**Introduction**

Diabetes mellitus is commonest endocrinal disorder leading to hyperglycemia which in turn causes vascular complications involving vital organs. Thus, results in sudden deaths due to coronary diseases increases enormously in man and women diabetics as compared to non-diabetic subjects. Therefore, the importance of diabetes increased so much so that cardio-diabetology has become a fast emerging super specialty through out the globe. This disease results in malfunctioning of almost all organs of the body including renal, nervous, cardiovascular as well as immunological system of body and the eyes.

Diabetic state is characterized by persistent hyperglycemia associated either with insulin deficiency or impaired insulin action. The major pathological factor for all complications of diabetes is hyperglycemia leading to glycation of number of proteins and enzymes. Hence, the control of blood sugar to standard normoglycemic range is of vital importance.

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**Ultra Dynamised and Undynamised Dilutions of Alloxan at Micro-doses Influence Selective Pancreatic Beta Cell and Hormonal Profile: An Experimental Approach**

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Dynamised and Undynamised preparations of Alloxan 6x, 30x, 200x, and 1000x were examined for its anti-diabetic activities in Alloxan induced diabetes mellitus albino rats. Oral administration of dynamised potencies of Alloxan 6x, 30x, 200x, and 1000x at dose level of 50 μg/100 g.b.w. daily for 30 days regularly exhibited slow and steady fall in blood sugar level accompanied with perceptible increase in serum growth hormone (GH) i.e. p < 0.01 (less significant) and p < 0.001 (significant) respectively when compared to dynamised and Undynamised control as well as Undynamised Alloxan fed groups under identical conditions. Histological and histo-morphometric studies also revealed reactivation of Pancreatic β-cells. Dynamised dilutions of Alloxan acts steadily through hypothalamo-hypophysial-pancreatic β-cells axis producing selective reactivation of β-cells at micro-doses, steadily viz 6x < 30x < 200x < 1000x. The drug may indirectly release Releasing factors (RF) from hypothalamic neurons, stimulating the secretion of growth hormone which in turn triggers optimum insulin secretion from β-cells. The therapeutic action of the test drug in dynamised dilutions at micro dose and relatively high dilutions on pancreatic β-cells confirms the phenomenon of “Potentization” & “Similia Similibus Curentur” and lack of acute and sub-acute toxicity at fairly large dosage may open up new prospects in the treatment of diabetes mellitus and throw light in elucidating the mechanism of action at higher dilutions. It was noticed that the dynamised dilutions of alcohol fed control group is more toxic and lethal to animals than the dynamised and Undynamised dilutions of Alloxan and Undynamised alcohol fed control groups. Furthermore, it was also discernible that blood sugar and growth hormone levels were stabilized even after withdrawal of test drug in its 30x, 200x and 1000x potencies.

**Key words:** alloxan; ultra dynamised dilutions; anti-diabetic activity in albino rats.

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importance for the prevention of these complications. There are many treatment modalities available for diabetes or “Madhumeh” in homoeopathy. Some of these medicines have been evaluated clinically as well. Many of these preparations are found to be most effective; however, their proper evaluation has not been done using modern scientific parameters so as to acceptable at the global level.

Homoeopathic drugs act by the principle of “Similia Similibus Curentur”. The drugs, Thyroidinum and Lac defloratum have been reported to cause and cure diabetes mellitus. About 100 remedies for the cure of diabetes mellitus and insipidus, out of these “A” grade remedies are Lyco., Phos., Plb., Syzyg., Tarent., Ther., Uran-N are reported.

Besides these, Cephalendra indica mother tincture was also proved to be most affective and anti-diabetic drug tested in alloxan induced diabetes rats. Dynamised dilution of alloxan 30C in alloxan induced diabetes rats through controlled experimental trials and it was discerned that that potentised dilutions 30C possessed perceptible anti-diabetic activity as compared to undynamised sample dilution of the same and vehicle fed control.

Homoeopathic drug preparations are made either in the liquid or in the solid form by a process called Potentisation and Trituration. In Potentisation 3 scales are used, Decimal scale denoted by letter ‘X’ (1/10). The first potency should contain one tenth part of the original drug and each succeeding potency should contain one tenth part of the potency preceding it. Centesimal scale denoted by letter ‘C’. The first potency should contain one hundredth part of the original drug and each succeeding potency should contain one hundredth part of the potency preceding it. Fifty millesimal scale, the fifty millesimal scale is based on the principle that 100 globules weighing sixty five mg (l grain) and such 500 globules, can hardly absorb one drop for their saturation. So the proportion of medicine to alcohol will be 1 to 50,000 (1/500 X 100). In all the three scales the successive strengths are raised by mechanically or manual successions strictly 10 times with 15lb pressure uniformly.

The present experiment was designed with a view to discern the therapeutic efficacy of dynamised and undynamised dilutions of Alloxan, a chemical, its commercial name is Mesoxalyurea 5-Oxobarbituric acid and IUPAC name is 1,3-Diazinane-2, 4, 5,6-tetrone with molecular formula C₄H₂N₂O₄, molecular weight 142.07, purity 98.5% Fig. 1.

![Fig. 1: 1, 3-Diazinane-2, 4, 5,6-tetrone](https://example.com/diazinane.png)

It is chiefly used in its ability to produce diabetes mellitus in experimental animals. Keeping in view of Homoeopathic principle of “Similia Similibus Curentur” and “Potentization”, an attempt has been made to discern the curative characteristics / therapeutic potentiality of potentised Alloxan in its 6x,30x, 200x and 1000x potencies, with special reference to biological/hormonal aspects and phenomenon of minimum dose.

Materials and Methods

To study the hypoglycaemic activity of dynamised and undynamised drug, vehicle and normal saline, albino rats of either sex weighing 150±25g were acclimatized 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 acclimatized 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.

Each animal was weighed and blood was collected from the tail vein for blood sugar and serum Growth hormone estimations before commencement of the experiments. All the animals were administered intra-peritoneal injection of Alloxan 12 mg/100g.b.w. dissolved in distilled water in three doses (Sigma Chemical Co., U.S.A.) at an interval of 7 days in order to avoid trauma formation.

On 30th day after alloxan injection, blood from all the animals were analyzed and when blood glucose level elevated beyond 250 mg/dl. The potentised form of Alloxan 6x, 30x, 200x and 1000x as well as equivalent concentration of vehicle i.e. 90% v/v alcohol, 0.9% physiological saline and dynamised/ undynamised preparations were made as per Homoeopathic Pharmacopoeia of India (Fig. 2). The diabetised rats were divided into groups of 10 each as follows.
**Experimental Groups for Diabetised Rats**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Normal Control</th>
<th>Fed on 50μl/100g.b.w. of saline once in a day for 30 days orally</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP-I</td>
<td>0.9% (w/v) Saline</td>
<td></td>
</tr>
</tbody>
</table>

**Dynamised Dilutions:**

<table>
<thead>
<tr>
<th>GROUP -II</th>
<th>Control: 90% (v/v) Alcohol</th>
<th>Fed on 50μl/100g.b.w. of dynamised Alloxan 6x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

**Test Drug,**

<table>
<thead>
<tr>
<th>Group – III</th>
<th>Alloxan 6x (v/v)</th>
<th>Fed on 50μl/100g.b.w. of dynamised Alloxan 6x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Group – IV</th>
<th>Alloxan 30x (v/v)</th>
<th>Fed on 50μl/100g.b.w. of dynamised Alloxan 30x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Group – V</th>
<th>Alloxan 200x (v/v)</th>
<th>Fed on 50μl/100g.b.w. of dynamised Alloxan 200x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

| Group – VI | Alloxan 1000x (v/v) | Fed on 50μl/100g.b.w. of dynamised Alloxan 1000x once in a day for 30 days orally. |

**Undynamised Dilutions**

<table>
<thead>
<tr>
<th>GROUP -VII</th>
<th>Control: 90% (v/v) Alcohol</th>
<th>Fed on 50μl/100g.b.w. of undynamised Alcohol once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Test Drug,</th>
<th>Group – VIII</th>
<th>Alloxan 6x (v/v)</th>
<th>Fed on 50μl/100g.b.w. of undynamised Alloxan 6x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Test Drug,</th>
<th>Group – IX</th>
<th>Alloxan 30x (v/v)</th>
<th>Fed on 50μl/100g.b.w. of undynamised Alloxan 30x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Test Drug,</th>
<th>Group – X</th>
<th>Alloxan 200x (v/v)</th>
<th>Fed on 50μl/100g.b.w. of undynamised Alloxan 200x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Test Drug,</th>
<th>Group – XI</th>
<th>Alloxan 1000x (v/v)</th>
<th>Fed on 50μl/100g.b.w. of undynamised Alloxan 1000x once in a day for 30 days orally.</th>
</tr>
</thead>
</table>

**Fig. 2: Method of Test Drug Preparation**

1g. (Alloxan) = 1g. sugar of milk

↓

Mix and rub for 6 min (a)

↓

Scrap from mortar and pestle and stir the mixture for 4 min. (b)

↓

Again rub for 6 min. And stir for 4 min. After scrapping

↓

Add 3g. of sugar of milk

↓

Repeat process (a) and (b) twice in the same order

↓

Add 5g. of sugar of milk

↓

Repeat Process (a) and (b) twice in the same order

↓

1X
The experiment was conducted over 45 days but the drug, vehicle & saline were administered regularly for first 30 days once in a day to each animals. Blood samples were collected from the tail vein on 15th & 30th day of treatment for determination of blood glucose and serum GH level. All the blood sugar estimations were done after 12 hours fasted animals using Spectrophotometers, Serum GH estimations were determined by Chemiluminescence immuno assay (Luminometer LB9501/16) using the kits supplied by Nicholas Institute Diagnostic, USA.

Two animals of all the groups were sacrificed on 30th day of treatment and pancreatic tissue were dissected out, quickly fixed in Bouin’s fixative. The paraffin sections 4 mm thicknesses were stained in Haemotoxylin-eosin (HE) and Gomoris Aldehyde Fuchsin (GAF) stain.

**Table 1: Influence of Dynamised & Undynamised State of Alloxan on Blood Sugar and Growth Hormone Levels in Diabetised Albino Rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Blood Sugar Level on 1st Day (fasting in mg/dil.)</th>
<th>Blood Sugar Level on 30th Day</th>
<th>Initial growth hormone (in ng/m)</th>
<th>Growth Hormone Level on 30th Day (in ng./ml)</th>
<th>Beta Cell counts islet area in cross section (mm$^2$ x 350) on 30th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP-1 0.9% Saline (w/v)</td>
<td>50m1/100g.</td>
<td>340±5</td>
<td>350±5</td>
<td>0.320±0.005</td>
<td>0.220±0.04</td>
<td>12±2</td>
</tr>
<tr>
<td>GP-2 Control 90% Alcohol (v/v)</td>
<td>50m1/100g.</td>
<td>330±4</td>
<td>340±4</td>
<td>0.302±0.002</td>
<td>0.288±0.022</td>
<td>16±3</td>
</tr>
<tr>
<td>GP-3 Alloxan 6x (v/v)</td>
<td>50m1/100g.</td>
<td>310±5</td>
<td>140±5**</td>
<td>0.295±0.003</td>
<td>0.452±0.001**</td>
<td>20±3**</td>
</tr>
<tr>
<td>GP-4 Alloxan 30x (v/v)</td>
<td>50m1/100g.</td>
<td>320±4</td>
<td>125±3**</td>
<td>0.312±0.002</td>
<td>0.589±0.042**</td>
<td>24±2*</td>
</tr>
<tr>
<td>GP-5 Alloxan 200x (v/v)</td>
<td>50m1/100g.</td>
<td>330±5</td>
<td>110±3*</td>
<td>0.327±0.001</td>
<td>0.612±0.004*</td>
<td>30±3*</td>
</tr>
<tr>
<td>GP-6 Alloxan 1000x (v/v)</td>
<td>50m1/100g.</td>
<td>330±3</td>
<td>95±4*</td>
<td>0.340±0.002</td>
<td>0.765±0.002*</td>
<td>32±4*</td>
</tr>
<tr>
<td>GP-7 Control 90% Alcohol (v/v)</td>
<td>50m1/100g.</td>
<td>335±5</td>
<td>350±5</td>
<td>0.258±0.0019</td>
<td>0.188±0.002</td>
<td>18±2</td>
</tr>
<tr>
<td>GP-8 Alloxan 6x (v/v)</td>
<td>50m1/100g.</td>
<td>325±4</td>
<td>330±5</td>
<td>0.284±0.012</td>
<td>0.109±0.042</td>
<td>16±3</td>
</tr>
<tr>
<td>GP-9 Alloxan 30x (v/v)</td>
<td>50m1/100g.</td>
<td>330±5</td>
<td>340±6</td>
<td>0.350±0.002</td>
<td>0.218±0.014</td>
<td>15±2</td>
</tr>
<tr>
<td>GP-10 Alloxan 200x (v/v)</td>
<td>50m1/100g.</td>
<td>340±5</td>
<td>345±4</td>
<td>0.390±0.021</td>
<td>0.180±0.002</td>
<td>14±3</td>
</tr>
<tr>
<td>GP-11 Alloxan 1000x (v/v)</td>
<td>50m1/100g.</td>
<td>325±6</td>
<td>335±6</td>
<td>0.310±0.011</td>
<td>0.205±0.002</td>
<td>19±2</td>
</tr>
</tbody>
</table>

*P< 0.001 versus Control: Significant Value
**P< 0.001 versus Control: Less Significant Value
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**HISTOGRAM 2**

**EFFECT OF DYNAMISED & UNDYNAMISED DILUTIONS OF ALLOXAN ON GROWTH HORMONE LEVELS IN DIABETISED ALBINO RATS**

* Group 1 normal saline
* Group 2 90% alcohol
* Group 3 Alloxan 6x
* Group 4 Alloxan 30x
* Group 5 Alloxan 200x
* Group 6 Alloxan 1000x
* Group 7 90% alcohol
* Group 8 Alloxan 6x
* Group 9 Alloxan 30x
* Group 10 Alloxan 200x
* Group 11 Alloxan 1000x

**Fig. 4: GRAPHICAL ILLUSTRATION SHOWING INFLUENCE OF DYNAMISED & UNDYNAMISED DILUTIONS OF ALLOXAN ON GROWTH HORMONE LEVELS IN DIABETISED ALBINO RATS**

*P < 0.001 versus Control: Significant Value
**P < 0.01 versus Control: Less Significant Value
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HISTOGRAM: 3

EFFECT OF DYNAMISED & UNDYNAMISED DILUTIONS OF ALLOXAN ON BETA CELL COUNTS IN DIABETISED ALBINO RATS

- Beta cell count on 30th day

Group 1: normal saline
Group 2: 80% alcohol
Group 3: Alloxan 6x
Group 4: Alloxan 30x
Group 5: Alloxan 100x
Group 6: Alloxan 200x
Group 7: Alloxan 1000x
Group 8: Dose attempted 50µl/100g.b.w once in a day for 30 days orally

*D < 0.001 versus Control: Significant Value
**P < 0.01 versus Control: Less Significant Value

Fig. 5: GRAPHICAL ILLUSTRATION SHOWING INFLUENCE OF DYNAMISED & UNDYNAMISED DILUTIONS OF ALLOXAN ON BETA CELL COUNTS IN DIABETISED ALBINO RATS

- Beta cell count on 30th day

Group 1: normal saline
Group 2: 80% alcohol
Group 3: Alloxan 6x
Group 4: Alloxan 30x
Group 5: Alloxan 100x
Group 6: Alloxan 200x
Group 7: Alloxan 1000x
Group 8: Dose attempted 50µl/100g.b.w once in a day for 30 days orally

*P < 0.001 versus Control: Significant Value
**P < 0.01 versus Control: Less Significant Value
Further, it was also observed that hypoglycaemic potentiality and growth hormone profile of Dynamised dilutions of Alloxan are more pronounced and perceptible in 200x and 1000x, test of significance P<0.001 as compared to 6x, and 30x and P<0.01 were noticed

The acute and sub-acute toxicity studies indicate that dynamised vehicle control group show more toxic effects and finally lethal to animals when compared to dynamised and undynamised dilutions of Alloxan, undynamised vehicle and saline. Histo-morphometrical studies of brain also discern the involvement of Hypothalamo-hypophysial pancreatic β-cells axis. The blood sugar stabilization studies of dynamised dilution of 30x, 200x and 1000x potencies exhibited stabilization of blood sugar after withdrawal of dynamised test drug for 10-25 days. The β-cells count, blood sugar and growth hormone level in various groups of alloxanised rats are reflected in table I. It is evident from the table that alloxan brings about necrosis of β-cells and reduces the β-cell counts along with an increase in blood sugar and decrease in growth hormone level. Whereas, dynamised dilutions of Alloxan brings about β-cell counts, blood sugar and growth hormone levels within normal range at a dose level of 50μg/100 g,b.w. Histo-pathological and histo-morphometric studies (plate. 1) of brain revealed certain hypothalamic neurons having specific structural peculiarities and containing neuro-secretory material (nsm) with prominently nucleated bipolar-neurons. These structural specificities were identified by special techniques of tinctorial affinities (developed in our laboratory by combination of Periodic-Acidic Schiff's & Acid Fuschin Stains) (pAS+AF).These neurons are juxtasomal with blood vessels and responsible for the secretion of releasing factors (RF) or inhibitory factors (IF) for growth hormones (GR). Hypo-secretion of GH probably inhibits insulin secretion and produce diabetes mellitus by damaging the b-cells.

However, in the present experimentation it was noticed that the dynamised dilution of Alloxan had brought about hypertrophy and degranulation in
hypothalamic neurons and probably release the GH - RF factors which in turn release the growth hormone secretion from the adenohypophysis. This may have resulted in the restoration/reactivation of β-cell counts. Whereas, vehicle fed control, normal saline and undynamised dilutions had shown hypotrophy and granulation in hypothalamic neurons and release the GF-IF factors which in turn inhibited growth hormone secretion from the adenohypophysis and finally damaged the pancreatic β-cells.

The dynamised dilutions of Alloxan stabilized blood sugar level, β-cell counts and growth hormone profile even after withdrawal of drug for 10 -25 days in 65% cases at micro-doses of Alloxan 30x < 200x< 1000x and test of significance i.e. P: < 0.01, < 0.001 respectively were discernible.

Discussion

The Histopathological studies of dynamised dilutions of 6x, 30x, 200x and 1000x potencies of Alloxan exhibited mitosis in β-cells which in turn shows 50-60% of b-cells count along with perceptible decrease in blood sugar and an increase in growth hormone level. On the contrary, the documented report of drug induced β-cell regeneration was observed with Homoeopathic drug, Cephalandra indica Q in diabetised rats⁵. Furthermore, similar phenomenon of selective β-cell regenerative potentiality and production of β-cells against necrotic effect with Pterocarpus marsupium in diabetised rats were discemed⁷-⁸.

Sulphonylurea compound produced hypoglycaemic conditions III normal animals by stimulating the pancreatic β-cells to produce more insulin and thereby increasing the glycogen deposition in the liver. These drugs however, do not decrease blood glucose in Alloxan diabetic animals. In contrast to the oral anti-diabetic agents, the exogenous administration of insulin is well known to produce hypo glycaemia in Alloxan- diabetic subjects. It is therefore, conceivable that hypoglycaemic principles in Momordica charantia Q extract a direct effect in diabetic - rabbits probably by a mechanism similar to insulin⁹.

The undynamised dilutions of test drug, vehicle, saline and dynamised vehicle did not show any hypoglycaemic potentiality on examination of histopathological parameters of cellular and neuronal components and biochemical estimation of blood. These observations clearly indicate that the mechanical potentization decreases with the material quantity of the solute. While potentiising, the energy supplied by the agitation / vigorous strokes, activates the solvent system / diluent medium to acquire and mimic the chemical specificity of original drug molecule and then act as therapeutic agent. However, the above observations are in close conformity with the earlier findings⁶.

This implies two hypotheses; firstly the action of Homoeopathic potencies will alternate in two opposite directions either “Inhibitory” or “Stimulatory” in Bio-system depending upon whether the potency imitates the solutes to represent 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 mimic the chemical specificity of Alloxan. It was also confirmed that anti-diabetic activity of alloxan induced diabetised rats were influenced significantly by alloxan 30X, 200X and 1000X potencies¹⁰. The present probe confirms the Homoeopathic principle of “Similia Similibus Curentur” and “Potentization” in having the therapeutic potentiality as an anti-diabetic agent in dynamised dilutions of 30X, 200X and 1000X of Alloxan in diabetised rats and also demonstrates the phenomenon of minimum dose.

Further, probe in this area would be rewarding in order to confirm the probable mechanism of action of Homoeopathic dilutions beyond Avogadro’s number i.e. 6.023x10²³ molecule.

Acknowledgement

The authors are grateful to Central Council for Research in Homoeopathy, New Delhi, Min. of Health & Family Welfare, Govt .of India for generously financing the project for completion of present investigation. Authors are also thankful to Dr. Ramesh, RO (C), Mrs. Anshu Rathi, SRF (C) Mrs. Bhawana Bajpai, SRF (Phg.) and Sri Divya Saurabh Kushwaha, SRF (Chem.) for compilation of technical data and preparation of histograms etc.

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