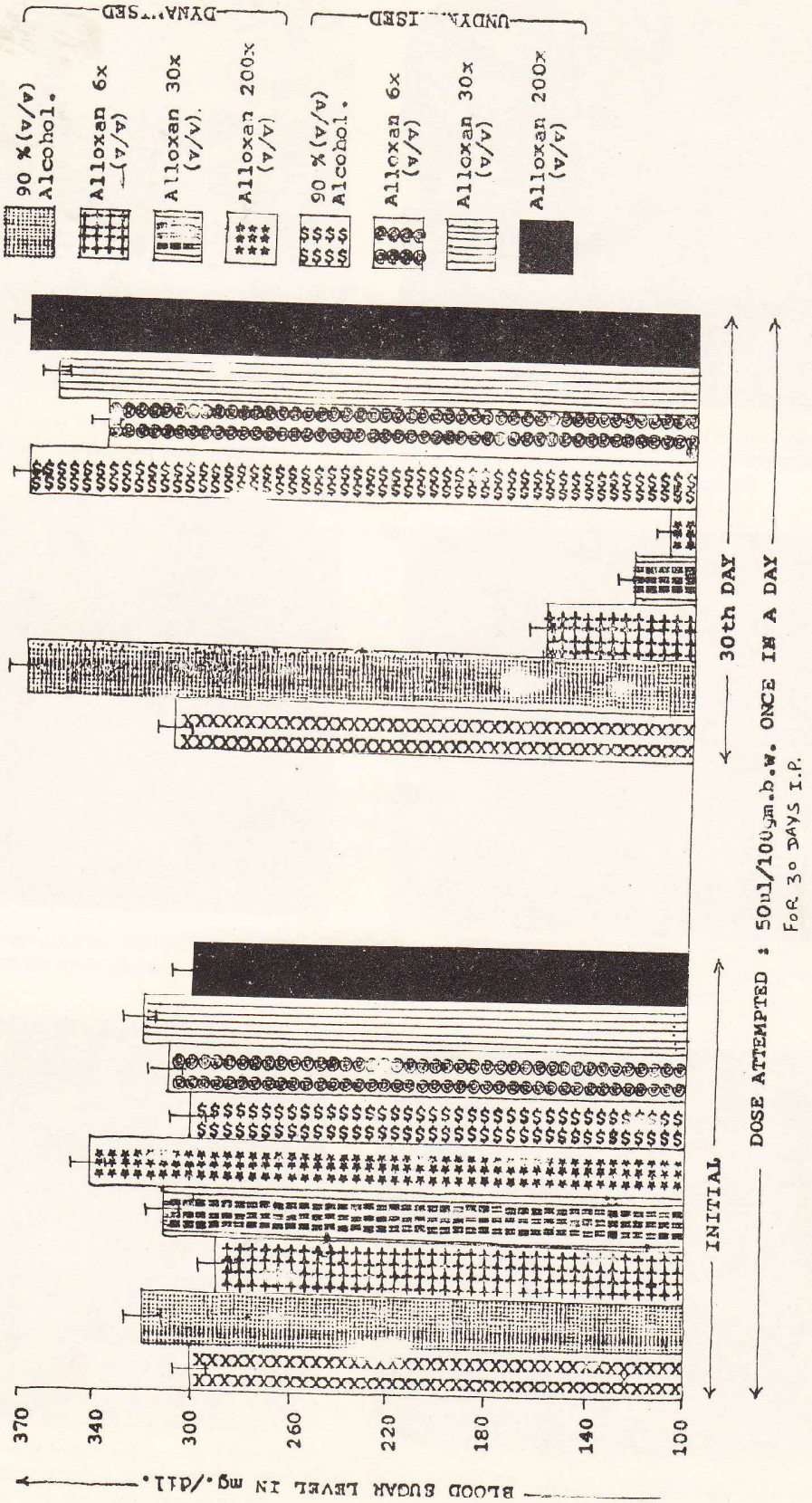
BLOOD SUGAR LEVEL/ β CELL COUNTS.	DOSE ATTEMPTED : 50µl/100gm.b.w. ONCE IN A DAY FOR 30 DAYS. I.P.									P VALUE
	NORMAL CONTROL.	DYNAMISED DILUTIONS.				unDYNAMISED DILUTIONS.				
	GP-I	GP-II	GP-III	GP-IV	GP-V	GP-VI	GP-VII	GP-VIII	GP-IX	
	0.9 % SALINE. (w/v)	CONTROL. 0 % ALCOHOL. (v/v)	TEST DRUG ALLOXAN 6x (v/v)	TEST DRUG ALLOXAN 30x (v/v)	TEST DRUG ALLOXAN 200x (v/v)	CONTROL 90 % ALCOHOL (v/v)	TEST DRUG ALLOXAN 6x (v/v)	TEST DRUG ALLOXAN 30x (v/v)	TEST DRUG ALLOXAN 200x (v/v)	
BLOOD SUGAR LEVEL (Fasting in mg/dl) ON 30th DAY OF ADMN. OF SALINE, VEHICLE & TEST DRUG.	310 ±4.0 A_1	370 ±5.2 B_1	160 ±4.2 C_1	125 ±4.5 D_1	110 ±5.3 E_1	370 ±6.5 F_1	340 ±5.2 G_1	360 ±5.2 H_1	370 ±6.2 I_1	A1 : B1 : NS A1 : C1 : < 0.01 A1 : D1 : < 0.00 A1 : E1 : < 0.00 A1 : F1 : NS A1 : G1 : NS A1 : H1 : NS A1 : I1 : NS
BETA CELL COUNTS PER ISLET AREA IN CROSS SECTION (mm^2 X 350) ON 30th DAY OF ADMN. OF SALINE, VEHICLE & TEST DRUG.	16 ±2.6 A_2	18 ±3.2 B_2	24** 4.5 C_2	28* ±3.5 D_2	30* ±2.8 E_2	16 ±4.4 F_2	18 ±3.2 G_2	14 ±2.2 H_2	16 ±2.5 I_2	A2 : B2 : NS A2 : C2 : < 0.01 A2 : D2 : < 0.00 A2 : E2 : < 0.00 A2 : F2 : NS A2 : G2 : NS A2 : H2 : NS A2 : I2 : NS

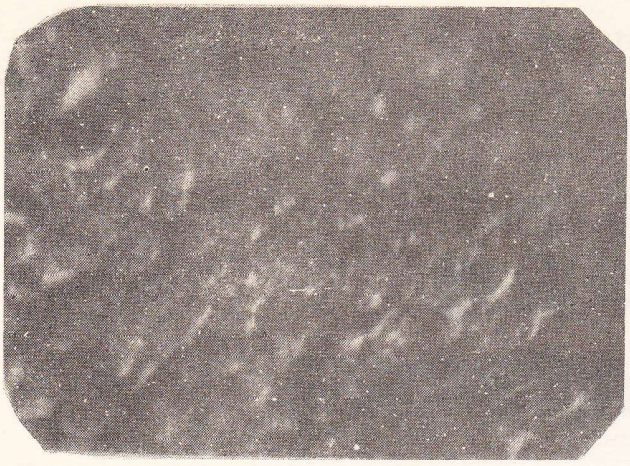
NS : NOT SIGNIFICANT VALUE.
* = P< 0.001 - VERSUS CONTROL - SIGNIFICANT VALUE.
** = P< 0.01 - VERSUS CONTROL ↓ LESS SIGNIFICANT VALUE.

HISTOGRAM SHOWING PANCREATIC BETA CELL COUNTS PER ISLET AREA AFTER 30 DAYS OF TREATMENT WITH DYNAMISED & UNDYNAMISED STATES OF ALLOXAN IN DIABETISED ALBINO RATS.

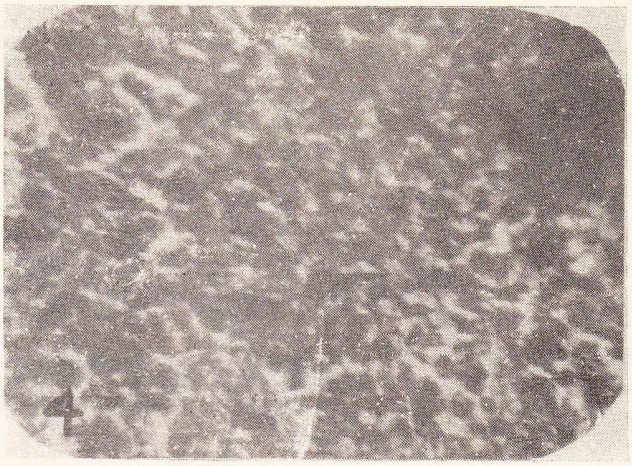


HISTOGRAM SHOWING
INFLUENCE OF DYNAMISED & UNDYNAMISED STATE OF ALLOXAN
ON BLOOD SUGAR LEVEL IN DIABETISED ALBINO RATS.





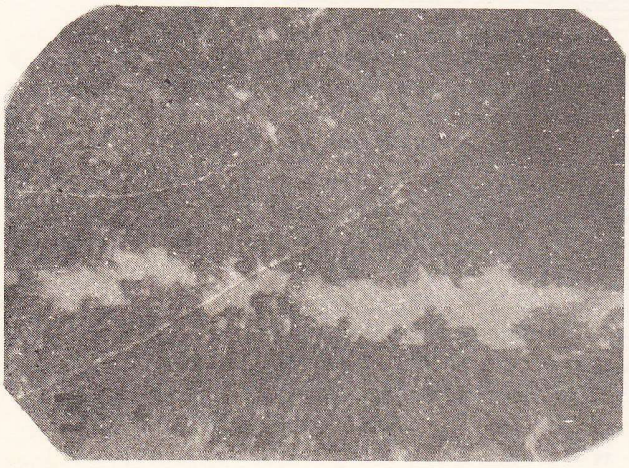
Control successive stages of pancreatic beta cells exhibited necrotic effect with degenerative changes.



Dynamised Alloxan 30x treated after 30th day with few functional pancreatic beta cell. Gomori's aldehyde fuchsin X 500.

Control pancreatic beta cell exhibited prominent necrotic effects with degenerative changes.

Dynamised Alloxan 200x treated after 30th day showing functional pancreatic beta cell. Gomori's aldehyde fuchsin X 500.



Degenerative changes with marked pancreatic beta cell necrosis after Alloxan treatment. Gomori's aldehyde fuchsin X 500.

Dynamised Alloxan 200x treated after 30th day S.S. of brain passing through hypothalamus showing perceptible secretory activity with prominent neurons. Gomori's aldehyde fuchsin X 500.